Novel Mutation p.A64D in the Serpina7 Gene as a Cause of Partial Thyroxine-binding Globulin Deficiency Associated with Increases Affinity in Transthyretin by a Known p.A109T Mutation in the TTR Gene#

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- transthyretin
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- TTR gene
- mutation

Abstract

Partial thyroxine-binding globulin deficiency (TBG-PD) is an endocrine defect with a prevalence of 1:4000 in newborns. Due to the presence of a single TBG gene on the X chromosome, most familial TBG defects follow an X-linked inheritance pattern. Abnormal T4 binding to T4-binding prealbumin (TTR) is a rare cause of euthyroid hyperthyroxinemia, which is transmitted by autosomal dominant inheritance. The purpose of the present study was to identify and characterize new mutations in the Serpina7 and TTR genes in a complete family with typical TBG-PD. All patients underwent clinical and biochemical evaluation. Sequencing of DNA, population screening by (SSCP) analysis, and bioinformatics studies were performed. Molecular studies revealed a novel p.A64D mutation in the exon 1 of Serpina7 gene associated with the previously reported p.A109T mutation in the exon 4 of TTR gene. To our knowledge, this is the first report of a patient with a TBG-PD by a mutation in Serpina7 that was coincident with a mutation in TTR gene that increased affinity of TTR for T_4. This work contributes to elucidate the molecular basis of the defects of thyroid hormone transport in serum and the improvement of the diagnosis avoiding unnecessary therapy.

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Introduction

Circulating thyroid hormones are bound to 3 classes of plasma proteins: thyroxine binding globulin (TBG), transthyretin (TTR; also termed thyroxine-binding prealbumin, TBPA), and albumin.

TBG, the principal thyroid hormone transport protein in the man, is a 54 KD single polypeptide chain synthesized by the liver and secreted into the blood. The preprotein is composed of a 20 amino acids signal peptide followed by a 395-residue polypeptide that contains 4 asparagines-linked oligosaccharide chains. TBG transports 75% of total T_4 (TT_4) and T_3 (TT_3) present in the serum. Serine protease inhibitor (Serpina) 7 gene, formerly known as TBG gene, is located on the long arm of the chromosome Xq22.2 spanning 5.5 kb of genomic DNA, which includes 5 exons [1]. Its structural organization is similar to that of other members of the Serpina superfamily [2]. The monomer is composed of a 20-amino acid signal peptide. The first exon is a noncoding exon, named exon 0, located at 1.62 Kb upstream from the first coding exon 1. The 5′ flanking region includes cis-acting transcriptional regulatory elements as the hepatocyte nuclear factor (HNF)-1 binding motif. Clinically TBG defects are classified according to the level of TBG in serum of affected hemizygotes: complete TBG deficiency (TBG-CD), partial TBG deficiency (TBG-PD), and TBG excess (TBG-E) [3]. Due to the presence of a single TBG gene on the X chromosome, most familial TBG defects follow an X-linked inheritance pattern [4]. In families with TBG-CD, affected males have no detectable TBG and carrier females have on the average half the normal TBG concentration. In families with partially TBG deficient males, the mean TBG concentration in heterozygous females is usually above half the normal. Serum TBG concentration in males with TBG-E is 2- to 4-fold the normal mean and that in the corresponding carrier females, is slightly higher than half that of the affected males. The prevalence of TBG-PD is 1:4000 in newborns. Twenty-nine distinct mutations have been identified and characterized in the human TBG: 3 splice site mutation (g.IVS1+2_3insT, g.IVS2-2

In memory of Professor Hugo Niepomniszcze.

* These authors contributed equally to this work.

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Table 1 Laboratory and molecular diagnosis data in the family group members.

	Gender	TBG[1] mg/l	TT3[2] ng/dl	TT4[2] µg/dl	FT4[2] ng/dl	FT4[1] ng/dl	TSH[2] µUI/ml	TBG Mutation	TTR Mutation
II-1	Male	<3	76	3.02	0.58	1.90	0.46	p.A64D	p.A109T
II-2	Female	3.6	86	3.6	0.72	1.74	4.85	p.A64D	p.A109T
II-4	Male	14.5	117	6.8	1.24	1.29	2.08	Wild type	p.A109T
II-5 (Propositus) under treatment	Male	<3	74	3.5	1.02	4.36	0.89	p.A64D	p.A109T
III-1	Male	ND	ND	ND	ND	ND	ND	p.A64D	Wild type
III-2	Male	ND	76	2.57	0.52	1.41	3.24	p.A64D	Wild type
III-3	Female	7.8	70	2.5	0.74	0.95	3.19	p.A64D	Wild type
III-4	Male	18.2	145	6.77	0.99	1.68	1.65	Wild type	p.A109T
Reference Range		17–27	80–200	4.87–11.72	0.9–1.70	0.8–1.9	0.3–4.26		

1: Immulite method; 2: Architect method

A>G, g.IVS4+5 G>A), 3 nonsense mutations (p.S23X, p.Q223X, p.W280X), 12 missense mutations (p. H-2Y, p.S23T, p.S52N, (p.I96N, p.A113P, p.D171N, p.A191T, p.L227P, p.N233I, p.Y309F, p.H331Y, p.P363L) and 11 deletions (p.D28fsX51, p.T38fsX51, p.P50fsX51, p.V165fsX168, p.D201fsX206, p.Y282fsX384, p. L283fsX301, p.A329fsX374, p.L352fsX374, p.382fsX384, p. L384fsX402) [4–32].

TTR is a homotetrameric secretory protein of 55 Kd synthesized by the liver, ephitelial cells of the choroid plexus, and the retinal pigment epithelium of the eye. It is involved in the transport of thyroid hormones in the plasma and in the cerebrospinal fluid, and also transports retinol in the plasma. The monomer is composed of a 20 amino acids signal peptide followed by a 127 residues polypeptide. TTR is coded by a single gene copy, 6.8 Kb long, which map on human chromosome 18q11.2-12.1, and contains 4 exons [33]. The HNF-1, HNF-3, HNF-4 are involved in the regulation of the TTR gene. To date, more than 80 different mutations in this gene have been reported; most mutations are related to extracellular amyloid deposition, affecting predominantly peripheral nerve and/or the heart, and a small portion of the gene mutations is nonamyloidogenic responsible for euthyroid hyperthyroxinemia. The first mutation identified in heterozygous state that has been associated with familial euthyroid hyperthyroxinemia was the p.A109T [34–37]. This mutation increased affinity of TTR for T_4 and subsequently increased serum TT_4 concentration. The trait was inherited in an autosomal dominant manner. The p.G6S, p.A109V and p.T119M mutations in TTR similarly led to dysprealbuminemic hyperthyroxinemia.

Here we report on a patient with typical TBG-PD caused by the novel mutation p.A64D in the exon 1 of Serpina7 gene. This mutation was associated with the previously reported p.A109T mutation in the exon 4 of TTR gene. To our knowledge, this is the first report of a patient with a TBG-PD by a mutation in Serpina7 that was coincident with a mutation in TTR gene, which increased affinity of TTR for T_4.

Patients and Methods

Patients

A 52-year-old man (II-5) was referred to the endocrine center for evaluation and management of his hypothyroidism. At the age of 22, he was misdiagnosed to have hyperthyroidism in another institution. So the patient underwent radioiodine therapy and total thyroidectomy. Permanent L-T_4 replacement therapy was indicated at a dose of 150 µg per day. At consultation he was clinically euthyroid. Blood analysis under treatment indicated low normal TSH (0.32 µIU/ml, reference range: 0.3–4.26), elevated free T_4 (FT_4) (3.21 ng/dl, reference range: 0.9–1.7) with low TT_3 (66 ng/dl, reference range: 80–200) and TT_4 (4.51 µg/dl, reference range: 4.87–11.72) (**Table 1**). As FT_4 remained high under treatment and to rule out to possibility of a persistent hyperthyroidism, his thyroid function was re-evaluated after 6 weeks of treatment withdrawal. He developed clinical and biochemical hypothyroidism (TSH was >100 µIU/ml, FT_4 <0.1 ng/dl, TT_3 <20 ng/dl) with normal TRab (4.1%) and negative anti-TPO and anti-T_4 antibodies. L-T_4 treatment was reinitiated with 125 µg per day. Of course, the pattern of normal serum TSH (3.37 µIU/ml) and euthyroid clinical with an increased serum FT_4 (3.05 ng/dl) had returned. Pituitary nuclear magnetic resonance imaging was performed and was considered normal. Serum TBG levels were extremely low (<3 mg/l reference range: 17–27), indicating a TBG deficiency (**Table 1**). Seven members of the family group were involved in this study. Written informed consent was obtained from the individuals investigated, and the research project was approved by the institutional review board.

Thyroid function testing

Serum TSH, serum TT3, serum TT4, and serum FT4 levels were determined by Architect (Abbott-QL). FT4 levels were measured by Immulite method similar to the TBG concentrations. Antithyroid antibodies (TRab, anti-TPO and anti-T4 antibodies) were determined by radioimmunoassay with commercially available kits (ICMA Immulite (Diagnostic Products Corporation, Los Angeles, CA, USA).

Protein binding studies

The proportion of ^{125}I-labeled T_4 bound to TBG, albumin, and TTR was carried out with slight modification from the originally reported assay [38] by the following method. Exogenous ^{125}I-T_4 (3.3 µCi; 10 µl) was incubated with 100 µl of the patients' serum at 37 °C for 30 min, after which the incubation was terminated by chilling at 1 °C. Serum (1 µl with bromphenol blue added as a visual marker) was separated by electrophoresis (12 g/l agarose, pH 8.7, with 40 mmol/l sodium borate buffer containing 1 mmol/l calcium lactate) in a vertical electrophoresis unit with an applied current of 20 mA for 2.5 h. The separated protein gel was dried for 1 h under a 500 W lamp. The dried gel was sliced into 3 mm fragments, and the radioactivity, representing bound T_4, of each fragment was quantified in a gamma counter and compared with exogenous binding protein calibrators.

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Table 2 Summary of TBG and TTR primers used for PCR amplification and sequencing, and PCR conditions.

Exon	Forward primers Position of 5′ end	Nucleotide sequence (5′→3′)	Amplicon (bp)	Forward primers Position of 5′ end	Nucleotide sequence (5′→ 3′)	Annealing temperature (°C)
TBG						
0	−267	agacactcttgccagcatgg	430	+163	gatgaccacacttagacctt	56
1A	6	CTTCCTTCCAAAATGTCACC[a]	224	229	ACAGGGGAAAAGAAGATGTT[a]	57
1B	220	TTTCCCCTGTGAGCATTTCT[a]	230	449	TGCCAGTGGTTTCAGATGCT[a]	56
1C	440	ACCACTGGCAAAGTTCTTGA[a]	238	+38	tgagccctagactgaaaatt[a]	56
2A	−57	cttgttggtactgataccgg[a]	247	190	AGAACAAAGAGTGCCAGAGC[a]	56
2B	181	TCTTTGTTCTTCCCAAGGAG[a]	230	+136	cttggcatattctagtgatc[a]	56
3	−30	attgccatgtgttcccttcc	237	+56	gactcctaattgctcttcc	57
4	−61	accttcccaaagactgttcc	352	291	AGCTCACATCAATCACACC	56
TTR						
1	29	AGGTTTGCAGTCAGATTGGC	262	85	tggttggcaaagctggaagg	57
2	−86	cgctccagatttctaatacc	297	80	atgtgagcctctctctacc	57
3	−88	atgtgtgttagttggtgggg	359	135	aggaaagggaacctttgg	58
4	−66	catgtgtgtcatctgtcacg	290	116	gcctggacttctaacatagc	58

Exon sequences are in capital letters, intron sequences are in lower-case letters. Primers are designated according to TBG and TTR gene reference sequences (TBG, GenBank Accession Number: NC_000023.10, TTR, GenBank Accession Number: NC_000018.9)

[a] Primers defined by Domingues et al. [19]. The intronic nucleotide position is numbered from the exon end: negative numbers start from the g of the ag splice acceptor site, positive numbers start from the g of the gt splice donor site

Genomic PCR amplification

Genomic DNA was isolated from peripheral blood leucocytes by using the cetyltrimethylammonium bromide (CTAB) method. All exons of the TBG and TTR genes including the intronic flanking regions from the affected patient and his family group were amplified using the primers and PCR conditions showed in **Table 2**. PCR was performed in 100 ml, using a standard PCR buffer (Promega, Madison, WI) containing 150–200 ng of genomic DNA, 200 mM of each deoxy (d)-NTP (dATP, dCTP, dTTP, and dGTP), 4% dimethyl sulfoxide, 0.5 U Taq polymerase (Promega, Madison, WI, USA), and 50 pmol of each forward and reverse primer. Samples were denatured at 94 °C for 3 min followed by 35 cycles of amplification. Each cycle consisted of denaturation at 94 °C for 30 s, primer annealing at 55–58 °C for 30 s, and primer extension at 72 °C for 1 min. After the last cycle, the samples were incubated for additional 10 min at 72 °C to ensure that the final extension step was complete. The amplified products were analyzed in 2% agarose gel, stained with ethidium bromide, and visualized under UV light.

DNA sequencing

TBG and TTR PCR fragments were sequenced using the same sense and antisense specific primers indicated in genomic PCR amplification, with the Big Dyedeoxyterminator Cycle Sequencing Kit (Applied Biosystems, Weiterstadt, Germany). The samples were analyzed on the ABI Prism 3100 DNA sequencer (Applied Biosystems).

Validation of c.251C>A [p.A64D] mutation

We validated the c.215C>A [p.A64D] mutation studying healthy unrelated individuals by SSCP using the same exonic primers indicated in **Table 2** to amplify the fragment 1B. The gel matrix contained 8% polyacrylamide (29:1) with glycerol. Samples were electrophoresed for 28 h at a constant temperature (4 °C). DNA was visualized by silver-staining according to standard procedures. In order to determinate if the alteration p.A64D was a known polymorphism we analyzed the database NHLBI GO Exome Sequencing Project (ESP) (http://evs.gs.washington.edu/EVS/).

Analysis of the protein secondary structure prediction

Wild type and mutant (p.A64D) sequences from human TBG were submitted for computer analysis of the protein secondary structure prediction to the Jpred 3 (http://www.compbio.dundee.ac.uk/www-jpred/) internet site. This algorithm provided a 3-state (α-helix, β-strand, and coil) prediction of secondary structure at 81.5% accuracy. For protein secondary structure comparison the position-specific scoring matrix output coming from Jpred 3 web system was performed.

Protein homology analysis

Amino acid sequence homology between TBG from different species was determined using the MegAlign (DNASTAR, Hauser University of California-SF) and Search for Conserved Domains (http://www.ncbi.nlm.nih.gov/BLAST) software programs. The amino acid sequences are based on the GenBank database: Homo sapiens TBG (CAA45509.1); *Mus musculus* TBG (AAS88726.1); *Cricetulus griseus* TBG (EGW13753.1); *Heterocephalus glaber* TBG (EHB09876.1); *Sus scrofa* TBG (AAT40589.1); *Bos taurus* TBG (AAF15301.1); *Ovis aries* TBG (CAA49450.1); *Canis lupus familiaris* TBG (AAW82006.1); *Spilogale putorius* TBG (AAW82014.1); *Callorhinus ursinus* TBG (AAW82016.1); *Mephitis* mephitis TBG (AAW82012.1); *Enhydra lutris* TBG (AAW82010.1); *Vulpes vulpes* TBG (AAW82008.1); *Ursus arctos* TBG (AAW82024.1); *Nandinia binotata* TBG (AAW82025.1); *Tremarctos ornatus* TBG (AAW82023.1); *Procyon lotor* TBG (AAW82021.1); *Phoca vitulina* TBG (AAW82019.1); *Zalophus californianus* TBG (AAW82017.1); *Odobenus rosmarus* TBG (AAW82015.1); *Mustela frenata* TBG (AAW82013.1); *Ailurus fulgens* TBG (AAW82005.1); *Lontra Canadensis* TBG (AAW82011.1); *Conepatus mesoleucus* TBG (AAW82009.1); *Canis rufus* TBG (AAW82007.1); *Ailuropoda melanoleuca* TBG (AAW82022.1); *Potos flavus* TBG (AAW82020.1); *Erignathus barbatus* TBG (AAW82018.1); *Viverra tangalunga* TBG (AAW82026.1).

In silico prediction analysis

The crystal structure of the reactive loop cleaved human thyroxine binding globulin complexed with thyroxine (PDB accession 2RIW_A, 2.04 Å resolution) was used to model the modified site in the mutant (p.A64D). We submitted the chosen model to

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"Swiss PDB Viewer" (spdbv) to determine potential structural and energetic differences between mutants and the wild type TPO [39]. The surface electrostatic potential of mutants and wild type structures, were computed by solving the Poisson–Boltzmann equation implemented in the program DelPhi [40]. This method has been shown to predict electrostatic potential energies reliably. The secondary structure picture was produced with the The PyMOL Molecular Graphics System (2010) (http://www.pymol.org).

Nucleotide and amino acid nomenclatures

The mutation nomenclature refers to the National Center for Biotechnology Information (NCBI) human Serpin 7 and TTR, reference nucleotide sequences NCBI accession number NM_000354.5 and NM_000371.3 respectively, and is expressed following the standard proposed by the Association for Molecular Pathology Training and Education Committee with the A of the ATG start codon denoted as +1 and the initiator methionine being codon 1.

Results

Thyroid function testing

The hormone concentrations and thyroid function tests of the index case (II-5) and the family members are shown in **Table 1**.

Protein binding studies

T_4 binding protein radioelectrophoresis confirmed that TBG-bound T_4 was 1.6 µg/dl whereas albumin-bound T_4 fraction was within their reference interval. Surprisingly, the TTR-bound T_4 was significantly increased (92.6 µg/dl, reference range: 48.8–70.4) indicating the presence of 2 abnormalities in binding proteins with opposite effects.

Mutational analysis

To test for the genetic alteration in TBG gene in the propositus (II-5), the entire coding region and adjacent exon-intron junctions of his TBG gene were sequenced. A novel germline TBG mutation was identified. A transvertion c.251C>A in exon 1B in a hemizygous form, leads to the replacement of alanine at position 64 with an aspartic acid (**Fig. 1**). In the family members, a brother (II-1) and 2 nephews (III-1 and III-2) carry the same hemizygous substitution. His sister (II-2) and his niece (III-3) were found to be heterozygous for the same mutation.

The sequencing of all the PCR products of TTR gene of II-5 showed a previously reported transition of guanine to adenine at nucleotide 385 in exon 4 (c.385G>A) in a heterozygous form, which replaces the wild-type alanine at codon 109 with threonine (p.A109T). Patient's brothers (II-1, II-4), sister (II-2), and a nephew (III-4) resulted as heterozygous carriers of p.A109T (**Fig. 1**).

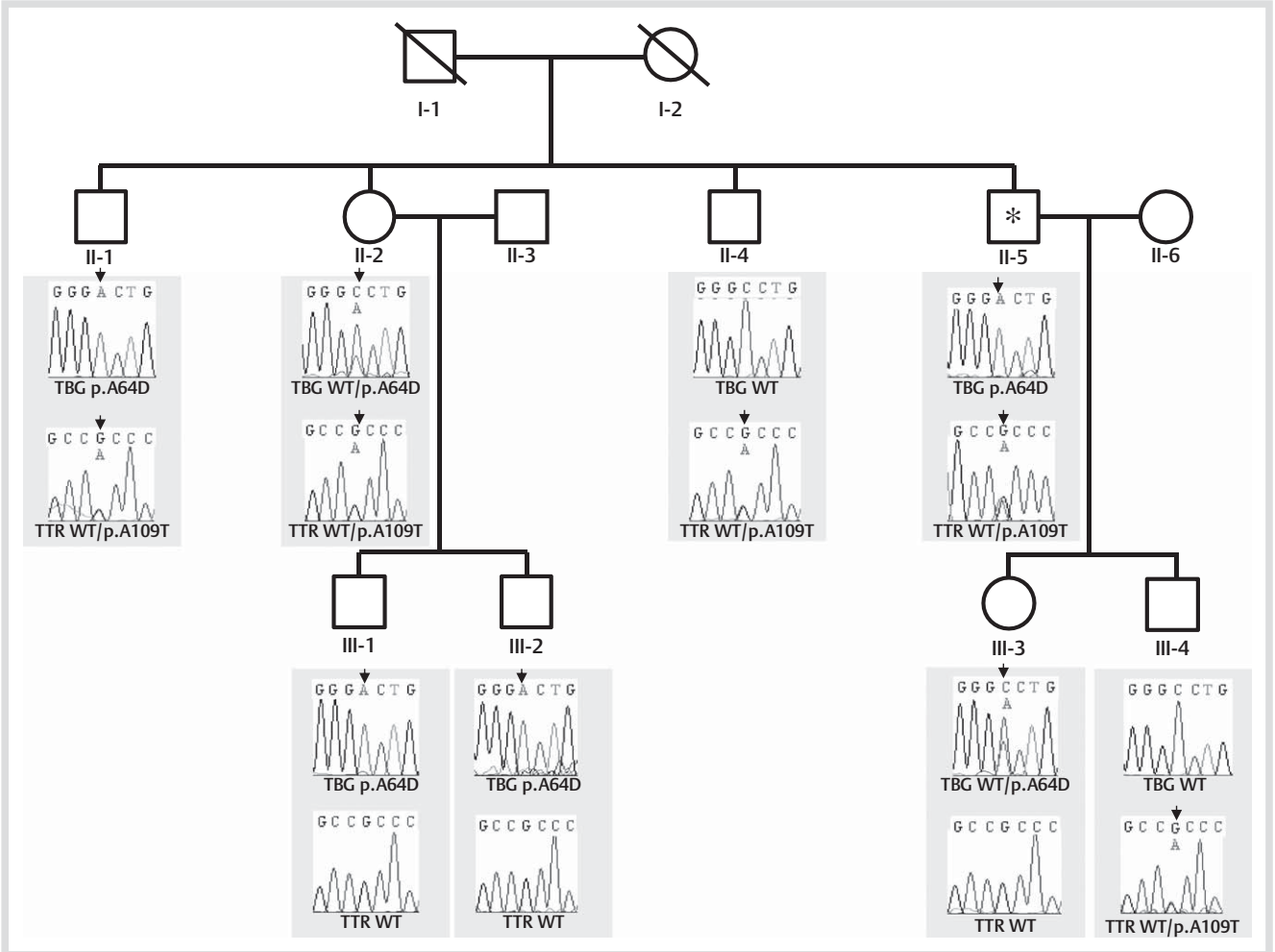

Fig. 1 Segregation analyses of the TBG and TTR mutated alleles in members of the family. Automated fluorescence-based sequencing chromatograms of exon 1B of TBG gene and exon 4 of TTR gene. II-1, II-2, II-3, II-4, II-5, II-6, III-1, III-2, III-3, III-4: family group. Arrows show the nucleotide changes for c.251C>A [p.A64D] and c.385G>A [p.A109T] mutations. The asterisk indicates the proband.

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In addition, sequencing analysis revealed a reported g.IVS3-18C>G polymorphism in TTR gene. The proband (II-5), one of his brothers (II-4) and his niece (III-3) carried the polymorphism in a heterozygous manner. The remaining members of the family included in this study resulted to be homozygous for the wild-type allele.

Validation of c.251C>A [p.A64D] mutation

We ruled out the possibility that the c.251C>A mutation could be a polymorphism as this was not detected in 102 chromosomes from the general population by SSCP analysis. Moreover, we determined that p.A64D was not a polymorphism because it is not reported in the database: NHLBI GO Exome Sequencing Project (ESP) (http://evs.gs.washington.edu/EVS/).

Analysis of the protein secondary structure prediction

Predictive analysis of the protein secondary structure spanning residues 1-765 showed that the mutation p.A64D induces a significant rearrangement (**Fig. 2**). The extension of 3 α-helixes was increased (^{105}MVEIQHGFQHLICSL^{119} → ^{105}MVEIQHGFQHLICSLN^{120}, ^{167}SAAKQEINSHVEMQT^{181} → ^{166}ISAAKQEINSHVEMQT^{181}, ^{286}SKTLKKWNRL^{295} → ^{286}SKTLKKWNRLL^{296}) whereas one β-strand segment was reduced (^{158}VFS^{160} → ^{159}F). A β-strand is missing at position 197 in the mutant TBG. A conversion has been observed from β-sheet to α-helix at position 206 and one from α-helix to β-sheet in the residue 200.

Protein homology analysis

The deleterious effect of the novel missense mutation p.A64D was therefore evaluated by assessing the degree of evolutionary conservation of the respective amino acid among human and several animal wild-type TBGs. Multiple sequence alignment using Clustal method, revealed that wild-type alanine residue at position 64 (excluding the signal peptide 20 amino acids) is conserved in all TBG species analyzed except in the species *Mus musculus* and *Cricetulus griseus* where the alanine is substituted by serine and in *Ailurus fulgens* and *Viverra tangalunga* where alanine is replaced by threonine (**Fig. 3**).

In silico prediction analysis

In silico studies were performed to elucidate a correlation between structural disturbances and putative functional

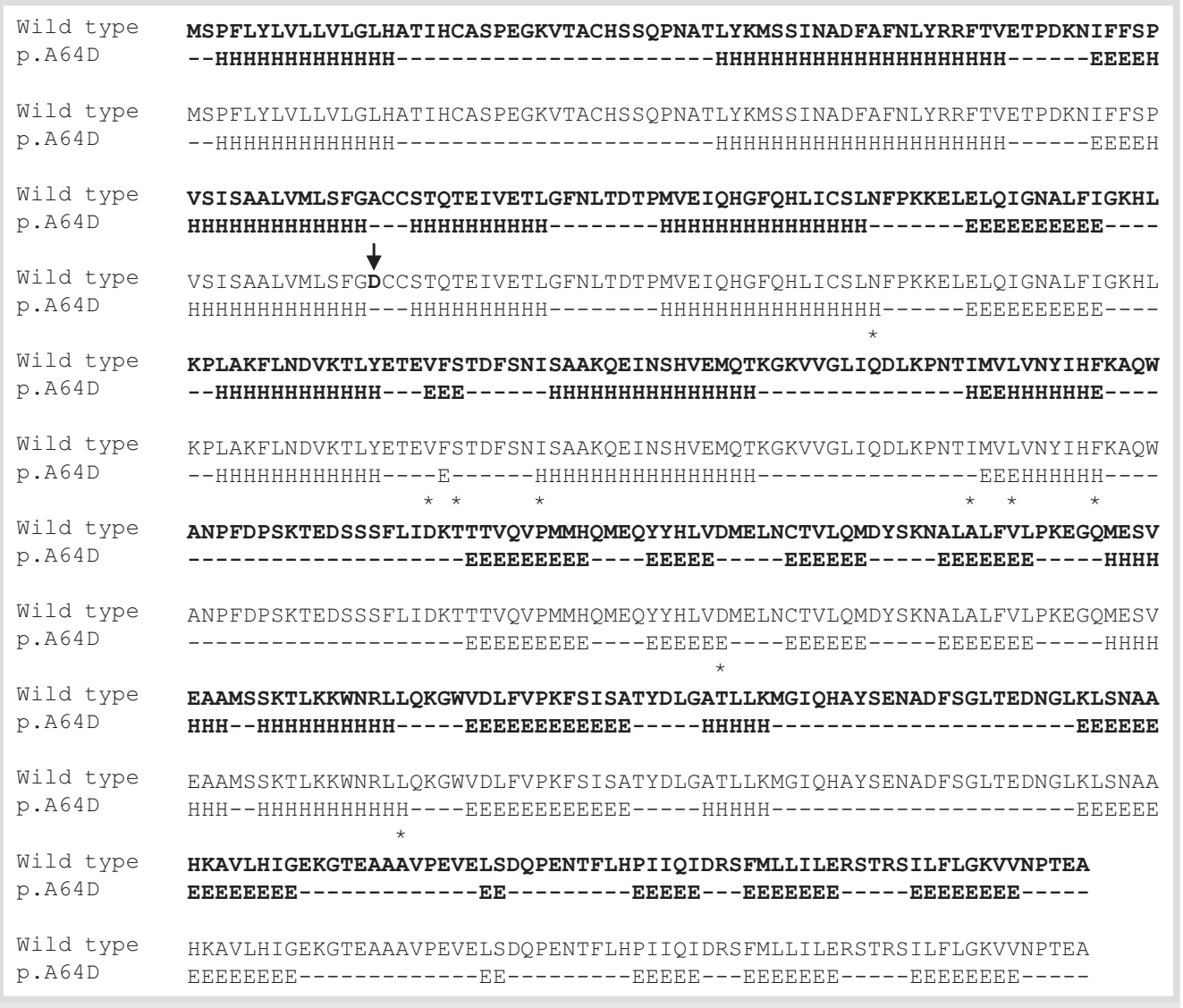

Fig. 2 Protein secondary structure analysis. E: β-sheet; H: helix; –: turn. Asterisks mark the differences in the protein secondary structure. The amino acids are indicated by the single-letter code and the position of the novel missense mutation in TBG gene (p.A64D) is shown.

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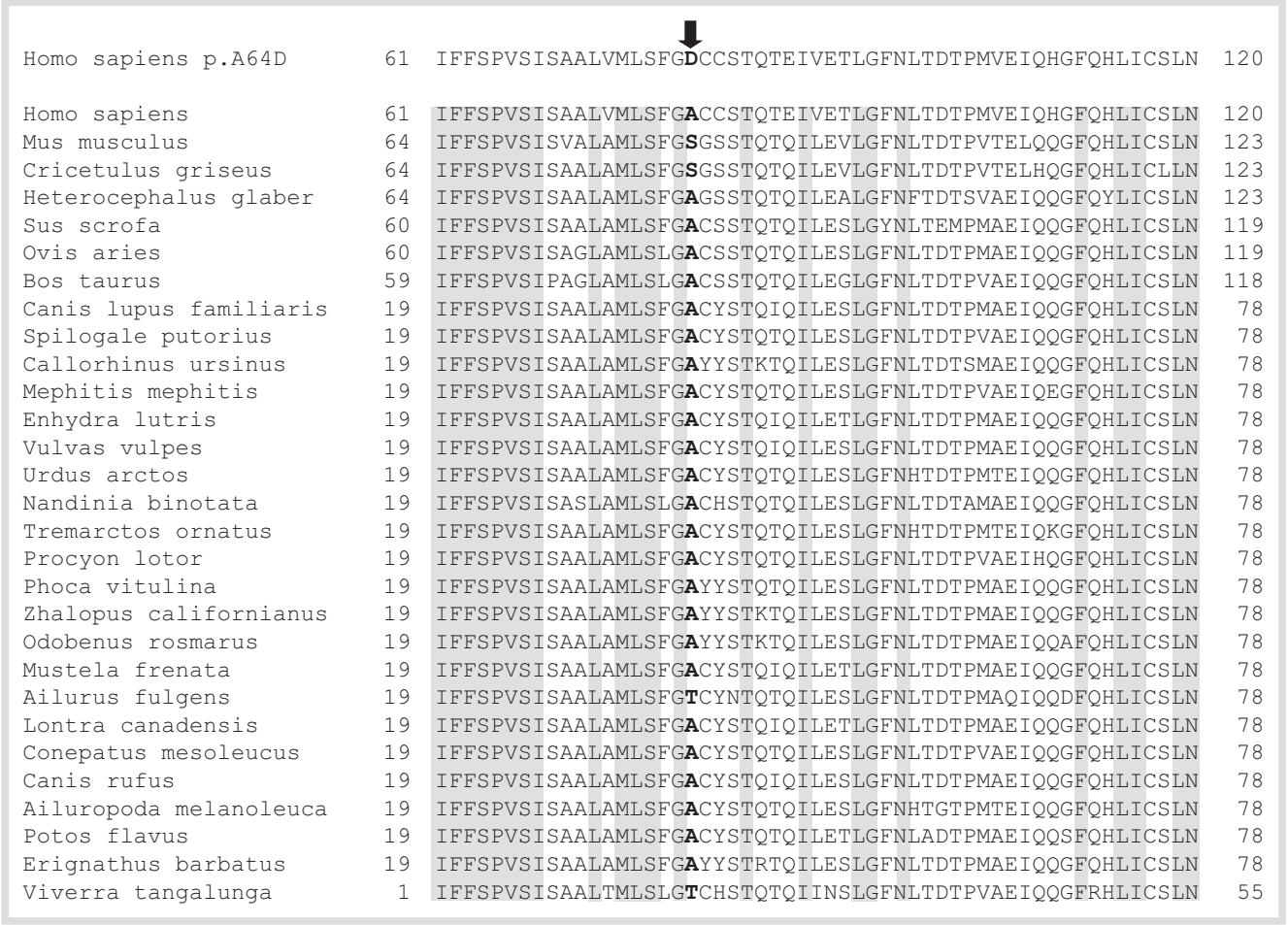

Fig. 3 Protein structure and evolutionary analysis of human TBG. Protein alignment of different species of wild-type TBG and human mutant TBG (p.A64D). The amino acids are indicated by the single letter code. Completely conserved residues are indicated in grey. Arrow points to the position of the amino acid substitution.

commitment achieving a possible explanation of the pathogenic mechanism of the novel missense mutation analyzed (**Fig. 4a, b**). p.A64D, showed the generation of 2 new favorable hydrogen bonds between the aspartic acid in position 64 and a serine located in 61 position and, on the other hand, between the same aspartic acid and a cysteine at position 65. In addition, an electrostatic surface alteration is produced resulting in a more electronegative region not only around the mutation but also far away in the molecular structure (**Fig. 4b**). The results obtained for the predicted minimization energy changes after this amino acid substitution define not only a local but a globally unfavorable structural condition, denoting a considerable energetic cost in global three-dimensional structure stabilization. The ΔG value is estimated as the difference between the energy of the wild type protein and that of the mutant enzyme and equals Δ39.66 kJ/mol. Computations were performed in vacuo with the GROMOS96 43B1, without reaction field.32.

Discussion

TBG is the main thyroid hormone transport protein in serum. Inherited TBG defects lead to a complete (TBG-CD) or a partial (TBG-PD) deficiency and have a diagenenic transmission, being clinically fully expressed only in hemizygous males and in homozygous females [29]. Another defect of thyroid hormone transport in serum is the named euthyroid hyperthyroxinemia, which are often incorrectly diagnosed in patients, who were then subjected to unnecessary laboratory tests and inappropriate therapy. Several investigators have demonstrated increased total T_4 in clinically euthyroid subjects who have marked elevations of circulating transthyretin [41].

Here we report a novel mutation in the TGB gene in codon 64 resulting in the substitution of alanine by aspartic acid. Co-segregation of this new variant with significantly diminished TBG levels in a hemizygous individual and failure to recognize the same variant in control alleles, made us to consider it as the underlying cause TBG-PD. The wild-type alanine 64 is conserved in 25 of 29 species for which suitable TBG sequences have been reported (**Fig. 3**). However, none of these 4 species that do not carry alanine, also do not present aspartic acid at that position. Additionally, computer analysis of the protein secondary structure showed that the p.A64D mutation induces a significant change. Using the crystal structure of the reactive loop cleaved human thyroxine binding globulin complexed with thyroxine, we could predict some structural and energetic commitments, which could explain functional impairment in patients presenting the mentioned mutation. The p.A64D consists in the replacement of a neutral nonpolar residue like alanine by a polar and negative residue like aspartic acid at position 64, which is

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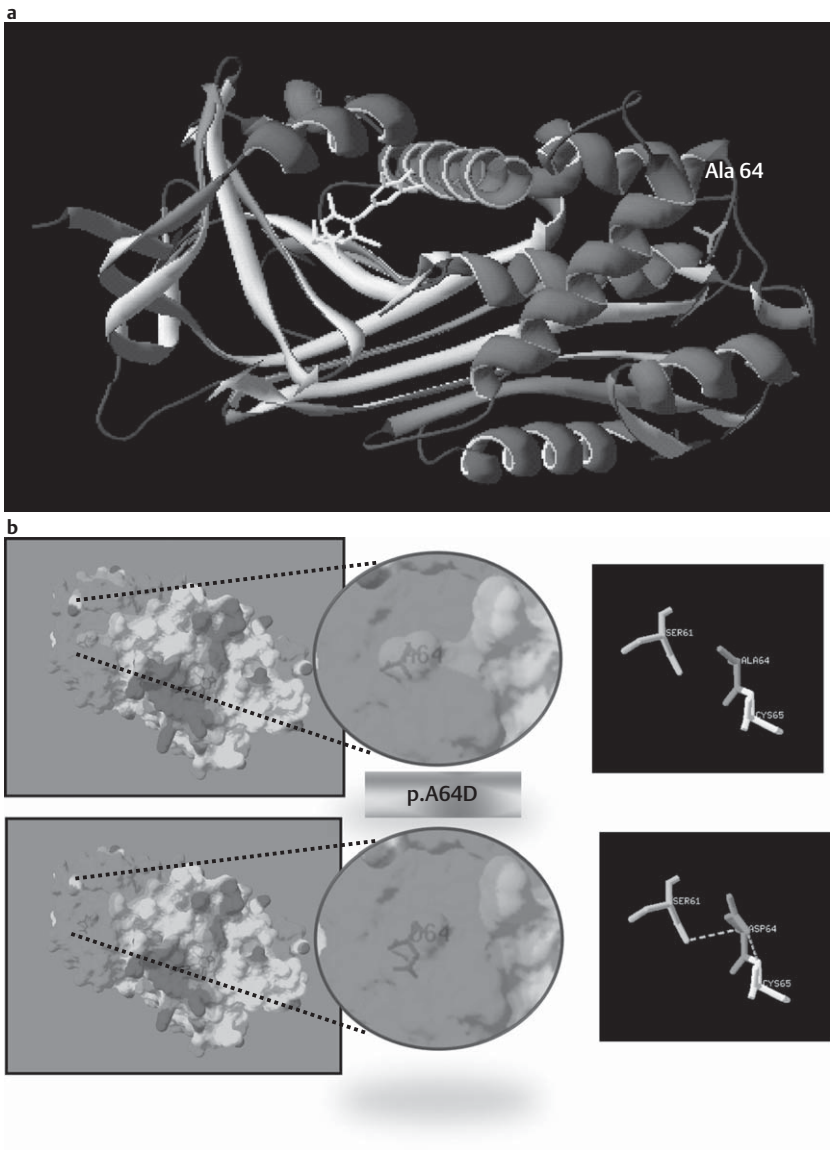

Fig. 4 Structural analysis. **a** Ribbon representation of the reactive loop cleaved human TBG complexed with thyroxine. Residue implicated in the novel missense mutation identified (A64) is indicated. **b** Surface electrostatics of the wild type A64 and mutant D64 TBG. Acidic regions are depicted in red and basic ones in blue.

expected to affect amino acid interactions among closer residues of the protein and a large electrostatic environment disturbance on the molecular surface (**Fig. 4b**).

This mutant TBG was not detectable by immunologic methods and was significantly diminished by binding methods. So, it is likely that this mutation results in abnormal intracellular transport or rapid degradation. TBG could be cleared from the circulation rapidly.

Up to now, no rearrangements or large deletions within the coding and noncoding regions of the TBG gene have been reported. The majority of complete TBG defects are associated with nonsense mutations and generating precocious stop codons and truncated proteins; partial TBG defects are typically associated to single missense mutations [42]. Also, there are no hot spots for mutations leading to TBG-CD and there no correlations between the degree of TBG deficiency and the location of the mutation.

The p.A64D variant of the TBG reported in this study is the first partial TBG deficiency associated to a previously identified p.A109T mutation in TTR. Three family members (II-1, II-2, II-5) carry both mutations, 3 (III-1, III-2, III-3) carry only the p.A64D and 2 (II-4, III-4) have only the p.A109T. All family members were born and live in an iodide-sufficient region. Based on the available biochemical and clinical data, we show that the 2 affected males (II-1, II-5) carrying the p.A64D mutation in a manner hemizygous and the additional heterozygous p.A109T present undetectable TBG levels, whereas the 2 females heterozygous for both p.A64D and p.A109T (II-2) or heterozygous for p.A64D and homozygous wild-type TTR allele (III-3) present detectable low TBG levels. As it is expected males carrying the hemizygous wild-type TBG allele and the heterozygous p.A109T showed normal levels (II-4, III-4).

On the other hand, patients with TTR mutation show moderately higher TT_4 levels than patients carrying only TBG mutation or both mutations in TBG and TTR. These findings are due to an increasing binding of T_4 to a mutant TTR with markedly elevated affinity for T_4 [35].

Coexistence of 2 inherited defects of thyroid hormone transport proteins produce atypical thyroid function test abnormalities which can be misinterpreted as thyroid hormone dysfunction. So, 3 cases of familial dysalbuminemic hyperthyroxinemia (FDH) have been identified where mutations in the albumin gene

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coexisted with mutations in TBG gene (first case) and mutations in the TTR gene (second and third cases). FDH is characterized by the presence of a variant albumin-like protein with increased affinity for iodothyronines. In the first case due to TBG deficiency the TT4 values were normal [43]. The second and third cases carrying TTR variants with approximately 4-fold increased T4-binding affinity showed the highest TT4 levels [44,45].

This work demonstrates the importance of the molecular analyses for the correct diagnosis of a patient who was initially misdiagnosed to have hyperthyroidism and thus was treated unnecessarily with thyroidectomy and radiotherapy.

In conclusion, molecular analysis of an Argentinian family with partial TBG deficiency reveals a mutant TBG p.A64D associated with a previously identified p.A109T TTR mutant that increased affinity of TTR for T_4. This work contributes to elucidate the molecular basis of the defects of thyroid hormone transport in serum and the improvement of the diagnosis avoiding unnecessary therapy. To our knowledge, this is the first report of a patient with a TBG-PD by a mutation in Serpina7 gene that was coincident with a mutation in TTR gene.

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Conflict of Interest

M. C. Olcese is a research fellow of the Universidad de Buenos Aires. H. M. Targovnik and C. M. Rivolta are established investigators of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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