Two Novel Mutations in the *Serpina7* Gene Are Associated with Complete Deficiency of Thyroxine-Binding Globulin

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Key Words

Thyroid · Thyroxine · Thyroxine-binding globulin · *Serpina7*

Abstract

Background: Thyroxine-binding globulin (TBG) is the main transport protein for T₄ in blood. Until now, 22 mutations leading to complete TBG deficiency (TBG-CD) have been reported. **Objective:** We report two mutations associated with TBG-CD found in patients from Andrews, S.C., USA (TBG-CD-Andrews), and Berlin, Germany (TBG-CD-Berlin). **Methods:** Automated chemiluminescence immunoassays were used for the determination of TSH, free and total T₄ and T₃ (fT₄, TT₄, TT₃) and TBG. Direct DNA sequencing was used to identify the TBG mutations in the probands. **Results:** TBG-CD-Andrews was found in a 1-month-old boy who was euthyroid with normal TSH and fT₄, but reduced TT₄, indicating TBG deficiency. TBG was not detectable, confirming TBG-CD. No mutation in the coding region and the promoter of the TBG gene was found, but a single nucleotide substitution in intron 1 disrupts the donor splice site of exon 0 (IVS1+2T>C). Another mutation was found in an 11-year-old boy. He was also euthyroid with normal fT₄ and TSH. However, TT₄ and TT₃ were low, suggesting TBG-CD. Sequencing revealed a 79-nucleotide deletion, ranging from intron 3

into exon 3. **Conclusion:** We report two novel mutations of the TBG gene associated with TBG-CD. Whereas most TBG-CDs are caused by small deletions, in TBG-CD-Andrews the disruption of a donor splice site was detected, whilst in TBG-CD-Berlin the largest deletion in the *Serpina7* gene to date was found.

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Introduction

In serum, thyroid hormone (TH) is mainly bound to transport proteins. The major proportion of circulating TH, 75% of T₄ and 70% of T₃ is bound to thyroxine-binding globulin [TBG; HUGO Gene Nomenclature Committee approved name: serpin peptidase inhibitor, clade A (alpha-1 antiprotease, antitrypsin), member 7, *Serpina7*]. TBG is encoded by a single gene located on the long arm of the X chromosome (Xq21–22) and synthesized in the liver as a 54-kDa protein of 415 amino acids [1, 2]. The mature protein consists of 395 amino acids after cleavage of a 20-amino-acid signal peptide.

By determination of TBG concentrations, TBG variants can be classified as excess and partial or complete

Fig. 1. **a** DNA sequence of TBG-CD-Andrews (end of exon 0 and beginning of intron 1). The single nucleotide substitution found in the proband is printed in bold underneath the wild-type sequence. **b** The pedigree of the TBG deficiency family from Andrews. The mother is heterozygous for the T>C mutation, the proband is hemizygous and the daughter inherited the normal allele (the arrows indicate the mutation).

deficiency, the latter defined by serum levels of TBG in radioimmunoassay or T_4 -binding analysis below the current limit of detection [3]. Free hormone levels are normal, but routinely used assays may give erroneous results in the absence of TBG. TBG deficiency follows an X-linked pattern of inheritance [4]. Heterozygous carrier females for a complete TBG mutation usually have about half the normal TBG concentration. The prevalence of complete TBG deficiency (TBG-CD) is approximately 1:15,000 newborn males [4]. So far, 22 mutations in the *Serpina7* gene leading to TBG-CD have been described. Here, we report two novel mutations associated with TBG-CD, a donor splice site mutation found in a boy from Andrews, S.C., USA, and a 79-nucleotide deletion in a boy from Berlin, Germany.

Material and Methods

Thyroid Function Tests

Automated chemiluminescence assays were used for the determination of TSH, free T_4 , free T_3 , total T_4 and total T_3 (fT₄, fT₃, TT₄ and TT₃; Andrews: Advia Centaur, Siemens, Malvern, Pa., USA; Berlin: Roche Elecsys, Basel, Switzerland) and TBG (ARUP Laboratories, Salt Lake City, Utah, USA).

Sequencing of the TBG Gene

Genomic DNA was isolated from peripheral blood leukocytes after informed consent was obtained. The promoter, the noncoding exon 0, the coding exons 1–4 and adjacent exon-intron junctions of the TBG gene were amplified by PCR (primer sequences are available upon request). The PCR products were used for automated sequencing with fluorescent dye terminators (ABI Prism System 377, Applied Biosystems, Foster City, Calif., USA). The sequences were compared with the published TBG DNA sequence (NG 021252; <http://www.ncbi.nlm.nih.gov/>).

Results

Patients and *Serpina7* Mutations

A discrepancy for free and total TH, indicating TBG deficiency, was found in a 1-month-old boy from Andrews, S.C., USA, who was euthyroid with normal TSH 2.4 mIU/l (normal 0.35–5.50) and fT₄ 1.2 ng/dl (normal 0.8–1.9) but reduced TT₄ 1.6 µg/dl (normal 4.5–12.0; table 1). TBG was not detectable at <3.5 µg/ml (normal 13–30), confirming TBG-CD. No mutation in the coding region and the promoter of the TBG gene was found, but a single nucleotide substitution in intron 1 (IVS1 + 2T>C) disrupts the donor splice site of exon 0 (fig. 1a). The pro-

Fig. 2. Above: the intron 3/exon 3 boundary region of the TBG gene. Below: deletion of 79 nucleotides found in a patient from Berlin.

Table 1. Thyroid function tests of the probands and their mothers

	Propositus Andrews	Mother Andrews	Reference range Andrews	Propositus Berlin	Mother Berlin	Reference range Berlin
TT ₄ , µg/dl	1.6 ^a	3.0 ^a	4.5–12.0	2.2 ^a	4.3 ^a	6.2–14
TT ₃ , ng/ml				0.56 ^a	0.55 ^a	1.0–2.16
fT ₄ , ng/dl	1.2	1.0	0.8–1.9	2.0 ^a	0.78	0.7–1.9
fT ₃						
TSH, mIU/l	2.4	1.86	0.35–5.5	0.9	2.6	0.3–4.0
TBG, µg/ml	<3.5		13–30			

^a Abnormal values.

positus' mother is heterozygous for this mutation and his sister inherited the normal allele (fig. 1b). His mother was euthyroid (TSH 1.86 mIU/l, 0.35–5.50; fT₄ 1.0 ng/dl, 0.9–1.8), but her TT₄ was also below the reference range (3.0 µg/dl, 4.5–12.0).

A different mutation was found in an 11-year-old boy from Berlin, Germany. He was euthyroid with a normal fT₄ 2.0 ng/dl (normal 0.7–1.9) and TSH 0.9 mIU/l (normal 0.3–4). TT₄ and TT₃ were low at 2.2 µg/dl and 0.56 ng/ml (normal 6.2–14 and 1.0–2.16), respectively, suggesting TBG-CD. Examination of the boy's family showed that the mother was euthyroid with TSH 2.6 mIU/l (normal 0.3–4) and fT₄ 0.78 ng/dl (normal 0.78–1.8) with low total thyroid hormone levels, TT₄ 4.3 ng/dl (normal 5.3–11) and TT₃ 0.55 ng/dl (normal 1.0–2.16), compatible with heterozygosity regarding the TBG mutation. Sequencing revealed a 79-nucleotide deletion, including the last 28 nucleotides of intron 3 and the first 51 nucleotides of exon 3 (fig. 2).

Discussion

TBG deficiency is usually suspected when discrepant free and total TH are found. As the *Serpina7* gene is located on the X chromosome, affected males are hemizygous for the underlying mutation and the discrepancy between free and total TH is more pronounced than in heterozygous females. It is therefore typical that the two mutations reported here were detected in boys. The mothers of both probands showed a milder biochemical phenotype with a slightly reduced TT₄, about double that of their affected sons, which is expected in heterozygosity for a TBG deficiency mutation.

To date 22 mutations have been reported in TBG-CD, reviewed in detail by Refetoff [4]. Among them are 4 splice site mutations (g.IVS1+2_insT [5], g.IVS2-2A>G [6], g.IVS4+2 [4], g.IVS4+5G>A [7]). In the patient from Andrews, thymine at the +2 position of the 5' donor splice site of intron 1 is changed to cytosine (g.IVS1+2T>C).

This represents a significant deviation from the donor splice site consensus, as thymine occurs in that position in >99% of splice sites [8]. Consequently, the splice site score described by Shapiro and Senapathy [8] to calculate the probability of splicing at a certain site is reduced from 86 for the wild-type sequence to 68 for the mutant sequence. For various splice site mutants tested in the rabbit beta globulin gene, no splicing was observed for sites with a score ≤ 70 . This mutation also closely resembles two previously reported mutations in TBG-CD: one found in a patient from Milan, Italy (TBG-Milano), with a thymine insertion at the less crucial position +3 of the same donor splice site [5], and one found in a patient from Lisle, Ill., USA, where the thymine at the +2 position of a donor splice site was also replaced (to a guanine, IVS4) [4]. The TBG-Milano and TBG-Andrews mutations are expected to disrupt the correct splicing of TBG RNA, possibly leading to either instable RNA, elongated by up to 1,621 nucleotides of unspliced intron 1, or to a severely disturbed start of translation in exon 1, resulting in TBG-CD.

Besides 4 nonsense mutations (p.S23X [9], p.Q223X [9], 2 different mutations in codon 280, p.W280X [10, 11]), 4 missense mutations have been reported (p.S52N [10], p.L227P [3], p.N233I [12], p.S370F [13]). Among the known mutations associated with TBG-CD are 10 deletions. In 8 cases, a single nucleotide was deleted, leading to frameshifts and premature stops (p.T38fsX51 [14], p.P50fsX51 [5, 15], p.V165fsX168 [16], p.D201fsX206 [17], p.L283fsX301 [18], p.A329fsX374 [18], p.352fsX374 [19], p.D28fsX51 [20]). An exception is TBG-CD-Neulsenburg, in which the deletion of 2 nucleotides led to a frame shift and elongation of the protein by 7 amino acids (p.L384fsX402 [21]). A 19-nucleotide deletion was found in TBG-CD-Harwichport [18]. The 79-nucleotide deletion in TBG-CD-Berlin, involving intron 3 and exon 3, represents the largest deletion so far associated with TBG-CD.

The biochemical definition of TBG-CD requires a serum TBG concentration below 0.005 mg/dl or 0.003% of

the average normal in an affected hemizygous male. While we had no laboratory test with sufficient sensitivity available, it is reasonable to assume that no functional TBG can be produced when the only allele lacks 79 bp involving an intron/exon border. Also, donor splice site mutations either at a less crucial position of the same splice site or of the same nucleotide and position in another intron have already provided explanations for TBG-CD in the past.

TBG deficiency changes the level of total but not of free TH and does not alter thyroid function status. The condition therefore has no clinical relevance and individuals with TBG deficiency are healthy. The free thyroid hormone levels estimated by routinely used techniques usually remain within the range of normal. However, sometimes severe TBG deficiency can affect even the free hormone estimates and lead to erroneous results. This is demonstrated by the elevated FT₄ of the propositus from Berlin (table 1). Given its prevalence of approximately 1:15,000 newborn males the possibility of TBG deficiency and incorrect free hormone estimates should be considered in the case of elevated free thyroid hormones in the presence of normal TSH, and should be excluded before assuming resistance to thyroid hormone or a TSHoma. In selected cases it may help to measure total TH or determine the free TH concentration by more direct methods, such as equilibrium dialysis, ultrafiltration or, potentially, mass spectrometry.

Acknowledgements

This work was supported by Deutsche Forschungsgemeinschaft DFG grants BI893/5-1 to H. Biebertmann, FU356/8-1 to D. Führer and MO1018/2-1 to L.C. Moeller.

Disclosure Statement

The authors declare that no competing financial interests exist.

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