Molecular Genetics and Metabolism Reports 8 (2016) 13–16

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Case Report

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

Fahimeh Soheilipour [a], Hassan Fazilaty [b,1], Fatemeh Jesmi [c], William A. Gahl [d,e], Babak Behnam [b,d,e,*,2]

[a] Endocrine Research Center, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences, Tehran, Iran
[b] Department of Medical Genetics and Molecular Biology, Iran University of Medical Sciences (IUMS), Tehran, Iran
[c] Minimally Invasive Surgery Research Center, Iran University of Medical Sciences, Tehran, Iran
[d] NIH Undiagnosed Diseases Program, Common Fund, Office of the Director, NIH, Bethesda, MD, USA
[e] Office of the Clinical Director, NHGRI, National Institutes of Health, Bethesda, MD, USA

ARTICLE INFO

Article history:
Received 11 April 2016
Received in revised form 6 June 2016
Accepted 6 June 2016
Available online 11 June 2016

Keywords:
TBG
SERPINA7
Mutation
Iran

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 TGB 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.

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 TGB 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.

* Corresponding author at: NIH Undiagnosed Diseases Program, National Human Genome Research Institute (NHGRI), National Institute of Health (NIH), Bethesda, MD 20892, USA.
E-mail addresses: Babak.Behnam@nih.gov, b_behnam@yahoo.com (B. Behnam).
[1] Present address: Instituto de Nuerociencias CSIC-UMH, San Juan de Alicante, Spain.
[2] Present address: NIH Undiagnosed Diseases Program, Office of the Clinical Director, NHGRI, National Institutes of Health, Bethesda, MD, USA.

http://dx.doi.org/10.1016/j.ymgmr.2016.06.001

Table 1
Primer pairs for *SERPINA7*.

Primer symbol	Primer sequence
SERPINA-1F	TGCATCTCCTGTTTTTCAAGG
SERPINA-1R	TGTCCAGTGGAGCAGATCAC
SERPINA-2F	TGGGAGACCATGACAAATGAC
SERPINA-2R	GTTTGTTGATGTGCTTGGATG
SERPINA-3F	CCCTACTCCCAGTGCTTCAG
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

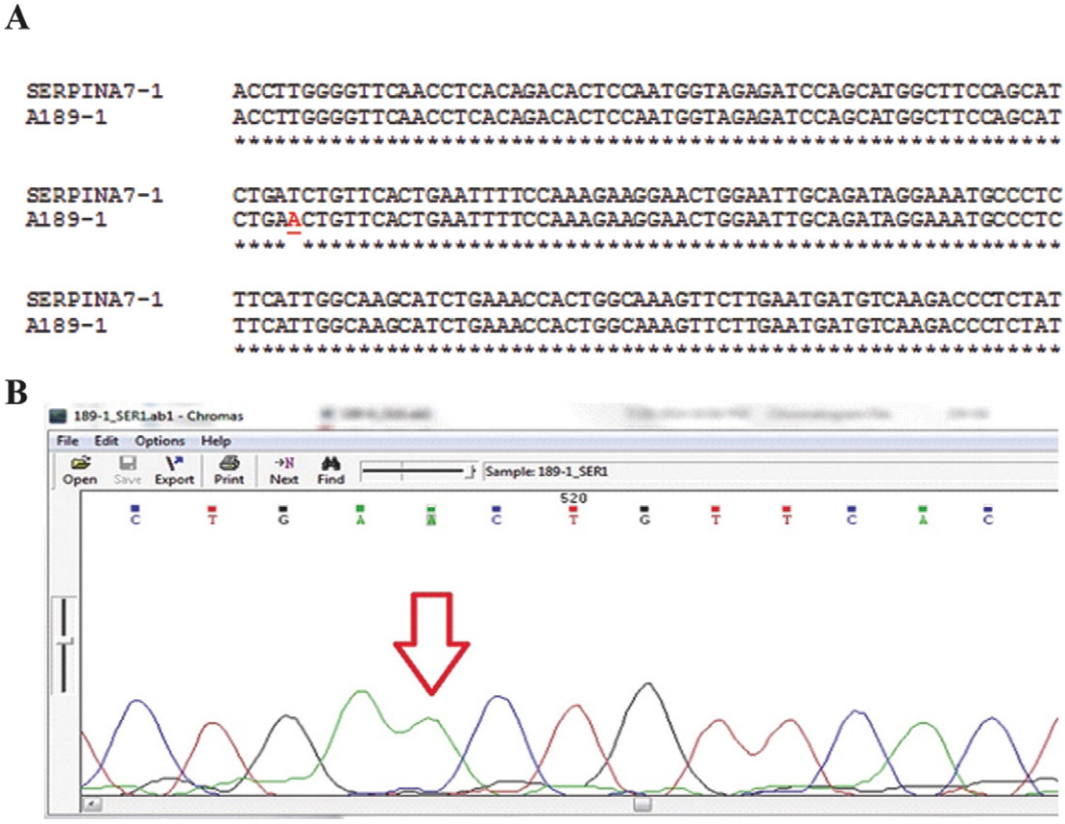

Fig. 1. Analysis of *SERPINA7* exon 1 sequencing. A. Aligned sequence of the patient with published template (ENSG00000123561) in Clustal Omega software. B. Corresponding chromatogram (Chromas software version 2.4.1) for the region containing alterations. Red arrow shows the substituted nucleotide.