CASE REPORT

Hereditary Complete Thyroxine-Binding Globulin Deficiency: Identification by T₃ Resin Uptake Test and DNA Analysis

Tsukasa Noguchi, Ikuo Yamamori*, Junta Takamatsu**, Tokuo Nakajima, Yuichi Mori*** and Yuichi Kumahara

Complete thyroxine-binding globulin deficiency (TBG-CD) was uncovered in a subject receiving a comprehensive health examination. The subject had an abnormally high T₃ resin uptake. A family study showed that the TBG abnormality had been inherited by X-chromosome linkage. Genetic analysis revealed single nucleotide deletion, common among Japanese with TBG-CD, from the allele specific amplification of the TBG genes of the family.

(International Medicine 32: 6-9, 1993)

Key words: comprehensive health examination, allele specific amplification

Introduction

Thyroxine-binding globulin (TBG), a glycoprotein consisting of 395 amino acids and 4 oligosaccharide chains, is synthesized in the liver (1). It serves as a major carrier protein of thyroid hormones in serum, and binds 70% of both thyroxine (T₄) and triiodothyronine (T₃) in serum (2). Hereditary TBG deficiency, characterized by an inborn defect of TBG, was first reported by Nicoloff et al in 1964 (3). The X-linked codominant mode of inheritance has been demonstrated (4–6), which necessitates heterozygosity only in females.

We have been performing T₃ resin uptake test as part of a screening program, which is a cost-effective way of finding abnormalities in thyroid function. It also reveals subjects with defective TBG (7). Through this program, we found a subject who had an abnormally high T₃ resin uptake. He was subsequently diagnosed by further examinations, including family studies, as having hereditary complete TBG deficiency (TBG-CD). Accordingly, we carried out genetic analysis of the patient and the family with allele specific amplification (ASA) of the TBG gene, and single nucleotide deletion, which is common in TBG-CD among Japanese, was demonstrated.

Case Report

A 48-year-old man born in Hyogo Prefecture came to Sakuragaoka Hospital for a comprehensive health examination. He had been healthy all his life and was not on any medication. His height was 165 cm, and weight was 65 kg. His physical findings were as follows: body temperature 35.9°C, heart rate 65/min and regular, blood pressure 100/70 mmHg. He was physically euthyroid. There were no findings of exophthalmos, macroglossia, goiter, finger tremor, or facial or pretibial edema. Laboratory data were as shown in Table 1. Blood chemistry and urinalysis were normal. ECG, Chest X-ray, upper gastrointestinal series, and ultrasonography of the abdomen gave normal findings. However, his thyroid function was further examined because the T₃ resin uptake was abnormally high (Table 1).

Methods

Thyroid function assessment

Commercially available kits were used to measure serum concentrations of thyroid hormones as follows: for T₃ and T₄, a double antibody radioimmunoassay (RIA) (Eiken kit T₃ and T₄, Eiken, Tokyo); for free T₃ (FT₃) and free T₄ (FT₄), an analogue method RIA (Amerlex-M FT₃ and FT₄, Amersham International, Tokyo); for thyrotropin (TSH), and an immunoradiometric assay (Spac TSH, Daiichi Isotope Co., Tokyo). Serum TBG was determined by RIAgnost TBG kit (Hoechst Japan, Tokyo) and by a highly sensitive en-
DNA Analysis of a TBG Deficient Family

zyme immunoassay kit kindly provided by Amano Pharmaceutical Co., Aichi, Japan.

Analysis of TBG gene

Genomic DNAs were extracted from peripheral white blood cells as described elsewhere (8). TBG genes of the subjects were amplified by polymerase chain reaction (PCR) (9). Three primers (C, N, and M) were chemically synthesized (DNA synthesizer, model 381A, Applied Biosystems, Foster City, CA) to detect the nucleotide deletion we reported in the TBG genes of six unrelated Japanese families with TBG-CD (10). PCR with primers C and N give a 297 base pair fragment exclusively for the normal TBG gene, while the mutant allele lacking cytosine at the first base of codon 352 is amplified only in the combination of primers C and M (Table 2). Amplification will occur with both sets of primers in heterozygotes, who have both normal and mutant alleles. PCR, polyacrylamide gel electrophoresis, staining and visualization of the amplified fragment were carried out as described previously (10).

Results

Serum FT4 and TSH levels of the subject were within normal ranges, although total T4 and total T3 were abnormally low (Table 3, No. 2). Serum FT3 was mildly decreased. Serum TBG concentration determined with RIAgnost TBG kit was less than 5.0 mg/l. It was even less than 0.1 mg/l according to a highly sensitive enzyme immunoassay. Thus, the family study was carried out, as shown in Fig. 1.

The serum TBG level of his older brother (Table 3, No. 1) was around the detection limit according to conventional RIA, but was in fact, undetectable by a highly sensitive EIA. He was thus supposed to be complete TBG deficient as the proband was. He also showed an abnormally high T3 resin uptake. His serum total T4 level was low. Serum TSH was in the normal range. The data for one of two younger sisters were all normal (Table 3, No. 3). The serum TBG of the other sister (Table 3, No. 4) was detectable but it was about half of

<table>
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<th>No.</th>
<th>Sex</th>
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<th>FreeT4</th>
<th>FreeT3</th>
<th>TotalT4</th>
<th>TotalT3</th>
<th>TBG (RIA)</th>
<th>TBG (EIA)</th>
<th>TSH mU/l</th>
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<td>M</td>
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<td>12–30</td>
<td>15.6–29.1</td>
<td>0.24–3.70</td>
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![Fig. 1. Pedigree of the family. Symbols used are as follows: □, male; ○, female; ■, serum TBG undetectable; ●, serum TBG within normal range; ●, serum TBG decreased; □, ○, not examined. The proband is indicated by an arrow. Numbers given for each person correspond to those in Table 3.](image)
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Fig. 2. Polyacrylamide gel electrophoresis results. N and M denote the primer specific to the normal and mutant allele, respectively. Molecular weight marker (φX174 digested by Hae III) is also shown in the leftmost lane. The size of each fragment is as follows: 1353, 1078, 872, 603, 310, 271, 234, 194, 118, and 72 base pairs. The last 3 fragments are not visualized on this photo.

that of normal subjects. Her serum total T₄ and total T₃ were at the lower limits of the respective normal ranges. T₃ resin uptake was within the normal range. The serum TSH levels of all the subjects were normal.

PCR resulted in amplification of the 297 base pair fragment only with the mutant primer in the proband and in his brother, whose serum TBG were undetectable (Fig. 2). In contrast, the DNA fragment was amplified only with the normal primer in his younger sister who showed a normal serum TBG value. In the youngest sister, whose serum TBG value was moderately low, clear bands were visualized both with normal and mutant primers.

Discussion

T₃ resin uptake testing as a part of a comprehensive health examination led to the diagnosis of hereditary TBG-CD in an otherwise healthy middle-aged man. He was considered euthyroid because his serum TSH was normal. The precise mechanism for the borderline elevation of serum FT₄ despite the mild depression of FT₃ remains unclear. Lack of TBG, the major transporter of T₄ in serum, may give an erroneously high value for FT₄ in analogue method RIA. The discrepancy between FT₄ and FT₃ values may be explained by the difference in the affinities of TBG to T₄ and T₃ (2). Since the serum TBG concentration determined with EIA was undetectable, it was thought that he was TBG-CD, not partial deficiency of TBG. The X-linked mode of inheritance was supported by the determination of serum TBG concentrations of the family members. Based on the X-linked transmission of the disease, his mother was thought to be heterozygous.

The TBG molecule consists of 395 amino acids and 4 asparagine-linked oligosaccharide chains (1). Its gene is located on the long arm of the X chromosome (6). The nucleotide sequence of its cDNA was reported by Flink et al (1). The molecular biological bases of hereditary TBG-CD have been studied in French Canadian (11), English (12), and Japanese (10) subjects, with the mutations reported being single nucleotide substitution at codon 227 (11) and single nucleotide deletion at codon 165 (12) or at codon 352 (10), respectively. These findings suggest that diversity exists in the molecular biological mechanisms of this hereditary condition (13).

Yamamori et al reported a single nucleotide deletion at codon 352 in six unrelated Japanese families with TBG-CD (10). No other mutations of the TBG gene are known to exhibit TBG-CD in Japanese. We analyzed for the presence of this mutation and demonstrated that it exists in this family. Consanguinity was not found between the current and previously reported families.

Based on neonatal screening programs for hypothyroidism, the incidence of TBG-CD is more common in Japanese (1:1,200 to 1:1,900) (14, 15) than in Caucasians (1:5,000 to 1:13,000) (16–18). The difference in the incidence of TBG-CD among races may simply reflect the time when each mutation appeared.

The ASA we applied in this work is based on the concept that a mismatch in the 3’end of the primer with the genomic DNA leads to unsuccessful amplification. It is an efficient method of screening for the presence of known mutations. It can also be applied to investigations of sickle cell anemia (19) or familial amyloidotic polyneuropathy (20). Only a small proportion of all mutations is detectable through restriction fragment length polymorphism, because it necessitates generation or disappearance of a recognition sequence for specific restriction endonucleases. ASA is advantageous in the screening of known mutations because it does not have such limitations.

The thyroid function of subjects with TBG-CD is thought to be normal with no abnormal signs and symptoms, and thus they may not visit medical facilities. At first, the present case was identified by a routine T₃ resin uptake test in a comprehensive health examination. This test is less accurate than newly developed thyroid function tests, but reveals the abnormal increase in patients with hyperthyroidism, TBG deficiency, or with generalized resistance to thyroid hormones (7). Although serum free thyroid hormone concentrations must be measured to evaluate thyroid function abnormalities, TBG-CD cannot be detected without determining serum total T₄, TBG or T₃ resin uptake. The T₃ resin uptake test is the only method available for evaluating thyroid function and binding protein at the same time. It is also simple and cost effective. Thus, despite the limitation that subjects with a mild decrease
of TBG may be overlooked, determination of T3 resin uptake is useful as a screening test (7).

Acknowledgements: We thank Mr. Shigeki Kimura at Amano Pharmaceutical Co. for preparing and providing the EIA kit for TBG determination. We also thank Mrs. Judy Noguchi for her helpful editing.

References

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