Case report

The IGSF1 Deficiency Syndrome May Present with Normal Free T4 Levels, Severe Obesity, or Premature Testicular Growth

IGSF1 Deficiency with Borderline Low FT4

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What is already known on this topic?

• Almost all individuals with IGSF1 deficiency have central hypothyroidism
• Almost all individuals with IGSF1 deficiency show disharmonious pubertal development and macroorchidism in adulthood, but premature testicular growth is rare
• Individuals with IGSF1 deficiency tend to be overweight, but extreme early-onset obesity has only been reported once

What this study adds?

• IGSF1 deficiency can present with FT4 levels above the lower limit of normal
• Premature testicular growth without elevated serum testosterone can be a sign of IGSF1 deficiency
• The extreme weight gain in this and a previous case suggests that this is part of the clinical spectrum of IGSF1 deficiency syndrome

Abstract

Our objective is to further expand the spectrum of clinical characteristics of the IGSF1 deficiency syndrome in affected males, which so far includes congenital central hypothyroidism, disharmonious pubertal development (normally timed testicular growth, but delayed rise of serum testosterone), macroorchidism, increased body mass index, decreased attentional control and a variable proportion of prolactin deficiency, transient partial growth hormone deficiency in childhood and increased growth hormone secretion in adulthood. We present a family in which the proband was diagnosed with central hypothyroidism (CeH) and low serum prolactin. Severe weight gain started at 2 years, up to a BMI of 42.3 at 13.9 years. Testicular enlargement (5-6 ml, 3.8-4.3 SDS) started at 3 years. A pathogenic variant was found in the IGSF1 gene: c.3411_3412del, p.(Tyr1137*). His brother was referred for short stature at age 13 years and was diagnosed with CeH, normal serum prolactin and IGF-1, and disharmonious puberty. In the four male relatives (the proband’s brother and three cousins) who carried the variant (one adult), FT4 was below the lower limit of the reference range in two, and just above this limit in the other two cases. Three were overweight or obese, adolescents had disharmonious pubertal development and the adult had profound macroorchidism. In conclusion, male hemizygous carriers of a pathogenic IGSF1 variant can present with FT4 levels above the lower limit of the reference range, and severe early-onset obesity or premature testicular growth are part of the phenotypic spectrum.

Keywords: IGSF1 deficiency; hypothyroidism; macroorchidism; obesity; prolactin

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Introduction
Central hypothyroidism (CeH) is a rare disorder characterized by a low serum free thyroxine (FT4) concentration and inappropriately low or normal TSH levels (1). Acquired CeH is often part of multiple pituitary deficiency, for example due to compressive lesions, cranial surgery or irradiation, or injury. Congenital CeH is usually part of multiple pituitary hormone deficiency, but can also be isolated; both forms can be caused by genetic defects (1, 2).

Usually, cases with the congenital form are not diagnosed shortly after birth, because most newborn screening programs in the world are based on the detection of elevated TSH values only. Even in the few countries and states where screening programs are based on a combination of TSH and T4, some cases of congenital CeH can remain undiagnosed for many years, because of borderline low T4 levels (3).

Recently, immunoglobulin superfamily, member 1 (IGSF1) deficiency (MIM #300888) emerged as a novel cause of congenital CeH (4), and is now considered the most prevalent cause of congenital CeH (5). IGSF1 encodes a glycoprotein that is located on the membrane of various cell types, its transcript is abundantly expressed in the rodent pituitary gland and testis (4, 6). Deficiency of this protein in the human male causes X-linked CeH, disharmonious pubertal development (normal timing of testicular growth, but delayed rise of testosterone) and macroorchidism. In a variable proportion of affected males other features are observed, including prolactin deficiency, partial and transient growth hormone (GH) deficiency in childhood, increased body mass index (BMI), and decreased attentional control (4, 7-19).

We present here a male index case with CeH, premature testicular growth, and severe early-onset obesity, associated with a hemizygous nonsense variant in IGSF1 [c.3411_3412del, p.(Tyr1137*)], as well as variable phenotypes observed in four male relatives carrying the variant, including normal FT4 concentrations. The large variation in clinical and biochemical findings in affected males in this family shows that the phenotype of IGSF1 deficiency is even broader than estimated so far (4, 7-19), and may include a serum FT4 concentration above the lower limit of the reference range, premature testicular growth and severe early-onset obesity.

Case Presentation

Proband
The proband was born full term after an uncomplicated vaginal delivery, with a birth weight of 4082 grams (1.1 SDS) and length of 52.1 cm (0.8 SDS) (22). He was admitted to the neonatal unit for hyperbilirubinemia 4 days after birth. Newborn screening using TSH and T4 showed CeH (T4 <77.2 nmol/L and TSH <20 mIU/L (cut-off limits in screening program), which was confirmed by a blood draw at 4 weeks of age (T4: 52.9 nmol/L, reference range 58.1-154.8 nmol/L; TSH 1.7 mIU/L, reference range 0.8-19.2 mIU/L). At 7 weeks old, thyroid hormone replacement was started at 7.4 µg/kg/day. An extensive pituitary examination was performed, showing an undetectable serum prolactin level and normal serum levels of IGF-1 and cortisol. No structural abnormalities were observed with magnetic resonance imaging of the pituitary.

At 2.4 years of age, he showed obesity (BMI 20.3 kg/m², 2.4 SDS (22)). At follow-up, normal linear growth and further excessive weight gain was noted with a maximum at 13.9 years, when weight was 125.2 kg with a BMI of 42.3 [163% of the 95th US percentile (22) and 4.2 SDS for a 1980 European reference (23), Figures 1A and 1B]. Thyroid hormone treatment was interrupted between 2.8 and 3.4 years due to proband’s mother stopping medication, but has been administered since. Serum FT4 levels have been maintained in the lower half of the reference range (Table 1). His testicular volume assessed with Prader orchidometer was 2 ml at 2.4 years (0.9 SDS (24), but showed a remarkable enlargement to 5-6 ml at a follow-up visit at 3.4 years old (3.8-4.3 SDS) (Figure 2A), without pubic hair appearance or linear growth acceleration. At 6.3 years, a testicular ultrasound was performed, showing right and left testicular volumes of 6.3 and 5.8 ml [4.2 SDS, reference range at that age 0.3 to 1.1 ml (24)], still in the absence of pubic hair, and with undetectable plasma testosterone. A GnRH stimulation test showed a pre-pubertal pituitary response (baseline and peak LH of 0.09 and 3 IU/L; baseline and peak FSH of 0.95 and 16 IU/L). A borderline pubertal response to GnRH was first observed at 9.4 years old (baseline and stimulated serum LH of 0.3 and 7 IU/L; FSH of 2 and 17.9 IU/L), when his testicular size was 10-12 ml (3.5-3.8 SDS).

However, plasma testosterone was still undetectable, and baseline and stimulated FSH secretion surpassed LH secretion. Further clinical and laboratory data are shown in Table 1. Reference data for serum testosterone in minipuberty (1-3 months) and Tanner stage were derived from the literature (25, 26). A thyroid ultrasound at 12.5 years showed a symmetrically small gland, with an estimated volume of less than 1 ml for each lobe. Bone age was close to chronological age (27).

Genetic analysis
Fragile X testing was negative, the methylation pattern of SNRPN was not suggestive of Prader-Willi syndrome, and the array comparative genomic hybridization (CGH) was normal. However, a pathogenic variant (nonsense mutation) in the IGSF1 gene [NM_00117096.1: c.3411_3412del, p.(Tyr1137*)] was detected by Sanger sequencing (4, 10, 11). Segregation analysis showed that the mutation was also present in three female and five male family members (Fig. 2B).
The proband's older brother (case III-1) was seen at the clinic at the age of 13 years, after the proband's initial findings. He had a normal TSH and FT4 newborn screening result. At presentation, besides CeH [FT4 9.0 pmol/L (reference range 11.6-20.6), TSH 1.54 mIU/L (reference range 0.5-4.3)], he had a low plasma testosterone level in contrast to a testicular size of 25-28 ml (2.1-2.3 SD). His serum prolactin and IGF-1 concentrations levels were normal, as was his height and BMI, and he did not have any manifestations of long-standing untreated hypothyroidism. He subsequently started levothyroxine at 1 μg/kg/day, which led to normalization of his FT4 levels. The first male cousin (case III-3, 20 years) showed CeH, obesity, macroorchidism (50 ml), and attention issues at school. Initial thyroid function studies showed a low FT4 (9.7 pmol/L (reference range: 11.9-20.6]) and inappropriately low TSH (1.01 mIU/L). Thyroid function studies were repeated showing a slightly higher FT4 (10.68 pmol/L), which was just above the lower limit of reference range of the FT4 assay in use at the time (10.55-22.78 pmol/L), at a TSH of 0.715 mIU/L. The patient did not start levothyroxine treatment. The second affected male cousin (case III-6, 14.3 years) showed a FT4 just above the lower limit of the reference range (12.0 pmol/L, reference range: 11.9-20.6) with normal TSH levels, and obesity. At 14.3 years his testes were of appropriate size for age (10-12 ml, -0.8 to -0.4 SDS). At ultrasound testicular volumes were 17 and 12 ml, 2.4 and 1.4 SDS) (24), which contrasted with the low plasma testosterone. The ultrasound also showed some peri-testicular fluid and microcyst. The third affected male cousin (case III-7, 11.4 years) also showed a serum FT4 just above the lower limit of the reference range (12.7 pmol/L, reference range: 11.9-20.6) with normal TSH levels, and was also obese. He had difficulties at school with reading comprehension. At 11.4 years of age, his testicular volume (5-6 ml) was larger than expected for the pre-pubertal pubic hair Tanner stage. The proband’s mother and female cousin, who were heterozygous for the IGSF1 variant, showed a serum FT4 of 12.9 pmol/L and 16.7 pmol/L, respectively (reference range: 11.9-20.6). Their menarcheal ages were 10 years and 11.5 years, respectively, and they both had normal menstrual cycles. Clinical data for one male patient and one female carrier were not available (cases I-1 and II-2). For all patients discussed in manuscript, blood work and examinations were done within the normal standard of care. Informed consent (and assent, if appropriate) was obtained for all genetic testing that was completed. For patients below the age of 18 years, an informed assent and consent was obtained, as appropriate. For patients above 18 years of age, an informed consent was obtained. Limited data of the proband, his elder brother and his mother were previously published as part of a large case series (11), and did not include the proband’s early obesity or very early testicular growth.

Discussion
Here, we describe a patient who presented initially with neonatal jaundice, central hypothyroidism and low serum prolactin, and later showed severe obesity, premature testicular growth, disharmonious pubertal development, disruptive behavior at school, and macroorchidism. Genetic evaluation showed a pathogenic variant in the IGSF1 gene [c.3411_3412del, p.(Tyr1137*)]. Further evaluation of the family showed that the proband’s brother and three out of four male maternal cousins carried the same variant. Their FT4 levels were just below or, in three cases, just above the lower limit of the reference range at first or second testing. Macroorchidism was also present in two young adult male relatives. In the two adolescents, testicular volume was large compared to Tanner stage and plasma testosterone, consistent with disharmonious pubertal development. Serum prolactin was decreased only in the proband, compared with approximately 60% of patients in earlier reports (11). Birth weight was between 0.8 and 2.1 SDS in male carriers. In this family, there are three clinical observations that are unusual compared to previously reported families. First, in virtually all patients with IGSF1 deficiency reported so far, the serum FT4 concentration is decreased in combination with normal or low serum TSH, while the occurrence of other clinical and laboratory features is more variable (4, 7-12, 14, 16-18). Serum FT4 levels have been reported to be just above the lower limit of reference range (13, 15) or fluctuating around it (19) in only two and one male patients, respectively. In the present report, we show that out of four male relatives of the proband, FT4 levels were just above the lower limit of normal in two of them, and fluctuated around the lower limit in a third case, suggesting that this may occur more often in males with IGSF1 deficiency than previously assumed.

Second, the start of testicular growth in the proband from 2 years of age was unusually early, possibly associated with relatively high serum FSH concentrations before and after stimulation by GnRH. A similarly early onset, but less extreme testicular growth (3 ml with the Prader orchidometer) with increased FSH levels has been recorded only once in a 3 year old boy with a hemizygous IGSF1 deletion (28). In adult male patients, 24-hour FSH secretion is generally increased, although within the normal range (11). Third, although obesity has been observed in 21% of children and 17% of adult males (11), an extreme early weight gain as in the proband is unusual. Only one previous report of a 2-year-old patient with IGSF1 deficiency showed severe obesity (4.2 SDS) (16). In our report, obesity was also observed in three of the four proband’s relatives carrying the IGSF1 variant. It is currently unknown why IGSF1 deficiency is associated with increased BMI, and in rare cases also with extreme body weight gain. One might speculate that intracellular thyroid hormone concentration or thyroid hormone dependent gene translation are defective in the adipose tissue of these patients. Alternatively, obesity may be caused by decreased fat-burning non-shivering thermogenesis given the role of TRH in the central regulation of brown adipose fat functioning and the decreased expression of the pituitary TRH receptor in IGSF1 knockout mice (4). We have not been able to formally test signs of tissue hypothyroidism or thermogenesis.

The occurrence of other clinical features, such as relatively high birth weight, prolonged neonatal jaundice, as well as the variability of clinical features within one family, are consistent with previous reports (4, 7-19). For example, birth weight was relatively high (0.8-2.1 SDS), in the upper half of what has been reported for other cohorts (9, 11).
Macroorchidism was seen in the three adult males and disharmonious puberty in the two adolescents, as reported in virtually all cases so far (11). Mild neurological findings, such as attention deficit and difficulties with reading and focusing, were present in three cases in this family, in line with observations in a larger cohort (12). The phenotypic differences within this family are intriguing, and this has been observed in all previously published families (11). In the proband, CGH and testing for Prader-Willi and fragile X syndrome were normal. Whole exome/genome sequencing did not reveal other genetic defects that explain these differences. We speculate that polymorphisms or (variable penetrance of) epigenetic changes account for the phenotypic differences.

The pathophysiology of the IGSF1 deficiency syndrome is currently unknown, but may include reduced expression of the receptor for thyrotropin-releasing hormone (TRH) and impaired TRH stimulation of thyrotropin secretion (29). The macroorchidism seen in these patients may be associated with increased FSH secretion (20). Alternatively, the usual T3 dependent pubertal increase in LH receptors and cessation of Sertoli cell proliferation could be affected in these patients (despite treatment with levothyroxine), causing a delay in pubertal testosterone rise in the presence of macroorchidism (30). The prolactin and GH deficiency may be related to effects on pituitary transcription factors, such as Pit-1 or TRH receptivity (2). The cause of the obesity seen in these patients has not been elucidated, and there seems to be no association between FT4 levels and the degree of overweight (11). Similarly, it is uncertain whether the issues with attentional control seen in these patients may be associated with timing of levothyroxine treatment (11).

In conclusion, this paper shows that male hemizygous carriers of a pathogenic IGSF1 variant can present with FT4 levels above the lower limit of the reference range; that premature testicular growth without increased testosterone concentrations may be part of the spectrum of clinical features; and that severe early onset obesity may be part of the phenotypic spectrum. This would imply that testing for IGSF1 should not only be considered in patients who have CeH of unknown cause and low prolactin, macroorchidism, or delayed puberty (11), but also when serum FT4 is slightly above the lower limit of normal and in cases with premature testicular growth or unexplained early-onset obesity. We maintain the advice to test family members in a pattern consistent with X-linked inheritance, if a proband is diagnosed with IGSF1 deficiency, because of the indirect evidence that levothyroxine treatment has a positive effect (11) and in order to obtain more information about the scope of the clinical and laboratory characteristics of this still enigmatic syndrome.

Disclosure Statement
No disclosures for Steven Ghanny, Aliza Zidell, Helio Pedro, Sjoerd D. Joustra, Monique Losekoot, Jan M. Wit and Javier Aisenberg.

Author Contributions Statement
SG was involved in aspects of patient care, as well as manuscript preparation. AZ and HP were part of the genetics team that evaluated families. They also assisted in manuscript preparation. SDJ was involved in advice on the clinical assessment of the proband and relatives, as well in revising subsequent versions of the manuscript. ML was responsible for genetic testing. JMW advised on the clinical assessment of the proband and relatives, and revised subsequent versions of the manuscript. JA was involved in aspects of patient care, as well as manuscript preparation. All authors agreed with the submitted version.

Ethics
Ethics Committee Approval: See attached letter
Informed Consent: Informed consent (and assent, if appropriate) was obtained for all genetic testing that was completed.

Authorship Contributions
Surgical and Medical Practices: Javier Aisenberg, Steven Ghanny, Aliza Zidell, Helio Pedro, Monique Losekoot
Concept: Javier Aisenberg, Steven Ghanny
Design: Javier Aisenberg, Steven Ghanny, Jan M Wit, Sjoerd D Joustra
Data Collection or Processing: Javier Aisenberg, Steven Ghanny, Aliza Zidell, Helio Pedro
Analysis or Interpretation: Javier Aisenberg, Steven Ghanny, Aliza Zidell, Helio Pedro, Jan M Wit, Sjoerd D Joustra
Literature Search: Javier Aisenberg, Steven Ghanny, Jan M Wit, Sjoerd D Joustra
Writing: Javier Aisenberg, Steven Ghanny, Jan M Wit, Sjoerd D Joustra

Conflict of Interest: No conflict of interest
Financial Disclosure: No financial disclosure

References


Uncategorized References
Table 1. Longitudinal clinical, laboratory, radiological and medication data in the proband

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<th>0.92</th>
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<th>3.40</th>
<th>6.30</th>
<th>7.60</th>
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<td>6.1</td>
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<td>27.3</td>
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<td>BMI SDS®</td>
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<td>3.9</td>
<td>2.9</td>
<td>2.6</td>
<td>2.4</td>
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<td>FT4 (pmol/L)</td>
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<td>29.6</td>
<td>11.6</td>
<td>10.0</td>
<td>15.4</td>
<td>18.9</td>
<td>14.2</td>
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<td>3.2</td>
<td>0.02</td>
<td>0.46</td>
<td>1.4</td>
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<td></td>
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<td></td>
<td></td>
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<td>&lt;0.2f</td>
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<td>Levothyroxine dose (ug/kg/day)</td>
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<td>5.5</td>
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<td>1.7</td>
<td>1.1</td>
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* Assessed by the atlas of Greulich and Pyle [27]
® Based on CDC reference [22]
c Assessed by the Prader orchidometer

d Sample taken before start of L-thyroxine treatment

e For minipuberty (1-3 months) the reference is 7.2±2.4 nmol/L [25]
f For Tanner stage 1 the reference range is <0.3-0.5 nmol/L [27]
Table 2: Clinical and laboratory findings in the proband and relatives carrying the pathogenic *IGSF1* variant (hemizygous in males, heterozygous in females)

<table>
<thead>
<tr>
<th>Position in pedigree</th>
<th>Proband</th>
<th>Brother</th>
<th>Mother</th>
<th>Cousin (M)</th>
<th>Cousin (F)</th>
<th>Cousin (M)</th>
<th>Cousin (M)</th>
<th>Reference Range</th>
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<tr>
<td>Age (years)*</td>
<td>15.9</td>
<td>13.7</td>
<td>43</td>
<td>20</td>
<td>18</td>
<td>14.3</td>
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<td>1.8</td>
<td>2.1</td>
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<td>Tanner stage (P)</td>
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<td>3</td>
<td>5</td>
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<td>5</td>
<td>3</td>
<td>2</td>
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<td>Height SDS c</td>
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<td>0.6</td>
<td>N/A</td>
<td>1.4</td>
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<td>BMI SDS d</td>
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<td>N/A</td>
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<td>1.7</td>
<td>2.1</td>
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<td>Testicular volume (mL) e</td>
<td>&gt;50cc</td>
<td>25-28</td>
<td>N/A</td>
<td>50</td>
<td>N/A</td>
<td>10-12‡</td>
<td>5-6</td>
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<td>Developmental issues</td>
<td>Disruptive behavior at school</td>
<td>Attention Deficit Disorder</td>
<td>None</td>
<td>Focusing difficulties</td>
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<td>None</td>
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<td>Free T4 (pmol/L)</td>
<td>See Table 1</td>
<td>9.0 e</td>
<td>12.9</td>
<td>9.7</td>
<td>16.7</td>
<td>12.0</td>
<td>12.7</td>
<td>11.9-20.6 e</td>
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<td>See Table 1</td>
<td>1.54</td>
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<tr>
<td>Testosterone (nmol/L)</td>
<td>14.7 f</td>
<td>2.5 g</td>
<td>N/A</td>
<td>12.5 f</td>
<td>N/A</td>
<td>3 g</td>
<td>Dependent on age and Tanner stage</td>
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Abbreviations: N/A, not applicable; SDS, standard deviation score
* Age at investigation
b Based on CDC references [22]
* Assessed with Prader orchidometer
e Ultrasonographic testicular volume of largest testis was 25 ml (0.87 SDS). Bilateral testicular microlithiasis was also noted.
* For patient III-1 the laboratory provided a reference range of 11.6-20.6 pmol/L
f For G5 the reference range is 12.2-21.3 nmol/L [26]
Figure 1. Height (panel A) and BMI (panel B) for age of proband plotted on CDC charts (22).
Figure 2. Panel A: Testicular growth as assessed by orchidometer in the proband plotted on reference charts according to Joustra et al. (24). Panel B: Pedigree. The arrow indicates the proband. Solid squares indicate hemizygous carriers. Black dots signify heterozygous female carriers.