A Novel Pathogenic IGSF1 Variant in a Patient with GH and TSH Deficiency Diagnosed by High IGF-I Values at Transition to Adult Care

Short Title: Novel pathogenic IGSF1 variant associated with transient GH deficiency

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What is already known on this topic?
IGSF1 deficiency is a recently discovered disorder and data on long-term follow-up are scarce. Patients are known to have central hypothyroidism (CeH), prolactin deficiency, disharmonious pubertal development, and adult macroorchidism. Some of the patients exhibit short stature and growth hormone (GH) deficiency. However, GH deficiency is transient, and adult patients may have increased GH secretion and acromegaly-like features.

What this study adds?
Genetic analysis revealed a novel c.3559C>T (p.Q1187*) variant causing a stop codon in IGSF1. We suggest genetic analysis of IGSF1 in patients with CeH, especially when accompanied by the clinical and laboratory features associated with the syndrome. Notably, children with CeH and GHD who show high IGF-I levels at transition to adult care, should prompt analysis of IGSF1.

ABSTRACT
IGSF1 deficiency is a rare X-linked condition characterized by central hypothyroidism (CeH) and a wide variety of other clinical features with variable prevalence, including a delayed pubertal testosterone rise and growth spurt in contrast to normal or accelerated testicular growth, and adult macroorchidism with relatively low serum testosterone concentrations. Other features include increased waist circumference, attention deficit, prolactin deficiency and transient partial growth hormone (GH) deficiency in childhood in contrast to an increased GH secretion in adulthood. Patients with this disorder are not detected if neonatal screening programs are based on thyroid-stimulating hormone (TSH) concentrations.

A 13.2-year-old male patient was referred to the pediatric endocrinology clinic for evaluation of short stature. He was born large for gestational age into a nonconsanguineous family. During work-up for short stature, deficiencies of TSH, prolactin and GH were detected, leading to treatment with levothyroxine and GH. At 16.9 years, GH treatment was stopped and during transition to adult care, his IGF-I level was above the normal range. This prompted an analysis of IGSF1, in which a novel hemizygous variant causing a stop codon c.3559C>T (p.Q1187*) was found, confirming the diagnosis of the IGSF1 deficiency syndrome. In this report, we describe his clinical and hormonal characteristics at presentation and during long-term follow-up.

Keywords: IGSF1, central hypothyroidism, short stature, large for gestational age, growth hormone deficiency, prolactin deficiency

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INTRODUCTION
Immunoglobulin superfamily member 1 (IGSF1) is a plasma membrane glycoprotein encoded by IGSF1. It consists of 20 exons and is located in the Xq26.1 region (1,2). Pathogenic IGSF1 variants cause the X-linked IGSF1 deficiency syndrome (MIM #300888) and the characteristic features in hemizygous male patients include central hypothyroidism (CeH), delayed pubertal testosterone rise and growth spurt in contrast to normal or accelerated testicular growth (disharmonious pubertal development) and adult macroorchidism with relatively low serum testosterone concentrations. A variable proportion of affected males show prolactin deficiency, increased waist circumference, decreased attentional control, with short stature and in 16% growth hormone (GH) deficiency (3). However, GH deficiency is transient, and adult patients may exhibit increased GH secretion (4). The degree and age at presentation of CeH are variable, with some patients presenting in infancy or childhood and some patients being diagnosed with mild hypothyroidism at old age based on family studies (2,3,5,6). Given the rarity of this syndrome, the full spectrum of its phenotype as well as its pathophysiology remain subject of investigation (7).

Here we report a patient who presented with short stature, CeH and GH deficiency treated with levothyroxine and GH, in whom the high plasma insulin-like growth factor-1 (IGF-I) levels during transition to adult care ultimately led to the
identification of a novel IGSF1 variant. We present longitudinal data of the patient with respect to pubertal development and anthropometric measurements, as well as clinical and laboratory features from his parents and brother.

CASE REPORT

A 13.2-year-old male patient was referred to the endocrinology clinic for evaluation of short stature. He was born as the second child into a nonconsanguineous family at 38 gestational weeks by caesarean section, with a birth weight of 4400 gr (3.0 SDS) (8). The first child died one day after birth due to respiratory distress. Neonatal screening for hypothyroidism based on thyroid-stimulating hormone (TSH) measurement was normal. Neuromotor development was normal and except for allergic bronchiolitis, the medical history revealed no abnormalities.

At presentation, the initial physical examination was normal (Table 1). His height was 1.44 m (-1.9 SDS), considerably shorter than target height (0.0 SDS). Body proportions and weight were normal. Bilateral testicular volume assessed by the Prader orchidometer was 5 mL (-1.1 SDS, (9)) and pubic hair was at Tanner stage 2 (-0.7 SDS according to Dutch references) (10).

During workup for short stature (Table 2), his total blood count and biochemistry including electrolytes and lipids were within reference ranges. Plasma free thyroxine (FT4) concentration was below the normal range, while plasma TSH concentration was normal, hence CeH was diagnosed. The adrenocorticotropic hormone (ACTH) stimulation test showed a normal peak cortisol of 19.5 mcg/dL. Lевothyroxine treatment was started and led to normalisation of FT4. Low levels of plasma IGF-I (180 ng/mL, -2.0 SDS) (11), and prolactin were detected. Low IGF-I levels 168 ng/mL (-2.3 SDS) persisted after levothyroxine treatment had started. Cranial and pituitary magnetic resonance images did not reveal any pathology. At follow-up his growth velocity decreased (4.3 cm/year, -1.1 SDS) and GH stimulation tests were performed, showing a peak GH level of 6.87 ng/mL after insulin and 2.67 ng/mL after clonidine. Serum IGF-I was 168 ng/mL (-2.3 SDS). GH treatment was started and consequently growth velocity increased to 8.9 cm/year (1.5 SDS) in the first year. Molecular analysis for PROP1 and POU1F1 genes was normal.

During pubertal evaluation, orchiometric testicular volume was 6 mL (-1.5 SDS) at 14 years, 15 mL (-1.7 SDS) at 16 years and 25 mL (0.0 SDS) at 17 years. At 15 years of age patient’s FSH level was 5.5 mIU/mL, LH level was 24 mIU/mL and testosterone level was 4.96 ng/mL. At 17.9 years testicular volume was at least 25 mL at both sides (25 mL, being the largest bead on the orchidometer) and measured 6 cm in length. Pubic hair was at Tanner stage 3 and testosterone level was 16.9 nmol/L. A testis ultrasound, performed at 22.8 years and using a similar formula to calculate testicular volume as used for the reference population, resulted in a normal testicular volume (right testis 16.2 mL (0.9 SDS), left testis 12.9 mL (-0.1 SDS)) (9). We performed a dual-energy X-ray absorptiometry scan and L1-L4 bone mineral density was 0.891 g/cm² (Z-score -1.8 SDS). GH was stopped at 16.9 years. Anthropometric measurements and pubertal stages of the patient are presented in Table 1.

At transition to adult care, 6 years after the cessation of GH therapy, plasma IGF-I level was increased to 2.6 SDS (396 ng/mL) (Table 2), rising the suspicion of a possible IGSF1 variant. Genetic analysis was performed and indeed a novel hemizygous variant causing a premature stop codon (c.3559C>T, p.Gln1187*) was found (Figure 1). We established the diagnosis of the IGSF1 deficiency syndrome, consistent with the combination of CeH, hypoprolactinemia, transient GH deficiency, short stature, large for gestational age (LGA) at birth and high IGF-I levels in adulthood. The patient complained of clumsiness but did not report any attention problems, and currently works as a teacher. He had subtle facial coarsening without facial puffiness. At 22.8 years his waist circumference was 1.9 SDS (12) and BMI was 1.2 SDS (13).

The patient’s mother is a heterozygous carrier for the IGSF1 variant and her serum prolactin and FT4 levels were in the lower end of the reference range, lipid profile was normal. Her age at menarche was 13 years. Her height was 1.58 m (-0.9 SDS) and weight was 94.8 kg (3.8 SDS). Her younger brother did not consent to genetic analysis, but showed a FT4 at the lower end of the reference range. His prolactin and lipid profile were in normal ranges. His height was 1.89 m (2.0 SDS) and weight was 120 kg (3.3 SDS). His father’s thyroid function tests, prolactin levels and lipid profile did not reveal any pathology and he had a normal weight and height.

Molecular Analysis

Written informed consent was obtained from the patient and parents. Genomic DNA was extracted according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). IGSF1 gene coding exon sequencing was performed on the MiSeq platform (Illumina inc, San Diego, CA, USA) with 150-bp pair-end reads.

DISCUSSION

We identified a novel, hemizygous IGSF1 mutation variant in a male patient who initially presented with borderline short stature and a history of weight birth weight, in whom further investigations elucidated CeH, GH deficiency and low serum prolactin. At that time, the IGSF1 deficiency syndrome had not yet been uncovered (2), and only recently information became available about the surprisingly broad phenotypic spectrum of the IGSF1 deficiency syndrome (3). If such patients would be identified already, the combination of CeH (100%), prolactin deficiency (61%), increased birth weight (26%), and partial transient GH deficiency (16%) would probably have led to genetic testing of IGSF1. In our case, the suspicion of IGSF1 deficiency syndrome was triggered by a relatively novel and unusual observation, elevated plasma IGF-I in adulthood (observed in 20%) (3,4).

Interestingly, several relatively frequent features of the syndrome were absent in this patient. The most prominent of these is the normal testicular development in terms of testicular size and serum testosterone, while in the cohort of 69 males (3) low-normal T concentrations and macroorchidism in adulthood were reported in 88%, and a delayed pubertal testosterone rise and early/normal timing of testicular growth in 75%. Other features that were absent included mild problems with attentional control (reported in 75%), a small thyroid gland (74%), increased waist circumference in adults (59%) and decreased DHEA(S) (40-50%).

Regarding the reported mild problems with attentional control in the IGSF1 deficiency syndrome (14), it has been hypothesized that this may be related to inadequate prenatal T4 concentrations during critical periods of brain maturation (15,16). On the other hand, Igsf1 is expressed (although in low quantity) in the adult rat cerebral cortex, striatum, subfornical organ, amygdala and glial cells of the hypothalamus, possibly having a direct effect on executive functioning (17). Our
patient did not mention attention deficits. However, he did complain of clumsiness, which has also been described previously (3).

CeH is a rare condition and can result from dysfunctioning of the hypothalamus or pituitary gland. It can be part of multiple pituitary hormone deficiencies or be isolated. Isolated CeH is very rare and genetic causes include variants in TSHβ, TRHR, TBLIX or IGSF1, the latter being the most common. CeH and prolactin deficiency are particularly suspected for IGSF1 deficiency, but are also observed in TRH receptor variants and may be a part of multiple hormone deficiency caused by a variant in POU1F1, PROP1, HESX1 or LHX3 (18). IGSF1 deficiency is the most frequent inherited cause of CeH (18). The incidence of CeH caused by IGSF1 deficiency is estimated to be 1:100,000 in the Netherlands and 1:80,000 in Japan (5,19).

We therefore suggest to perform genetic analysis for this gene in any case of non-acquired CeH, but of course particularly in infants. There are additional suggestive features of this syndrome (3). Detection of CeH by neonatal screening programs requires both FT4 and TSH determinations (20).

In the Netherlands, neonatal screening for congenital hypothyroidism consists of T4, TSH, and thyroxine-binding globulin since 1995. Our patient demonstrates the importance of determining both FT4 and TSH in screening and diagnosing all forms of hypothyroidism.

Growth of patients with the IGSF1 deficiency syndrome resembles the growth pattern of constitutional delay of growth and puberty (CDGP), as characterized by growth retardation in early adolescence and a late pubertal growth spurt (3,6), and in both conditions circulating testosterone levels are low. However, in IGSF1 deficiency syndrome there is a normal or even early start of testicular growth, while this is delayed in CDGP. It is unclear whether the slow growth in adolescence of males with IGSF1 deficiency can be completely explained by the low circulating testosterone concentrations, or whether the partial GH deficiency also plays a role. Using current criteria for GHD, this was identified in 16% of children in IGSF1 deficiency (3,4), but in all cases GHD was transient. Similarly, a recently published Turkish patient with IGSF1 deficiency was treated with GH for two years, but during adolescence his GHD proved transient (21). Remarkably, in adult IGSF1 deficient patients IGF-I levels are generally in the upper half or even above the reference range, with increased GH secretion and variable mild acromegalic features in late adulthood (3,4,6).

During childhood, our patient had short stature and low GH responses to pharmacological stimuli, but in adulthood plasma IGF-I level was high. He had normal facial coarsening however he did not have other acromegalic features. The underlying mechanism of transient GH deficiency and somatotropic hyperfunction in adulthood is unknown. Because IGSF1 is expressed in rat hypothalamus, including ~19% of Ghrh-expressing neurons, as well as in pituitary somatotropes, it may be implicated either in hypothalamic regulation of GH or play a somatotrope-specific role in GH production or secretion (4). As pathogenic IGSF1 variants were not prevalent in a cohort of patients with CDGP without CeH or macroorchidism, testing for IGSF1 variants in the absence of those symptoms is not recommended (22).

Delayed testosterone production and macroorchidism after adolescence are characteristic features of IGSF1 deficiency (2). At ultrasonographic examination, most pediatric patients had testicular volumes in the upper half of the reference range, and 87% of adults showed macroorchidism (3,6). Our patient was an early stage of puberty at presentation and it took about 2.5 years for his testes to enlarge from 4.5 ml to at least 25 ml at 22 years (an increase from -1.1 to +1.5 SDS). Thus, true macroorchidism was not diagnosed with the orchidometer in this patient. However, the upper limit of normal testicular volume in adults is 32 ml (9), and as the largest bead of a typical orchidometer is 25 ml, this device is not suitable to diagnose adult macroorchidism. At physical examination, the length of the patient’s adult testis was 6 cm. When assuming a corresponding width of 3.5 cm, the orchidometric volume would be estimated at 38.5 ml (π/6 x length x width² (9)), thus above the reference range. The reasons for a lower Z-score measured with ultrasound is unclear.

It is not known why macroorchidism occurs in IGSF1 deficient patients. It has been suggested that the increased FSH to LH ratio plays a role (23), although both are generally within normal ranges, as was the case in our patient. An alternative explanation may be that FSH action is increased through increased sensitivity of gonadotropins to TRH (24) or by hypothyroidism which lengthens the period of Sertoli cell proliferation and leads to increase in Sertoli cell number and testes size (25). However, macroorchidism is also observed in IGSF1 deficient patients treated with levothyroxine since birth. Although IGSF1 deficient patients without macroorchidism have been reported (26), testicular growth in this condition typically starts at a normal or even advanced age, and shows a faster and longer growth period. Remarkably, testosterone secretion, pubic hair development and growth spurt start late. In all patients fertility was preserved, except one patient who was diagnosed with azoospermia and was resistant to treatment with FSH and LH (3).

Increased waist circumference and obesity are common in patients with IGSF1 deficiency (3). In a previous report, an untreated patient with IGSF1 deficiency showed profound hypercholesterolemia, with improvement after starting levothyroxine (27). Also, seven out of 69 male IGSF1 patients were diagnosed with dyslipidemia, two of whom while on levothyroxine treatment (3). Our patient had a normal BMI and lipid profile before and after the levothyroxine was started. Heterozygous female carriers reported to have milder phenotypes with low FT4, mild prolactin deficiency, increased BMI, and increased waist circumference. No fertility issues were reported among the female carriers (3). Patient’s mother was a carrier, and her prolactin and FT4 levels were at the lower tertile of the reference range and she did not have problems while lactating.

Since IGSF1 deficiency is only known for 10 years, data on long-term follow-up are scarce. Phenotype and genotype relation of patients with IGSF1 deficiency has not been determined yet and clinical findings varied with the same mutation within families (3). Recently, Roche et al reported the largest family of IGSF1 deficiency (c.2318T>C, p.L773P) with ten hemizygous males and 11 heterozygous females. Patients had classical endocrine manifestations of IGSF1 deficiency however the penetrance of thyroid dysfunction (mild to moderate) and prolactin deficiency were variable (28).

In this article, we report a patient with a novel pathogenic IGSF1 variant who presented with CeH, hypoprolactinaemia, borderline short stature and normal pubertal development with an increasing SDS of testicular volume over time, as well as a striking reversal of GH deficiency in adolescence to increased IGF-I levels in adulthood. We suggest genetic analysis of IGSF1 in any patient with CeH, especially when accompanied by any of the clinical and laboratory features associated with the syndrome. Clinicians should keep in mind that these patients may show high IGF-I levels, acromegaly-like features, and macroorchidism in adulthood.
Informed Consent: Written informed consent was obtained from the patient and the family.

Authorship Contributions
Concept: Aslı Derya Kardelen, Firdevs Baş, Şikaran Poyrazoğlu, Feyza Darendeliler
Design: Aslı Derya Kardelen, Feyza Darendeliler
Data Collection or Processing: Aslı Derya Kardelen, Esin Karakılıç Özturan, Firdevs Baş, Serdar Ceylaner
Analysis or Interpretation: Aslı Derya Kardelen, Feyza Darendeliler, Sjoerd D Joustra, Jan M Wit
Literature Search: Aslı Derya Kardelen, Sjoerd D Joustra, Jan M Wit
Writing: Aslı Derya Kardelen, Sjoerd D Joustra, Jan M Wit

References

Figure 1. Next gene sequencing image of the novel hemizygous c.3559C>T (p.Q1187*) change in IGSF1 gene of the patient.
### Table 1: Anthropometric measurements and pubertal stage of the patient

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>13.2</th>
<th>15.9</th>
<th>16.9</th>
<th>22.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height, m (SDS)</td>
<td>1.44 (-1.9)</td>
<td>1.63 (-1.5)</td>
<td>1.69 (-0.9)</td>
<td>1.77 (0.2)</td>
</tr>
<tr>
<td>Weight, kg (SDS)</td>
<td>46.5 (0.4)</td>
<td>60.5 (-0.6)</td>
<td>60.7 (-0.9)</td>
<td>86.3 (1.3)</td>
</tr>
<tr>
<td>BMI, kg/m² (SDS)</td>
<td>22.4 (0.7)</td>
<td>22.7 (0.3)</td>
<td>21.3 (-0.4)</td>
<td>27.5 (1.2)</td>
</tr>
<tr>
<td>SH/Height (SDS)</td>
<td>0.54 (1.1)</td>
<td>0.52 (-0.6)</td>
<td>0.52 (-0.8)</td>
<td>0.51 (-1.2)</td>
</tr>
<tr>
<td>Arm span, cm</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>180.5</td>
</tr>
<tr>
<td>Bone age, years</td>
<td>12.5</td>
<td>14</td>
<td>14</td>
<td>NA</td>
</tr>
<tr>
<td>PAH, m</td>
<td>1.69</td>
<td>1.76</td>
<td>1.82</td>
<td>NA</td>
</tr>
<tr>
<td>Tanner stage</td>
<td>T 5/5 mL</td>
<td>T 15/15 mL</td>
<td>T 25/25 mL</td>
<td>T 30/25 mL</td>
</tr>
<tr>
<td></td>
<td>PH 2</td>
<td>PH 4</td>
<td>PH 5</td>
<td>PH 5</td>
</tr>
<tr>
<td></td>
<td>SPL 4 cm</td>
<td>SPL 8 cm</td>
<td>SPL 8 cm</td>
<td>SPL 9 cm</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, Body mass index; NA, not available; PAH, predicted adult height; PH, pubic hair; SH, Sitting height; SDS, Standard deviation score; SPL, stretched penile length; T, testis volume by orchidometer

### Table 2: Hormonal evaluation of the patient

<table>
<thead>
<tr>
<th>At presentation (13.2 years)</th>
<th>Last evaluation (22.8 years)</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolactin (ng/mL)</td>
<td>2.3</td>
<td>2.9</td>
</tr>
<tr>
<td>TSH (mIU/L)</td>
<td>3.2</td>
<td>&lt;0.015*</td>
</tr>
<tr>
<td>FT4 (pmol/L)</td>
<td>10.1</td>
<td>13.3*</td>
</tr>
<tr>
<td>Cortisol (µg/dL)</td>
<td>11.5</td>
<td>11.8</td>
</tr>
<tr>
<td>DHEAS (µmol/L)</td>
<td>287</td>
<td>NA</td>
</tr>
</tbody>
</table>
| IGF-I (ng/mL) | 180a | 396b | 183-850
| IGFBP-3 (µg/mL) | 10.6a | 7.36b | 3.1-9.5
| FSH (IU/L) | NA | 5.8 | 2.6-11 |
| LH (IU/L) | NA | 2.8 | 0.4-7.0 |
| Testosterone (nmol/L) | NA | 21.6 | 6.1-27.1 |
| SHBG (nmol/L) | NA | 40.5 | 14.5-48.4 |
| AMH (ng/mL) | NA | 7.78 | 0.73-16.05 |

Abbreviations: AMH, anti-mullerian hormone; BMD, bone mineral density; DHEAS, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; FT4, free thyroxine; IGF-I, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein 3; LH, luteinizing hormone; SHBG, sex hormone binding globulin; TSH, thyroid stimulating hormone.

*During suppletion with 150 µg levothyroxine once daily.