Novel Insights from Clinical Practice

HORMONE RESEARCH IN PÆDIATRICS

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Normal Growth despite Combined Pituitary Hormone Deficiency

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Established Facts

- In some case reports, growth during childhood in the absence of growth hormone (GH) has been described.
- In such cases, hyperinsulinemia, hyperprolactinemia, GH/insulin-like growth factor (IGF) variants, and mutations of genes such as *HESX1* have been proposed as explanations for the observed normal linear growth. However, the mode of action remains unclear. Moreover, in most cases, no cause has been identified and the involvement of as-yet unidentified growth-promoting factors has been proposed.

Novel Insights

- Normal growth can be observed in teenagers with hypopituitarism and an absence of hyperinsulinemia, hyperprolactinemia, or circulating growth-promoting factors.
- Extensive hormonal and genetic analyses suggested a *HESX1* mutation, probably associated with mutations of other genes involved in the regulation of intracellular signaling pathways, potentially accounting for the maintenance of normal growth.

Keywords
Growth · Insulin-like growth factors · IGF/IGFBP-3 molar ratio · Combined pituitary hormone deficiency

Abstract
Background: The paradox of normal growth despite a lack of growth hormone (GH) is an unexplained phenomenon described in some pathological (sellar, suprasellar, and hypo-

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thalamic disorders) and overgrowth syndromes. It has been suggested that the paradoxical growth is due to other GH variants, GH-like moieties, prolactin, insulin, insulin-like growth factors (IGFs), and unidentified serum factors or growth mechanisms. The objective of this study was to determine the mechanism underlying this normal growth without GH. ***Case Description:*** We describe here growth, hormonal, and genetic analyses for an adolescent boy with panhypopituitarism who achieved an adult height above his genetic potential. ***Results:*** Normal growth was observed despite low serum GH, IGF-I, IGF-II, IGF binding protein 3 (IGFBP-3) and acid labile subunit (ALS) concentrations, but the IGF-II/IGFBP-3 molar ratio was slightly high. Panhypopituitarism was associated with a heterozygous missense mutation of *HESX1*, with variable penetrance in heterozygous relatives. Exome analysis detected heterozygous missense mutations of various genes involved in intracellular signaling pathways. The growth-promoting activity of the patient's serum was unable to induce AKT phosphorylation in the MCF-7 cell line. ***Conclusion:*** The high IGF-II/IGFBP-3 molar ratio was not the cause of the sustained high growth velocity, due to the low affinity of IGF-II for IGF type 1 receptor. The key finding was the *HESX1* mutation, as similar cases have been described before, suggesting a common mechanism for growth without GH. However, the variable penetrance of this variant in heterozygous relatives suggests that modifier genes or mechanisms involving combinations with mutations of other genes involved in intracellular signaling pathways might be responsible.

Introduction

Normal linear growth in children is a complex process dependent on genetic background, nutrition, environment, and hormonal status, including the growth hormone/insulin-like growth factor 1 (GH/IGF-I) axis, in particular, but also thyroid hormones, gonadal steroids, insulin, and probably many other unknown factors [1, 2].

Postnatal growth in the absence of GH has been described in patients with organic combined pituitary hormone deficiency (CPHD), following the resection of craniopharyngiomas and hypothalamic tumors, and also in septo-optic dysplasia [3–13]. Hyperinsulinemia [5, 7, 9, 10, 14], hyperprolactinemia [1, 9, 14], hyperleptinemia [8, 10, 15], normal or high levels of IGF, bioavailable GH/IGF variants, or sex steroid sensitivity and local aromatase activity have been put forward as explanations for linear growth [11, 16]. In some cases, unexplained normal growth has been reported despite GH/IGF-I deficiency, suggesting a role for other unidentified growth-promoting factors [3, 6, 8, 12, 17]. Some patients with clinical signs, such as acromegaly, gigantism, or overgrowth syndrome, but without excess GH, have been described, and genetic abnormalities, such as an *NSD1* mutation, have been reported in these patients [18].

We describe here the growth pattern and the results of numerous hormonal and genetic analyses for an adolescent boy with panhypopituitarism, who was diagnosed and followed up in our Pediatrics Department between 2005 and 2013, who achieved an adult height beyond his genetic potential despite GH/IGF-I deficiency.

Case Report

The patient was referred to the Pediatric Endocrinology Unit of Ain Shams University Hospital, Cairo, at the age of 18.5 years, for evaluation due to excessive enlargement of his hands and feet, already noted by his family 2.5 years earlier. The patient was born at term. His birth was complicated by Erb's palsy, due to breech delivery. He weighed 2.5 kg at birth, but no information was available concerning his length and head circumference at birth or growth during childhood before the consultation. However, he and his parents said that he was always as tall as his classmates at school. At the age of 16 years, he had first noticed that he was becoming taller than his friends and that his hands and feet were excessively large. He was taller than all but one of the boys in his secondary school class. On admission, physical examination (at the age of 18.5 years) revealed mild acromegalic facial features (prominent supraorbital ridges in frontal view but not in profile, a long face, prognathism, and large ears) associated with particularly large measurements for both his hands and feet, exceeding the 97th percentile (Fig. 1). The patient was 176.3 cm tall (SDS = +0.25; mid-parental target height = 173.8 cm). His BMI was 22.5 (SDS = +0.3 SDS) and head circumference 58.6 cm (+2 SD). His body proportions were normal (upper-to-lower segment ratio = 0.98), but the measurements for both his hands and feet were >97th percentile. The patient was still prepubertal and, at initial presentation (at 18.5 years), both testes had a volume of 3 mL. Orchidopexy was performed 1 month before this first presentation. Three years later, the left testis had a volume of 1 mL, subsequently becoming impalpable, indicating testicular atrophy. The penis was of normal size (6 cm). Bone age assessment according to Greulich and Pyle standards revealed a delay in bone maturation (i.e., a bone age of 13.5 years). His whole-body bone mineral density *Z* score was –3.7. The only other abnormality noted was mild scoliosis. No delay in mental development was observed, and the patient had a normal IQ. Clinical cardiovascular examination and electrocardiogram results were normal. There was no history of consanguinity. Biochemical tests (Table 1) and extensive hormonal investigations were carried out, and karyotyping was performed. These tests revealed the existence of several pituitary deficiencies (Table 2). MRI revealed a hypoplastic anterior pituitary stalk interruption and an ectopic posterior pituitary. In the 6 months between the age of 18.5 and 19 years, the patient grew by 3.7 cm. Remarkably, this patient

Color version available online

Fig. 1. Photographs of the patient. **a** At 2 years of age. **b** At 10 of age. **c** At 16 years of age. **d** Whole-body and facial features at 19 years of age.

Table 1. Serum biochemistry results (normal values)

Lipid profile	
Cholesterol, g/L	2.63 (<2)
LDL-cholesterol, g/L	2.04 (<1.6)
HDL-cholesterol, g/L	0.35 (>0.6)
Triglycerides, g/L	1.17 (<1.5)
Liver functions	
ASAT, IUI/L	39 (5–34)
ALAT, IU/L	54 (<55)
Kidney functions	
Creatinine, mg/L	12.2 (7.2–12.5)
Electrolytes	
Na^{+}, mmol/L	139 (136–145)
K^{+}, mmol/L	4.0 (3.5–5.1)
Cl^{-}, mmol/L	101 (97–111)

had an almost normal life, suffering only from effort intolerance before the initiation of treatment, but not after hormonal substitution. Thyroid, adrenal, and testosterone deficiencies were discovered during his first medical consultation (at the age of 18.5 years). Hormonal replacement therapy was initiated at the age of 19 years and consisted of L-thyroxine, hydrocortisone, and testosterone. The patient's insulin sensitivity was assessed before sex steroid replacement and followed during treatment, due to the strong family history of type 2 diabetes (in his father, uncles, aunts, and grandmothers) (Fig. 2). Written informed consent was obtained from the patient for hormonal and genetic studies, and for photography. A DNA sample was stored for subsequent genetic analysis.

Hormonal Evaluation

See supplementary Table 1 for details (for all online suppl. material, see www.karger.com/doi/10.1159/000/499318).

GH secretion status was evaluated by stimulation with GHRH, arginine, insulin, and L-DOPA (hGH ELISA kit, GenWay, Inc.). Serum IGF-I concentration was determined after separating IGF from binding proteins by ultrafiltration, as previously described [19]. Another radioimmunoassay for IGF-I (Biosource, Nivelles, Belgium) was also carried out.

Serum IGFBP-3 concentration was determined by immunoassay (BioChem Immuno Syst, Bologna, Italy). IGFBP-3 levels were also determined with the Nichols Advantage IGFBP-3 assay (Nichols Institute Diagnostics, San Clemente, CA, USA). This assay systematically gives values much lower than those obtained with other kits (personal data) and was therefore not used for the determination of the IGF/IGFBP-3 molar ratio.

GHBP concentration was determined with the DSL kit (Diagnostic System Laboratories [DSL], Webster, TX, USA). Serum IGF-II concentration was determined by the IGF-II IRMA DSL-9100 with extraction kit (DSL). IGF-II immunoblotting was performed as previously described [20, 21].

Serum acid labile subunit (ALS) concentration was determined with the total ALS DSL 10–8200 ELISA kit (DSL). Serum leptin concentration was determined with the human leptin RIA HL-81HK kit (Linco Research, St Charles, MO, USA). Serum insulin concentration was determined after overnight fasting, in basal conditions, and following oral glucose loading, with the AxSYM Insulin MEIA assay (Axis-Shield Diagnostics, Ltd., Dundee, UK, for Abbott Diagnostics Division). Serum concentrations of LH, FSH, free T4 (fT4), cortisol, prolactin, and ACTH were determined with an Abbott Axsym analyzer.

Growth-Promoting Activity of the Patient's Serum

GH-mediated growth-promoting activity was assessed with the Nb2 bioassay [22] and AKT phosphorylation assays, as follows: MCF-7 breast cancer cells were cultured in DMEM supplemented with 10% FBS. The cells were plated in complete medium in 96-well plates (5,000 cells/well), and then left to expand overnight. The medium was removed, and the cells were placed in phenol red-free and serum-free DMEM supplemented with 10 nM fulvestrant, a competitive estrogen receptor (ER) antagonist, for 24 h. The cells were then exposed to a 2% dilution of serum from the patient or an age-matched control in DMEM for 15 min. We then assessed pAKT levels in quadruplicate wells, after lysis, by homogeneous time-resolved energy transfer (HTRF®), with a Phospho AKT (ser 473) HTRF kit (Cis Bio France, 30200 Codolet).

Genetic Analysis

Molecular Diagnosis of Overgrowth Syndromes

Methylation of the *H19/IGF2* and *KCNQ1OT1* genes on chromosome 11p15 was analyzed, as previously described [23], for the diagnosis of Beckwith-Wiedemann syndrome. The 23 coding exons and intron-exon junctions of the *NSD1* gene were subjected to direct bidirectional sequencing to test for Sotos syndrome [24].

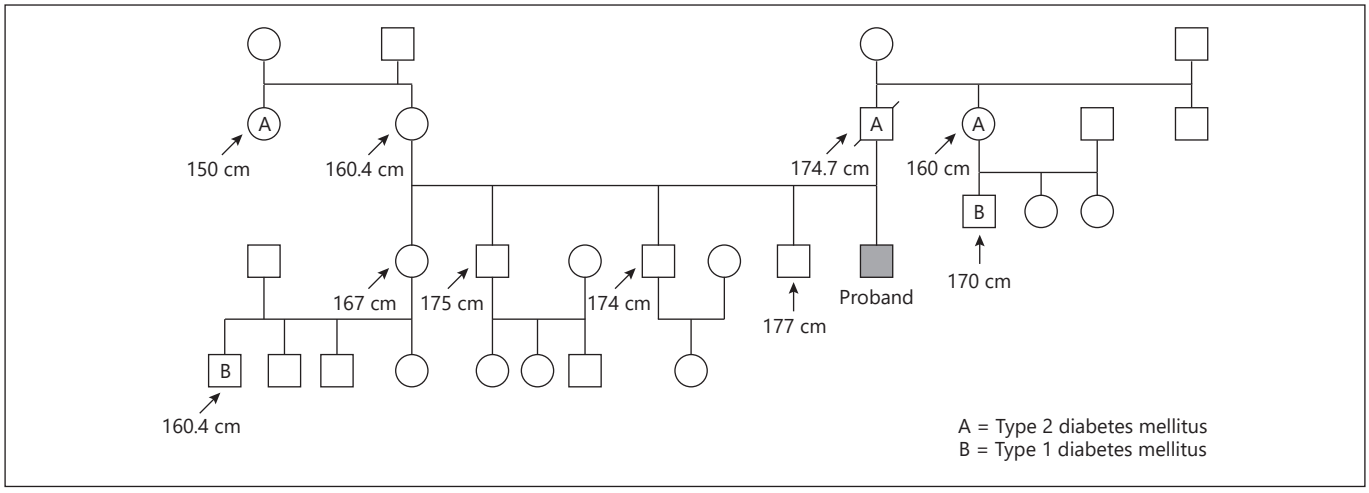

Fig. 2. Pedigree of the patient (arrows indicate the height data available for these members of the family).

Table 2. Patient hormonal determinations and normal values according to age and pubertal stage

TSH, mIU/mL	2.3 (0.35–5.5)
Free T4, pmol/L	8.1 (11–21)
Peak growth hormones	
ITT, ng/mL	0.2 (>7)
GHRH + arginine, ng/mL	2.7 (>7)
L-DOPA, ng/mL	0.1 (>7)
Peak cortisol, ng/mL (ITT)	2 (40–380 [a.m.])
Peak LH, mIU/mL (LHRH test)	0.1 (Tanner I: 0.04–3.6)
Peak FSH, mIU/mL (LHRH test)	0.2 (Tanner I: 1.4–5.9)
Basal prolactin, ng/mL	37.5 (2.1–17.7)
ACTH, pg/mL	21 (<65)
Testosterone, ng/mL	0.1 (prepubertal: <0.03–0.1)
Estradiol[a], pg/mL	<10 (prepubertal <20)
GH-Nb2[b], ng/mL	8.3 (>6.7)
IGF-I	
In-house method, ng/mL (polyclonal antibody)	25 (200–520 according to age; 110–350 according to prepubertal stage)
RIA Biosource kit, ng/mL	44 (normal values: 100–500)
IGFBP-3	
Immunoassay, µg/mL (BioChem Immuno Syst)	1.6 (1.5–6)
Nichols Advantage[c], µg/mL	0.85 (1.67–3.25 according to age and prepubertal stage)
ALS, µg/mL	7 (8–35)
GHBP, pmol/L	2,959 (350–3,435)
IGF-II, ng/mL (kit IRMA DSL)	522 (582–1,034)
Leptin, ng/mL (RIA kit, Linco)	6.8 (0.7–5.3)

All serum samples were evaluated before starting replacement therapy.

[a] 28 pg/mL 6 months after testosterone replacement.

[b] Measures GH biological activity.

[c] This assay systematically gives values much lower than those obtained with other kits (personal data), and so was not used for the determination of the IGF/IGFBP-3 molar ratio.

Array Platform

Oligonucleotide single-nucleotide polymorphism (SNP) array analysis was performed with the Human CytoSNP-12 BeadChip (Illumina). This array contains approximately 300,000 probes, including approximately 200,000 tagged SNPs, and has a mean resolution of approximately 10 kb. SNP copy numbers (log ratio) and allelic composition (B allele frequencies) were assessed with Genome Studio v2010. 2 (Illumina, Inc.) and CNV partition v2.4.4 software. The IGF type 1 receptor (IGF-1R) region was analyzed from SNP 96,978,785 to SNP 97,356,782 (hg18). This area covers approximately 378 kb with 57 SNPs, including 52 that are specific to the 315 kb of the IGF-1R (the mean distance between SNPs in the IGF-1R is approx. 6 kb).

Sequencing and/or MLPA Analysis of the *IGF 1*, *IGFBP3*, *IGFALS*, and *IGF1R* Genes

The sequences of the 5 coding exons and intron-exon junctions of the *IGF 1* gene and its cDNA were determined as previously described [25], together with the sequences of the 21 coding exons and intron-exon junctions of the *IGF1R* gene. MLPA analysis (MRC-Holland, Amsterdam, the Netherlands) was also performed for the *IGF1R*, *IGFBP3*, and *IGFALS* genes.

Whole-Exome Sequencing

Library preparation, exome capture, sequencing, and data analysis were carried out by IntegraGen SA (Evry, France). Sequence capture, enrichment, and elution were performed according to the kit manufacturer's instructions (SureSelect, Agilent), and we used the NEBNext® Ultra kit (New England Biolabs®) for library preparation. Each DNA sample was then sequenced on an Illumina HiSeq4000 as paired-end 75 bp reads. Image analysis and base calling were performed with Illumina real-time analysis, using the default parameters.

Bioinformatics Analysis of Sequencing Data Based on the Illumina Pipeline

CASAVA1.8.2 was used to align sequencing runs to a reference genome (hg19), to call the SNPs based on allele calls and read depth, and to detect variants (SNPs & indels). All targets were sequenced at >20× coverage. The alignment algorithm used was ELANDv2e (multiseed and gapped alignments). Only the positions included in the bait coordinates were conserved. Genetic variants were annotated with the IntegraGen in-house pipeline. We annotated genes (RefSeq) and known polymorphisms (dbSNP 132, 1,000 genomes [1,000 G], EVS), and characterized the mutations detected (exonic, intronic, silent, and nonsense). For each position, exomic frequencies (Homo & HTZ) were determined from all the exomes already sequenced at IntegraGen, and the exome results provided by 1,000 G, EVS, and HapMap. We required variants to be nonsynonymous and not present in dnSNP132, 1,000 G, EVS, and HapMap, with most bases scoring ≥Q30, to meet our filtering requirements.

Results

Growth Follow-Up

From diagnosis, when the patient was still prepubertal, to the start of sex steroid replacement therapy, there was a spontaneous growth velocity of 3.7 cm in 6 months. After the initiation of hormonal treatment, he attended regular follow-up visits including clinical and biological examinations, and his final height at the age of 22 years and 9 months was 187 cm (SDS +1.85), with a reduced growth spurt and complete epiphyseal fusion (Fig. 3). His bone mineral density was recently evaluated at 32 years, and completely normal *Z* scores were obtained for the spine, femur, and forearm.

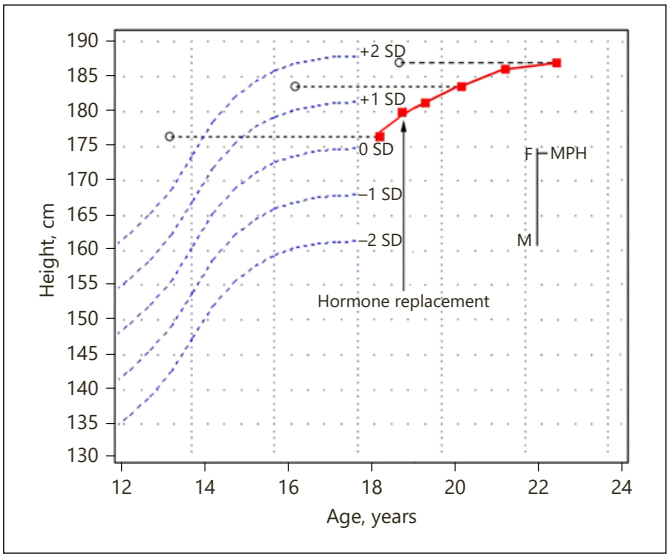

Fig. 3. Patient's growth chart from first visit until epiphyseal fusion. Open circles, bone ages; arrows, ages at which the patient underwent endocrine treatment. Hormone replacement: thyroxine, cortisol and testosterone. MPH, mid-parental height; F, father; M, mother.

Laboratory and Hormonal Evaluations

Blood biochemical parameters (Na^+, K^+, Cl^-, and creatinine) were normal, except for the lipid profile (Table 1). Endocrine evaluation (Table 2) before replacement therapy revealed severe GH and cortisol (without high ACTH levels) deficiencies, slightly low fT4 levels, and LH/FSH/testosterone deficiencies. Estradiol concentration was very low (<10 pg/mL) but rose to 28 pg/mL after 6 months of testosterone replacement. Basal prolactin and leptin concentrations were moderately high. The GH/IGF-I axis was evaluated in various assays and the 3 pituitary stimulation tests showed severe GH deficiency. IGF-I, IGF-II, IGFBP-3, and ALS levels were very low, even though GHBP levels were in the normal range. Western blot analyses excluded the possibility of high-molecular-weight forms of IGF-II precursors and confirmed the low levels of the mature 7-kDa IGF-II form

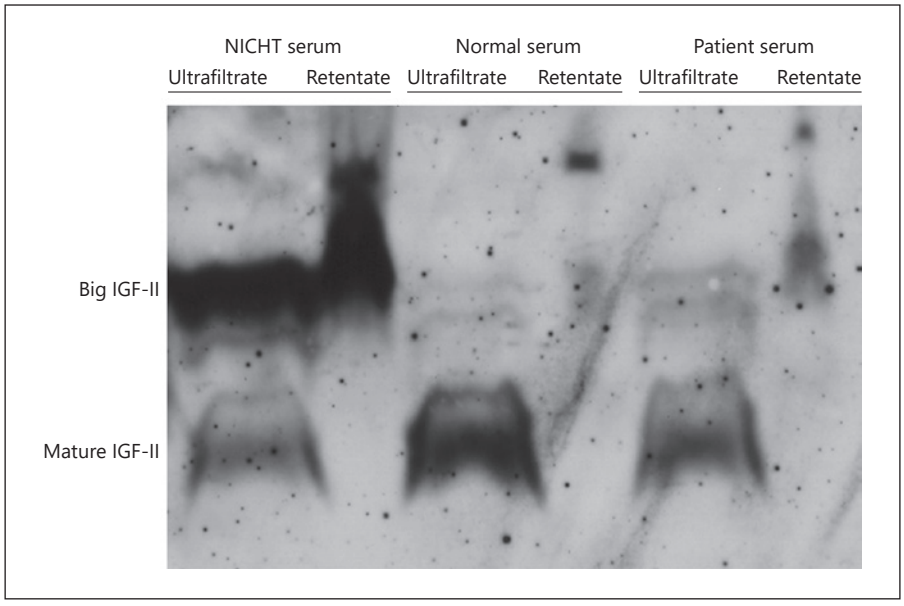

Fig. 4. IGF-II immunoblotting. Plasma samples were incubated in 2 mL 0.01 N HCl to separate IGF from IGFBP. The ultrafiltrate (containing the normal 7-kDa mature IGF-II) and the retentate (IGFBP) were lyophilized, and then dissolved and subjected to electrophoresis in a 15% polyacrylamide gel under nonreducing conditions. The resulting bands were electrotransferred onto a membrane. IGF-II was identified with a monoclonal anti-IGF-II antibody and visualized by chemiluminescence with a second anti-IgG antibody coupled with horseradish peroxidase. The pattern obtained for the patient was compared with that of normal serum (mature IGF-II) and serum from a nonislet cell hypoglycemia tumor (NICHT) containing high-molecular-weight forms of IGF-II (Big IGF-II).

detected by IRMA (Fig. 4). Serum GH biological activity, as assessed in the GH-NB2 assay, was surprisingly normal, but growth promotion activity, as assessed by evaluating AKT phosphorylation in the MCF-7 cell line, was barely detectable (Fig. 5).

The IGF-I/IGFBP-3 molar ratio was low, regardless of the IGF-I kit used: 0.06 and 0.10 (reference values, mean [range]: prepubertal stage = 0.16 [0.10–0.20], pubertal stage = 0.27 [0.19–0.39], and young adults = 0.28 [0.14–0.46]), whereas the IGF-II/IGFBP-3 ratio was moderately high: 1.24 (in-house reference values according to age = 0.84 ± 0.16).

Before replacement therapy, the patient had normal insulin sensitivity (fasting serum glucose concentration = 0.89 g/L and insulin level = 16.5 mIU/L). However, in the 6 months following cortisol and testosterone replacement treatment, he developed glucose intolerance (a fasting glucose concentration of 1.22 g/L and 2-h OGTT value of 1.50 g/L, with insulin levels of 38 and 161 mIU/L, respectively). Subsequent analyses during follow-up revealed a normalization of fasting and 2-h post-prandial glucose concentrations after OGTT.

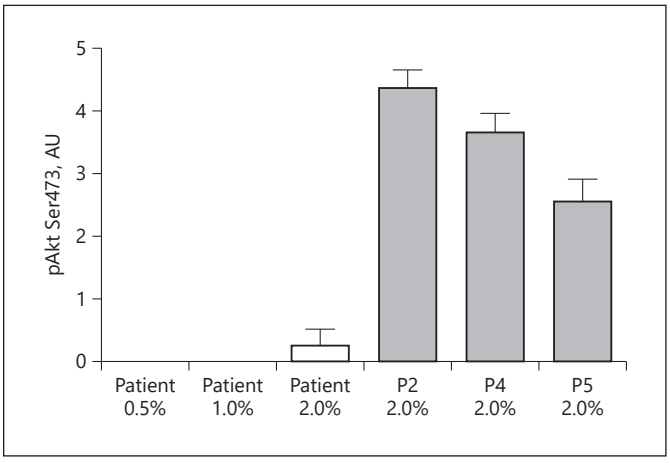

Fig. 5. Growth-promoting activity of the patient's serum. AKT phosphorylation was evaluated in MCF-7 breast cancer cells, as described in Methods. Cells were exposed to a 2% dilution of the patient's serum or serum from an age-matched normal subject for 15 min. The cells were then lysed, phosphorylated (p)AKT was added, and homogeneous time-resolved energy transfer (HTRF®) analyses were performed in quadruplicate cells with a kit. P2, P4, and P5 = control sera from individuals at Tanner stages 2, 4, and 5, respectively.

Genetic Analysis

Overgrowth Syndromes

The patient did not have the classical phenotype of overgrowth syndromes, such as Beckwith-Wiedemann or Sotos syndrome, but genetic and epigenetic analyses were carried out. These analyses revealed an absence of centromeric (*KCNQ1OT1*) and telomeric abnormalities (*H19/IGF2*) in the 11p15 region, and no paternal isodisomy or 11p15 imprinting errors. Tests for Sotos syndrome based on analysis of the *NSD1* gene revealed no deletions or mutations. No nucleotide polymorphisms, mutations, or deletions were detected on exome analysis for the following genes: *NSD1*, *CUL4B*, *EZH2*, *NFIX*, *PHF6*, *PTEN*,

UPF3B, *IGF1R*, *ACTHR*, and *ERα* or *ERβ*. However, 2 very frequent heterozygous missense polymorphisms were found for *GLI3* (rs929387 and rs846266), together with 1 frequent heterozygous missense for *MED12* (rs372765), and these polymorphisms were also detected in other members of the family. A very rare variant (0.0002%), a heterozygous missense *PTCH2* mutation, was present only in the proband (rs138324984). Polyphen2 predicted this variant to be benign and SIFT predicted that it would be tolerated.

IGF system analysis by gene sequencing, MLPA, SNP array, and exome analyses showed the *IGF 1* gene sequence to be normal, thereby ruling out the presence of an activating mutation, and it revealed numerous polymorphisms of the *IGF2*, *IGFBP3*, and *IGFALS* genes, which were also found to be present in other members of the family. Analyses of the *IGF1R* gene ruled out deletion-duplication of the IGF-1R region. The nucleotide sequence of the 21 exons and exon-intron junctions was normal, with several frequent polymorphisms in the heterozygous state. However, these nucleotide variants are also found in children with growth retardation. These variants were subsequently confirmed by exome analysis, which identified 3 variants in the proband: rs45461797 (also present in his brother, sister, and mother), rs2229765 (also present in his brother and sister), and rs17847203 (also present in his brother). The exonic mutations were synonymous. Finally, the R1353H activation mutation associated with extreme tallness [26] was not found in our patient.

Whole-Exome Sequencing

Whole-exome sequencing (WES) was performed for the proband, his mother, one of his brothers (the second male sibling and third child of the family in birth order; see pedigree), and his sister. Unfortunately, no DNA was available for the patient's father, who had already died, making the list of candidate genes longer. We therefore excluded some genes with heterozygous missense variants and unknown functions.

No significant gene function was identified for the homozygous mutations present only in the proband (the mother and/or siblings were heterozygous) or for the compound heterozygous mutations found only in the proband (the mother carried 1 heterozygous variant and the 2nd was probably inherited from the father).

No nonsense mutations relating to pathological growth were found among the rare heterozygous mutations present only in the proband (which were either inherited from the father or were de novo mutations).

- Only 1 heterozygous exonic nucleotide deletion was found, for *AKAP12* (NM_144497) (Chr6: 151673006 T/-).
- Some heterozygous missense mutations of interest were also detected: (i) *ARAP1* (NM_001040118) Chr11: 72437737 G>T, A146D amino-acid substitution (ii) *HS6ST1* (NM_004807), Chr12: 129025782 C>T, R397H amino-acid substitution (iii) *SH2B3* (NM_005475), Chr12: 111856572 A>G, E208G amino-acid substitution; this gene encodes a member of the SH2B adaptor family of proteins (iv) an IGF-IIBP-3 mRNA (rs150697725; frequency <0.2%) missense mutation (NM_006547:p.N359D) was found in the heterozygous state in the proband, but not in the other 3 family members tested. However, this mutation is not considered to be deleterious and has been predicted to be benign (Polyphen) or tolerated (SIFT).
- Other heterozygous missense mutations identified in the proband were also found in the other 3 members of the family tested: *CDKN2A* (rs116150891, LP_001182061.1:p.R144C), *GPR98* (NP_115495.3:p.G3346R, a new variant not previously reported in any database) and *PIK3C2G* (a 4-bp deletion leading to a truncated protein NP_004561.3:p.Gln448LeufsTer5, described in Exac but not in any dbSNP).
- Finally, one of the key genetic alterations detected was the *HESX1* heterozygous missense mutation (rs 121909173), resulting in a nonconservative Q6H substitution. This mutation was found in the proband and in other heterozygous family members, but its penetrance was variable, as the same mutation was detected in his sister and brother (the second male sibling, third in the birth order; see pedigree).

Discussion

We describe here the growth pattern of an adolescent boy with mild acromegaloid features, panhypopituitarism, a small anterior pituitary associated with a truncated stalk, and an ectopic posterior pituitary. The plasma concentrations of the components of the somatotropic axis, GH, IGF-I, IGF-II, IGFBP-3, and ALS were very low, contrasting with the lack of growth failure. The patient achieved an adult height above his genetic potential without GH replacement therapy.

Many similar cases of normal linear growth without GH have been reported in patients with organic CPHD or septo-optic dysplasia, and after the resection of craniopharyngiomas or hypothalamic tumors [5–8, 11–13, 27–

30]. As previously discussed [11], it is remarkable that these patients are able to lead an almost normal, problem-free life.

Several hypotheses have been put forward to account for the normal linear growth of patients with similar conditions, including hyperleptinemia, hyperinsulinemia, normal IGF-I levels despite GH deficiency, and hyperprolactinemia, but as-yet unidentified growth mechanisms have also been evoked in some cases [3, 6, 8–10, 12, 14, 15, 17].

However, our patient had leptin levels that were only slightly high, just above the upper limit of the reference range, and there was no evidence of hyperinsulinemia before replacement therapy. He had moderately high prolactin levels. His serum GH bioactivity with the Nb2 bioassay was normal (probably due to the effect of prolactin on the GH/prolactin receptor in the rat cell assay) [22, 31], but was not associated with IGF-I stimulation, so prolactin cannot account for his growth. Our findings thus indicate that leptin, insulin, and prolactin are unlikely to have been responsible for the stimulation of linear growth in this patient.

Our patient had very low levels of GH, and normal GHBP levels in a context of GH deficiency, as described in several previous patients [17, 32], together with low levels of IGF-I, IGFBP-3, ALS, and IGF-II in the absence of high-molecular-weight forms of IGF-II. Trisomy of 15q26qter and activating mutations of the *IGF1R* gene, which have been shown to cause tall stature [26, 33], were excluded in our patient by karyotyping, MLPA, SNP array analysis, sequencing, and exome analysis.

Despite low total IGF-I and IGF-II levels, the IGF-I/IGFBP-3 molar ratio did not confirm enhanced bioavailability, and the IGF-II/IGFBP-3 molar ratio was higher than the reference values for prepubertal boys or young adults. However, this higher IGF-II/IGFBP-3 molar ratio could not have triggered an IGF-I-like effect, given the lower affinity of IGF-II for the IGF-1R. The bioavailable IGF-II may instead have activated the signaling pathway involving isoform A of the insulin receptor, with proliferative effects [34]. However, clinical and biological arguments (the absence of hypoglycemia and AKT phosphorylation, respectively) do not support the implication of this high ratio in the maintenance of growth.

The patient's serum did not have a stimulatory effect in our cellular AKT phosphorylation assay. This finding contrasts with those of previous studies that reported an increase in the proliferation of erythroid progenitor cells in response to serum from patients [3, 6, 17], and a response in human osteoblast cells similar to that obtained with control serum [8] for cell counting and ^{3}H-thymidine incorporation. There thus appears to be another, as-yet unidentified growth mechanism at work that stimulates growth and proliferation via another signaling pathway. Alternatively, the low level of activity of our patient's serum in the AKT phosphorylation assay may have been due to inhibition by other overstimulated pathways, e.g., as observed in some patients with *SHP2* mutants inducing RAS/ERK1/2 hyperactivation associated with alterations to the JAK 2/STAT 5 or PI3K/AKT pathways [35–38].

Heterozygous missense mutations of *HESX* (encoding a promoter-specific transcriptional repressor) may be associated with mild clinical manifestations, with incomplete penetrance in heterozygous family members [13, 39, 40]. This situation applied to this family, which presented a heterozygous *HESX1* mutation (causing a Q6H substitution) in exon 1, previously described by Thomas et al. [40] and shown to be associated with GH, TSH, and LH/FSH deficits. Similar cases, in which *HESX1* mutations are associated with CPHD, suggest a possible common mechanism for growth without GH [13], but this mechanism of action remains unresolved. The point mutation causing the Q6H substitution in our patient has not been identified as a relatively common sequence variant in the global population. If this mutation is deleterious, its inheritance by unaffected members of the family would confirm the variable penetrance of *HESX1* mutations. This variable penetrance suggests that there must be another cause of hypopituitarism, acting in combination with the *HESX1* variant, and it remains possible that other modifier genes affect HESX1 function.

Some of the heterozygous exonic nucleotide deletions or heterozygous missense mutations revealed by the exome analysis concerned genes with activities related to G-protein signaling pathways and the activation of cAMP-dependent PKA (*AKAP12*), GPCR signaling, Rho GTPase signaling pathways (*ARAP1*), a range of signaling activities involving growth factor and cytokine receptors (*SH2B3*), inhibition of the G1 phase of the cell cycle (*CDKN2A*), inhibition of cell growth (*GPR98*), or regulation of the translation and production of IGF-II (*IGF 2* mRNA *binding protein 3*), suggesting a role in normal growth and development [41–43]. Interestingly, SH2B3 (associated with hypogonadotropic hypogonadism, in particular) mutations may have contributed to the hypopituitary phenotype of our patient, and PIK3C2G mutations (involved in diabetes type 2) may have contributed to the susceptibility of this family to diabetes.

Taken together, all these abnormalities associated with the *HESX1* mutation may account for the phenotype of our patient.

Conclusion

The normal growth despite CHPD was not mediated by GH, IGF-I, IGF-II, insulin, prolactin, or leptin, and the high IGF-II/IGFBP-3 molar ratio was probably not responsible for the sustained high growth velocity of this patient. The lack of growth-promoting activity of the patient's serum suggests that stimulation by an intracellular mechanism is more likely than an effect of circulating growth-promoting factors. The *HESX1* mutation detected in this patient may be one of the most important alterations, because several mutations of this gene have already been reported to be associated with CPHD. However, the penetrance of these mutations is variable, because it was also found in other healthy members of this family. This incomplete penetrance suggests a possible effect of modifier genes or of other associated genetic abnormalities. The exome analysis performed on this patient highlighted multiple variants of genes involved in intracellular signaling pathways that might act in combination with the *HESX1* mutation, and accounting for the observed phenotype. However, the mechanisms of linear growth in this patient remain unclear, despite the large panel of genetic and laboratory investigations performed.

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Statement of Ethics

The Research Ethics Committee at the Faculty of Medicine, Ain Shams University, reviewed and approved the study protocol.

Disclosure Statement

The authors have no conflicts of interest to declare relevant to this study.

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Author Contributions

M.E.K. and H.E. provided the familial cases and recorded their clinical and radiological data, carried out the stimulation tests, determined serum levels of GH, insulin, LH, FSH, and fT4. and participated in discussions concerning the manuscript. L.P. and Y.L.B. performed hormonal evaluations: serum concentrations of IGF-I, IGFBP-3, GHBP, IGF-II, ALS, and IGF-II immunoblot analysis. S.R., N.T., I.N., and Y.L.B. performed and analyzed BWS molecular diagnosis at the 11p15 level, IGF-I and IGF-1R sequencing and MLPA analysis. W.A.H. and Y.L.B. analyzed the WES data. M.B. assessed GH bioactivity. P.L. and F.G. performed AKT phosphorylation assays. S.C.-B. performed and interpreted the SNP microarray analysis. Y.L.B. designed and analyzed data, coordinated and supervised the study, and drafted the manuscript. All authors approved the final version.

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