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Sporadic Congenital Hyperthyroidism due to a Germline Mutation in the Thyrotropin Receptor Gene (Leu 512 Gln) in a Japanese Patient

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Abstract. Constitutively activating thyrotropin receptor (TSHR) germline mutations have been identified as a molecular cause of congenital hyperthyroidism. We here describe a Japanese woman who had presented with severe hyperthyroidism and advanced bone age as a neonate. She underwent neurosurgical intervention for craniosynostosis, and presented with perodactylia and mild mental retardation with hydrocephalus. Hyperthyroidism has been refractory to antithyroid drug therapy in the absence of antithyrotropin receptor antibodies during follow-up of 20 years, resulting in an enlarged goiter. Analysis of the patient's genomic DNA showed a heterozygous thymine-to-adenine point mutation in exon 10 of TSHR at position 1535 which was not present in the parents' DNA. This mutation, changing leucine to glutamine in codon 512 in the third transmembrane region, was previously identified as a somatic mutation in toxic thyroid nodules and was shown to increase basal cAMP production *in vitro*. To our knowledge, this is the first report of a germline mutation of TSHR causing sporadic congenital nonautoimmune hyperthyroidism in a Japanese patient.

Key words: Thyrotropin receptor, Germline mutation, Nonautoimmune hyperthyroidism, Congenital onset, Advanced bone age

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CONGENITAL hyperthyroidism is rare. Although most cases are caused by transplacental passage of maternal thyrotropin receptor (TSHR) antibodies in Graves' disease [1], leading to transient hyperthyroidism, some neonates have persistent hyperthyroidism without autoimmunity. Hyperthyroidism in such neonates may be severe and unremitting, and may be hereditary. The pathogenesis of these cases of hyperthyroidism remained obscure until constitutively activating germline mutations in the TSHR were identified as a cause of hereditary hyperthyroidism in two families [2] and of a sporadic case [3]. These mutant regions of TSHR which are the molecular basis for nonautoimmune hyperthyroidism overlap with those of toxic thyroid nodules, suggesting the same mechanism of constitutive activation of TSHR by permanent activation of the cAMP cascade that stimulates the growth and function of thyrocytes. *De novo* activating TSHR germline mutations have been reported in 9 cases as the cause of sporadic congenital nonautoimmune hyperthyroidism [3–11]. The clinical features of sporadic nonautoimmune hyperthyroidism are the earlier onset of thyrotoxicosis and more severe thyrotoxicosis, which is difficult to control with antithyroid drug therapy, than that of familial cases [12]. The severity of hyperthyroidism in sporadic cases may be attributed to the type of TSHR mutation because of no overlap between mutations in sporadic cases and those in familial cases, and has prevented familial transmission. Here, we describe a patient with sporadic congenital

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hyperthyroidism who presented an enlarged goiter and several complications during treatment with antithyroid drugs for 20 years, and in whom a novel TSHR germline mutation was detected.

Case Report

The patient was the third child of healthy unrelated parents born at the 32nd week of gestation. Birth weight was 1860 g, and APGAR score was 5/8 at 1 and 5 minutes. Tachycardia was observed 10 days after birth. Although no goiter was noted, thyroid function tests confirmed hyperthyroidism with a serum thyroxine (T4) level of 47 μg/dl (normal: 5.0–13.0 μg/dl), a serum triiodothyronine (T3) level of 737 ng/l (normal: 70–210 ng/l) and a resin triiodothyronine uptake rate of 55% (normal: 23%–33%). Hyperthyroidism was refractory to inorganic iodine therapy at the age of 5 months, when antithyroid drug treatment was initiated. At this time, technetium-99 scintigraphy showed a homogeneous and high uptake of 13% (normal: less than 3%). Antithyrotropin receptor antibodies were negative. Radiography of the hands and wrists showed an advanced bone age (corresponding to 5.0–6.0 years) with closure of the anterior fontanel. At the age of 1.5 years, neurosurgical intervention became necessary because of craniosynostosis. Epiphyseal closure was complete at the age of 12.5 years. Although motor development was grossly normal, goiter and mental retardation were already obvious during elementary school. The patient's intelligence was below normal. She graduated from junior high school, but was enrolled in a special education program. Once euthyroidism was achieved with antithyroid drug therapy, hyperthyroidism reoccurred whenever an attempt was made to reduce the dose of therapy, which had been maintained for 20 years, on and off in combination with L-thyroxine.

At the age of 20 years, she consulted our hospital while receiving thiamazole (20 mg/day) and L-thyroxine (50 μg/day). The patient was 150 cm tall and weighed 40 kg. The thyroid gland was enlarged (Fig. 1A). Perodactylia was present with bilaterally shorten forms of 5th metacarpus, and 3rd and 4th metatarsi on radiography (Fig. 1B, C, D). Thyroid function tests showed that serum thyrotropin (TSH) was 0.006 mIU/l (normal: 0.30–5.00 mIU/l), free T4 was 0.73 ng/dl (normal: 0.70–1.60 ng/dl), and free T3 was 11.50 pg/ml (normal: 1.70–3.70 pg/ml), presenting T3-predominant type. Serum anti-thyroglobulin antibodies, antithyroid peroxidase antibodies, thyrotropin-binding inhibitory immunoglobulins, and thyroid-stimulating antibodies were negative. Computed tomography (CT) of the neck revealed a diffuse goiter with a thyroid volume of 370 ml (Fig. 1E), which was estimated by using the equation for an ellipsoid: pi/6 (length × height × width). CT of the head showed dilatation of the cerebral ventricles (Fig. 1F).

Molecular analysis of the patient's TSHR gene revealed a *de novo* heterozygous point mutation in exon 10 at position 1535, a thymine-to-adenine shift, which is shown as a reverse sequence in Fig. 2A. This mutation leads to an amino acid substitution from leucine to glutamine at codon 512 (Leu512Gln). The mutation was not detected in her mother (Fig. 2B) or father (data not shown). Although total thyroidectomy was recommended, she preferred to receive outpatient radioiodine therapy. Six months after treatment of 13 mCi radioiodine, thyroid function tests showed subclinical hyperthyroidism (TSH: 0.129 mIU/l, free T4: 0.57 ng/dl, free T3: 3.53 pg/ml), while receiving thiamazole (15 mg/day) and L-thyroxine (50 μg/day). There was no history of thyroid disorders in other family members.

Methods

Laboratory evaluation of thyroid function and autoimmunity

Concentrations of TSH, free T4, and free FT3 were measured with enzyme immunoassays (AxSYM TSH, AxSYM FT4, and AxSYM FT3, respectively, Abbott Japan Co., Tokyo, Japan). Thyrotropin-binding inhibitory immunoglobulin activity was measured with commercial enzyme-linked immunoassay kit (RSR Ltd., Cardiff, UK). Thyroid-stimulating antibodies were measured by a commercial radioimmunoassay kit (Yamasa Co., Chiba, Japan). Anti-thyroglobulin antibodies and anti-thyroid peroxidase antibodies were measured with a commercial RIA kit (RSR Ltd.).

DNA sequencing

Genomic DNA was extracted from peripheral leukocytes obtained from the patient and her parents. Exon 10 of the TSHR encoding the entire intracellular and

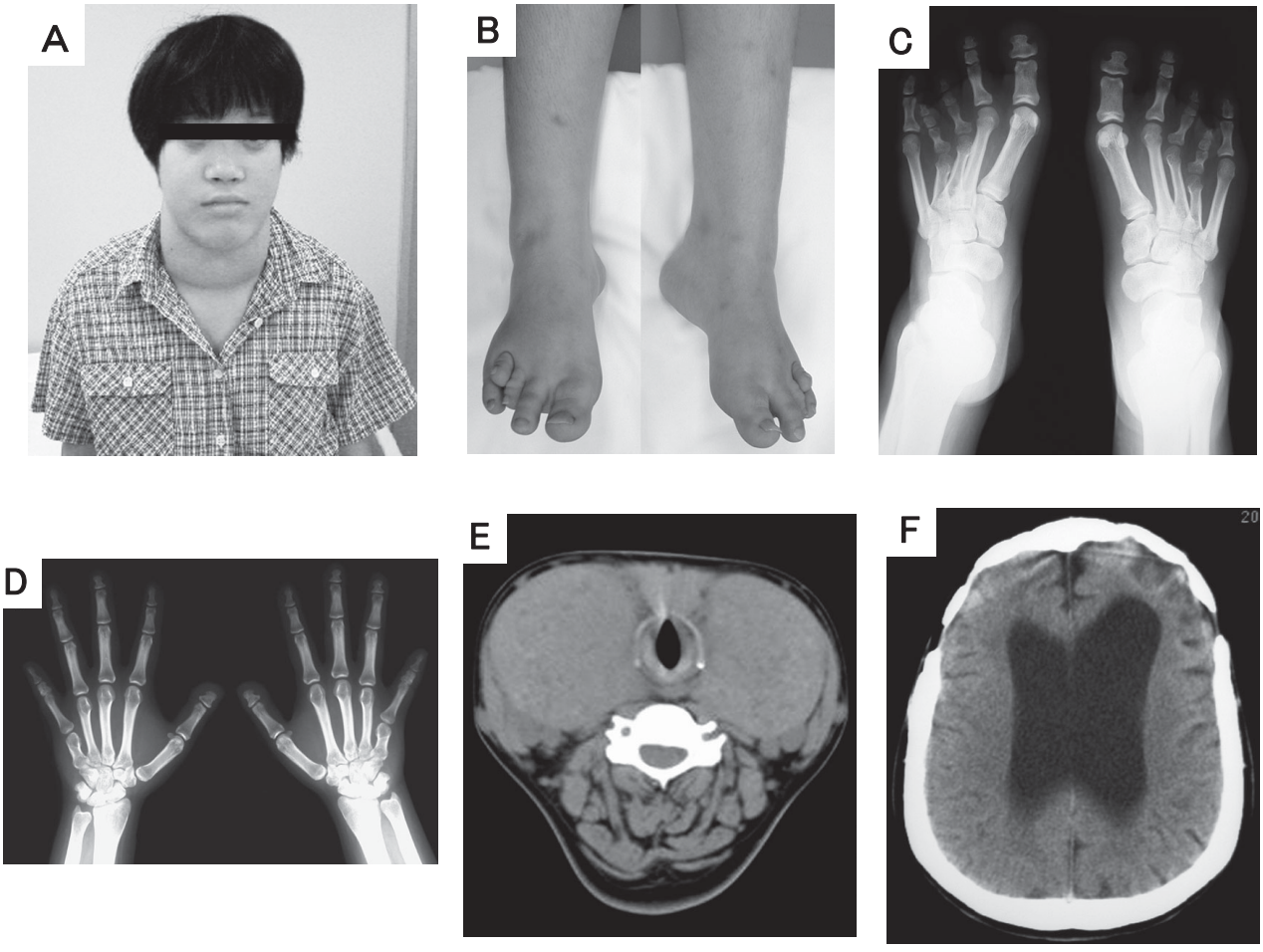

Fig. 1. A. Patient's facial appearance with a large goiter. B. Perodactylia in the bilateral fourth dactyli. C. Bilaterally shorten form of 5th metacarpus on radiography of the hands. D. Bilaterally shorten form of 3rd and 4th metatarsi on radiography of the feet. E. The diffusely enlarged thyroid gland shown on neck CT. F. Dilatation of the ventricle on head CT.

transmembrane regions and part of the proximal extracellular domain of the TSHR was amplified with one set of primers by the polymerase chain reaction. Conditions were as follows: initial denaturation for 2 min (94°C), followed by 30 cycles of denaturation for 30 s (94°C), annealing for 30 s (55°C), and elongation for 90 s and 30 s (72°C) with a final elongation step of 7 min (72°C). The forward primer was 5'-TAG GCT CAA GCA ATC CAC CTG-3'. The reverse primer was 5'-GTG TCA TGG GAT TGG AAT GC-3'. Direct sequencing of PCR products was performed using the Bigdye® terminator v1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA), and an automatic ABI 310 sequencer (Applied Biosystems), using either the same primers or internal primers (5'-ACT GTC TTT GCA AGC GAG TT-3' as a forward primer and 5'-GTC CAT GGG CAG GCA GAT AC-3' as a reverse primer). The present study was approved by the ethical committee of Kuma Hospital, and informed consent was obtained from the patient and her parents for the use of samples for research purposes.

Discussion

We have reported a case of sporadic congenital nonautoimmune hyperthyroidism with an activating TSHR germline mutation in a Japanese patient. Molecular analysis of the TSHR gene using genomic DNA from the patient revealed a heterozygous substitution in the third transmembrane domain of the TSHR, changing leucine 512 to glutamine, that was not present in the parents' DNA. Somatic mutations of residue 512 have been described in three cases of toxic thyroid nodules

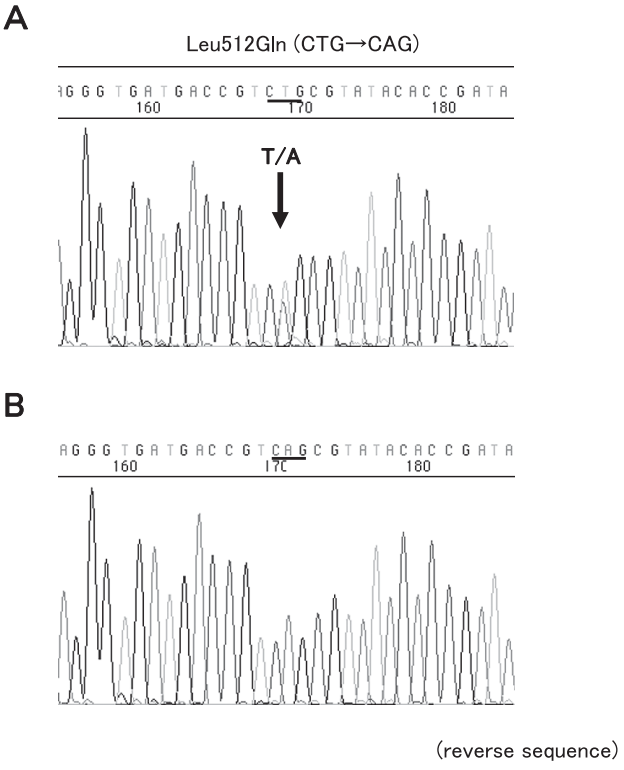

Fig. 2. Sequencing analysis of TSHR exon 10 in genomic DNA extracted from peripheral blood leukocytes of the patient and the patient's mother (reverse sequence shown). A. In the patient, a heterozygous thymine-to-adenine point mutation was identified at position 1535, resulting in an amino acid substitution of leucine for glutamine at codon 512. B. In the mother, who had no documented history of thyroid disease, only the wild-type TSHR was found. Arrow indicates a heterozygous thymine-to-adenine point mutation at position 1535.

[13, 14]; however, a germline mutation of this residue has not been reported previously in sporadic or familial cases of nonautoimmune hyperthyroidism. The third transmembrane domain of the TSHR from all mammalian species are identical amino acid sequences [15–20], and plays a key role in the activation of the receptor by a conformational change in coordination with seven transmembrane domains [21], suggesting the structural and/or functional importance of this domain of the TSHR. Indeed, the functionality of this mutated receptor was investigated by transient transfection in COS-7 cells. The receptor with a Leu 512 Gln mutation showed a 5-fold increase in basal cAMP level compared with that in the wild type transfectant [14]. This evidence implies that constitutive activation of TSHR by the Leu 512 Gln mutation is a direct cause of severe hyperthyroidism in the present case.

Based on the previous reports of sporadic congenital nonautoimmune hyperthyroidism [3–11] (Table 1), almost all cases were marked by premature delivery and the onset of hyperthyroidism within 1 year. In cases who were poorly controlled with antithyroid therapy, surgery was performed before the age of 10 years [3, 5–10]. The most common clinical findings in these patients were marked bone age acceleration, causing abnormality of bone structure in several cases [5–7]. When the exophthalamus is detected at the onset of hyperthyroidism, it usually does not progress during the years of follow-up, but resolves after antithyroid drug therapy [4, 5, 7, 10]. Although it is still unclear whether the hyperthyroidism causes impaired neuronal

Table 1. Clinical Characteristics of Sporadic Congenital Hyperthyroidism due to TSHR Mutations

Cases	TSHR mutation	Onset of hyperthyroidism	Gestation (weeks)	Therapy		Clinical complications	References
				Surgery	Radioiodine		
1	Ser 281 Asn	neonatal	36	+	–	craniosynostosis	(6)
2	Met 453 Thr	neonatal	32	–	–	exophthalamus, hepatosplenomegaly	(4)
3	Met 453 Thr	8 months	36	+	+	exophthalamus, splenomegaly, mental retardation	(10)
4	Ser 505 Asn	11 months	40	–	–	atopic dermatitis	(5)
5	Ser 505 Asn	5 months	38	+	–	exophthalamus, craniosynostosis, speech disturbance	(7)
6	Ile 568 Thr	5.5 weeks	35	–	–	speech disturbance	(11)
7	Val 597 Leu	9 months	37	+	–	—	(9)
8	Phe 631 Leu	neonatal	32	+	+	mental retardation	(3)
9	Thr 632 Ile	neonatal	33	+	+	mental retardation	(8)
10	Leu 512 Gln	neonatal	32	–	+	craniosynostosis, mental retardation, perodactylia, hydrocephalus	present case

development [22], more than half of patients have impaired neuronal function, including mental retardation and speech disturbance [3, 5, 7, 8, 10, 11]. The clinical features of the present case are consistent with those of previous reports of sporadic congenital nonautoimmune hyperthyroidism, and the hydrocephalus might be related to the craniosynostosis, even though no signs of increased intracranial pressure were present after surgery [23–25].

In the Japanese population, one familial case of nonautoimmune hyperthyroidism in adults caused by a receptor with a Pro 639 Ala mutation has been reported, but the functionality of this mutation has not been determined [26]; another case showed no mutations of TSHR in spite of the frequent occurrence of hyperthyroidism without autoimmunity in a family [27]. Although congenital nonautoimmune hyperthyroidism is rare, further investigation and recognition of this disease are required for accurate diagnosis to distinguish it from autoimmune hyperthyroidism. Long-term treatment with antithyroid drugs did not prevent further thyroid enlargement in the present case. Delayed or inadequate treatment of hyperthyroidism, presumably present since an early state of fetal development, might lead to irreversible consequences, such as mental retardation. Therefore, it is important to perform aggressive therapy that may include surgery and ablation radiotherapy without delay after the diagnosis.

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