



# First report of inherited thyroxine-binding globulin deficiency in Iran caused by a known *de novo* mutation in *SERPINA7*



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## ABSTRACT

**Background:** Thyroxine-binding globulin (TBG) is the main transporter of thyroid hormones in human serum, encoded by the gene *TBG* (*SERPINA7*), located in long arm of X-chromosome (Xq21-q22). Deficiency of *SERPINA7* (serum protease inhibitor, clade A [alpha-1 antiproteinase, antitrypsin], member 7) leads to inherited TBG deficiency. Several mutations have been reported in the coding and noncoding regions of *SERPINA7* in association with TBG deficiency.

**Methods:** Automated chemiluminescence immunoassays were used to determine TSH, free and total T4 and T3 (fT4, TT4, TT3) and TBG. Direct DNA sequencing identified the mutation in *SERPINA7*.

**Results:** We present a 3 and 4/12 year old boy, born premature, who was mismanaged as hypothyroidism before referral to our center, and was diagnosed with TBG deficiency at our center with a hemizygous substitution in exon 1, position c.347T>A, leading to replacement of isoleucine for arginine in position 96 (considering the first 20 amino acid signal peptide).

**Conclusion:** This known mutation, reported as the first *SERPINA7* mutation in Iran, emphasizes the point that endocrinologists should pay more attention to inherited TBG to prevent unnecessary treatment.

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## 1. Introduction

Thyroxine-binding globulin (TBG) is the main transporter of thyroid hormones in human serum. TBG is encoded by the serum protease inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7 (*SERPINA7*), the *TBG* gene, located on the long arm of the X-chromosome (Xq21-q22). *SERPINA7* loss of function mutations leads to inherited abnormalities of serum TBG that are either a complete (TBG-CD), partial (TBG-PD), or excessive (TBG-E). Since *SERPINA7* resides on the X chromosome, women with homozygous inactivating mutations and men hemizygous for a deleterious mutation usually manifest as TBG-CD, and heterozygous women as partial [1,2]. Gene duplication or triplications have been associated with TBG-E [3].

Inherited defects of TBG do not cause thyroid disease or altered metabolism. Rather, total thyroid hormone (TH) concentrations in serum

are altered while free TH remains unchanged. Medical risks associated with TBG deficiency are minimal if free TH levels are monitored, but unrecognized deficiencies may lead to unsuitable treatment and complications [4]. Based on published reports, the frequency of TBG deficiency varies from 1 in 1200 to 15,000 newborns; the exact frequency is likely underestimated due to lack of ability to detect heterozygous females and males with TBG-PD [5].

TBG is a glycoprotein consisting of 415 amino acids with four N-linked units of oligosaccharide, synthesized in the liver. The *SERPINA7* gene (OMIM + 314,200) contains 4 coding exons, spanning ~7.5 kb in the human genome. Several mutations in the coding and noncoding regions, of *SERPINA7* have been reported in association with TBG deficiency [5,6]. In this article we present the first case report of TBG deficiency in Iran.

## 2. Case presentation

The patient was a 3-year-and-four-month old boy, when seen in the endocrinology clinic with thyroid dysfunction. At the first visit, he could talk, walk, and run; his height and weight were at the 10%, and he had near normal intelligence.

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**Table 1**  
Primer pairs for *SERPINA7*.

Primer symbol	Primer sequence
SERPINA-1F	TGCATCTCTGTTTTCAAGG
SERPINA-1R	TGTCCAGTGGAGCAGATCAC
SERPINA-2F	TGGGAGACCATGACAAATGAC
SERPINA-2R	GTTTGTGATGTGCTTGGATG
SERPINA-3F	CCCTACTCCCAGTGTCTCAG
SERPINA-4R	TCAGCCAGGGTTCAATCTTC

In his past medical history, the boy was born at 27 weeks and 6 days of gestation with a weight of 760 g and an Apgar of 5 and 7 at one and five minutes. He was resuscitated and received Continuous Positive Airway Pressure (CPAP) along with prophylactic surfactant due to respiratory distress syndrome (RDS) at birth. He underwent laser therapy for prematurity retinopathy and was hospitalized for 100 days in NICU after birth. The proband had a TSH of 2.4 mIU/mL (normal range: 0.2–8.0 mIU/mL) and a T4 of 2.1 µg/dL (normal range: 6–14.7 µg/dL) at one month of age, treated with 25 µm of levothyroxine daily. At 22 months, his TSH was 0.3 mIU/L and his T4 was 3.0 µg/dL; the values reached 1.6 mIU/L and 4.0 µg/dL, respectively, at 24 months of age. At 28 months, the TSH and T4 were 3.1 mIU/L and 2.4 µg/dL, respectively. A complete blood count (CBC) test in his 30-month age showed a normal pattern. The measured levels of thyroid hormones are demonstrated in Table 3. Thyroid hormones were measured by electrochemiluminescence method with Elecsys 2010 Roche HITACHI instrument.

Upon referral to our center, the TSH and T4 levels were 10.1 mIU/L and 3.0 µg/dL, respectively. After assuring that the patient and family were compliant, it was concluded that the levothyroxine dosage (25 mg four days a week) had no effect on serum levels of thyroid hormones; the TSH remained 4.4 mIU/L and the T4 2.9 µg/dL. This prompted an investigation for genetic disorders. This study was performed in the Molecular Genetics Laboratory of Ali-Asghar Children's Hospital, Iran University of Medical Sciences, in accordance with the Declaration of Helsinki. An informed consent was obtained from the parents on behalf of the child.

### 3. *SERPINA7* gene analysis

Genomic DNA was extracted from peripheral blood cells, using a standard phenol-chloroform protocol. All four coding exons of the *SERPINA7* gene, including intron-exon boundaries, were amplified by polymerase chain reaction (PCR) utilizing the primers listed in Table 1. Single strand sequencing was performed using standard ABI3730 system (Applied Biosystems, Macrogen, South Korea) with both forward and reverse primers. Sequencing results were analyzed using Chromas version 2.4.1 software, and were aligned to the published template (ENSG00000123561) using Clustal Omega software (EMBL-EBI). Result showed a hemizygous substitution in exon 1, position c.347T>A; p.I116N (p.I96N by numbering from the first amino acid of the mature protein and consider the signal peptide as –1 to –20). The chromatogram and aligned sequence are shown in Fig. 1A–B. This change leads to substitution of a hydrophobic amino acid (isoleucine) to a polar amino acid (asparagine). After diagnosis of this mutation, treatment for the

A

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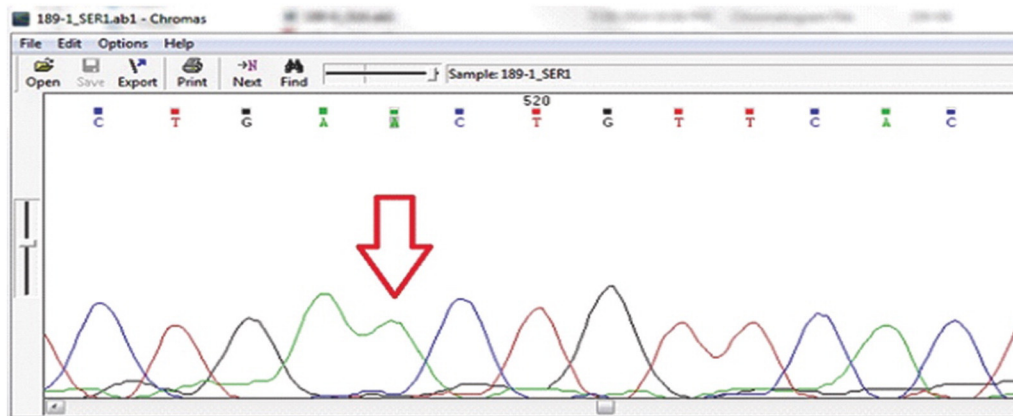
SERPINA7-1      ACCTTGGGGTTCAACCTCACAGACACTCCAATGGTAGAGATCCAGCATGGCTTCCAGCAT
A189-1          ACCTTGGGGTTCAACCTCACAGACACTCCAATGGTAGAGATCCAGCATGGCTTCCAGCAT
*****

SERPINA7-1      CTGATCTGTTCACTGAATTTTCCAAGAAGGAACTGGAATTGCAGATAGGAAATGCCCTC
A189-1          CTGAACTGTTCACTGAATTTTCCAAGAAGGAACTGGAATTGCAGATAGGAAATGCCCTC
****

SERPINA7-1      TTCATTGGCAAGCATCTGAAACCACTGGCAAAGTTCTTGAATGATGCAAGACCCCTCTAT
A189-1          TTCATTGGCAAGCATCTGAAACCACTGGCAAAGTTCTTGAATGATGCAAGACCCCTCTAT
*****

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B



**Fig. 1.** Analysis of *SERPINA7* exon 1 sequencing. A. Aligned sequence of the patient with published template (ENSG00000123561) in Clustal Omega software. B. Corresponding chromatogram (Chromas software version 2.4.1) for the region containing alterations. Red arrow shows the substituted nucleotide. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 2**  
Reported (non-large deletion) mutations in *SERPINA7* gene.

Region	Mutation	Reference	
Exon 1	S23T	[10]	
	S23X	[11]	
	D28fsX51	[12]	
	R35W	[32]	
	R35E	[33]	
	T38fsX51	[13]	
	P50fs51X	[28]	
	S52N	[14]	
	S52R	[31]	
	S61C	[31]	
	A64D	[27]	
	E74K	[31]	
	I96N	[8] & Present study	
	N112L	[31]	
	A113P	[15]	
	V165fsX168	[16]	
	D171N	[17]	
	188fsX195	[18]	
	Exon 2	T191A	[19]
		D201fsX206	[29]
V215G		[34]	
Q223X		[11]	
L227P		[20]	
N233I		[35]	
Exon 3		W280X	[11,14]
	W280fsX325	[9]	
	L283fsX321	[21]	
	L283F	[20]	
	Y309P	[22]	
Exon 4	A329fsX374	[21]	
	H331Y	[23]	
	K332fsX374	[31]	
	L352fsX374	[24]	
	P363L	[25,26]	
	D368G	[31]	
	S370F	[36]	
	Y381fsX396	[4]	
	R381G	[31]	
	S382R	[31]	
	382fsX384	[21]	
	L384fsX402	[30]	
	Non-coding	g.IVS1,+2_3insT	[5]
H(-2)Y		[9]	
+20 kb G > A		[6]	
IVS1 + 2 T > C		[30]	

patient was tapered and he is currently (after two years) not receiving levothyroxine treatment and is in good condition.

#### 4. Discussion

This study reports the first case of TBG deficiency in Iran with TBG-CD. Regarding the fact that the patient was born premature, decision

on low levels of serum T4 was not enough for initiating the treatment, as it may be due to physiologic hypothyroxinemia of prematurity [7]. For premature infants with puzzling thyroid hormone tests, FT4 is a better evaluation that should have been considered for the present patient to avoid unnecessary treatment for more than two years, which might unnecessarily predispose the infant to the adverse effects of the drug. When the patient was first visited by our team, molecular genetic analysis revealed a hemizygous substitution, c.347T>A; p.I116N (p.I96N in mature protein); thus, levothyroxine was tapered and discharged.

Isoleucine 96 resides in a highly conserved domain of the protein that is preserved among human, chimpanzee, mouse, rat, pig, bovine and sheep, and is a site of glycosylation. This variant, which is known as TBG-Gary, has been previously reported (<http://www.ncbi.nlm.nih.gov/clinvar/variation/9790/>) and associated with severe TBG deficiency. This alteration damages a potential glycosylation domain by creating a new site (Asn-Cys-Ser), therefore results in encoding a protein with impaired T4 binding ability and severe instability that causes rapid denaturation [8]. This mutation is known to cause TBG-PD, however in our hemizygote case, it leads to complete deficiency (TBG-CD).

In TBG-CD serum concentration for TBG is usually below 5 mg/L, while in TBG-PD it overlaps the normal concentration in heterozygote females. To date, about 45 mutations have been described to cause TBG deficiency, mostly in coding regions [5–6,8–36] (Table 2) (recently reviewed by Pappa et al. [31]). There does not appear to be a “hot spot”; missense and nonsense mutations are scattered in coding regions and splice sites. The majority of alterations that lead to TBG-CD are nonsense mutations that drastically change the amino acid sequence and consequently the proper function of protein [5]. Some reports also described mutations in non-coding regions, which mostly affect mRNA splicing [9, 18]. Recently, in four families that showed no mutations in *SERPINA7*, Ferrara and colleagues discovered a new variant 20 kb downstream of the gene. This alteration resides within a liver-specific enhancer, leading to reduction in the activity and consequently the expression of the *SERPINA7* [6].

#### Conflict of interest

The authors declare no conflict of interest.

#### Acknowledgements

BB and FS visited the patient, collected data, and drafted the manuscript; BB designed the experiment and confirmed the molecular analysis. FJ completed and edited the manuscript, and HF performed the PCR and analyzed the sequence data.

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**Table 3**  
The detailed thyroid hormones of the patient in different intervals.

Age	Lab item (NL range)							
	TSH, mIU/ml (0.2–8.0)	T4, µg/dl (6–14.7)	T3, ng/ml (0.75–2.0)	fT4, pmol/l (10.29–36.03)	T3RU, % (25–35%)	FTI (1.12–4.37)	T3RIA, ng/dL (80–200)	T4RIA, µg/dL (4.5–12.5)
1 month	1.5	–	–	12.87	–	–	40	1.9
2 months	2.4	2.1	0.99	25.74	31	–	70	3.3
4 months	5.8	2.2	0.6	–	35	–	–	–
19 months	0.1	5.0	0.9	41.0	–	–	–	–
22 months	0.3	3.0	0.7	23.4	37.7	1.13	–	–
24 months	1.6	4.0	–	30.2	40.1	1.70	–	–
28 months	3.1	2.4	0.6	–	–	–	–	–
30 months (4 weeks after stopping levothyroxine)	10.1	3.0	0.8	4.3	36.5	1.10	–	–
36 months (with 1/4 pills levothyroxine)	5.0	3.3	–	10.1	36.5	1.20	–	–
40 months	4.4	2.9	1.0	14.157	33	–	–	–

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