CASE STUDY

doi: 10.5455/medarh.2024.78.154-158
MED ARCH. 2024; 78(2): 154-158
RECEIVED: DEC 30, 2023
ACCEPTED: MAR 02, 2024

A Novel TSHR Gene Mutation in a Family with Non-autoimmune Hyperthyroidism

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ABSTRACT

Background: Familial non-autoimmune hyperthyroidism is a rare disorder characterized by the absence of thyroid autoimmunity, particularly TSH receptor antibody [TRAb]. **Objective**i The aim of this study was to describe a novel TSHR mutation identified in a family of two siblings and their father. **Methods:** Two siblings presented for endocrine assessment at ages 7 and 14 years with mild T3 toxicosis, and the father presented at 30 years of age with non-autoimmune thyrotoxicosis. Both siblings were treated with oral antithyroid therapy to achieve reasonable symptom control and thyroid function normalization. The father was treated with oral antithyroid therapy, radioactive iodine, thyroidectomy, and thyroid replacement therapy. Peripheral blood DNA was extracted from both affected siblings and father. Mutation analysis of TSHR was carried out by PCR and Sanger sequencing of both strands of the extracted DNA. **Results:** Both siblings and their father were heterozygous for the missense TSHR variant c.1855G>C, p.[Asp619His], in exon 10. **Conclusions:** This novel TSHR variant is associated with T3 toxicosis during childhood. Therefore, early identification and treatment may improve patient outcomes.

Keywords: Non-autoimmune, hyperthyroidism, familial, T3 toxicosis, gene mutation.

1. BACKGROUND

Familial non-autoimmune hyperthyroidism (FNAH) is a rare disorder characterized by the absence of thyroid autoimmunity, particularly thyroid stimulating hormone receptor (TSHR) antibody (TRAb). It results from a heterozygous activating germline mutation in the gene encoding the TSH receptor [TSHR; TSH Receptor Mutation Database III, OMIM 609152], which leads to continuous activation and consequent hyperthyroidism(1). Nearly all activating TSHR mutations occur in exon 10 and are located within the transmembrane domains (2).

2. OBJECTIVE

This study reports three cases of FNAH (two siblings and their father). The siblings, while incidentally diagnosed, showed subtle signs of thyrotoxicosis. Sequencing their germline DNA revealed a novel TSHR heterozygous missense variant (c.1855G>C, p.[Asp619His]) in exon 10, attributed to their T3 toxicosis. Their father, diagnosed with thyrotoxicosis at 30 years old, implies the potential progression of this condition from T3 to frank toxicosis if left untreated.

3. CASE STUDY

Case 1

A 7-year-old boy was found to have suppressed TSH levels, normal FT4 levels, and elevated FT3 levels (Table 1). This was an incidental finding during treatment with carbamazepine for partial complex seizures that persisted after discontinuing Carbamazepine two years later. During this time, he experienced relative growth spurt [height increased from the 75th PC to the 95th PC] without entering puberty. On review of symptomatology at the age of 9 years, he reported fatigue, sweating, and heart palpitations. He also became increasingly emotional and anxious. He denied having diarrhea, heat intolerance, or weight loss. He had a strong family history of hyperthyroidism, including his father [Case 3], paternal grandfather, aunt, uncles, and maternal grandmother.

On examination, the patient was slightly anxious and fidgious. He was sweaty and had fine tremors with no goiter or exophthalmos. His eyes showed mild weakness in convergence and a slight conjunctival injection. His pulse rate was 88/min and BP was 100/70 mm/Hg [appropriate for his age]. His height was in the 91st percentile, exceeding his mid-parental height [MPH 50th PC], his weight was in the 78th percentile, and his BMI was in the 54th percentile [healthy range].

Investigations demonstrated negative TRAb, antithyroglobulin antibodies, antithyroid peroxidase antibodies, and a normal urinary iodine level of 185 µg/L [>100 µg/L, not iodine deficient]. His bone age X-ray was accelerated to 10.5 years. Thyroid scan revealed a mildly enlarged thyroid gland with uniform technetium uptake in both lobes, which was highly normal at 4% [normal:1–5%] at 20 minutes. Carbimazole (5 mg) was started and then increased to 7.5 mg mane for better control (Table 1 and 2).

At 10.5 years, his sweaty palms and tremor persisted despite being biochemically euthyroid [TSH 1.0 mIU/L]. The pulse rate was 74 bpm, and BP was 110/80 mmHg. The patient commenced early puberty. Two years later, he was asymptomatic, except for intermittent palpitations. The pulse rate was 80 bpm, and BP was 126/70 mmHg. His bone age X-ray remained advanced to 14 years [CA 12.6 years].

At 16 years and 11 months, the patient remained asymptomatic. He showed good concentration and academic achievement at school. He was clinically euthyroid, but with fine tremor and subtle lid lag on upward gaze. His eye movements were normal with no lid lag or retraction. His height was in the 73rd percentile, slightly above his mid-parental height [MPH 50th PC], his weight was in the 60th percentile, and his BMI was in the 40th percentile [healthy range]. The thyroid function test results were normal (Table 1, 2). Echocardiography excluded dilated cardiomyopathy (1, 10). Bone mineral density measured by dual X-ray absorptiometry [DEXA] was normal [total BMD 1.1 SD, femur neck BMD 1.2 SD, lumbar spine BMD 0.8 SD for height].

	Case 1	Case 2	Case 3
Age at presentation	7 years	14.5 years	30 years
Diagnosis TSH	<0.04 mU/L	0.02 mU/L	<0.04 mU/L
FT4	19 pmol/L	17.9 pmol/L	31.5 pmol/L
FT3	8.8 pmol/L	7.2 pmol/L	ND
Treatment TSH	2.04 mU/L	0.27 mU/L	0.01 mU/L
FT4	13.2 pmol/L	15.2 pmol/L	17.7 pmol/L
FT3	5.6 pmol/L	6.3 pmol/L	6.1 pmol/L
Treatment	Carbimazole 15mg	Carbimazole 7.5mg	RAI, thyroidectomy Thyroxine

Table 1. Demographic Characteristics and Thyroid Function Before and After Treatment

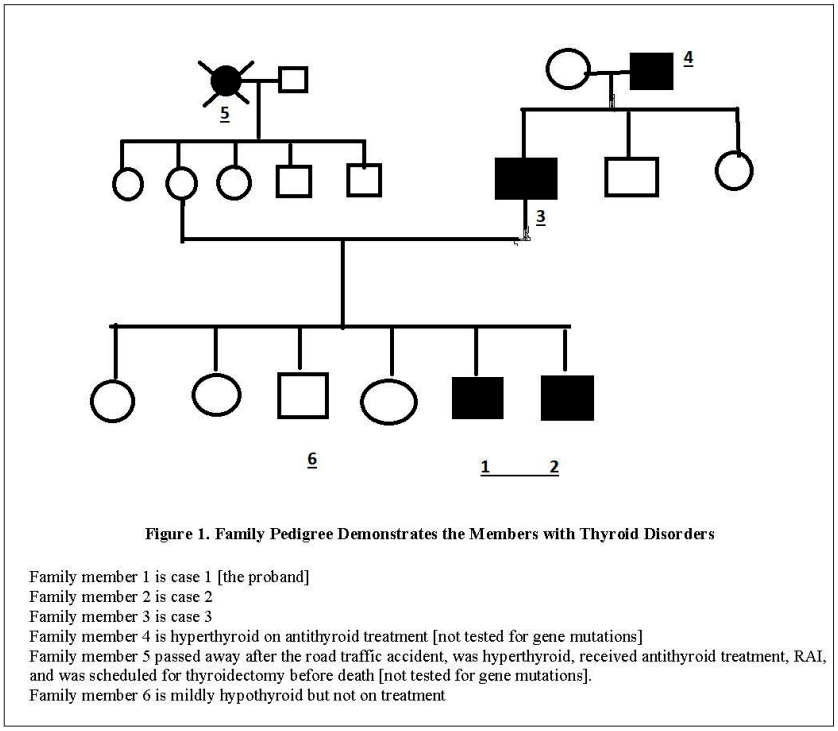

Figure 1. Family Pedigree Demonstrates the Members with Thyroid Disorders

Family member 1 is case 1 [the proband]
Family member 2 is case 2
Family member 3 is case 3
Family member 4 is hyperthyroid on antithyroid treatment [not tested for gene mutations]
Family member 5 passed away after the road traffic accident, was hyperthyroid, received antithyroid treatment, RAI, and was scheduled for thyroidectomy before death [not tested for gene mutations].
Family member 6 is mildly hypothyroid but not on treatment

Figure 1. Family Pedigree Demonstrates the Members with Thyroid Disorders

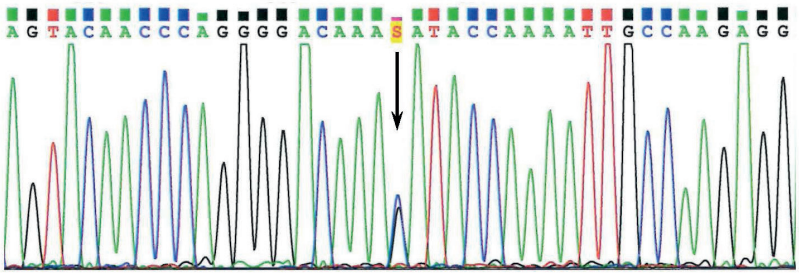

Figure 2. Sanger Sequence Electropherogram of Heterozygous Missense Variant TSHR c.1855G>C, p.[Asp619His] in Exon 10

Case 2

At 14 years and 7 months, his younger brother [case 2] was incidentally identified with abnormal thyroid function. He had a history of asthma. Tests for thyroid antibodies, including TRAb, were negative. On clinical review, he reported exertional dyspnea, occasional excercise-related chest pain, persistent sweating, and occasional mood swings. He denied palpitations, weight loss, changes in bowel habits, or heat intolerance. His school performance was on average with tendency to become easily distracted.

When examined, the patient looked clinically well and slim. He had fine resting tremor. Pulse rate was 72 bpm. No goiter or thyroid nodules were palpable, and there was no exophthalmos, lid lag, or lid retraction. His height was in the 83rd percentile, weight was in the 79th percentile, and BMI was in the 66th percentile. He was

Case 1 [Before treatment at 7 and 9 years, all others after treatment and dose escalation]												
Age [years]	7	9	10	10 6/12	12	13	14	15 7/12	15 8/12	15 11/12	16 6/12	16 11/12
TSH mIU/L	<0.04	<0.04	<0.04	1.3	0.49	0.38 [0.35-6]	3.4	0.01	0.01	0.38 [0.35-6]	4.01 [0.4-5]	2.04
FT4 pmol/L	19 [10-22]	19	16	16	13.8	16.0 [11-24]	15.5	24.9	17	16 [11-24]	12.8 [10-20]	13.2
FT3 pmol/L	8.8 [3.2-6.3]	10.3	6.7	5.3	6.0	7.0 [3.1-6.8]	7.1	9.9	6.4	**7.0** [3.1-6.8]	6.1 [3.5-6.5]	5.6
Carbimazole dose [mg]		started at 5	5 Dose increased	7.5	7.5	7.5	?7.5/5	7.5/5 Dose increased	7.5/7.5	7.5/7.5 Dose increased	10/7.5 Dose reduced	7.5/7.5

Case 2					
Age [years]	14 6/12	14 7/12	15	15 7/12	
TSH mIU/L	0.02 [0.35-6.0]	<0.03 [0.4 to 5]	0.06 [0.4- 5]	0.27 [0.4-5]	
FT4 pmol/L	17.9 [11-24]	15 [10-20]	14.4 [10-20]	15.2 [10-20]	
FT3 pmol/L	7.2 [3.5 - 6.5]	6.7 [3.5 to 6.5]	6.6 [3.5 to 6.5]	6.3 [3.5-6.5]	
Carbimazole dose		Started on 5 mg	5mg Dose increased	7.5mg	

Case 3										
Age [years]	30	30	31	31	31	32	32	32		36
TSH [0.2-3.5] mIU/L	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04		0.01
FT4 **[10-22] pmol/L**	31.5	32	24	19	28	33	36	33		17.7
FT3 [3.2-6.3] pmol/L				5.8			12.7	9.8		6.1 [2.6-6]

Table 2. Serial Thyroid Function Tests of the Cases 1-3

iodine replete, with a urinary iodine level of 332 µg/L. A thyroid scan revealed normal technetium trapping of 1.1% [0.4 4%] of the injected dose at 20 minutes. Thyroid ultrasound demonstrated a smooth and homogenous parenchymal texture with no solid nodules identified. There was a small colloid cyst located medially in the mid pole on the left, measuring 2 × 3 mm in diameter. Given his strong family history of hyperthyroidism, he was started on a low dose of carbimazole (5 mg), which was then increased to 7.5 mg.

At 15 years and 7 months, his symptoms partially improved, although hyperhidrosis persisted. He reported good energy levels, improved concentrations at school, and better sleep. Adherence to carbimazole was incomplete. When examined, he was clinically well and pubertal, with no palpable goiter. His heart rate was 69 beats/minute. His blood pressure was 128/66 mmHg. He had very fine resting tremor with sweaty palms. He had no lid lag, retraction, or exophthalmos. His height was 178.7 cm, 80th percentile; weight 71.3 kg, 84th percentile; BMI 22.3, 74th percentile]. Thyroid function testing revealed mildly suppressed TSH, normal free T3, and free T4 levels (Table 1, 2). Echocardiography and DEXA were normal: [total BMD 0.3 SD, femur neck BMD 0.5 SD, lumbar spine BMD 1.2 SD for height].

Case 3 [Father]

The father was incidentally diagnosed with hyperthyroidism at age 30, four years before Case 1. In retrospect, he reported symptoms like palpitations, fatigue, poor concentration, increased bowel movements (3/day), and eye swelling, but no weight loss. He displayed no goiter or exophthalmos, had elevated blood pressure (160/80 mmHg), increased Free T4 levels, and suppressed TSH levels, with negative results for TRAb, anti-TGB, and anti-TPO. Initially, he received carbimazole (5 mg BD), later increased to 10 mg BD, with incomplete adherence.

By age 32, his TSH remained suppressed, Free T4 levels were elevated at 33 pmol/L, and Free T3 levels were also elevated at 12.7 pmol/L (Table 1, 2). Treatment included radioactive iodine, followed by total thyroidectomy and thyroxine replacement (250 mg/day). His father had hyperthyroidism, and his father's nieces experienced thyroid issues (Figure 1). In his recent examination, when euthyroid, he exhibited a fine tremor with normal muscle power and eye exam. His blood pressure was 148/91 mmHg, heart rate 65 bpm, weight 113 kg, height 178.2 cm, and BMI 35.6 kg/m2.

Mutation analysis

For mutational analysis, informed consent was obtained from siblings‘ parents. Peripheral blood DNA was extracted from both affected siblings and their fathers. Mutation analysis was conducted using PCR and Sanger sequencing of TSHR coding exons 1-10 from both extracted DNA strands. This revealed a heterozygous missense variant c.1855G>C, p.[Asp619His] in exon 10 of the two siblings and father (Figure 2).

4. DISCUSSION

This report describes a novel TSHR mutation segregating into three cases of FNAH in an Australian family with varying severities of non-goitrous hyperthyroidism, and historically, an earlier generation may also have been affected.

Germline gain-of-function activation of TSHR is the most common cause of familial non-autoimmune hyperthyroidism (3). The first case was reported clinically in 1982 (4), and then twelve years later, a germline TSHR mutation [c.1526C>T, p.[Val509Ala]] was identified and confirmed to cause a 3-fold higher basal cAMP level compared to wild-type TSHR (5). Currently, 69 germline TSHR mutations associated with hyperthyroidism have been reported (6).

This autosomal dominant disorder exhibits variable phenotypic expression, with heterogeneity within families accross generations, based on disease severity and age of onset (7-11). This phenotypic expression can be altered by differences in iodine intake and modulation of this heterozygote mutation by epigenetic factors.

In this study, three family members with the same germline TSHR mutation showed differences in the onset and degree of thyroid dysfunction. Case 1 was first identified at the age of 7 years with mild T3 toxicosis; however, treatment was mostly asymptomatic during follow-up at the age of 17 years. He had a high normal Tc uptake on scanning, whereas his younger brother [Case 2] had a low normal Tc uptake and milder thyroid hyperfunction. In contrast, their father [Case 3] had more florid hyperthyroidism when diagnosed at the age of 30 years. Wide variations in age of onset have been found in previous kindred studies, such as 10-36 years [Nancy family], 2-21 years [Cardiff family], 18–53 years [Reims family] (4, 5, 12), and 4-60 years reported by Karges et al. (13)

Goiter was absent in the current cases aligning with other reports findings (14). The formation of goiter is mostly diffuse in childhood and may become nodular in adulthood (15, 16). In FNAH, goiter formation is manifested by the initiation of DNA synthesis at low concentrations of TSH.

Another recently published case report of a first multi-generation Slovenian family with FNAH due to a novel TSHR disease-causing variant described affected family members born between 1896 and 2017. Among the descendants, seven males and four females across four generations developed clinical features of hyperthyroidism, but did not show characteristics of autoimmune hyperthyroidism; all affected subjects presented with mild hyperthyroid symptoms. Nevertheless, members of the second generation, who were the initial family members clinically diagnosed with non-autoimmune hyperthyroidism, had cardiac complications. For this generation, it is not clear exactly when hyperthyroidism started, as they did not complain of specific hyperthyroid symptoms. Moreover, they all presented with a specific appearance, namely a scrawny build, aquiline nose, staring eyes, and long, thin fingers. In later generations, when hyperthyroidism was detected in childhood, family members typically had advanced stature compared with their chronological age. Two third-generation subjects underwent early thyroidectomy at 25 and 18 years of age (6).

Our reported cases 1 and 2 were detected incidentally but had subtle signs of thyrotoxicosis, in line with the recently published case report of the Slovenian family with FNAH who presented with mild hyperthyroid symptoms. Case 1 had a more severe disease, with accelerated growth and bone age. Case 2 had minimal symptoms and signs, but had sweatiness, poor concentration, and tremors. Some, but not all, of these symptoms improved when the patient became euthyroid. Even though not all the symptoms had resolved, case 1 showed good concentration and academic performance at school. Similarly, case 2 reported good energy levels and improved concentration at school and during sleep. In case 3, the diagnosis was made comparatively late, and the patient showed poor treatment adherence and eventually required thyroidectomy.

This report has limitations, such as uncertainty regarding the onset of thyrotoxicosis in each case and whether they had excess thyroid hormone at birth. Additionally, obtaining more clinical or diagnostic information for older family members with thyroid conditions was not feasible.

5. CONCLUSION

This report details two siblings who developed childhood T3 toxicosis due to a novel TSHR mutation. Their father, at 30 years old, also had the same variant and exhibited T3 and T4 toxicosis. This highlights a potential progression from T3 to severe toxicosis, emphasizing the importance of early detection and treatment for better clinical outcomes.

- **Ethical Approval and Consent**: This study was approved by the Executive of the Sydney Children's Hospital Network

Human Research Ethics Committee [HREC] project number CCR2020/40.

- **Consent Form Publication**: Written consent was obtained from the mother for publishing the clinical data of the children. The father provided separate consent for publishing his clinical data.
- **Availability of Data and Materials**: The datasets are available from the corresponding author upon reasonable request.
- **Author's Contribution**: All authors contributed to the study's conception, design, data acquisition, analysis, and interpretation. They were also involved in drafting, revising, and approving the final version of the article.
- **Conflicts of interest**: There are no conflicts of interest.
- **Financial support and sponsorship**: None.

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