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□ Case Report □

A Single Nucleotide Deletion resulting in Frameshift in Two Korean Neonates with Thyroxine-Binding Globulin Deficiency

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Abnormalities in the levels of thyroxine-binding globulin (TBG) are not associated with clinical disease and they do not require treatment. Congenital TBG deficiency is inherited in an X-linked manner. To date, some complete and partial TBG variants and one polymorphism have been identified by analysis of the TBG gene. Two male neonates were referred to us because of their low T_4 levels that were noted on the neonatal screening test. They showed normal levels of free T_4 and TSH. Their serum TBG was not detectable and those values of their parents were within the normal ranges. The genomic DNA was extracted from their white blood cells and the four coding exons of the TBG gene were amplified by using polymerase chain reaction. Sequencing of the four coding regions and all the intron/exon junctions revealed a single nucleotide deletion of the first base of the codon 352 of the mature protein in both of the neonates. This mutation resulted in a frameshift and a premature stop codon (TGA) 374. Their mothers were shown to be heterozygotes. We detected a single nucleotide deletion resulting in a frameshift in two male Korean neonates who had complete TBG deficiency. **(Korean J Pediatr 2005;48:1252-1255)**

Key Words : Thyroxine binding globulin deficiency, Deletion, Neonate

Introduction

Thyroxine-binding globulin (TBG) is a 54-kDa glycoprotein that is synthesized by the liver[1]. It is composed of a single polypeptide chain made of 395 amino acids. TBG is the major transport protein for thyroid hormones in the serum. The human TBG gene is located on the X chromosome (Xq22.2)[2], and it consists of five exons spanning 5.5 kbp. Its structure and sequence are homologous to those of the genes encoding plasma serine protease inhibitors and cortisol-binding globulin[3]. The first exon (exon 0) is a short noncoding sequence[4].

Hereditary TBG abnormalities are manifested as complete TBG deficiency, partial TBG deficiency or TBG excess. The prevalence of TBG deficiency varies from 1 : 3,000 to 1 : 15,000[5, 6]. By definition, complete TBG deficiency is the absence of detectable TBG in the serum of affected hemizygous males, as based on the currently available technology that can detect TBG levels as low as 0.03 % of the normal levels in adults (16 mg/L). Partial TBG deficiency is characterized by a diminished, but not absent TBG level. In subjects with TBG deficiency, the serum levels of total T_4 and T_3 are low, but free T_4, free T_3 and TSH levels are normal. Although TBG deficiency is a harmless condition, it may cause undue concern among parents and physicians, resulting in unnecessary evaluation and therapy for a presumed hypothyroid condition[7].

Several mutations have been identified in those people with TBG deficiency[8]. We detected a single nucleotide deletion in the coding region of the TBG gene that resulted in a frameshift and premature termination in two Korean neonates.

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Case Report

Korean male neonates were referred to Department of Pediatrics, The Catholic University of Korea, St. Mary's Hospital because of their low total T_4 levels. They were 3 weeks and 4 weeks old, respectively, and findings on physical examination were normal. They were diagnosed as having complete TBG deficiency. The diagnosis of TBG deficiency was based upon the following findings; clinical euthyroidism, low serum levels of total T_4 associated with normal serum TSH and free T_4 levels, and undetectable immunoreactive TBG in their serums (Table 1). The serum TBG was measured by employing a radioimmunoassy kit (RIA®-gnost TBG, CIS bio international, Cedex, France)

The genomic DNA was obtained by extraction it from the peripheral blood mononuclear cells. Four exons (exon 1-4) and the adjacent exon/intron junctions of the TBG gene were amplified by using a Gene AMP PCR System 2400 (PERKIN ELMER corporation, California, USA), and with using the oligonucleotide primers (Table 2) and PCR conditions as previously described[9]. The PCR products were isolated and subjected to direct DNA sequencing.

Sequencing of the four coding regions and all the intron/exon junctions revealed a single nucleotide deletion of the first base of the codon 352 of the mature protein in both of the neonates. This mutation resulted in a frameshift and a premature stop codon (TGA) 374 (Fig. 1). Their mothers were both tested and shown to be heterozygotes.

Table 1. Laboratory Findings of the Subjects

	Age (weeks)	Total T_4 (ng/dL)	Total T_3 (ng/dL)	Free T_4 (μg/dL)	TSH (mIU/L)	TBG (mg/L)
Patient 1	3	2.64	201	2.12	5.00	ND
Patient 2	4	1.69	172	1.86	2.03	ND

ND : not detectable

Table 2. Sequences and Locations of the Oligonucleotide Primers Used for Sequencing of the Thyroxine Binding Globulin (TBG) Gene

Primer	Sequence	Location
1A	5′-CCCTGATGAGCACATCATCA-3′	-151 to -132
1B	5′-CAGTGGAGCAGATCACTGTG-3′	-731 to 750
2A	5′-CCTTGATCCTCAGTVACTTG-3′	1524 to 1542
2B	5′-CTTGGCATATTCTAGTGATC-3′	2064 to 2083
3A	5′-AAGCTTGATATGGTGATTGC-3′	2626 to 2645
3B	5′-TCAGAACTGCATCTCACCA-3′	3071 to 3089
4A	5′-CGCCAGAGAATGATTGACTA-3′	3180 to 3199
4B	5′-CCATTGCAATACACACGTGC-3′	3580 to 3599

The numbers refer to exons and the letters indicate complementarity to the sense (B) or antisense (A) strand.

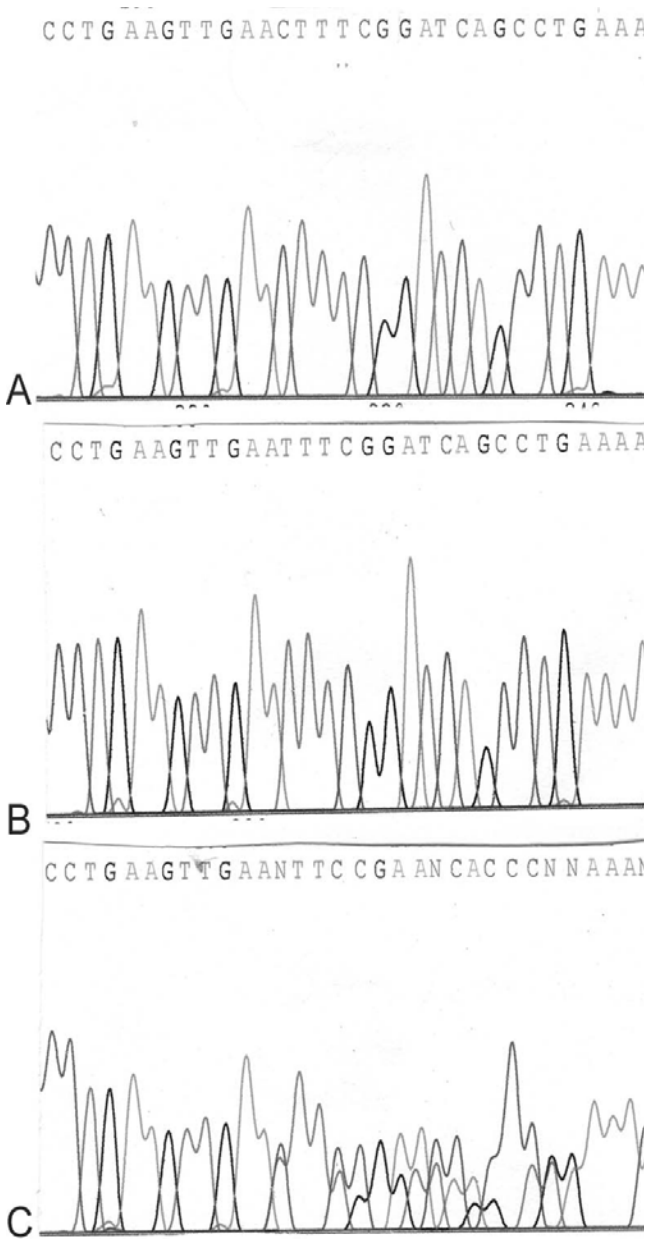

Fig. 1. Direct sequencing of the TBG gene exon 4 showing the codons from 348 through 358 (A, normal). The exonic mutation of the case consists of a single nucleotide deletion of the first base (arrow) of the codon for residue 352 of the mature protein (B). As a consequence of a frameshift, a termination codon (TGA) appeared on codon 374 (not shown here). The sequence of the mother is shown to be heterozygous (C).

Discussion

The TBG variants are inherited in an X-linked fashion, and this is consistent with the presence of a single TBG gene on the long arm of the X-chromosome (q21-22). Therefore, the defective TBG phenotypes are fully expressed in males. Meanwhile, the females carrying both the normal and variant genes exhibit TBG levels that are intermediate to those TBG levels found in the normal and affected hemizygotes.

Recent molecular studies of the TBG gene have found that there are no mutational hot spots for familial TBG deficiency, and there is no correlation between the degree of TBG deficiency and the location of the mutation[10]. To date, many TBG variants have been identified that produce TBG-CD. These include TBG-CD5, TBG-CD6, TBG-CDJ (Japan), TBG-CDY (Yonago), TBG-CDB (Buffalo), TBG-CDBe (Bedouin), TBG-CDN (Negev) and TBG-CDK (Kankakee). Variable phenotypes have been observed in association with the selective inactivation of the X-chromosome carrying one of the two alleles[11]. The defects included a single amino acid substitution that produced abnormal post-translational processing (TBG-CD5). The other five single nucleotide substitutions or nucleotide deletions produced truncated molecules that were caused by the early termination of translation[12].

Several variant partial TBG defects have been described that are associated with altered TBG binding of T_4. These defects, including TBG-SD, TBG-G, TBG-M, TBG-A, TBG-Q and TBG-PDJ, are characterized by decreased serum TBG concentrations, as measured by immunoassay, and increased concentrations of denatured TBG, as identified on the basis of the protein's decreased heat stability[13].

The mutation detected in our patients is the same mutation (TBG-CDJ) that has been described in six Japanese families with complete TBD deficiency[14]. This TBG mutant has a deletion at the first base of the codon for amino acid 352, and this leads to premature termination that produces a TBG molecule of 373 amino acids. Expression of the cDNA in the COS-1 cells revealed the complete absence of TBG secretion.

The increased prevalence of the genetic variants for TBG deficiency have been reported in certain populations (e.g. TBG-A in Australian Aborigines and TBG-S in African American). According to the results of conducting gene screening for the TBG deficiency in 50 Japanese subjects[15], all the male subjects manifesting complete TBG deficiency and all the female subjects manifesting complete TBG deficiency were demonstrated to be hemizygotes and heterozygotes for the TBG-CDJ mutation, respectively. Considering these results together with our results, TBG-CDJ is thought to be a rather common mutation in both Koreans and Japanese with TBG deficiency.

In Korea, several cases of TBG deficiency have been reported[16], but the prevalence of complete and partial TBG deficiency is not known. More extensive study will be necessary to reveal the prevalence and the genetic characteristics of TBG deficiency in the Korean population.

한 글 요 약

단일 뉴클레오타이드 결손으로 인한 Frameshift 돌연변이로 규명된 티록신결합글로불린 결핍증 1례

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TBG 결핍증은 X 염색체 장완의 TBG 유전자의 돌연변이에 의해서 발생하며, 낮은 총 T_4와 총 T_3, 정상 유리 T_4와 유리 T_3, 정상 TSH 농도를 특징으로 한다. 혈청 티록신글로불린 농도에 따라 완전 TBG 결핍증과 부분 TBG 결핍증으로 나눌 수 있으며, 적절하게 진단하지 못하면 불필요한 검사나 치료의 요인이 될 수 있다. 저자들의 완전 TBG 결핍증으로 진단된 2명의 남아에 대하여 TBG 유전자 분석을 시행하였다. 대상아들은 신생아 선별검사에서 측정된 낮은 총 T_4 농도 때문에 내원하였다. 진찰 소견은 정상이었으며, 갑상선 기능 검사 상 유리 T_4, TSH 농도는 정상이었다. 방사면역측정법에 의한 혈청 TBG는 측정되지 않았다. 중합효소연쇄반응을 이용하여 4개의 TBG 유전자 엑손을 증폭한 후 자동염기서열분석을 시행하였다. 두 대상아에서 모두 엑손 4의 352번째 codon의 첫 번째 단일 뉴클레오티드 C의 결손에 의한 frameshift 돌연변이로 374번째 codon에 termination codon이 나타난 것을 확인하였다. 대상아의 어머니들에게서는 돌연변이 대립유전자와 정상 대립유전자의 이형접합체를 확인하였다. 한국인 TBG 결핍증의 역학과 유전적 특성을 규명하기 위한 더 광범위한 연구가 필요할 것으로 생각된다.

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