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Case Report

A novel variant of *IGSF1* in siblings with congenital central hypothyroidism whose diagnosis was prompted by school health checkups

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Highlights

- We report C-CH in siblings whose diagnosis was prompted by school checkups.
- The siblings were carriers of a novel nonsense variant of *IGSF1*.
- School health checkups may prompt new C-CH diagnoses in Japan.

Abstract. Following the partial revision of the enforcement regulations of the School Health and Safety Act, school health checkups incorporated growth evaluation of schoolchildren in April 2016 using growth charts. We report cases of congenital central hypothyroidism (C-CH) in siblings with a novel nonsense variant in the immunoglobulin superfamily member 1 gene (*IGSF1*); their diagnoses were prompted by school health checkups. School checkups revealed that the older brother was overweight and had a reduced growth rate at the age of 11 yr, whereas the younger brother was overweight and had short stature at the age of 8 yr. They were diagnosed with C-CH because of normal thyroid-stimulating hormone (TSH) levels despite a low free thyroxine level and low TSH response in the thyrotropin-releasing hormone stress test. Only the older brother had prolactin deficiency and testicular growth without elevated testosterone levels. The siblings harbored a novel nonsense variant in exon 16 of *IGSF1* (NM_001555.5: c.3056G>A: p.Trp1019Ter) and were diagnosed with IGSF1 deficiency. In Japan, C-CH may be overlooked because TSH-based newborn screening alone is usually performed for patients with congenital hypothyroidism. The implementation of growth monitoring using growth charts in school health checkups may prompt new C-CH diagnoses.

Key words: immunoglobulin superfamily 1, congenital central hypothyroidism, prolactin

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Introduction

In April 2016, Japan's Ministry of Education, Culture, Sports, Science, and Technology promulgated a partial revision to the School Health and Safety Act. This revision recommends the use of growth charts to evaluate students' growth during school health examinations. The use of a growth chart allows for the detection of short stature, obesity, and reduced or increased growth rates.

Congenital central hypothyroidism (C-CH) is a rare form of hypothyroidism characterized by defective thyroid hormone production due to insufficient stimulation by thyroid-stimulating hormone (TSH) of an otherwise normal thyroid gland (1). Recent studies have indicated that the immunoglobulin superfamily 1 (*IGSF1*) is the most frequently implicated gene in isolated C-CH (2–4). The prevalence of C-CH caused by IGSF1 defects has been reported to be 1/80,000 to 1/100,000; however, the exact prevalence has not been determined (5, 6). IGSF1 defects cause X-linked C-CH, with some carriers also presenting with PRL deficiency, transient growth hormone deficiency, metabolic syndrome, a tendency towards pubertal delay, post-pubertal macroochidism, and attention deficit disorder (2, 7). *IGSF1* encodes a plasma membrane immunoglobulin superfamily glycoprotein, containing 12 C2-type immunoglobulin (Ig)-like domain loops, a transmembrane domain, and a short intracellular C tail. The protein is cotranslationally cleaved such that only the C-terminal domain, which contains seven Ig loops, reaches the plasma membrane (8). Human *IGSF1* and murine *Igsf1* mRNAs are highly expressed in Rathke's pouch, adult pituitary gland, and testes; however, the physiological role of IGSF1 in humans and the mechanism underlying the clinical manifestations of IGSF1 deficiency remains unclear (7). *Igsf1* knockout mice have reduced expression of the pituitary thyrotropin-releasing hormone receptor, which has been speculated to contribute to this phenotype (7). However, a recent study suggested that IGSF1 loss can impair TSH production, independent of alterations in TRHR levels or thyroid hormone activity (9).

Herein, we report the case of siblings who were diagnosed with C-CH caused by a novel nonsense variant in *IGSF1* after presenting with an overweight status and growth failure during school health checkups.

Patients and Methods

Case description

Patient 1: Patient 1 was born at 40 wk of gestation via normal vaginal delivery. His birth length and weight were 49.4 cm (–0.27 standard deviation (SD) for a normal Japanese boy) and 3,620 g (+1.2 SD for a normal Japanese boy), respectively. The neonatal hospital records did not indicate icterus. Congenital hypothyroidism screening in the city where the patient was born was based on TSH alone, and the results were normal. His psychomotor development was normal, and he did not have an attention-deficit disorder. He was the first child of non-consanguineous parents and lacked a family history of thyroid disease. At the age of 11 yr, he was overweight, as noted during a school health checkup. He was suspected to have C-CH based on low free T_4 (FT_4) and inappropriately normal TSH concentrations. The patient was then referred to our hospital for further evaluation. At that time, his height was 134.9 cm (–1.8 SD for a normal Japanese boy); weight, 42.2 kg (0 SD for a normal Japanese boy); and body mass index (BMI), 23.2 kg/m^2 (92nd percentile for a normal Japanese boy). His thyroid gland was not palpable. The patient's bilateral testicular volume was 4 mL. The growth chart revealed that he had been obese since age 3, with his growth rate decreasing from age 4 (**Fig. 1A**). He did not have constipation, bradycardia, or low body temperature. The laboratory findings were as follows (normal range in parentheses): serum TSH, 0.96 μIU/mL (0.35–4.94 μIU/mL); FT_4, 0.62 ng/dL (0.70–1.48 ng/dL); free triiodothyronine (FT_3), 2.80 ng/dL (1.68–3.67 ng/dL); total cholesterol (TC), 317 mg/dL (< 190 mg/dL); low-density lipoprotein cholesterol (LDL-C), 217 mg/dL (< 110 mg/dL); high-density lipoprotein cholesterol, 93 mg/dL (> 40 mg/dL); triglycerides (TGs), 128 mg/dL (31–108 mg/dL); IGF-1, 220 ng/mL (113–499 ng/mL) and testosterone, 0.52 nmol/L. The laboratory findings are summarized in **Table 1**. Magnetic resonance imaging revealed no abnormalities in the brain or the hypothalamic-pituitary regions. Ultrasonography revealed a small thyroid gland. Accordingly, the patient was diagnosed with central hypothyroidism, PRL deficiency, and a suspected IGSF1 deficiency. Thyroid hormone replacement therapy was initiated, which slightly improved hypercholesterolemia. Thereafter, at age 12.7, his bilateral testicular volume was 8 mL; however, his serum testosterone level was not elevated (testosterone 0.41 nmol/L).

Patient 2: Patient 2 was the younger brother of Patient 1. He was the third child in his family and was delivered vaginally at 40 wk of gestation. His birth length and weight were 51 cm (+0.8 SD for a normal Japanese boy) and 3,724 g (+1.2 SD for a normal Japanese boy), respectively. There was no record of icterus during the neonatal period. Congenital hypothyroidism screening in the city where the patient was born was based on TSH alone, and the results were normal. His psychomotor development was normal, and he did not have any attention disorders or hyperactivity. At the age of 8 yr, after his older brother's diagnosis, he presented with short stature and was overweight, as noted during a school health checkup (**Fig. 1B**). At that time, his height was 117.8 cm (–2.2 SD for a normal Japanese boy); weight, 28.3 kg (–0.1 SD for a normal Japanese boy); and BMI, 20.4 kg/m^2 (92nd percentile for a normal Japanese boy). The bilateral testicular volume was 3 mL. He did not have constipation, bradycardia, or low body temperature. The laboratory findings were as follows (normal range in parentheses): TSH, 1.44 μIU/mL (0.61–4.23 μIU/mL); FT_4, 0.83 ng/dL (0.90–1.70 ng/dL); FT_3, 3.4 ng/dL (2.3–4.0 ng/dL); TC, 221 mg/dL (< 190

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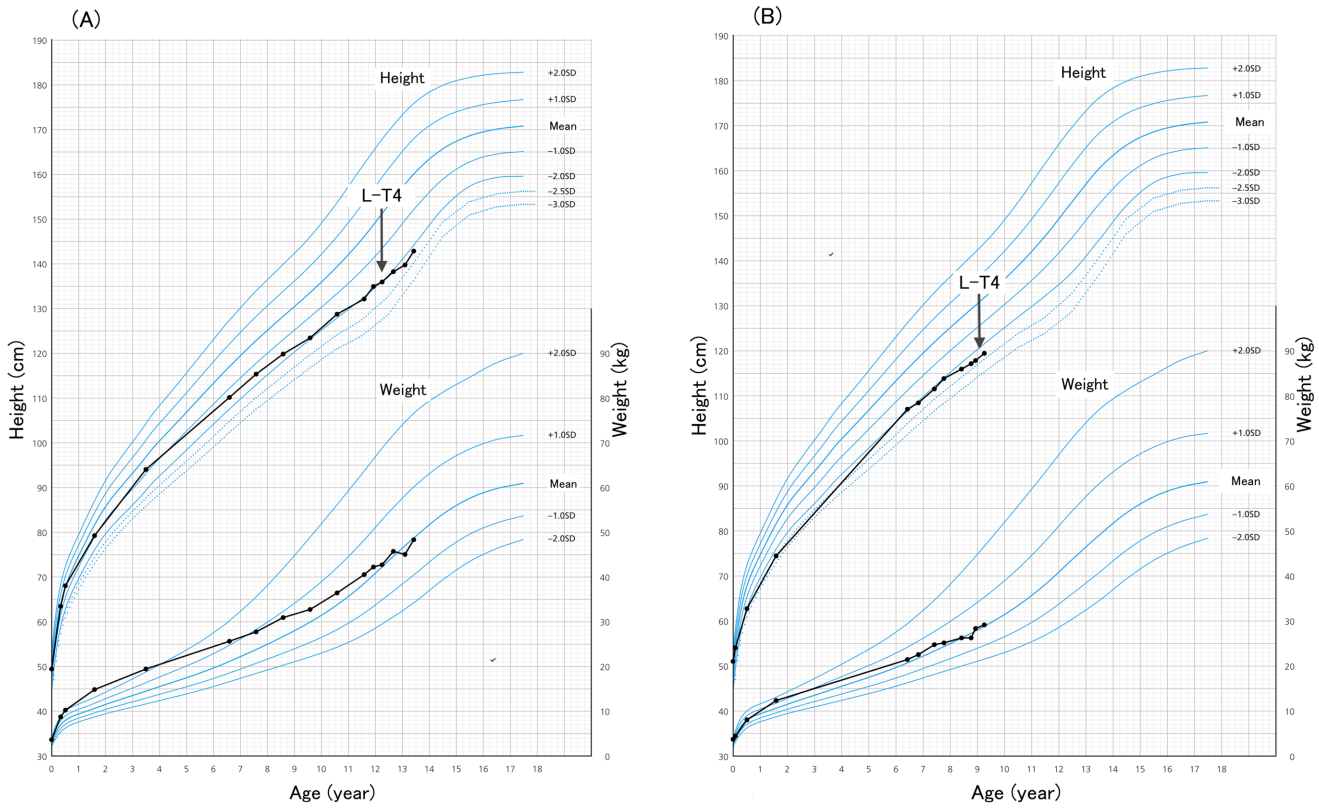

Fig. 1. Growth chart. (A) Older brother (Patient 1) and (B) younger brother (Patient 2).

mg/dL); LDL-C, 129 mg/dL (< 110 mg/dL); TG, 42 mg/dL (31–108 mg/dL); and IGF-1, 125 ng/mL (72–292 ng/mL). The laboratory findings are summarized in **Table 1**. Ultrasonography revealed a small thyroid gland. He was diagnosed with C-CH based on normal TSH levels despite a low FT_4 level, and a low TSH response in the thyrotropin-releasing hormone stress test. Treatment with levothyroxine was initiated, which improved hypercholesterolemia.

Molecular studies

Genomic DNA was extracted from peripheral blood samples. Genomic DNA obtained from Patient 1 was subjected to next-generation sequencing at the National Research Institute for Child Health and Development to examine multiple genes associated with pituitary dysfunction. Seventeen genes (*HESX1*, *LHX3*, *LHX4*, *OTX2*, *POU1F1*, *PROKR2*, *PROP1*, *SOX2*, *SOX3*, *CHD7*, *FGF8*, *FGFR1*, *GLI2*, *IGSF1*, *KISS1R*, *SOX10*, and *WDR11*) were screened. The identified *IGSF1* variant was confirmed by Sanger sequencing. Genomic DNA obtained from Patient 2 was subjected to Sanger sequencing.

Ethical consideration

Informed consent for genetic testing was obtained from the patients' parents. The parents approved the publication of the patient's clinical and genetic data. This study was approved by our institutional ethics committee (approval number: 21-48).

Results

Next-generation sequencing panel analysis revealed that Patient 1 had a hemizygous nonsense variant in exon 16 of *IGSF1* (NM_001555.5: c.3056G>A: p.Trp1019Ter). No other pathogenic variants were identified. The identified *IGSF1* variant was confirmed by Sanger sequencing. Sanger sequencing of *IGSF1* also revealed the aforementioned variant (p.Trp1019Ter) in Patient 2 (**Fig. 2**). This variant was located in the C-terminal region of IGSF1. This mutation was absent from the Tohoku Medical Megabank Organization database 8.3KJPN (http://jmorp.megabank.tohoku.ac.jp), the registry information of EAST Asians in the Genome Aggregation Database (https://gnomad.broadinstitute.org/), and the Human Genetic Variation Database (https://www.hgvd.genome.med.kyoto-u.ac.jp/). Evaluation of thyroid function and genetic analyses were not performed for the patients' mother, the second (male) child, and the fourth (female) child in the family.

Discussion

We identified a novel nonsense *IGFS1* variant (c.3056G>A: p.Trp1019Ter) in the siblings with C-CH. Regarding genetic defects of *IGSF1*, deletion, nonsense,

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Table 1. Laboratory finding

	Patient 1	Patient 2
TC (mg/dL)	317	221
LDL-C (mg/dL)	217	129
TGs (mg/dL)	128	42
IGF-1 (ng/mL)	220[a]	125[b]
testosterone (nmol/L)	0.52	0.13
TSH levels in TRH test (μU/mL)[c]		
basal	0.56[d]	1.44[e]
peak	3.67	6.35
FT_4 levels in TRH test (ng/dL)[c]		
basal	0.65[d]	0.83[e]
120 min	0.67	0.84
PRL levels in TRH test (ng/mL)[c]		
basal	< 0.60[f]	1.62[f]
peak	4.54	12.03
GH levels in arginine test (ng/mL)[c]		
basal	2.13	0.85
peak	6.54	22.1
LH levels in GnRH test (mIU/mL)[c]		
basal	< 0.10	< 0.10
peak	1.32	1.34
FSH levels in GnRH test (mIU/mL)[c]		
basal	1.96	1.41
peak	10.41	8.14
ACTH levels in CRF test (pg/mL)[c]		
basal	18	15.5
peak	31.3 (15 min)	41.7 (15 min)
Cortisol levels in CRF test (μg/dL)[c]		
basal	9.5	8.8
peak	11.4 (60 min)	14.4 (30 min)

[a]Reference range of this age is 113–499 ng/mL. [b]Reference range of this age is 72–292 ng/mL. [c]TRH, arginine, GnRH, and CRF tests were perfomed at the age of 12 yr in Patient 1 and at the age of 9 yr in Patient 2. [d]Reference range, TSH, 0.35–4.94 μU/mL, FT4, 0.7–1.48 ng/dL. [e]Reference range, TSH, 0.61–4.23 μU/mL, FT4, 0.90–1.70 ng/dL. Before the examination of Patient 2, our institution changed the method of measuring thyroid hormones and the reference range. [f]Basal reference range PRL for 12-yr-old, 1.3–10.8 ng/mL; basal reference range of PRL for 9-yr-old, 1.4–11.5 ng/mL. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; TGs, triglycerides; TSH, thyroid-stimulating hormone; TRH, thyrotropin-releasing hormone; ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor.

missense, and splicing mutations have been identified, mostly located in the C-terminal domain (10). *In vitro* functional studies of these *IGSF1* variants revealed that the encoded proteins migrate predominantly as immature glycoforms on SDS-PAGE and are largely retained in the endoplasmic reticulum, resulting in decreased membrane expression levels (11–14). Nakamura *et al*. indicated that two nonsense variants in the portion encoding the IGSF1-C-terminal domain cause a loss of normal IGSF1 function and/or expression (11). Our nonsense variant was predicted to result in the loss of normal protein function and/or expression in the pituitary gland through either protein truncation or nonsense-mediated mRNA decay, although no *in vitro* functional analysis was performed. The *IGSF1* p. Trp1019Ter variant qualifies as a pathogenic variant according to the PVS1, PM1, and PP1 criteria in the ACMG/AMP guidelines. Specifically, it meets the following criteria: its nonsense nature and loss of function are known mechanisms for central hypothyroidism (PVS1), which is located in the C-terminal region and is similar to the most established causative mutations for IGSF1 deficiency (PM1), and it shows co-segregation with the disease in multiple affected family members in a definitive causative gene (PP1) (15). The patients' clinical findings were consistent with IGSF1 deficiency.

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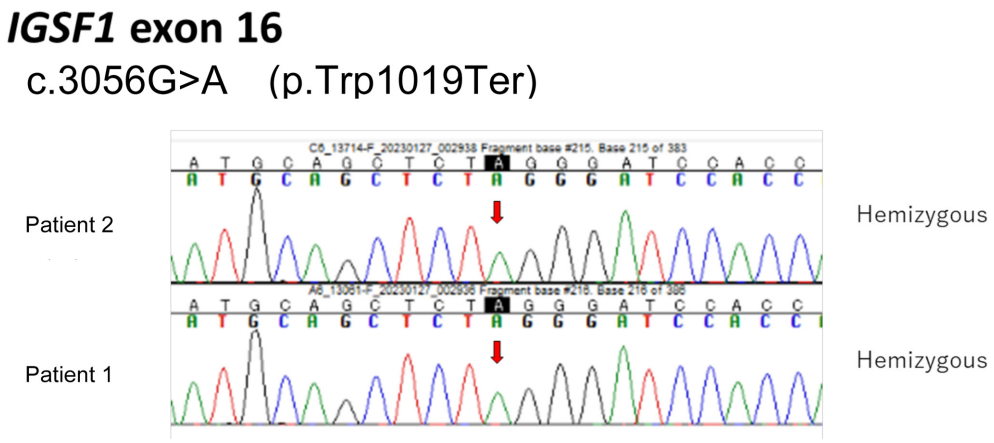

Fig. 2. Sanger sequencing showing the c.3056G>A (p.Trp1019Ter) (Red arrow).

Patient 1 showed C-CH and PRL deficiency, which are consistent with the characteristics of IGSF1 deficiency. Although Patient 2 lacked PRL deficiency, other studies have reported inconsistencies in PRL deficiency in family members with IGSF1 deficiency who have the same *IGSF1* mutation. Additionally, birth weights greater than the mean weight at gestational age, increased BMI, and dyslipidemia were also concordant with the symptoms of IGSF1 deficiency. IGSF1 deficiency is known to cause early or normal timing of testicular enlargement but delayed testosterone rise during puberty. Similarly, Patient 1 showed bilateral testicular volumes of 8 mL each at 12.7 yr but no testosterone elevation (testosterone 0.41 nmol/L). Patient 2 did not exhibit testicular enlargement. Both patients lacked a transient growth hormone deficiency or attention-deficit/hyperactivity disorder. Although dyslipidemia improved after levothyroxine supplementation, there were no clear improvements in BMI or growth velocity. Patients with IGSF1 deficiency can remain overweight even with appropriate thyroid hormone replacement (2). Therefore, C-CH may have been caused by the *IGSF1* p. Trp1019Ter variant.

School checkups led to a diagnosis of C-CH in these siblings. The severity of hypothyroidism varies among patients with an IGSF1 deficiency. Accordingly, the time of diagnosis and chief complaint also vary, including some cases with jaundice and failure to thrive from infancy, asymptomatic cases accidentally detected during family screening of the proband (16). IGSF1 deficiency, diagnosed based on increased BMI observed during a school health checkup, seems to be relatively mild. In Japan, growth charts are incorporated into school health checkups to assess student growth. This allows children with obesity and/or decreased growth velocity to seek medical consultations. In Japan, there are likely undiagnosed patients with C-CH who do not exclusively present with IGSF1 deficiency. One reason for this is that newborn screening for CH in Japan is usually based on TSH levels alone. Another reason is that C-CH is an easily missed clinical diagnosis even if it is a part of multiple pituitary hormone deficiencies (4). Therefore, school health checkups may prompt new diagnoses of C-CH. Pediatricians should check the thyroid function in children with obesity and/or decreased growth velocity. In addition, for male patients with C-CH, it is important to investigate PRL deficiency, discrepancy between the timing of testicular growth and rising testosterone levels, and high birth weight, considering IGSF1 deficiency.

Conclusion

We report the case of siblings who were diagnosed with C-CH caused by a novel nonsense variant (p.Trp1019Ter) during school health checkups that revealed overweight status and growth failure. Our findings demonstrated that implementation of growth monitoring using growth charts during school health checkups may prompt new C-CH diagnoses in Japan.

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