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Novel germline mutation (Leu512Met) in the thyrotropin receptor gene (*TSHR*) leading to sporadic non-autoimmune hyperthyroidism

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Abstract

Background—Primary nonautoimmune hyperthyroidism is a rare cause of neonatal hyperthyroidism. This results from an activating mutation in the thyrotropin-receptor (*TSHR*). It can be inherited in an autosomal dominant manner or occur sporadically as a de novo mutation. Affected individuals display a wide phenotype from severe neonatal to mild subclinical hyperthyroidism. We describe a 6-month-old boy with a de novo mutation in the *TSHR* gene who presented with accelerated growth, enlarging head circumference, tremor and thyrotoxicosis.

Methods—Genomic DNA from the patient's and parents' peripheral blood leukocytes was extracted. Exons 9 and 10 of the *TSHR* gene were amplified by PCR and sequenced.

Results—Sequencing exon 10 of the *TSHR* gene revealed a novel heterozygous missense mutation substituting cytosine to adenine at nucleotide position 1534 in the patient's peripheral blood leukocytes. This leads to a substitution of leucine to methionine at amino acid position 512.

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The mutation was absent in the parents. In silico modeling by PolyPhen-2 and SIFT predicted the mutation to be deleterious.

Conclusions—The p.Leu512Met mutation (c.1534C>A) of the *TSHR* gene has not been previously described in germline or somatic mutations. This case presentation highlights the possibility of mild thyrotoxicosis in affected individuals and contributes to the understanding of sporadic non-autoimmune primary hyperthyroidism.

Keywords

neonatal Graves' disease; thyroid; thyroid dysfunction; thyrotoxicosis; thyrotropin receptor action

Introduction

Neonatal hyperthyroidism occurs in 1: 25,000 to 1: 45,000 births. It is most commonly caused by transplacental passage of thyrotropin receptor stimulating antibodies (TSI) to a baby born from a mother with active Graves' disease or one previously treated with surgical thyroidectomy or radioiodine ablation. Outside of the neonatal period, infants present with failure to thrive, accelerated linear growth, advanced bone age, tachycardia, cranio-synostosis, irritability, and/or developmental delay [1]. In rare circumstances, a gain-of-function mutation in the thyrotropin-receptor (*TSHR*) gene can result in neonatal hyperthyroidism. Germline mutations, inherited in an autosomal dominant pattern, cause familial non-autoimmune hyperthyroidism (FNAH), whereas de novo mutations cause sporadic congenital non-autoimmune hyperthyroidism (SCNAH) [2]. Although rare in children, somatic mutations in the *TSHR* gene can also lead to an autonomous adenoma [3].

To date, approximately 21 familial and 12 sporadic gain-of-function mutations in the *TSHR* gene have been described [4–6]. The TSHR is encoded by the human *TSHR* gene which resides on the long arm of chromosome 14. The TSHR is a member of the G protein-coupled receptor superfamily, and stimulation leads to both increased growth of the thyroid follicular cells and hormonogenesis via stimulation activation of adenylyl cyclase. Gain-of-function mutations, resulting in constitutive activation of the TSHR, most commonly occur in exon 10. These mutations lie in the region encoding the β -subunit of the TSHR, with the highest frequency in the sixth transmembrane domain of the receptor [7]. The first case of SCNAH was described by Kopp et al. in a premature male infant with a goiter and antibody-negative thyrotoxicosis who failed to remit off anti-thyroid drug therapy and was found to have a Phe631Leu substitution in the sixth transmembrane domain [8].

Of the cases that have been reported, there is no genotype-phenotype correlation in children with gain-of-function mutations of *TSHR* gene. Children with these mutations display a wide phenotype from severe fetal hyperthyroidism to asymptomatic subclinical hyperthyroidism. The extent of thyromegaly also varies amongst mutations. Children with SCNAH tend to have a more severe course and are diagnosed at a younger age compared to the familial subtype. The degree of thyrotoxicosis or glandular growth can be difficult to control with antithyroid drugs or subtotal thyroidectomy [9]. Therefore, it is critical to identify, evaluate, and report the natural history of these mutations. In this report, we

describe a case of an infant with SCNAH due to a de novo germline mutation of *TSHR* gene that has not previously been described.

Materials and methods

Patient

The patient was a male infant born at term via spontaneous vaginal delivery to a 34-year-old G1P1 mother after an uncomplicated pregnancy. He was the first pregnancy to healthy, non-consanguineous parents. There was no history of maternal thyroid disease or autoimmune disorders. In utero monitoring did not reveal fetal tachycardia, intrauterine growth restriction or neonatal goiter. Anthropometric birth data revealed a weight of 3377 g [30th percentile, −0.45 standard deviation score (SDS)] and a length of 55.8 cm (50th percentile, +0.12 SDS). Newborn screening was normal for total thyroxine (T4) by dried blood spot. There was no extended family history of thyroid disease or autoimmune disorders. Somatic growth was normal until 4 months of age.

At 6 months of age, the patient was observed to have an exaggerated Moro reflex with a fine hand tremor. He was otherwise asymptomatic. Laboratory evaluation was notable for normal serum calcium and a thyrotropin (TSH) < 0.01 µIU/mL (1.36–8.80), free T4 (FT4) 2.1 ng/dL (1.1–2.0), total triiodothyronine (T3) 250 ng/dL (80–200). Thyrotropin receptor antibodies (TRAbs) <0.9 IU/L (< 1.75) and TSI 92% (–122%) were negative. Thyroglobulin antibody (Tg Ab) 5 IU/mL (0–33) and thyroperoxidase antibody (TPO Ab) <20 IU/mL (0–40) were also negative. By 9 months of age, length increased from the 50th to 95th percentile and head circumference increased from the 50th to 95th percentile while maintaining weight along the 50th percentile (Figure 1). Cognitive and motor skills were developmentally appropriate.

At 11 months of age, the patient presented to our pediatric endocrinology practice at which time his tremor had resolved. Physical examination revealed no cafe-au-lait macules, craniosynostosis, tachycardia, goiter, orbitopathy or hyperdefecation. Thyroid function testing was notable for a TSH < 0.005 µIU/mL (0.8–8.2), FT4 2.74 ng/dL (0.8–1.8), and T3 183 ng/dL (105–269). Quantitative beta-human chorionic gonadotropin (hCG) was normal. A third generation TSI was <1.0 (–1.3). An I-123 uptake and scan revealed autonomous, symmetric uptake of 23% at 4 h and 30% at 24 h (Figure 2). Parental thyroid function was normal, paternity confirmed, and maternal TRAbs were negative.

Low dose antithyroid drug (methimazole 0.1 mg/kg/day, Figure 1) was initiated, and free thyroxine normalized by 12 months of age. Interval thyroid function testing did not require any titration of methimazole and no adverse effects were observed (Table 1). Linear growth velocity and head circumference normalized, and the patient remained asymptomatic.

Methods

Genomic DNA was extracted from the peripheral blood leukocytes from the patient and his parents under informed consent. Exons 9 and 10 of the human *TSHR* gene were amplified by polymerase chain reaction and sequenced (QIAGEN, HotStarTaq DNA Polymerase Kit, Germantown, MD, USA). The primer sequences for exon 10, where the mutation was

identified, were as follows: 1F: 5'-GCCTGGCACT-GACTCTTTTC-3'; 1R: 5'-GCCCCATTATGTCTTCACACG-3'; 2F: 5'-TCAAAA-ACCCCCAGGAAGAG-3'; 2R: 5'-AGTGAAGAAACCAGCCGTGT-3'; 3F: 5'-TTTCTGCATGGGGATGTACC-3'; 3R: 5'-CTGGGTTGTACTGCGGATTT-3'; 4F: 5'-GTTTTTGTCTGACGCTCAAC-3'; 4R: 5'-TGCTGTTCTTTGGAGGAACC-3'; 5F: 5'-ATTTTCACCAAGGCCTTCCA-3'; 5R: 5'-AGCTATGTGTTGGGGGTGTC-3' (IDT, Inc. Coralville, IA, USA). PCR conditions included initial denaturation for 7 min at 95 °C, 35 cycles of denaturation for 45 s at 95 °C, annealing for 45 s at 55 °C, elongation for 105 s at 72 °C, and a final elongation step for 10 min at 72 °C [7]. DNA sequencing was performed and analyzed using Sequencher DNA analysis software (Gene Code, Ann Arbor, MI, USA).

Results

Sequencing of exon 10 of the *TSHR* gene revealed a novel heterozygous missense mutation substituting cytosine to adenine at nucleotide position 1534 in the patient's peripheral blood leukocytes. This mutation leads to a substitution of leucine to methionine at amino acid position 512 (p.Leu512Met). DNA sequencing indicated the absence of these mutations in the parents (Figure 3). No SNPs have been reported at this amino acid position [10]. In silico prediction modeling by Polyphen2 and SIFT both predicted the mutation to be damaging. The PolyPhen-2 score was 1.0 (range 0–1) and the SIFT score was 0 (range 0–1). Both of these are maximum scores that would predict the mutation to interfere with the protein function [11, 12].

Discussion

In this study, we present a history of an infant with a mild clinical presentation of SCNAH that was found to have a novel germline, likely gain-of-function mutation of the human *TSHR* gene, Leu512Met, located within the third transmembrane domain. The absence of the mutation in the parents indicates a de novo mutation, Leu512Met has not been previously reported either as a germline or somatic mutation; however, this amino acid position has been the site of gain-of-function mutations from Leu512Gln and Leu512Arg [13–16]. This variant would be classified as pathogenic according to the American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines, meeting criteria PS2, PM1, PM2, PM5, and PP5 [17]. Sequencing of the entire gene was not performed due to the high prevalence of previously reported mutations in exons 9 and 10; therefore, other rare genetic variants or polymorphisms that may alter TSHR activity cannot be excluded. In silico modeling predicts our patient's mutation to be pathogenic; however, in vitro studies would be needed to fully confirm causality of the genetic variant. Loss of function mutations at amino acid position 512 have not been described in association with hypothyroidism.

A de novo germline mutation at amino acid position 512 from leucine to glutamine (Leu512Gln) has been previously described in a premature infant with severe thyrotoxicosis. This patient presented with craniosynostosis, cognitive deficits, and hydrocephalus that required surgical intervention. She had refractory thyrotoxicosis while on antithyroid drug therapy and a large goiter in the absence of TRAbs [18]. This mutation has been previously described and proven to be an activating mutation at the somatic level in toxic adenomas

[13, 14, 19–21]. The functionality of this mutation via cotransfection into COS-7 cells was found to increase basal cAMP levels via activation of the G_s pathway [13]. The role of the G_q –PLC cascade has been less investigated [2].

Some gain-of-function mutations are shared between FNAH, SCNAH, and autonomous adenomas. The earlier onset of SCNAH has led to the hypothesis that sporadic germline mutations in the *TSHR* gene may be more constitutively active. Interestingly, in SCNAH, there is no apparent correlation of a patient's mutation with the level of basal cyclic adenosine monophosphate activity. There are more shared mutations between SCNAH and somatic mutations compared to SCNAH and FNAH. SCNAH mutations also display higher active in vitro activities than familial *TSHR* gene mutations. This is likely because strong germline mutations are negatively selected and may result in spontaneous abortions [22, 23].

Gain-of-function mutations of the *TSHR* gene lead to increased function and thyroid glandular growth. Our patient had a milder presentation and did not present with a goiter, possibly due to the early presentation. Although goiter size can be variable, especially in young patients, its continuous growth can present challenges in management. Our patient is currently well controlled with a low dose of antithyroid drug. Long-term definitive therapy such as near total thyroidectomy or radioactive iodine ablation will likely be required. More severe cases in young children may require high doses of antithyroid drug, which may result in the need to replace with physiologic doses of thyroid hormone. When diagnosis is delayed and the degree of thyrotoxicosis is more severe, cognitive development may be affected, further emphasizing the need for early recognition and diagnosis.

This case report contributes to the understanding of sporadic non-autoimmune primary hyperthyroidism. No genotype-phenotype relationship has been reported in patients with germline *TSHR* gene mutations suggesting that other epigenetic and/or environmental factors may be involved [23]. Further characterization is needed, emphasizing the need for these cases to be identified and reported. While the sixth transmembrane domain has been deemed a hotspot for mutations, continued contributions may alter our understanding of areas of the *TSHR* genes prone to mutation. Early diagnosis in childhood is important so as to rapidly initiate antithyroid therapy and manage the thyrotoxicosis, to potentially avoid an enlarging goiter, and to prevent neurocognitive delays in young children with nonautoimmune hyperthyroidism.

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Figure 1.
Growth charts from time of clinical presentation. Arrow indicates initiation of methimazole (0.5 mg/kg/day).

Figure 2.

Patient's I-123 uptake and scan.

In the setting of a suppressed TSH, scintigraphy confirms autonomy with diffuse, symmetric, uptake of 23% at 4 h and 30% at 24 h.

Figure 3.

Forward DNA sequencing results from the patient and his parents.

At nucleotide position 1534, the patient is heterozygous, expressing both cytosine and adenine, leading to a missense mutation of methionine for leucine at amino acid position 512. The absence of this mutation in the parents, as shown here, indicates this sporadic germline mutation.

Table 1

Laboratory data of the patient with sporadic congenital nonautoimmune hyperthyroidism.

Age, months	TSH, μ IU/mL	FT4, ng/dL	TT3, ng/dL	Methimazole dose, mg/kg/day
6	< 0.01 (1.36–8.80)	2.1 (1.1–2.0)	218 (80–200)	
7	< 0.01 (1.36–8.80)	2.3 (1.1–2.0)	209 (80–200)	
8	< 0.01 (1.36–8.80)	2.2 (1.1–2.0)	199 (80–200)	
9	< 0.01 (1.36–8.80)	2.6 (1.1–2.0)	221 (80–200)	
10	< 0.01 (1.36–8.8)	2.6 (1.1–2.0)	221 (80–200)	
11	< 0.005 (0.8–8.20)	2.7 (0.8–1.8)	183 (105–269)	0.1
12	0.08 (0.85–6.50)	1.3 (0.9–1.7)	128 (80–200)	0.1
13	1.18 (0.85–6.50)	1.2 (0.9–1.7)	127 (80–200)	0.1
14	0.21 (0.85–6.50)	1.4 (0.9–1.7)	93 (80–200)	0.1
18	0.34 (0.85–6.50)	1.5 (0.9–1.7)	138 (80–200)	0.1
22	0.42 (0.85–6.50)	1.5 (0.9–1.7)	154 (80–200)	0.1
26	0.77 (0.85–6.50)	1.5 (0.9–1.7)	158 (80–200)	0.1

Reference ranges for different assays provided in parentheses.