Mass Screening for Complete Deficiency of Thyroxine-Binding Globulin in Adult Japanese by Comprehensive Health Examination

Tsukasa Noguchi, Tetsuro Miki*, Junta Takamatsu**, Tokuo Nakajima and Yuichi Kumahara

The incidence of complete thyroxine-binding globulin (TBG) deficiency (TBG-CD) was determined for a Japanese population from a comprehensive health examination, in which a T3 resin uptake test of the upper 5% (78 subjects from among 1,589 men) was the screening line for TBG-CD. Further analysis of the known mutation in TBG-CD gene of the Japanese population (reported as TBG-CDJ with codon 352 deletion) was performed on 72 subjects, and three were found to have TBG-CDJ, two of whom were siblings. Only those three subjects had a serum TBG concentration of less than 5 mg/l. The six subjects for whom the DNA analysis was not performed, did not have a serum TBG level of less than 5 mg/l. From these findings, the gene frequency of TBG-CDJ was calculated to be 0.13%. The incidence of TBG-CDJ in the total Japanese population is suggested to be 0.09%.

Key words: T3 resin uptake test, polymerase chain reaction, thyroid hormones, mutation, gene frequency, DNA analysis

Introduction

Thyroxine-binding globulin (TBG), the major transport protein for thyroid hormones in human serum, is synthesized in the liver. It consists of 395 amino acids and 4 asparagine-linked oligosaccharide chains (1), and binds 70% of the thyroxine (T4) and triiodothyronine (T3) in human serum (2). The TBG gene is located on the long arm of the X chromosome (3), which is composed of five exons (4, 5).

Complete TBG deficiency (TBG-CD) was first reported by Nicoloff et al in 1964 (6). Yamamori et al reported a single nucleotide deletion at codon 352 (TBG-CDJ) in six unrelated Japanese families with TBG-CD (7). A frameshift by a nucleotide deletion at codon 352 of TBG-CDJ results in a 22 amino acid truncation and a further 22 amino acid divergence at the C terminus. This mutation causes loss of biological activity. The mechanism has been evaluated in an expression experiment (8). TBG-CDJ is synthesized as a truncated molecule and retained within the rough endoplasmic reticulum, resulting in complete absence of secretion.

The incidence of TBG-CD has been investigated in neonatal screening programs for hypothyroidism (9–13), in which TT4 has been used. TBG-CD was found in the group of low TT4.

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However, in these studies, molecular genetic analysis for TBG-CD was not carried out. The genotype of TBG-CD was not investigated. We previously reported a single family with hereditary TBG-CD from data of an abnormally elevated T3 resin uptake test that had been included as a screening for thyroid function (14). Subsequently, we performed TBG screening for the adult Japanese population by performing the T3 resin uptake test in comprehensive health examinations, which was the aim of the present study. Further, we carried out molecular genetic analysis related to TBG-CD and determined the genotype of TBG-CD.

Materials and Methods

Comprehensive health examination program

From April 1990 to March 1991, 1,589 men underwent comprehensive health examinations at Sakuragaoka Hospital. As part of a screening program, we included the T3 resin uptake test in these examinations. Among the subjects showing a high T3 resin uptake test, the upper 5% (78 males) were selected for...
measurement of serum TBG concentration and for molecular genetic analysis by polymerase chain reaction (PCR) (15) of the TBG gene.

**Thyroid function tests**

Serum concentrations of free T4 and TSH were measured, as well as titers of anti-thyroglobulin antibody and anti-thyroid microsomal antibody for 3 cases whose serum TBG concentrations were less than 5.0 mg/l. The serum TBG concentrations were determined by RIAgnot TBG kit (Hoechst Japan, Tokyo). Serum free T4 concentrations were determined by analogue method RIA (Amerlex-M FT4, Amersham International, Tokyo). Serum TSH concentrations were determined by immunoradiometric assay (Spac TSH, Daiichi Isotope Co., Tokyo). The titers of anti-thyroglobulin antibody were determined by gelatin-particle agglutination test (Serodia-ATG, Fujirebio Inc., Tokyo). The titers of anti-thyroid microsomal antibody were determined by gelatin-particle agglutination test (Serodia-AMC, Fujirebio Inc.). In three cases where the serum TBG concentrations were less than 5.0 mg/l in the RIA, the TBG concentration was further measured by a highly sensitive chemiluminescent enzyme immunoassay (CLEIA) (Lumino-master TBG, Sankyo Co., Tokyo).

**DNA analysis**

The DNA analysis was performed by a method which was different from the method reported previously (14). Genomic DNA was extracted from peripheral white blood cells as described elsewhere (4). Oligonucleotide primers (F and R) were designed to amplify the part of exon 4 including codon 352 (7). With oligonucleotide primers F and R, a DNA fragment of 95 or 94 base pairs was amplified by PCR. The PCR products were digested with EcoRI, electrophoresed in 8% polyacrylamide gels, and visualized with UV light after staining with ethidium bromide.

**Results**

**Serum concentration of TBG and DNA analysis**

As shown in Table 1, serum TBG concentrations were determined for 63 males. The remaining 15 subjects could not be examined due to sample loss. Serum TBG concentrations of less than 5.0 mg/l were obtained for three subjects and, as shown in Fig. 1, the PCR products were digested with EcoRI in all

<table>
<thead>
<tr>
<th>No.</th>
<th>T3 resin uptake (RIA) %</th>
<th>TBG (RIA) mg/l</th>
<th>Free T4 pmol/l</th>
<th>TSH mU/l</th>
<th>Anti-thyroid microsomal antibody X</th>
<th>Anti-thyroglobulin antibody X</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;50.0</td>
<td>&lt;5.0</td>
<td>24.0</td>
<td>1.85</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>2</td>
<td>&gt;50.0</td>
<td>&lt;5.0*</td>
<td>27.0</td>
<td>1.52</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>3</td>
<td>&gt;50.0</td>
<td>&lt;5.0</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
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<tr>
<td>Normal range</td>
<td>22–35</td>
<td>12–30</td>
<td>10–27</td>
<td>0.24–3.70</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
</tbody>
</table>

No. 2 and No. 3 were brothers. NM: not measured. *Serum TBG concentration of No. 2 by CLEIA was less than 0.5 mg/l.

**Table 1. Gene Analysis of Complete TBG Deficiency and Serum TBG Concentrations in Subjects with High T3 Resin Uptake Test from a Comprehensive Health Examination Program. The Subjects were Separated Based on Whether or Not Digestion with EcoRI Occurred**

<table>
<thead>
<tr>
<th>DNA analysis performed</th>
<th>Serum TBG (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>digested</td>
<td>&lt;5  &gt;5  N.M.</td>
</tr>
<tr>
<td>not digested</td>
<td>3    3    0    0</td>
</tr>
<tr>
<td>DNA analysis not performed</td>
<td>6   0   54   15</td>
</tr>
</tbody>
</table>

N.M.: indicates not measured.

**Figure 1. Polyacrylamide gel electrophoresis.** P, PCR products; E, fragments after EcoRI digestion; M, size markers of HindIII digested φX174. No. 1, 2, 4 and 5 were the results of TBG-C, in which the 95 bp fragments were not digested with EcoRI. No. 3 was the result of TBG-CDJ, in which the 94 bp fragments were digested with EcoRI, presenting a single band of 74 bp.
cases. The serum TBG concentrations of 60 subjects were more than 5.0 mg/l. Among them, DNA analysis was performed in 54 cases. Their PCR products were not digested with EcoRI. DNA analysis was not performed in the remaining 6 cases due to loss of peripheral white blood cell samples. In the remaining 15 subjects whose serum TBG concentrations were not determined, the PCR products were not digested with EcoRI.

**Thyroid function in subjects with TBG deficiency**

As shown in Table 2, of the three subjects with serum TBG concentrations of less than 5.0 mg/l, two were brothers (No. 2 and No. 3). T3 resin uptake tests of the three subjects gave results of more than 50%. Serum TSH, FT4, anti-thyroid microsomal antibody and anti-thyroglobulin antibody titers were determined for two subjects (No. 1 and No. 2). Their serum TSH and FT4 levels were within normal ranges, as were their anti-thyroid microsomal antibody and anti-thyroglobulin antibody titers. The thyroid function of the third subject was not examined. By CLEIA, the serum TBG concentration of one male (No. 2) was less than 0.5 mg/l.

**Discussion**

Two methods have been reported for identifying the deletion of the first base c at codon 352 of the TBG gene. One method, used here, employs primers designed to form the restriction site of EcoRI in the PCR product from the mutant gene (7). The other method uses allele-specific primers (14, 16).

The presence of the same mutation with TBG-CDJ was first checked by PCR for the three cases with TBG concentrations of less than 5.0 mg/l. When the same mutation with TBG-CDJ could not be confirmed, all of the nucleotide sequences of the TBG gene had to be determined. But the three subjects were TBG-CDJ, which is reported to be a common cause of TBG-CD in Japanese (16). The 60 males whose serum TBG concentrations were greater than 5.0 mg/l, could not have been TBG-CD. The PCR products of the 15 cases with unknown serum TBG concentrations were not digested with EcoRI, indicating that these cases were not TBG-CDJ. In such cases, there may be unknown types of TBG-CD gene other than TBG-CDJ. However, the possibility of a subject having a serum TBG concentration of less than 5.0 mg/l, is less than one (15×(3/63)) based on the data that the serum TBG concentration was less than 5.0 mg/l in 3 cases among 63 patients.

The molecular biological bases of hereditary TBG-CD have been studied in French Canadian (17), English (18), and Japanese (7) subjects, with the mutations reported being a single nucleotide deletion at codon 352 (7), respectively. All of the TBG-CD in Japanese for whom genetic analysis was done, including our 3 TBG-CD cases, harbored the single nucleotide deletion at codon 352. Furthermore, this has been detected only in Japanese, suggesting that the mutation appeared in the ancestors of the Japanese after the divergence of the human races and spread among Japanese descendants by the founder effect. The linkage disequilibrium by haplotype analysis should clarify the founder effect, which has been investigated in other diseases including myotonic dystrophy (19, 20) and cystic fibrosis (21).

As two of the three TBG-CDJ males were siblings, the calculated gene frequency was 2 in 1,588, or 0.13%. We could not inquire the number of siblings in the denominator. But, even if there were 100 pairs of siblings, the re-calculated gene frequency should be 0.13% (2/1,488). As our comprehensive health examination is a workshop examination, the rate of siblings may be even smaller. Consequently, the calculated gene frequency of TBG-CDJ is expected to be virtually the true frequency.

In evaluating the incidence of complete TBG deficiency, it should be recognized that TBG-CD is inherited in the X-linked codominant mode, and thus females carrying TBG-CD alleles are usually recognized as having TBG partial deficiency due to the heterozygousness. Homozygous females can be recognized as having TBG complete deficiency, but such a probability is almost negligible, being (0.0013)^2 = 0.00000169 from the data of gene frequency. The ratio of both sexes in the Japanese in the 1990’s, was almost 96.5:100 (male:female) (22). Accordingly, we corrected the number of females based on the ratio among the Japanese in this period, i.e., the number of males multiplied by 100/96.5 was 1,647. This 1,647 multiplied by 0.00000169 was 0.0028, which is extremely small. By adding 1,647 to the denominator, the incidence of TBG-CDJ was calculated to be 0.09%. This incidence is almost equal to that found in neonatal screening of the Japanese (1:1,200 to 1:1,900) (9, 10), and is higher than in Caucasians (1:5,000 to 1:13,000) (11–13).

TBG-CDs of previous reports of neonatal screening in Japan were not studied further regarding genotypes. Thus some other types of mutant TBG-CD genes may exist in such subjects. However, in the present study, all subjects with TBG deficiency had the TBG-CDJ gene. This suggests that TBG-CD with a mutation of the TBG gene other than TBG-CDJ must be extremely rare. Thus, the incidence of TBG-CD seems to be almost identical with that of TBG-CDJ in the Japanese population.

**Acknowledgements:** We thank Judy Noguchi for her helpful editing.

**References**


TBG Deficiency in Japanese


