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ORIGINAL ARTICLE

A novel variant in *Serpina7* gene in a family with thyroxine-binding globulin deficiency

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Abstract Thyroxine-binding globulin (TBG) carries approximately 75% of serum T4 and T3. This protein is encoded by *serpina7* gene, formerly known as *TBG* gene, localized on X-chromosome (Xq22.2). A deficiency in TBG is suspected when abnormally low serum total T4 and T3 are encountered in clinically euthyroid subjects in the presence of normal serum TSH. This condition has been associated with different *serpina7* gene mutations resulting in amino acid substitutions or truncations in the mature protein. Herein, we report a new *serpina7* gene variant in three members of the same family. It results in the replacement of the normal asparagine 233 by isoleucine and, subsequently, in disruption of a glycosylation site. Co-segregation of this new variant with undetectable levels of TBG in the hemizygous man studied and failure to recognize the same variant in 100 alleles at random, made us to consider it as the underlying cause of the TBG deficiency.

Keywords Thyroid · Thyroxine-binding globulin · Thyroid hormones · *Serpina7* gene variant

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Introduction

The main proteins that carry thyroid hormones are thyroxine-binding globulin (TBG), transthyretin (TTR or prealbumin), and albumin. TBG carries approximately 75% of serum T4 and T3 [1]. Inherited or acquired variations in the concentration and/or affinity of these proteins may produce changes in serum total thyroid hormones.

TBG is a member of the serine protease inhibitor (SERPIN) superfamily. It is a 54 kDa acidic glycoprotein, consisting of a single polypeptide chain of 395 amino acids and four oligosaccharide chains; it is synthesized in the liver [2] and is encoded by *serpina7* gene (formerly known as *TBG* gene). The gene locus is on X-chromosome (Xq22.2) [3].

The complete sequence of human *TBG* gene was first described in 1986 [4], and since then several variants have been associated with TBG defects. A review of all *serpina7* mutations reported in the literature was recently published [5].

TBG deficiency is suspected when abnormally low levels of total T4 and T3 are observed in clinically euthyroid subjects in the presence of normal TSH [6]. It is defined as complete (TBG-CD) or partial (TBG-PD), depending on serum values [6]. The majority of complete TBG defects are associated with nonsense mutations

generating precocious stop codons and truncated proteins; partial TBG defects are typically associated to single missense mutations [7].

Herein, we report a novel missense mutation in *serpina7* identified in three members (two females and one male) of the same family. According to the X-linked transmission of the defect [8], females showed a reduction in TBG levels whereas the single male lacked TBG.

Results

A novel variant in exon 2 of *serpina7* gene involving codon 233 (AAC → ATC; Asn → Ile) was identified in the three family members (Fig. 1a, b). The females presented a heterozygous condition while the male presented a hemizygous condition.

Analysis of 100 alleles, randomly chosen, revealed that all were homozygous for the wild variant as illustrated in Fig. 1b.

Discussion

Mutations randomly distributed throughout the *serpina7* gene are the underlying cause of hereditary TBG deficiency [9, 10]. Since this gene is located on the long arm of the X-chromosome (Xq22.2), familial TBG variants follow an X-linked pattern. Thus, TBG abnormalities are fully manifested in hemizygous males whereas heterozygous females may present a phenotype indistinct of normal individuals with TBG levels overlapping the normal range [8]. Nonetheless, a selective X inactivation may respond for identical phenotypes among women and men [11]. Females with XO Turner's syndrome may also express only the mutant allele [12].

So far, the studies of affected families have identified 27 different mutations in *serpina7* [5, 13] resulting in defective synthesis or changes in the physical properties or biological function of the protein. In one family, low-serum TBG concentrations were autosomally transmitted by an unknown mechanism and the patients' TBG gene was normal [14].

Herein, we report an additional missense mutation, AAC → ATC, in codon 233. This variant results in the substitution of asparagine by isoleucine thus, disrupting a consensus sequence (Asn-X-Ser/Thr) corresponding to a glycosylation site. A missense mutation in codon 52 resulting in the substitution of serine by asparagine originating a potential glycosylation site has already been reported [15].

Considering that the carbohydrate chains are important for the correct post-translational folding, secretion, and degradation of the molecule [9, 16], it is likely that the present mutation might respond for an abnormal secretion and/or rapid degradation. An altered ability to bind either T4 or anti-TBG antibody can not be ruled out. Co-segregation of this new variant with undetectable levels of TBG in the hemizygous man and failure to recognize the same variant in 100 alleles at random, made us to consider it as the underlying cause of the TBG deficiency.

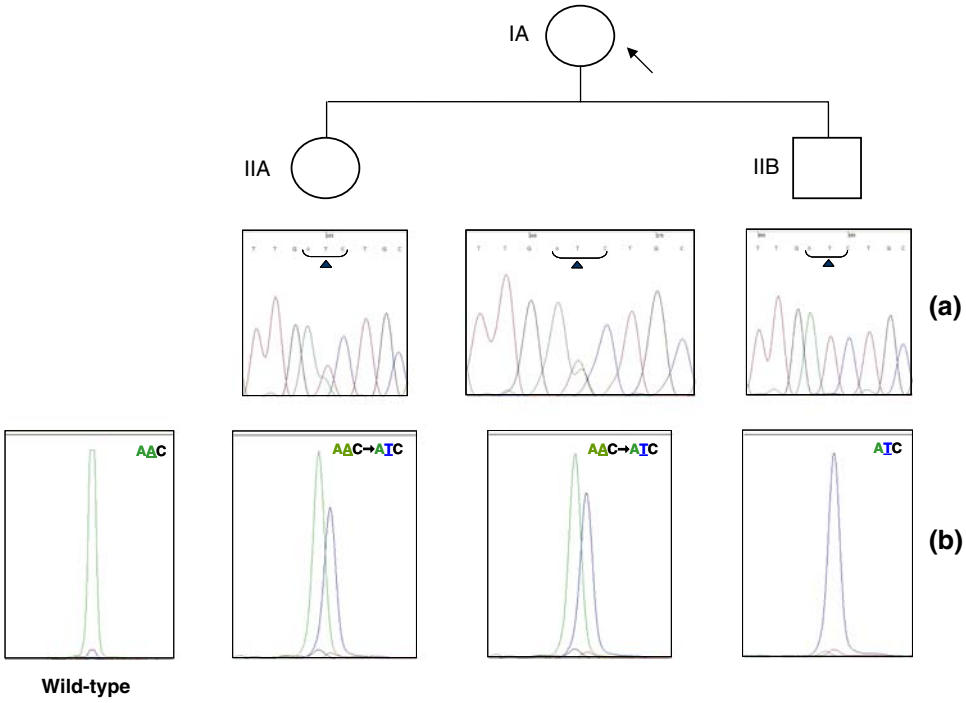

Fig. 1 Molecular features of the target family. **a** Direct sequencing analysis. Individuals IA and IIA are heterozygous for the new variant; individual IIB is hemizygous for the new variant. **b** Results of a minisequencing reaction for the AAC → ATC variant at codon 233. Wild-type (one peak occurs in the electropherogram, adenine); IA and IIA: heterozygous (two different peaks occur, adenine and thymine); IIB: hemizygous (one peak occurs, thymine)

Table 1 Thyroid function and TBG levels

Individuals	TT4	TT3	TSH	FT4	FT3	TBG
IA	38.6[a]	1.3	3.4[a]	9.0	6.3	75
IIA	78.5	1.9	2.1	12.9	6.5	233
IIB	38.6	1.3	2.3	10.3	9.4	<45
	69–142 nmol l^{-1}	0.8–2.4 nmol l^{-1}	0.5–4.7 mU l^{-1}	10–28 pmol l^{-1}	4.3–8.1 pmol l^{-1}	240–555 nmol l^{-1}

[a] Initial results for the index case; all other results were subsequently obtained

Noteworthy, is the co-segregation of the goiter trait. Thyroid enlargement is not frequent in patients with inherited TBG abnormalities. Some authors suggest that defects in TBG might enhance goiter in predisposed individuals while others consider this observation as a coincidental association [17]. In this particular family, unexpectedly, we observed low-levels of free T4. Recently, Gudmundsson et al. [18] reported a variant on 9q22.33 associated with a higher susceptibility to develop thyroid cancer as well as low levels of free T4. Taken together, it is therefore possible that besides the *serpina7* mutation, this family might carry the above variant justifying further studies in the future.

Patients and methods

The propositus, a 45-year-old Caucasian female, was referred to our outpatient clinic for evaluation of a nodular goiter. Physical examination revealed a smooth and soft left-sided nodular goiter with a dominant nodule of 3.3 cm. There was no cervical lymphadenopathy. Blood tests revealed low serum total T4 and total T3 concentrations and normal TSH. The diagnosis of TBG deficiency was subsequently confirmed (Table 1). All family members were born and live in an iodine-sufficient region. TBG was assayed using a commercial kit (IMMULITE TBG, Siemens). The patient underwent left lobectomy, following a cytological diagnosis of follicular neoplasm. The histological diagnosis was follicular adenoma.

Blood samples from the patient's daughter (a 20-year-old girl) and son (a 16-year-old boy) were obtained, for studies of thyroid function (Table 1) and genotyping. Later on, it was noticed that the girl had a thyroid nodule with approximately 1.5 cm on the left lobe corresponding to a benign cytological diagnosis (colloid nodule) whereas the boy had a thyroid nodule on the right lobe with approximately 1.5 cm corresponding to a suspicious cytological diagnosis. In both cases, the ultrasonography excluded cervical lymphadenopathy. The boy was submitted to a right lobectomy and the histological examination assigned the diagnosis of nodular hyperplasia.

DNA extraction, from peripheral blood, was performed using Puragene™ DNA Purification System Blood Kit (Gentra, USA).

Coding regions of the *serpina7* gene were amplified using intronic primers and conditions previously described [19]. PCR purified products (GFX™ PCR DNA and Gel Band Purification Kit, GE Healthcare, UK) were directly sequenced using BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA).

The minisequencing method with a specific primer (5′-cct agt gga tat gga att ga-3′) was used to screen for the *novel* variant in 100 alleles randomly chosen. Reactions were performed using the SNaPshot® Multiplex Kit (Applied Biosystems).

Separation of the sequencing and minisequencing products were performed using ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The peak signals were analyzed with proper software (Applied Biosystems).

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