CHAPTER 6

The IGSF1 deficiency syndrome: characteristics of male and female patients

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ABSTRACT

Context

Ig superfamily member 1 (IGSF1) deficiency was recently discovered as a novel X-linked cause of central hypothyroidism (CeH) and macroorchidism. However, clinical and biochemical data regarding growth, puberty, and metabolic outcome, as well as features of female carriers, are scarce.

Objective

Our objective was to investigate clinical and biochemical characteristics associated with IGSF1 deficiency in both sexes.

Methods

All patients (n = 42, 24 males) from 10 families examined in the university clinics of Leiden, Amsterdam, Cambridge, and Milan were included in this case series. Detailed clinical data were collected with an identical protocol, and biochemical measurements were performed in a central laboratory.

Results

Male patients (age 0-87 years, 17 index cases and 7 from family studies) showed CeH (100%), hypoprolactinemia (n = 16, 67%) and transient partial GH deficiency (n = 3, 13%). Pubertal testosterone production was delayed, as were the growth spurt and pubic hair development. However, testicular growth started at a normal age and attained macro-orchid size in all evaluable adults. BMI, fat percentage and waist circumference tended to be elevated. The metabolic syndrome was present in 4 of 5 patients over 55 years of age. Heterozygous female carriers (age 32-80 years) showed CeH in 6 out of 18 cases (33%), hypoprolactinemia in 2 (11%), and GH deficiency in none. As in men, body mass index, fat percentage, and waist circumference were relatively high, and the metabolic syndrome was present in 3 cases.

Conclusion

In male patients, the X-linked IGSF1 deficiency syndrome is characterized by CeH, hypoprolactinemia, delayed puberty, macro-orchidism, and increased body weight. A subset of female carriers also exhibits CeH.

CONTEXT

We recently described a novel X-linked syndrome caused by loss-of-function mutations or deletions in Ig superfamily member 1 (*IGSF1*, OMIM 300888) (1). The main clinical characteristics of the IGSF1 deficiency syndrome are congenital hypothyroidism of central origin (central hypothyroidism [CeH]) and macro-orchidism. In a variable proportion of affected males, other features were observed, including prolactin deficiency (69%), partial and transient GH deficiency (22%), disharmonious pubertal development (normal timing of testicular growth but delayed rise of testosterone), and increased body mass index (BMI) (1). More recently, 4 Japanese patients with similar phenotypes were described (2;3).

IGSF1 is a plasma membrane glycoprotein, highly expressed in Rathke's pouch and the adult anterior pituitary gland. Pathogenic *IGSF1* mutations impair protein trafficking to the cell surface in heterologous HEK293 cells. *Igsf1*-deficient male mice show diminished pituitary and serum TSH concentrations, decreased serum T_3 concentrations and increased body mass. These observations appear secondary to reduced pituitary TRH receptor expression (1). However, the exact pathophysiology of the various clinical features remains unknown (4).

Here, we provide additional clinical and biochemical information of the IGSF1 deficiency syndrome, including neonatal screening data, longitudinal data on pubertal development, serum IGF-1 concentrations, body composition, and elements of the metabolic syndrome in male patients, as well as characteristics of heterozygous female carriers.

SUBJECTS AND METHODS

Subjects

We collected data on 24 hemizygous male carriers of *IGSF1* mutations or deletions from 10 unrelated families. We previously showed that all intragenic mutations cause trafficking defects in the IGSF1 protein when expressed in heterologous HEK293 cells (1). Seven families were studied in the Leiden University Medical Center or the Academic Medical Center in Amsterdam (The Netherlands) (families A, C-G). One family was investigated at Addenbrooke's Hospital in Cambridge, Great Ormond Street Hospital for Children and the UCL Institute of Child Health in London (United Kingdom), and the Fremantle Hospital Unit of the University of Western Australia (family B). The remaining two families were investigated at the Endocrine Unit of the University of Milan (Italy) (family I and J) (1). Since female carriers of an *IGSF1* mutation might be symptomatic due to skewed inactivation of the X-chromosomes, we studied female relatives (see

supplementary file of Sun et al. (1) for pedigrees of all families). Out of the 14 mothers of the 17 index cases (obligate carriers), heterozygosity was confirmed in 11 and assumed in the remaining 3. In addition, 7 other potential carriers were tested, of whom 5 were positive. One confirmed carrier (C-II.2) did not consent to phenotyping studies and was excluded from further analysis. Thus, 18 female carriers were included in this study. The Medical Ethics Committee of the Leiden University Medical Center approved the study. All subjects gave written informed consent for clinical investigation and publication.

Methods

Study design

In this descriptive case series, we present data on the clinical and biochemical characteristics of patients with hemizygous (males) or heterozygous (females) mutations or deletions in *IGSF1*. Data were collected from patient records and complemented with additional measurements. Data are presented separately for index cases (n = 17 males), nonindex males (n = 7) and females (n = 18).

Endocrine data

In the Netherlands, neonatal screening for congenital hypothyroidism consists of the sequential measurement of T_4, TSH and (since 1995) T_4-binding globulin (TBG) concentrations in filter paper dried blood spots collected shortly after birth. In the Italian region of family J, screening consists of simultaneous TSH and T_4 measurement (5). Screening data, as well as confirmatory pretreatment thyroid function data obtained via venipuncture, were retrieved from patient records and compared with in-house (except for T_4 (6)) age-specific reference intervals. Family members of index patients were screened for pituitary deficiencies by standard functional testing, as previously described (1).

CeH was defined as a plasma free T_4 (FT_4) concentration below the age-specific reference interval (<12 pmol/L in neonates and <10 pmol/L in older individuals) in combination with a nonelevated plasma TSH concentration (7). Prolactin deficiency was defined as a plasma prolactin below the lower limit of the reference interval. The diagnosis of GH deficiency (GHD) was based on a combination of clinical characteristics (eg, growth failure), bone age, serum IGF-1 concentrations, and maximal GH concentrations in stimulation tests or a 12-hour GH profile [for details, see Ref. 1]. ACTH deficiency was defined as an insufficient increase in cortisol levels (<0.55 μmol/liter) after a CRH stimulation or insulin tolerance test. Hormonal replacement therapy and medication for dyslipidemia, hypertension, or diabetes mellitus were started at the discretion of the treating physician.

Growth and pubertal development

Growth and pubertal development were evaluated in all male patients with a minimal age of 16 years who had been followed up by a pediatric endocrinologist since childhood. All available longitudinal data on height, pubertal stage, testicular volume (assessed with the Prader orchidometer), and plasma testosterone were retrieved from the hospital's patient records. Height standard deviations scores (SDSs) were calculated with the Growth Analyzer Research Calculation Tool 4.0 (Growth Analyser BV), using the Dutch reference values of 2010 (8). Pubertal stage SDS was calculated using the Dutch reference values of 1997 (9) in Puberty Plot (Netherlands Organisation for Applied Scientific Research TNO) (10).

Metabolic parameters

Height and weight were measured using a wall-mounted stadiometer and a digital floor scale, respectively. BMI SDS was calculated using Dutch reference values of 2009 (11). Body fat percentage was evaluated with bioelectrical impedance analysis, using the Bodystat 1500MDD (Bodystat Limited, Isle of Man, British Isles). Results were compared with reference data provided by Bodystat. In B-III.7, B-III.8, and B-I.8, fat percentage was measured with bioelectrical impedance analysis using the Tanita body composition analyzer (TBF-300, Arlington Heights, USA), and dual-energy X-ray absorptiometry was used in B-II.13, B-II.8, and B-II.11 (Lunar Prodigy, GE Healthcare, USA) and B-I.4 (Discovery A, Hologic, USA). For B-III.7 and B-III.8, age-specific normal values (12) were used as reference interval, and for adults from family B the Lunar Prodigy UK reference values.

The metabolic syndrome was defined according to criteria proposed by the International Diabetes Federation (13;14), ie, an elevated waist circumference and any 2 of the following: elevated triglycerides, reduced high-density lipoprotein (HDL)-cholesterol, elevated blood pressure (BP), elevated fasting glucose, or specific treatment for these abnormalities. For adults, cut-off values as defined by the International Diabetes Federation were used (13). For children older than 10 years, reference data were used to determine gender- and age-specific +2 SDS cut-offs for waist circumference (15), HDL-cholesterol (16), triglycerides (16), glucose (3.3-5.6 mmol/L, in-house), and BP (17).

Ultrasonographic measurements

Using standard ultrasonographic imaging, an experienced radiologist examined the thyroid gland and ovaries. Thyroid gland size was compared with normative values (18). Ultrasonographic testicular volumes were reported previously (1).

Laboratory measurements

Plasma LH and FSH were analyzed by an automated assay on the Roche Analytics E170. Plasma FT_4, FT_3, TSH, prolactin and GH were analyzed by a fluoroimmunoassay (Delfia

1232 Fluorometer, Perkin Elmer), T_4, T_3, and rT_3 by in-house RIA, thyroglobulin (TG) by a chemoluminiscence assay (BRAHMS, ThermoFisher), and TBG with an enzyme immunometric assay (Monobind Inc, Lake Forest, IL USA). Newborn screening for T_4, TBG, and TSH was performed by standard immunoassays. ACTH, cortisol, and IGF-1 were analyzed by a chemoluminescence assay (Immulite 2000, Siemens). IGF-1 bioactivity of 10-fold diluted serum samples was measured using a validated IGF-1 receptor kinase receptor activation assay (19;20). The intra-assay CV was 5.6%. The interassay CVs were 6.8% and 12.6%, which averaged 414 ± 28 and 1146 ± 144 pmol/L, respectively. Plasma testosterone (in-house) and estradiol were measured by RIA (Siemens), and SHBG by IRMA (Orion Diagnostica). Cholesterol (including the fractions), triglycerides, and glucose were measured with routine clinical chemical methods. In Italy, neonatal screening for TSH and T_4 was performed with Autodelfia (PerkinElmer, Waltham, USA). The analytical sensitivities were 2 µU/mL and 1.5 µg/dL, respectively, and the intra-assay variations were 6% and 10%, respectively. Age-specific reference values for children were used for testosterone (21) and IGF-1 (22). All other parameters were assessed in one hospital (Academic Medical Center Amsterdam); in-house reference intervals from the Academic Medical Center Endocrine Laboratory were used.

X chromosome inactivation

X-chromosome inactivation (XCI) is the transcriptional silencing (via DNA methylation) of 1 of the 2 X-chromosomes in female mammals, creating a functional mosaic of maternally and paternally derived X chromosomes, usually in a 50:50 distribution. Skewed XCI is arbitrarily defined, usually as preferential activation or inactivation of 1 X-chromosome in 75-90% of cells (23;24), and has been linked to the severity of female phenotypes in X-linked disorders (25). To evaluate XCI, DNA was extracted from peripheral blood cells according to standard procedures. The X inactivation pattern was estimated by an assay based on methylation-sensitive restriction sites located in exon 1 of the androgen receptor (AR) gene, using *Hpa*II (New England Biolabs) or *Cfo*I enzymes (Roche). After digestion, the samples were subjected to PCR-mediated analysis for the *AR* gene. PCR products were separated on an ABI3500 sequencer (Life Technologies) and the amount of each product was evaluated by Genemapper software. X-inactivation skewing was calculated as described in Lau et al (26). A skewed population was defined as a cell population with more than 90% silencing of one of the *AR* alleles.

RESULTS

Male patients

Endocrine and metabolic status

Seventeen male hemizygous carriers of an IGSF1 defect (age range 0-27 years, 6 adults) were detected either through neonatal screening (n = 12) or on clinical grounds (2 presented with CeH in infancy and 3 with GHD in childhood, 2 of whom were Dutch and had a negative screening for congenital hypothyroidism). The neonatal screening results of the former 12 index patients are displayed in Table 1. In all cases, T4 concentrations were below the reference interval, and TSH concentrations were nonelevated in all (data missing for 1 patient). At confirmatory testing, the mean FT4 concentration was 73.2 ± 12.2% of the lower limit of the age-specific reference interval, with normal TSH in all subjects. Seven males (age range 43–87 years) with hemizygous IGSF1 defects were discovered through family studies. None of them were screened for congenital hypothyroidism in the neonatal period, since the screening program was not available at their year of birth.

Table 1. Neonatal screening results of hemizygous male index cases diagnosed with central hypothyroidism shortly after birth (n = 12)[a]

	Neonatal screening results			Thyroid hormone state at diagnosis					
	T_4, SDS	TSH	T_4/TBG ratio	Age, wk	FT_4	FT_4	T_4[b]	TSH	TBG
Normal values		≤7 mU/L	>8.5		12-30 pmol/L	%LL		0.8-10.0 mU/L	330-660 nmol/L
A-III.11	-2.4	5.0	-	3	↓8.3	69.2	↓65	3.5	350
C-III.1	-2.7	5.0	-	3	↓10.4	86.7	↓73	1.4	-
D-III.3	-2.9	5.0	↓5.5	3	↓5.1	42.5	-	4.8	-
D-III.4	-3.1	-	-	1	↓9.6	80.0	↓91	2.9	480
E-IV.1	-2.4	<5.0[c]	-	2	↓9.0	75.0	↓75	5.6	444
E-IV.3	-2.2	8.4[c]	-	2.5	↓9.8	81.7	↓80	3.7	427
F-IV.1	-2.6	2.0	↓4.9	3	↓7.3	60.8	-	4.2	-
F-IV.2	-3.7	2.0	↓5.8	3	↓9.4	78.3	-	1.5	-
G-III.1	-2.4	5.0[c]	-	5	↓10.1	84.2	-	1.1	-
G-III.3	-3.0	5.0[c]	-	2.5	↓8.0	66.7	105	2.2	496
J-III.1	-2.9	<2	-	3	↓11	79.1	-	2.5	-
J-III.2	-2.4	3.0	-	2	↓10.3	74.1	-	3.2	-
Mean ± SD	-2.7 ± 0.4	2.7 ± 1.6	5.4 ± 0.5		9.0 ± 1.6	73.2 ± 12.2	82 ± 14	3.1 ± 1.4	439 ± 57

Abbreviation: %LL, percentage of lower limit of the reference interval.

[a]A downward (↓) indicates a value below the reference interval. [b]Age-specific neonatal normal ranges for T_4 are 125-255 nmol/L (6-9 days), 100-250 nmol/L (10-13 days), 95-225 nmol/L (14-20 days, interpolated), 90-200 nmol/L (3-8 weeks) (6). [c]Calculated from results expressed as µU/blood spot, by multiplying by 167.

TRH stimulation tests showed a decreased TSH response in infants, and a response in the lower normal range for older individuals (1).

At the most recent evaluation, all male patients (n = 24) had CeH and 16 hypoprolactinemia (Table 2). Thyroid size was measured with ultrasound in 7 index cases and 2 nonindex cases and was normal in all but 1 (F-IV.1, 0.6 mL at 12.7 years). All index patients were treated with T_4. In 3 nonindex patients, T_4 treatment was started after diagnosis (A-I.4, B-II.11, G-I.1), whereas 4 chose not to be treated because of absence of symptoms

Table 2. Pituitary function of hemizygous male patients (*n* = 24)

Case ID	Case (I or N)	Nucleotide alteration	Age at most recent Evaluation, y	Pituitary deficiencies	PRL, µg/L[a]
A-III.11	I	c.2137_2163del	17.64	TSH	8.5
A-III.7	I		21.36	TSH, PRL, GH[b]	↓<1.0
A-II.4	N		52.40	TSH	5.5
A-I.4	N		86.70	TSH, ACTH	11.8
B-III.7	I	c.2931G>A	10.51	TSH, PRL	↓1.9
B-III.8	I		7.94	TSH	14.8
B-II.11	N		43.91	TSH, PRL	↓3.2
B-I.4	N		66.37	TSH	5.0
C-III.1	I	c.2248delG	17.39	TSH, PRL	↓<1.0
D-III.3	I	c.2248delG	10.46	TSH, PRL	↓<1.0
D-III.4	I		3.79	TSH, PRL	↓1.0
D-I.3	N		62.75	TSH	6.5
E-IV.1	I	126-kb deletion	21.86	TSH, PRL	↓3.5
E-IV.3	I		22.37	TSH	12.5
F-IV.1	I	328-kb deletion	12.70	TSH	5.5
F-IV.2	I		9.44	TSH, PRL	↓<1.0
F-II.8	N		58.24	TSH, PRL	↓3.0
G-III.1	I	c.2588C>T	27.52	TSH, PRL	↓<1.0
G-III.3	I		23.08	TSH, PRL	↓3.5
G-I.1	N		87.49	TSH, PRL	↓<1.0
I-III.2	I	c.3518G>A	16.69	TSH, PRL, GH[b]	↓<1.0
J-III.1	I	c.3596dupT	3.26	TSH, PRL	↓2.3[c]
J-III.2	I		0.16	TSH, PRL	↓2.1 [c]
K-II.3	I	c.2309G>A	26.54	TSH, PRL, GH[b]	↓<1.0

Abbreviations: I, index case; N, non-index case; PRL, prolactin.

[a]Range of normal values for prolactin for adults is 4.0-15.0 µg/L. A downward arrow (↓) indicates a value below the reference interval.

[b]Partial GHD, successfully treated with GH (for further details, see Ref. 1).

[c]At retesting in the Academic Medical Center Amsterdam, PRL levels were not decreased (5.2 µg/L in J-III.1 [3.77 years] and 86.7 µg/L in J-III.2 [0.67 years, reference interval 5.1-98.7 µg/L] (27)), possibly due to stressful venipuncture.

of hypothyroidism. Patients were generally highly educated, and no signs of cognitive impairment were observed. As reported previously (1), in 3 cases magnetic resonance imaging, performed because of macrocephaly (A-III.11), central hypothyroidism (C-III.1) and GHD (K-II.3), was abnormal, showing a frontoparietal hygroma, hypoplasia of the corpus callosum and small stalk lesion, respectively. In 8 other cases MRIs were normal.

IGF-1 concentrations were generally within the reference interval in childhood (Figure 1A), although three index cases were diagnosed with GHD (K-II.3, I-III.2 and A-III.7) and treated with GH (IGF-1 during therapy not shown). In adolescence and adulthood, IGF-1 concentrations were mostly in the upper half of the reference interval. Bioactive IGF-1 showed a similar pattern (data not shown). Two GHD patients (K-II.3 and A-III.7) discontinued GH treatment after a normal provoked GH peak in late adolescence [for details on the diagnosis, treatment, and follow-up of these cases with GHD, see Ref. 1]. Based on abnormal CRH and metyrapone testing results, one patient (A-I.4) was diagnosed with partial ACTH deficiency and was started on hydrocortisone treatment.

Metabolic parameter of children and adolescents (n = 11, all index cases) are shown in Supplementary Table 1. BMI was increased in 3 and fat percentage was elevated in 5 of 7 evaluable cases (>2 SDS for age- and sex-matched reference values). Waist circumference was >2 SDS in 3 children, and between 0 and 2 SDS in all others. The other metabolic parameters were generally normal. In one infant (J-III.2, 0.16 years old), waist circumference and HDL-cholesterol appeared mildly affected.

In adult cases (n = 13, 6 index cases, Supplementary Table 1), BMI was elevated in 10, fat percentage in 8 (not performed in G-I.1), and waist circumference in 7 patients. Triglycerides were increased in 1, BP in 6, fasting glucose in 2, and HDL-cholesterol was decreased in 4. The metabolic syndrome was diagnosed in 4 patients, all >55 years of age.

Growth and pubertal development

Cross-sectional data on testis volume assessed by ultrasound were described previously (1), and plotted in Figure 2. Macro-orchidism was observed in all male patients aged ≥12 years. In our earlier paper (1), we showed that plasma LH and FSH were within reference ranges in most cases, but plasma FSH was always higher than LH and above the reference range in 6 cases. Inhibin B concentrations tended to be high (elevated in 4 subjects), and anti-Mullerian hormone concentrations relatively low (decreased in 5 subjects) relative to the reference range (1).

Longitudinal growth and pubertal development data could be retrieved in 8 out of 9 index cases aged 16 years or older. All had disharmonious puberty, ie, start of testicular growth at a normal age, whereas testosterone secretion, pubertal growth spurt, and pubic hair development were delayed. In one case, no data on testosterone were available, but growth spurt and pubic hair development were delayed and preceded by

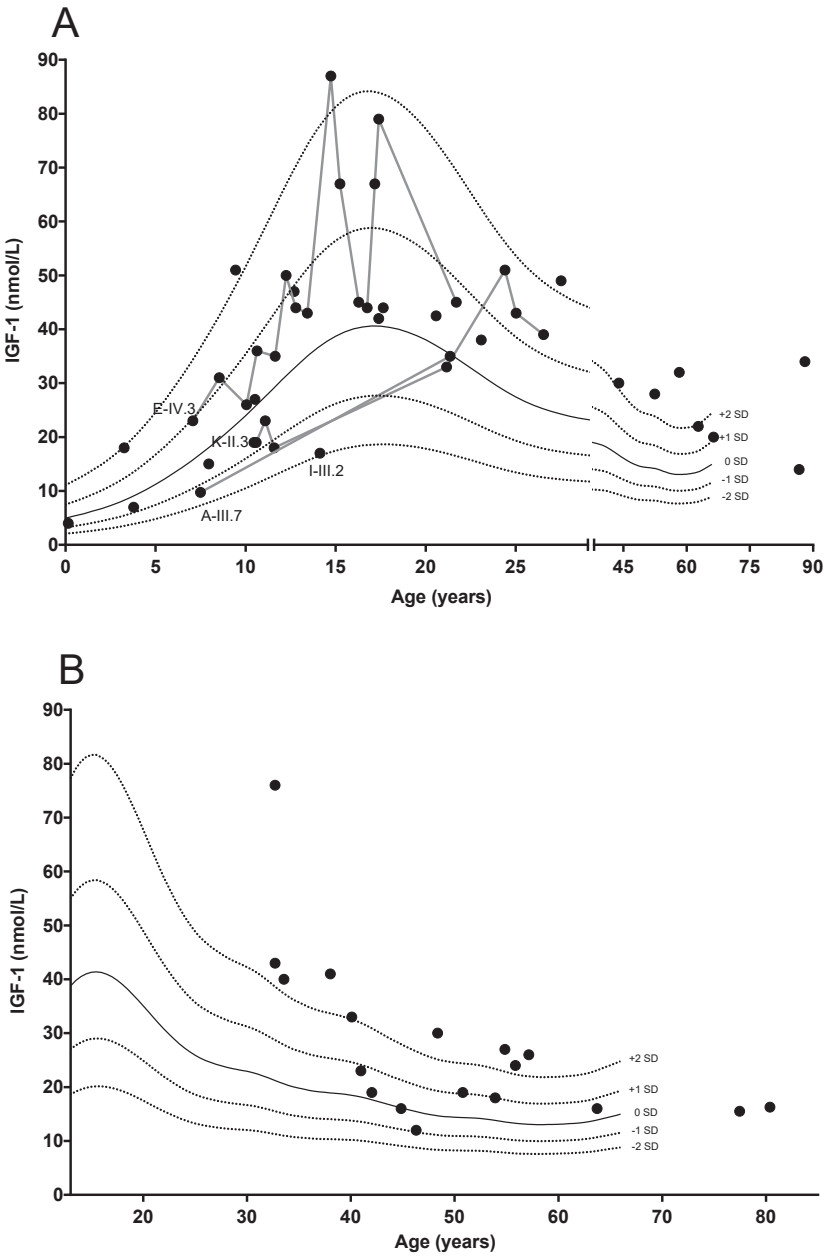


Figure 1. A, IGF-1 concentrations in 24 hemizygous male patients with IGSF1 deficiency syndrome. Index cases: 0-27 years old, non-index cases: 42-87 years old. In three patients (A-III.7, E-IV.3, and K-II.3), longitudinal data were available. IGF-1 levels under GH replacement therapy were excluded (in A-III.7, I-III.2, and K-II.3). Reference values for IGF-1 are derived from Rikken et al. (22) and in-house normal values from the University Medical Center Utrecht, The Netherlands. B, IGF-1 levels in 18 heterozygous female carriers of an *IGSF1* mutation. Reference values for IGF-1 are derived from in-house normal values from the University Medical Center Utrecht, The Netherlands.

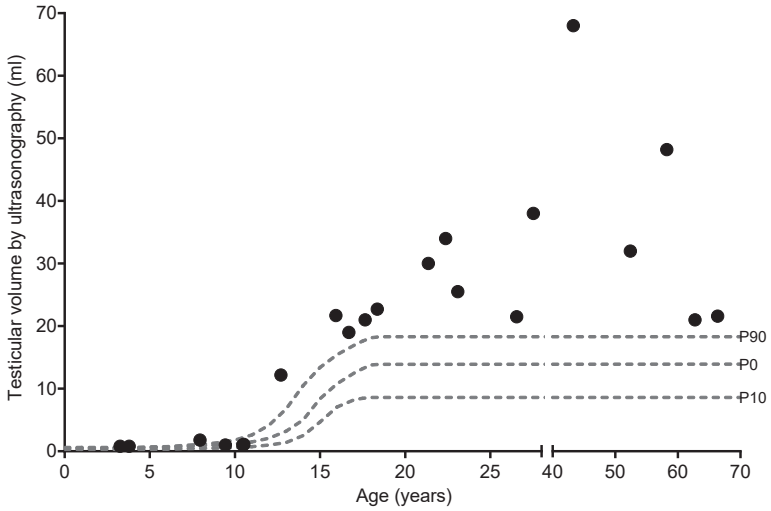


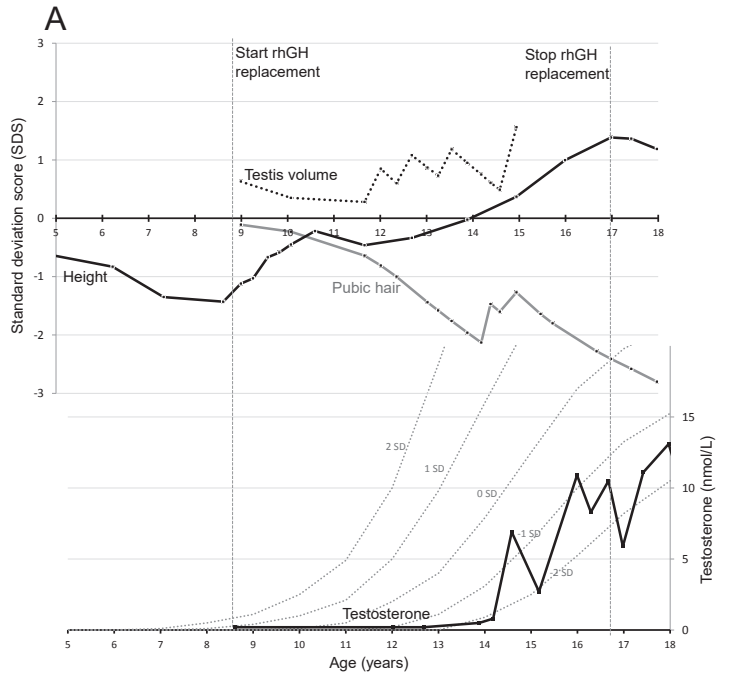
Figure 2. Testicular volume, measured with ultrasonography, adapted from Sun et al (1). The P10-90 represent the 10th-90th percentile of the age-specific reference range (28).

testicular growth. For illustrative purposes, we present data on the three patients from whom most data were available (Figures 3, A-C).

Patient A-III.7 (Figure 3A) showed a decreasing height SDS in childhood. Because of GHD, GH treatment was started at the age of 8.8 years, inducing rapid catch-up growth. Testosterone production did not start until the age of 14 years, and remained in the lower part of the reference interval throughout puberty. Subsequently, pubic hair SDS decreased to almost -3 SDS. In contrast, the pubertal increase in testicular size started at a normal age, and its volume remained between 0 and +1.5 SDS in the following years. GH treatment was discontinued at 17.5 years of age, and IGF-1 levels remained between +1.0 and +2.0 SDS.

In patient A-III.11 (Figure 3B), height and pubic hair SDS decreased throughout childhood and puberty, falling more and more behind compared with the reference population. No deviations from the reference population were observed in the pubertal increase in testicular volume. So far, endogenous testosterone production has not been observed, and therapy with im testosterone esters was started at 15 years.

Patient C-III.1 (Figure 3C) showed normal growth during childhood, although his height SDS slightly decreased in early adolescence and caught up again at the age of 13 years. Pubic hair SDS also decreased and did not catch up until the age of 15 years. This probably results from a relatively late start of testosterone production (around 13 years old). Testicular volume, however, started to increase at a normal age. Indeed, it increased at such a high rate that his testicular volume SDS exceeded +1.5 SDS from 13.5 years onward.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Testis volume
Height
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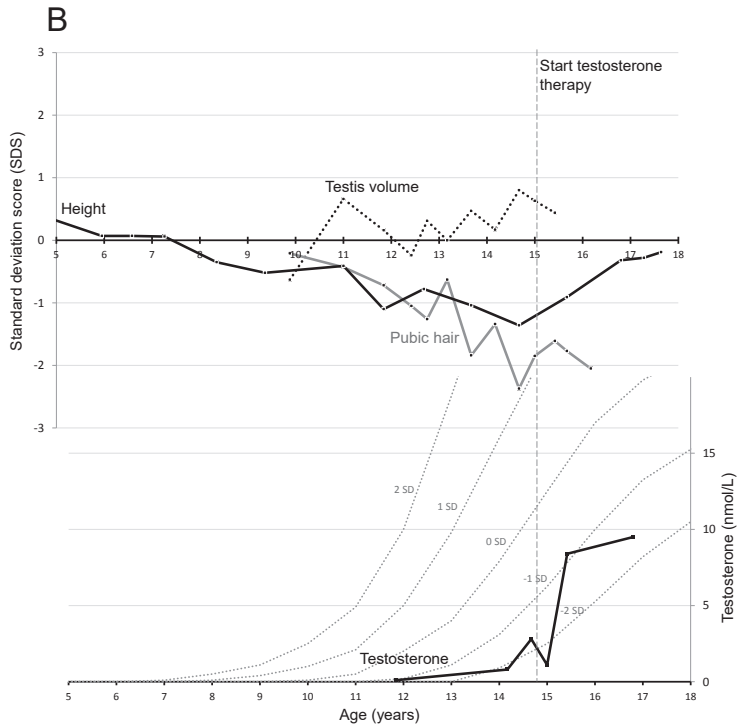
B
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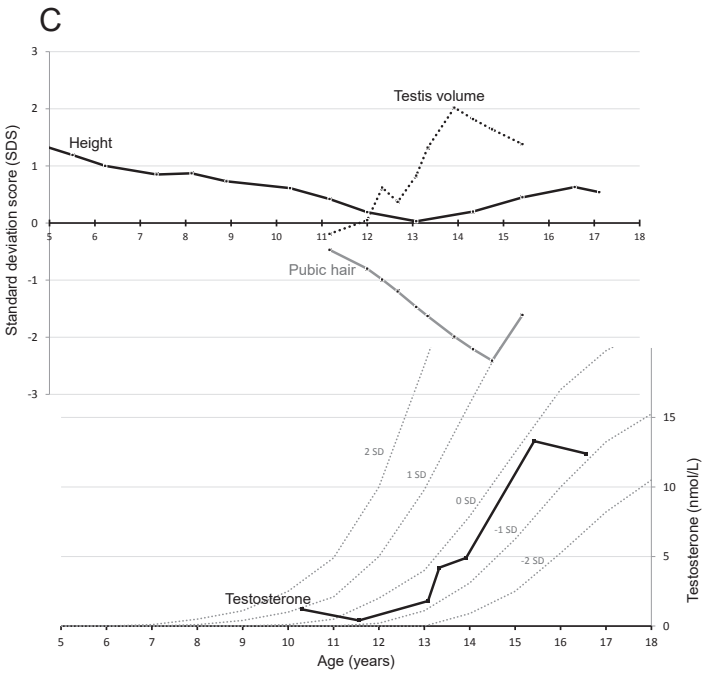

Figure 3. Longitudinal data on linear growth, testis volume (measured with the Prader orchidometer), and pubic hair stage, expressed in standard deviation scores, and testosterone levels in nmol/L. Normal values for testosterone are derived from Andersson et al. (21). Abbreviation: rhGH, recombinant human GH. A, patient A-III.7; B, patient A–III.11; C, patient C-III.1.

Female patients

Family studies detected 18 heterozygous female carriers of an *IGSF1* defect (age range 32-80 years). FT_4, FT_3 and TSH concentrations of untreated cases were generally in the lower half of the reference interval (average 12.0 ± 2.0 pmol/L, 4.4 ± 1.2 pmol/L, and 1.6 ± 0.8 mU/L, respectively) (Table 3). Six females were biochemically hypothyroid, of whom 3 were diagnosed previously and treated with levothyroxine, and 2 were prolactin deficient. Like in men, IGF-1 concentrations of female carriers at middle age were around or above (n=9) the upper limit of the reference interval (average 28.9 ± 15.7 nmol/L) (Figure 1B). Growth and pubertal development were generally normal, including normal reported age at menarche (median 14 years, range 11-19 years, delayed in E-II.14) (9). Four females underwent oophorectomy for ovarian cysts (B-I.8, B-II.8, B-II.13, and F-III.4). Thyroid size was measured with ultrasound in 6 females, and was normal in all.

X chromosome inactivation analysis was not informative (for both probes) in 7 female patients. In 4 of the remaining 11 females, significant skewing of X-inactivation was observed with either of the 2 probes (F-III.4: 100/100%, F-III.5: 91/80%, E-II.14: 92/88%, and possibly K-I.2: 88/80%). Their plasma FT_4 concentrations were 9.8, 9.9, 15.2, and 16.0 pmol/L, respectively.

Table 3. Thyroid function and X-inactivation in heterozygous female carriers (N = 18)

Case	Age,y	FT_4	TSH	T_3	FT_3	rT_3	Tg	PRL	XCI[b] (%)
Normal range		10-23 pmol/L	0.8-6.0 mU/L	1.3-2.7 nmol/L	3.3-8.2 pmol/L	0.11-0.44 nmol/L	<1-45 pmol/L	5.0-23.0 µg/L	
F-III.4[c]	32.71	↓9.8[c]	↓0.49[c]	1.8[c]	-	-	4[c]	12.9[c]	100 / 100
F-III.5[d]	32.71	↓9.9[d]	↓0.40[d]	-	-	-	7[d]	6.5[d]	91 / 80
F-III.2	33.56	13.2	1.20	2.7	↓2.8	0.24	5	18.0	70 / 85
D-II.11	38.03	10.6	0.97	1.7	4.4	0.23	28	9.0	NI
J-II.2	42.02	12.7	1.15	1.8	5.3	0.20	5	↓2.7	NI / 52
D-II.9	40.10	↓9.6	2.10	2.0	3.8	0.18	31	5.0	NI
B-II.13	40.96	12.1	2.02	2.6	3.6	0.24	25	5.2	NI
I-II.3	44.85	13.4	1.81	2.1	3.4	0.30	42	11.0	NI
A-II.14	46.29	10.5	↓0.33	2.1	3.5	0.20	↑79	14.0	NI
B-II.8	48.72	↓9.6	2.63	2.5	5.0	0.17	↑51	9.0	52 / 54
E-III.4	50.78	13.2	1.50	↑3.0	4.5	0.16	24	9.5	55 / 71
G-II.4	54.85	13.7	2.40	1.8	5.7	0.13	20	↓4.0	63 / 76
A-II.10	53.91	12.0	2.76	1.4	3.5	0.25	7	↑23.5	NI
G-II.2[e]	55.86	-	2.00[e]	-	-	-	8[e]	2.0[e]	NI
E-III.2	57.15	13.0	3.10	1.7	4.8	0.21	8	11.0	54 / 65
K-I.2	63.73	16.0	1.53	2.5	3.9	0.35	↑49	5.5	88 / 80
B-I.8	78.00	↓9.4	1.17	1.7	4.8	0.07	41	9.0	57 / 61
E-II.14	80.38	15.2	0.92	2.0	7.6	0.25	14	12.5	92 / 88
Mean ± SD	49.7 ± 14.0	12.0 ± 2.0	1.6 ± 0.8	2.1 ± 0.4	4.4 ± 1.2	0.2 ± 0.07	24.9 ± 20.8	9.5 ± 5.5	

Abbreviation: NI, noninformatie; PRL, prolactin; Tg, thyroglobulin. [a]Arrows indicatie below (↓) or above (↑) reference interval. [b]Expressed as percentage of skewing using either the *HpaII* or the *CfoI* probe. [c]Diagnosed with CeH at 24 years and treated with levothyroxine 250 µg. Presented FT_4, TSH, T_3, and PRL are before treatment (23 years), and Tg is during undertreatment (32 years). FT_4 and TSH during treatment (32 years) were 7.1 pmol/L and 0.02 mU/L, respectively. [d]Diagnosed with CeH at 29 years and treated with levothyroxine 75 µg. Presented FT_4 and TSH are before treatment (29 years), and Tg and PRL are during undertreatment (32 years). FT_4 and TSH during treatment (32 years) were 8.2 pmol/L and <0.01 mU/L, respectively. [e]Diagnosed with CeH at 39 years and treated with levothyroxine. Presented TSH is before treatment (39 years, total T_4 was 62 nmol/L (reference 70-150 nmol/L)), and Tg and PRL are during overtreatment (55 years). FT_4 and TSH during treatment (55 years) were 24.7 pmol/L and <0.01 mU/L, respectively

Similarly to hemizygous male carriers, female carriers tended to be overweight (Supplementary Table 2), with an increased BMI in 11 of 18, increased fat percentages in 7 of 13 (not performed in 5), and increased waist circumference in 10 of 17. Triglycerides were increased in 1, HDL-cholesterol mildly decreased in 1, BP increased in 4, and glucose was borderline increased in 2 females. Three females (48, 54 and 80 years old) fulfilled the criteria for the metabolic syndrome. As in male patients, no signs of cognitive impairment were observed in females.

DISCUSSION

The IGSF1 deficiency syndrome in 24 hemizygous male patients is characterized by CeH and macro-orchidism, preceded by disharmonious puberty in all evaluable cases, hypoprolactinemia (67%), and transient partial GHD in childhood (13%). Most patients show increased BMI (63%) and/or fat percentage (89%). There is no difference in the frequency of pituitary deficiencies between index cases and cases discovered through family studies. Heterozygous female carriers showed CeH in 6 (33%) and hypoprolactinemia in 2 (11%) of 18 cases.

Delay of pubertal testosterone production, growth spurt and pubic hair development was consistently observed in all 8 evaluable patients in whom CeH was diagnosed in childhood. In contrast, testicular enlargement started at a normal age and continued well into adulthood, such that macroorchid testes were observed in all cases. The pathophysiology of this unusual disharmoniously delayed puberty remains to be resolved (4). The recently reported 21-year-old Japanese male with a novel mutation in *IGSF1* had CeH, prolactin deficiency, and delayed puberty, but no macro-orchidism (2). We speculate that his testicular size may still increase with age, whereas population reference intervals for testicular size in Japanese men were reported to be almost half of those in Caucasians (29;30).

BMI, fat percentage, and waist circumference were increased in almost all patients. However, with the increasing trend for (abdominal) obesity in Western countries, the reference intervals that were used might be outdated. Furthermore, other metabolic parameters such as triglycerides, HDL-cholesterol, fasting glucose, and blood pressure were infrequently affected before the age of 55 years. It is therefore uncertain whether an adverse metabolic profile is part of this syndrome, and if so, whether it is a consequence of the associated endocrinopathies. IGF-1 concentrations in childhood were generally normal, although 3 patients were diagnosed with partial GHD in childhood (1). When retested at adult age, 2 of them showed a normal stimulated GH secretion; the third patient has not been retested yet and is still on GH replacement therapy. Circulating IGF-1 tended to increase with age relative to the age-matched reference interval. In our current cross-sectional analysis, bioavailable IGF-1 concentration was normal. Intriguingly, in several patients, acromegaloid features were observed in late adulthood.

In female carriers, CeH was present in 6 out of 18 cases, and FT_4 was in the lower half of the reference interval in the remainder (range 10.5-16.0 pmol/L). One of the most affected females (F-III.4) was also operated for ovarian cysts, and showed skewing of X chromosome inactivation (100/100%), suggesting skewing towards the mutant allele. Two others (K-I.2 and E-II.14) also showed (possible) skewing (88/80% and 92/88%, respectively), but had the highest FT_4 of all females in which XCI analysis was informative (16.0 and 15.2 pmol/L, respectively), suggesting skewing towards the wild-type allele. Growth and pubertal development was normal in all female cases, but ovarian cysts were resected in 4

of them. As in men, female carriers showed on average an increased BMI, fat percentage, and waist circumference, with other metabolic parameters relatively unaffected.

In most male patients, CeH was profound and detected through neonatal screening (The Netherlands and Italy, where screening includes T_4 and TSH) or on the basis of clinical symptoms (1). In these cases, failure of detection and subsequent treatment of hypothyroidism might have placed them at risk of neurodevelopmental delay (31). However, in several family members of index cases, hypothyroxinemia was less profound. Although they might report differently after being treated, these patients did not show overt signs or symptoms of hypothyroidism, and did not report reduced quality of life. To prevent unnecessary medicalization, future studies will need to assess the need for T_4 treatment in IGSF1 deficiency syndrome when signs or symptoms of hypothyroidism are absent.

We recently speculated on the pathophysiology of this novel syndrome (4). The main features of the syndrome include pituitary dysfunction (particularly CeH) and enlarged testes. Because *IGSF1* is expressed in murine pituitary thyrotropes, lactotropes, and somatotropes (1), and *Trhr* is down-regulated in the pituitaries of *Igsf1*-deficient mice, decreased TRH signalling might explain the pituitary phenotype of IGSF1-deficient patients. This, however, does not explain the disharmoniously delayed pubertal development, which is not observed in patients with *TRHR* mutations (32) or in other genetic mutations associated with isolated congenital CeH, such as in *TSHB* (33) and *PROP1* (34). With respect to macro-orchidism, it is tempting to assume an association with hypothyroidism, as Sertoli cell number (which determines testis size) is increased when circulating T_3 is low (35). In addition, relatively increased FSH (35), or impaired communication between germ cells or nonhormonal factors and Sertoli cells might alter proliferation or function of Sertoli cells (36) in this syndrome. More research is needed to determine IGSF1's function in pituitary and testis in order to gain a more definitive understanding of the pathophysiology of these patients.

In conclusion, the X-linked IGSF1 deficiency syndrome is characterized in males by CeH, delayed puberty, macro-orchidism, hypoprolactinemia, transient partial GHD and increased BMI both in index patients and their affected family members. CeH was present in 6 out of 18 female carriers. Screening for *IGSF1* mutations is indicated in patients with CeH and/or macro-orchidism. Positive tests should be followed by family studies.

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SUPPLEMENTARY DATA

Supplementary table 1a. Cross-sectional metabolic parameters in hemizygous male children and adolescents (index cases)

Case	Age	BMI	Fat%†	WC (cm)	TG (mmol/L)	HDL (mmol/L)	BP (mmHg)	FG (mmol/L)	MS‡
J-III.2	0.16	↑19.1	-	↑46	1.08	↓0.95	100/65↑	4.6	*
J-III.1	3.26	17.5	-	56	0.56	1.30	90/60	4.2	*
D-III.4	3.79	16.9	-	52	0.79	1.24	[1]	4.9	*
B-III.8	7.94	19.2	18.5	64	0.51	1.20	106/64	3.6	*
F-IV.2[2]	9.44	↑22.9	↑30.9	↑78	0.60	1.58	111/71	5.2	*
D-III.3	10.46	17.6	↑21.1	66	0.57	1.53	108/71	5.2	N
B-III.7	10.51	20.4	21.1	75	0.41	1.62	101/51	4.6	N
F-IV.1	12.70	23.4	↑28.5	78	0.74	1.32	115/66	5.4	N
I-III.2	16.69	25.6	↑20.3	80	1.69	1.05	109/57	4.9	N
C-III.1	17.39	26.2	-	90	0.50	1.33	111/56	5.2	N
A-III.11	17.64	↑29.1	↑19.7	↑94	0.58	1.18	135/66	5.1	N

WC, waist circumference; TG, triglycerides; HDL, high-density lipoprotein; BP, blood pressure; FG, fasting glucose; MS, metabolic syndrome; -, missing value. [1]Not assessable due to distress during blood pressure measurement. [2]Patient was diagnosed with diabetes mellitus at age 4.4 years and uses insulin aspart and risperidon (possible side-effects include weight gain). The DM type is unknown, but antibodies (anti-IA2, anti-GAD65, anti-insulin, anti-islet cell) and MODY 1 and 3 testing were negative and the diabetes is rather well controlled with only small doses of insulin before meals, so type 2 is more likely than type 1. †Derived from bioelectric impedance. ‡Increased waist circumference and two of the following: increased TG, BP, FG or decreased HDL. ↓↑below or above 2 SDS of age- and gender-specific reference values. *Metabolic syndrome is undefined in children <10 years old. Normal values for fat percentage: 12-15% (5-9yr), 12-18% (10-30yr). Fat percentage in B-III.7 was approximately 0.8 SDS and in B-III.8 approximately 0.3 SDS (12). Age specific normal ranges were obtained from the following reports: BMI (11), waist circumference (15), HDL (16), triglycerides (16), glucose (3.3-5.6 mmol/L, in-house), and blood pressure (17).

Supplementary table 1b. Cross-sectional metabolic parameters in hemizygous male adults

Case	Case	Age	BMI	Fat%	WC	TG	HDL	BP	FG	MS
IDF cut-off values				†	< 94 cm	< 1.7 mmol/L	≥ 1.03 mmol/L	SBP <130 or DBP <85 mmHg	< 5.6 mmol/L	‡
E-IV.1	I	21.86	23.6	14.2	80	1.00	1.45	124/80	5.2	N
A-III.7	I	21.36	↑25.2	15.8	↑97	0.82	1.13	↑130/74	4.3	N
E-IV.3	I	22.37	↑27.3	↑20.7	89	1.6	↓0.78	124/67	4.1	N
G-III.3	I	23.08	24.7	↑26.8	88	0.99	1.14	↑141/82	5.1	N
K-II.3	I	26.54	23.8	↑20.0	84	0.34	1.65	105/70	4.2	N
G-III.1	I	27.52	↑28.6	↑30.1	↑101	0.99	1.24	128/76	4.4	N
B-II.11	N	43.91	↑28.2	20.6	90	0.93	1.23	124/68	5.4	N
A-II.4	N	52.40	↑25.9	↑22.8	↑101	1.13	↓1.00	125/75	5.4	N
F-II.8[1]	N	58.24	↑38.7	↑41.2	↑127	1.07	1.19	↑154/91↑	↑8.5	Y
D-I.3[2]	N	62.75	↑36.9	↑34.1	↑116	↑3.48	↓0.89	110/82	4.8	Y
B-I.4	N	66.37	↑25.8	22.4	92	0.46	1.73	↑153/90↑	5.3	N
A-I.4	N	86.70	↑30.1	↑31.2	↑128	0.88	1.36	↑130/70	↑5.7	Y
G-I.1[2]	N	88.09	↑26.6	-	↑110	1.33	↓0.86	↑135/75	5.3	Y
Mean ± SD			28.1 ± 4.7	25.0 ± 7.9	100.2 ± 15.7	1.2 ± 0.8	1.2 ± 0.3	130 ± 14 / 77 ± 8	5.2 ± 1.2	

I, index case; N, non-index case; WC, waist circumference; TG, triglycerides; HDL, high-density lipoproteins; BP, blood pressure; FG, fasting glucose; MS, metabolic syndrome; -, missing value. [1]Patient uses medication for hypertension and type 2 diabetes mellitus. [2]Patient uses medication for hypertension. †Derived from bioelectric impedance, except for B-I.4 and B-II.11, in whom DXA was used. ‡Increased waist circumference and two of the following: increased TG, BP, FG or decreased HDL. ↓↑below or above the reference interval or IDF cut-off values for normality (13). Normal values for fat percentage: 12-18% (10-30yr), 13-19% (31-40yr), 14-20% (41-50yr), 16-20% (51-60yr), 17-21% (>61yr). Fat percentage in B-II.11 was -0.1 SDS and in B-I.4 approximately 0 SDS according to Lunar Prodigy United Kingdom reference values.

Supplementary table 2. Cross-sectional metabolic parameters in heterozygous female carriers (*n* = 18)

Case	Age	BMI	Fat%†	WC	TG	HDL	BP	FG	MS
IDF cut-off for normal				< 80 cm	< 1.7 mmol/L	≥ 1.29 mmol/L	SBP <130 or DBP <85 mmHg	< 5.6 mmol/L	‡
F-III.4	32.71	22.0	-	73	0.51	3.60	107/69	4.5	N
F-III.5	32.71	23.5	-	75	0.84	2.88	122/55	4.5	N
F-III.2	33.56	↑29.8	-	76	0.75	4.10	120/70	4.2	N
D-II.11	38.03	21.6	26.2	72	0.42	2.40	115/66	4.8	N
J-II.2	42.02	23.6	-	↑86	0.50	1.90	120/70	4.6	N
D-II.9	40.10	22.8	↑30.7	72	0.50	2.21	110/60	4.9	N
B-II.13	40.98	↑26.9	45.8	↑94	0.47	2.93	114/62	5.5	N
I-II.3	44.85	↑25.5	↑35.4	77	0.77	3.08	125/74	4.6	N
A-II.14	46.29	24.1	25.4	↑86	0.99	2.40	110/75	3.9	N
B-II.8	48.72	↑25.9	34.0	↑80	1.27	1.78	↑134/73	↑5.6	Y
E-III.4	49.54	↑27.4	↑36.7	↑95	1.18	1.55	129/85↑	5.0	N
A-II.10	53.91	↑27.2	↑29.9	74	0.38	3.31	122/78	4.5	N
G-II.4	53.60	↑32.1	-	↑105	0.70	1.48	106/84	↑5.6	N
G-II.2[1]	54.62	↑26.4	↑37.1	↑109	↑2.26	1.31	126/71	5.5[1]	Y
E-III.2	55.90	↑25.5	↑31.5	↑92	0.52	1.96	114/72	4.5	N
K-I.2[2]	63.73	23.1	30.0	76	0.52	3.18	115/70	4.6	N
B-I.8[2]	78.00	↑25.4	43.9	↑86	0.84	1.66	↑176/82	5.1	N
E-II.14	80.38	↑26.1	↑43.3	↑87	1.25	↓1.26	↑130/70	5.5	Y
Mean ± SD	49.4 ± 13.9	25.5 ± 2.7	34.6 ± 6.6	84.2 ± 11.3	0.8 ± 0.5	2.4 ± 0.9	122 ± 16 / 71 ± 8	4.9 ± 0.5	

WC, waist circumference; TG, triglycerides; HDL, high-density lipoprotein; BP, blood pressure; FG, fasting glucose; MS, metabolic syndrome; -, missing value. N.a., not applicable. [1]Patient has diabetes mellitus type 2 and uses metformin. The data on metabolic parameters were obtained while she had received thyroxine treatment since 12 years. Her most recent plasma FT_4 of 24.7 pmol/L and TSH of <0.01 mU/L suggested overtreatment. [2]Patient uses antihypertensive medication. †Derived from bioelectric impedance, expect for B-II.13 and B-II.89, in whom DXA was used. ‡Increased waist circumference plus 2 of the following: increased TG, BP, FG or decreased HDL. ↓↑below or above the reference interval or IDF cut-off values for normality (13). Normal ranges for fat percentage: 21-27% (31-40yr), 22-28% (41-50yr), 22-30% (51-60yr), 22-31% (>61yr). Fat percentage in B-II.13 was +1.7 SDS, in B-II.8 -0.1 SDS, and in B-I.8 approximately +1.0 SDS, according to Lunar Prodigy United Kingdom reference values.