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The decrease of T3 / T4 is not hypothyroidism – a new mutation of Serpina7 gene results in partial thyroglobulin deficiency

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To explore an unusual cause of the decrease of T3/T4 through a new mutation of TBG gene in a family, so as to avoid habitual thinking and reduce subsequent over treatment. TSH, free total T4, T3 and free T4, T3 were determined by automatic chemiluminescence immunoassay. The TBG mutation was identified by direct DNA sequencing. A frameshift mutation of p.I372ffs * 32 was found in the TBG gene (c.1114delc) of the patient by direct DNA sequencing, and the proband of the family was heterozygous. In vitro expression showed that the affinity of TBG for T4 decreased. Further examination of the family members showed that T3 and T4 were decreased, while FT3, FT4 and TSH were normal. If the patients with low TT4 and TT3 but normal TSH are found, the serum TBG level and related genes should be detected to determine whether it is TBG deficiency and avoid wrong treatment.

1. Introduction

Circulating thyroid hormone (TH) mainly binds to three serum transporters, including thyroxine binding globulin (TBG), thyroxine transporter (TTR) and human serum albumin (HSA). Only 0.03% of T4 and 0.3% of T3 are free. TBG is the most important binding protein, binding about 75% and 70% of T4 and T3. It is a member of serpin superfamily and has the strongest affinity for th, which is 50 times and 7000 times higher than TTR and HSA, respectively. TBG is encoded by serpina7 gene located on the long arm of X chromosome (Xq21 – 22), which contains five exons and is synthesized into a 54kDa protein containing 415 amino acids in liver (Mori et al. 1995). After cleavage of 20 amino acid signal peptide, the mature protein was composed of 395 amino acids.

The main function of TBG is to help maintain the stability of thyroid hormone in serum. The study estimates that the amount already in the serum can be used in just a few hours without producing new thyroid hormones. However, in the presence of TBG, T4 will only decrease by 10%, T3 will only decrease by 40%. Therefore, TBG can also play a role in the prevention of thyroid hormone fluctuations (Refetoff et al. 2015). When TBG decreased, the content of free T4 increased, which negatively inhibited TSH, resulting in the decrease of total T4. Therefore, total T4 decreased and free T4 in serum returned to normal level (Franklyn et al. 2000).

2. Case presentation

A female, born in February 1983. In December 2016, physical examination found thyroid dysfunction (TT3, TT4 decreased, FT3, FT4, TSH normal), no fatigue, stomach and weight changes, no limb edema, etc. The local hospital was treated with levothyroxine tablets 50 μg/day. In February 2017, the total triiodothyronine (TT3) and total thyroxine (TT4) were still lower than normal, and the TSH decreased. At the same time, the patient stopped levothyroxine tablets because of weight loss, palpitation, fear of heat and sweating. In August 2017, TSH was normal, TT3 and TT4 were lower than normal, FT3 and FT4 were normal, and TBG was 5.57 mg/l (see Table for thyroid function examination and treatment). The patient had no history of thyroid surgery. TT4 and TT3 of the mother and two sons were lower than normal, while FT3, FT4 and TSH were normal. The thyroid function of the elder sister and younger brother of the patient was within the normal range. Physical examination: T: 36.4 °C, P: 76 times/min, R: 20 times/min, BP: 122 / 76 mmHg, height (cm): 162, weight (kg): 64, BMI: 24.38 kg/m^2. Clear mind, symmetrical figure, no enlargement of bilateral thyroid, no palpable nodules, arrhythmia, no murmur, no enlargement of liver and spleen, no abdominal mass, no tenderness, no edema. The study was approved by the Dongyang People's Hospital. Obtain oral informed consent.

TSH, anti TPO and TBG:TSH, anti TPO and TBG were measured by chemiluminescence immunoassay (Roche). The precision of the method was 4.3%, 5.8% and 3.2%, respectively. The normal range of TSH was 0.27-4.2 UIU/ml, the positive value of TPOAb was more than 5.61 IU/ml, and the normal range of TBG was 13-39 mg/L.

Thyroid function of patients and their families is shown in the Table

Abbreviations:

TBG: Thyroid binding globulin; TTR: transthyroxine; HSA: human serum albumin; T4: thyroxine; TH: thyroid hormone; TBG-CD: TBG deficiency; TBG-PD: partial TBG deficiency; TBG-E: excessive TBG.

3. SERPINA7 gene analysis

Genomic DNA was isolated from peripheral blood leukocytes by salting out blood DNA extraction kit I, then PCR amplification and direct sequencing were performed. Serpina 7 gene was amplified by PCR and sequenced by Illumina sequencer. The detection interval included one related gene, and the four coding regions contained 1248 bases in total. The average coverage depth was 236 ± 11x, and the coverage area greater than 10x accounted for 100.0%, and the coverage area greater than 20x accounted for 100.0%.

Results: there was a deletion of cytosine at base 1114 and codon 372. This mutation results in the replacement of normal leucine (CTT) with phenylalanine (TTT).

Table: Thyroid function and antibody of patients and their families

	Test date	TT3 nmol/l	TT4 nmol/l	FT3 pmol/l	FT4 pmol/l	TSH uIU/ml	TBG	TPOAb IU/ml	treatment
							mg/l		
Reference value		1.2-3.1 nmol/l	66-181 nmol/l	3.1-6.8 pmol/l	12-22 pmol/l	0.27-4.2 uIU/ml	13-39 mg/l	0-5.61 IU/ml	Levothyroxine sodium(μg)
Proband	2016.12	0.92↓	43.91↓	4.01	18.91	1.98	/	/	50
	2017.2	0.75↓	40.45↓	2.97	13.24	0.005↓	11.02	/	50
	2017.3	0.84↓	44.39↓	3.72	14.85	0.132↓	/	/	0
	2017.8	0.66↓	39.16↓	3.59	17.24	1.82	5.57	31.1	0
	2018.4	0.62↓	28.95↓	3.38	13.25	1.64	/	/	0
	2018.7	0.82↓	43.42↓	4.46	19.25	1.58	/	/	0
	2019.2	0.78↓	46.02↓	3.59	18.93	1.67	8.16	23.1	0
Proband's son (16 years old)	2019.8	0.81↓	24.8↓	3.93	14	2.78	10.57	16.2 (<34U/ml)	0
Proband's son (26 months)	2019.8	0.57↓	18.6↓	3.01↓	11↓	2.69	13	/	0
Mother of proband	2019.8	0.68↓	47.37↓	2.95	11.45	2.64	11.34	0.51	0
Brother of the proband	2019.8	1.31	83.09	4.89	12.8	1.18	/	1.37	0
Sister of the proband	2019.8	1.7	91.42	4.4	15.86	2.27	/	17.8	0

Note: TBG: thyroxine binding globulin: TPOAb: thyroid peroxidase antibody; TT3: total triiodothyronine; TT4: total thyroxine; TSH: thyrotropin; FT3: free triiodothyronine; FT4: free thyroxine

Gene sequencing was performed in Shanghai Jiahe inspection center. The proband, her mother and son were heterozygotes of c.1114delc (p.l372ffs * 32) mutation. Other family members were normal (Figs. 1, 2).

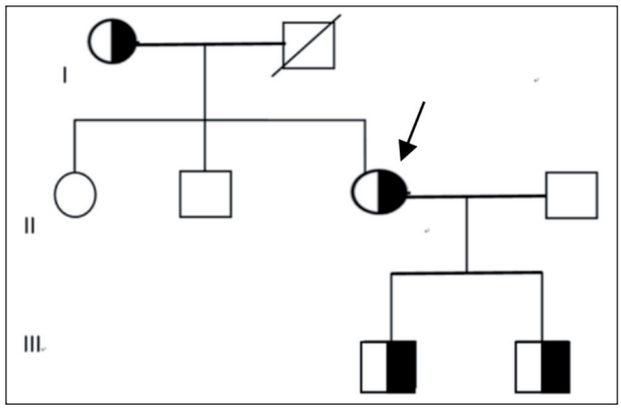

Fig. 1: pedigree of the family: the arrow indicates the proband. The semifilling sign is heterozygote.

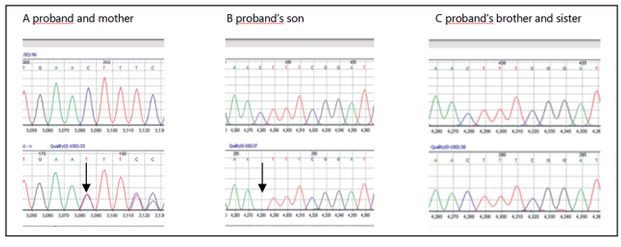

Fig. 2: Detection of serpina 7 gene in patients and family members; a and B are chromatograms containing altered regions, with arrows indicating substituted nucleotides. C is the normal gene chromatogram of family members.

4. Discussion

Circulating TH mainly binds to three serum transporters, including TBG, TTR and HSA. Only 0.03% of T4 and 0.3% of T3 are free. TBG is the most important binding protein, binding about 75% and 70% of T4 and T3. It is a member of serpin superfamily and has the strongest affinity for th, which is 50 times and 7000 times higher than TTR and HSA, respectively. TBG is encoded by serpina7 gene located on the long arm of X chromosome (Xq21–22), which contains five exons and is synthesized into a 54kDa protein containing 415 amino acids in liver (Mori et al. 1995). After cleavage of 20 amino acid signal peptide, the mature protein was composed of 395 amino acids.

The main function of TBG is to help maintain the stability of thyroid hormone in serum (Refetoff et al. 2015). When TBG decreased, the content of free T4 increased, which negatively inhibited TSH, resulting in the decrease of total T4. Therefore, total T4 decreased and free T4 in serum returned to normal level (Franklyn et al. 2000).

The concentration of TBG in normal people was 13-39 mg/L. Serpina7 gene mutation can lead to genetic abnormalities of serum TBG, including total TBG deficiency (TBG-CD), partial TBG deficiency (TBG-PD) or excessive TBG (TBG-E). Serpina7 is located on the X chromosome. Women with homozygous inactivation mutation and heterozygous men usually show complete deficiency. The TBG of affected men is usually lower than the detection range, while the serum TBG of women is about half of that of normal people. Heterozygous women show partial deficiency, and the TBG level is higher than half of that of normal people, which is usually within the normal range (Refetoff et al. 1996). Gene duplication or triploidy is associated with excessive TBG (Mori et al. 1999).

TBG-CD requires that the serum concentration of TBG in affected hemizygous men be lower than 5 mg/L (0.9 nmol/L) or 0.003% of the mean normal value (Refetoff 1989). So far, 27 mutations of TBG gene causing TBG-CD have been identified (Refetoff 1989). There are 18 truncated molecules due to frame shift caused by single nucleotide substitution or deletion. TBG-PD is the most common form of hereditary TBG deficiency (neonatal prevalence is 1:4000). The serum TBG level of heterozygous women usually overlaps with that of normal people. In contrast to TBG-CD, all TBG PDs are caused by missense mutations.

Hereditary TBG deficiency will not lead to thyroid disease or metabolic changes, but will lead to abnormal serum total thyroid hormone (TH), which is easily misjudged as central hypothyroidism; however, the levels of free TH and TSH remain unchanged. If the free th level is measured, the medical risk associated with TBG deficiency is small, but the unrecognized defects may lead to inappropriate treatment and complications (Refetoff and Selenkow 1968).

This patient, his mother and two sons showed the decrease of T3 and T4, while FT3, FT4 and TSH were normal, which suggested

that TBG deficiency was hereditary. Combined with TBG concentration, partial TBG deficiency was diagnosed. All previously reported TBG PDs were caused by missense mutations. A frameshift mutation c.1114delc (p.l372ffs * 32) of serpina 7 gene was found in this family, which has not been reported previously. This mutation resulted in the replacement of normal leucine (CTT) with phenylalanine (TTT). Leucine is a highly conserved protein domain and a glycosylation site. Therefore, this frameshift mutation destroys a potential glycosylation domain, resulting in the decrease of T4 binding ability and stability of the encoded protein, resulting in rapid denaturation (Mori et al. 1989). The clinical manifestations of this family were completely consistent with the partial TBG deficiency reported in the literature (Ferrara et al. 2015; Sklate et al. 2014; Ferrara et al. 2013; Pappa et al. 2017). Thyroid function examination showed that TT4 decreased, FT4, FT3 and TSH were in normal range; even in men, the serum TBG concentration was only slightly lower than normal.

The deficiency of TBG leads to the decrease of serum thyroid hormone bound with protein, but the concentration of FT4 and FT3 are not affected by the binding protein, so there is no clinical manifestation of hypothyroidism, and no drug replacement therapy is needed; the normal level of FT4 and FT3 is the key point to distinguish TBG deficiency from central hypothyroidism.

Therefore, if TT4 and TT3 are found to be low, but TSH is normal, FT3 and FT4 should be further examined to confirm whether they are suffering from central hypothyroidism. Serum TBG levels and related genes were detected in ft3ft4 normal subjects to determine whether it is TBG deficiency, avoid wrong treatment and bring adverse effects to patients.

Ethics approval and consent to participate: The study was approved by the Dongyang People's Hospital. Obtain oral informed consent.

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