IGF1 haploinsufficiency in children with short stature: a case series

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Abstract

Context: Short stature in children is a common reason for referral to pediatric endocrinologists. The underlying cause of short stature remains unclear in many cases and patients often receive unsatisfactory, descriptive diagnoses. While textbooks underline the rarity of genetic causes of growth hormone (GH) insensitivity and the severity of its associated growth failure, increased genetic testing in patients with short stature of unclear origin has revealed gene defects in the GH/insulin-like growth factor (IGF-I) axis associated with milder phenotypes. As such, heterozygous *IGF1* gene defects have been reported as a cause of mild and severe short stature. Here, we aimed to describe the clinical and hormonal profile of children with *IGF1* haploinsufficiency and their short-term response to growth hormone treatment (GHT).

Case descriptions: We describe five patients presenting with short stature, microcephaly, and in four out of five born small for gestational age diagnosed with *IGF1* haploinsufficiency. The phenotype of these patients resembles that of previously described cases with similar gene defects. In our series, segregation of the short stature with the *IGF1* deletion is evident from the pedigrees and our data suggests a modest response to GHT.

Conclusions: This study is the first case series of complete heterozygous *IGF1* deletions in children. The specific genetic defects provide a clear image of the phenotype of *IGF1* haploinsufficiency – unbiased by heterozygous mutations with possible dominant negative effects on IGF-I function. We increase the evidence for *IGF1* haploinsufficiency as a cause of short stature, microcephaly, and SGA.

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Introduction

Short stature is a common indication for consulting pediatric endocrinologists. However, in 50–90% of cases, the underlying cause of the short stature remains unclear after clinical and laboratory evaluation (1). Subsequently, these patients often get unsatisfactory descriptive diagnoses of being born small for gestational age (SGA) without catch-up growth, constitutional delay of growth and puberty, familial short stature, or idiopathic short stature (2). Advances in genetics, in particular copy number variation (CNV) analysis, targeted gene panels, and whole-exome or genome sequencing, have increased the diagnostic yield (1). Loss-of-function mutations in genes of the GH/IGF-I axis have been recognized as implicated in growth failure. In patients with severe growth failure,

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biallelic mutations or heterozygous mutations with dominant negative effect, leading to complete or nearly complete loss of protein function, have been reported in growth hormone (*GH*) and growth hormone-releasing hormone (*GHRH*) (3), growth hormone receptor (*GHR*) (4), insulin-like growth factor-1 (*IGF1*) (5), insulin-like growth factor-1 receptor (*IGF1R*) (6), Signal Transducer And Activator Of Transcription 5B (*STAT5B*) (7) and Pappalysin 2 (*PAPPA2*) genes (8). In patients with mild short stature, biallelic inactivating mutations of the IGF Binding Protein Acid Labile Subunit (*IGFALS*) gene have been reported (9). However, mutations leading to partially reduced activity or haploinsufficiency leading to reduced protein levels remain underdiagnosed in patients with a milder phenotype and in individual cases, it is often difficult to conclude that such mutations are causally related to the short stature. Complete heterozygous deletion of the *IGF1* gene was previously described in one patient with postnatal growth failure (10). Here, we describe the clinical features of five patients with complete heterozygous *IGF1* deletion and their response to GH treatment (GHT) after 2–4 years.

Subjects and methods

Patients

Five patients with heterozygous deletions of the entire *IGF1* gene were diagnosed in four different pediatric endocrinology units in Belgium. The probands were born between 2008 and 2015 and followed for short stature. Height, weight, and head circumference (HC) were measured using standard techniques (11). Standard deviation scores (SDSs) for height, weight, HC, target height, and BMI were calculated from the Flemish Growth Charts 2004 standards (12). Target height was calculated from the mean of parental heights +6.5 cm for boys and −6.5 cm for girls (11). Birth weight (BW), length (BL), and head circumference (BHC) were expressed as SDSs adjusted for gestational age based on Niklasson *et al.* (13). SDSs for body proportions were calculated from Dutch references with the sitting height/height (SH/H) ratio based on Frederiks *et al.* (14) and the armspan/height (AS/H) ratio on Gerver *et al.* (15). Bone age reading of an X-ray of the left hand and wrist was done using the Greulich and Pyle method (16) and bone age delay is presented as chronological age (CA) minus bone age (BA). We compare the growth response to GHT with that in short children born SGA treated with a dose of 0.033 mg/kg/day as reported by de Zegher *et al.* (17). The patients and their parents provided written informed consent for publication, and the study was conducted in accordance with institutional guidelines. The study was approved by the UZ Brussel ethical committee (File 2021/037, BUN 1432021000405).

Laboratory tests

Serum IGF-I and GH levels were measured by sandwich chemiluminescence immunoassays using either the DiaSorin (three patients), IDS (one patient), or Immulite 2000 kit (one patient). Glucagon stimulation tests were done by intramuscular injection of 1 mg glucagon followed by collecting blood samples 0, 30, 60, 90, 120, 150 and 180 min afterwards for dosing GH levels. Peak GH cut-offs for a normal response were >10 µg/L before 2011 or >7 µg/L after 2011 based on a change in the GH assay used in all implicated laboratories. IGF-I generation tests were done by collecting a baseline IGF-I sample and 1 after 7 days of daily subcutaneous GH injection (0.05 mg/kg/day). IGF-I response was considered normal when the difference between the simulated value and the baseline was >15 ng/mL (18). All IGF-I dosages for the IGF-I generation tests were centralized and determined by automated chemiluminescence with the IDS-iSYS IGF-I assay. SDS calculations for serum IGF-I levels are based on the normative data of Bidlingmaier *et al.* (19) and those for IGFBP-3 on Friedrich *et al.* (20).

Genetic analyses

DNA samples were obtained from probands and selected biological relatives. Array comparative genomic hybridization (CGH) analysis was done using an ISCA 60K or 180K oligonucleotide microarray according to the manufacturer's instructions (Agilent). Briefly, 1 mg of patient and reference DNA (pool of seven male DNA samples: Promega) were digested with AluI and RsaI (5 units each) and subsequently labeled respectively with Cyanine-5 dUTP and Cyanine-3 dUTP using the BioArray Genomic DNA Labelling System (Enzo, Farmingdale, NY, USA).

Case report

Patient 1

Patient 1 is a 4-year-old boy. He was born by vaginal delivery after an uncomplicated pregnancy at 39 weeks and 5 days of gestation. The boy was SGA with a BW at −2.9 SDS, BL at −4.0 SDS, and BHC at −2.0 SDS. He stayed briefly in the neonatal intensive care unit (NICU) during

the first days of life for a suspected neonatal infection which resolved with antibiotic treatment. At age 1, he had a unilateral orchidopexy but otherwise no surgical or medical antecedents. His parents are of Sicilian origin and not consanguineous. The father has a height at −3.0 SDS and HC at −1.0 SDS. The mother has a height at −3.1 SDS and her HC is at −2.7 SDS. Both parents being short, his target height is −2.7 SDS. An older brother of 11 years and 5 months is in good health and has a low-normal height at −1.9 SDS. Patient 1 was referred to a pediatric endocrinology unit at the age of 21 months for short stature. At physical examination, his height was −4.4 SDS, weight −5.7 SDS, and BMI −3.7 SDS. A severe microcephaly with a HC at −4.1 SDS and clinodactyly of the fifth finger were noted. The SH/H SDS was −1.6 SDS and the AS/H −0.3 SDS. His psychomotor development was age-appropriate and hearing tests were normal. The GH stimulation test by glucagon injection documented a normal GH response (peak 7.4 µg/L). IGFPB-3 levels were normal at −0.2 SDS, serum IGF-I was low-normal at −2.2 SDS, and glycemia was normal. There was no IGF-I increase during an IGF-I generation test (differential value 4 ng/mL, gain of +0.1 SDS) (Table 1). At the start of the IGF-I generation test, serum ALS concentration was normal (865 U/L, reference: 390–1166 U/L), and at the end of the test, an elevated level was measured (1230 U/L) (Table 1). Genetic analysis by CGH array revealed a 2.26 Mb deletion at 12q23.1q23.3 comprising the *IGF1* gene (Fig. 1A and E). The result in

Table 1 Overview of clinical characteristics in the reported patients.

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex	Male	Male	Female	Male	Female
Birth					
Gestational age, week	39.7	38.0	41.0	38.0	40.1
Weight SDS	−2.9	−3.2	−2.8	−1.4	−3.4
Length SDS	−4.0	−4.0	−2.7	−0.9	−2.5
HC SDS	−2.0	−2.4	−2.0	−3.0	−0.7
First presentation					
Age, year	1.8	8.8	1.0	5.2	4.1
Height SDS	−4.4	−3.1	−4.3	−4.2	−3.6
Weight SDS	−5.7	−3.3	−5.2	−4.5	−3.9
BMI SDS	−3.7	−1.3	−2.7	−1.0	−1.6
HC SDS	−4.1	−4.7	−2.7	−3.1	−3.9
SH-H SDS	−1.6	−1.0	–	–	+0.4
AS-H SDS	−0.3	−0.2	–	+0.2	−0.5
Bone age delay					
Baseline CA-BA, year	1.1	1.8	–	1.9	1.9
Endocrine data					
GH peak[a] (µg-ml)	7.5*	–	7.9*	15.1*	–
Basal IGF-I (SDS)	−2.2	−1.8	−1.6	−1.4	−0.4
Stimulated IGF-I[b] (SDS)	−2.1	–	–	+0.3	+0.4
Basal ALS (U-l)	865	–	–	1499	2023
Reference	390–1166			528–1440	705–1818
Delta ALS[b] (U-l)	365	–	–	836	349
IGFBP-3 (SDS)	−0.2	–	–	+0.9	+0.9
Phenotypic features					
Walking age, month	16	12	18	9	–
Education	Normal	Special	Normal	Normal	Normal
Specific needs	No	Multiple	Multiple	No	Multiple
Hearing	Normal	Normal	Normal	Normal	Normal
Clinodactyly	Present	Present	Absent	Present	Present
Parents					
Inheritance	Maternal	Paternal	*De novo*	Unknown	Maternal
Nonaffected parent height SDS	−3.0	−1.4	−0.8 to +1.0	–	−1.0
Affected parent height SDS	−3.1	−3.1	–	–	−3.3
Nonaffected parent HC SDS	−1.0	–	–	–	+0.2
Affected parent HC SDS	−2.7	−2.6	–	–	0.3
Target height SDS	−2.7	−2.0	+ 0.1	−2.1	−2.1

*Normal values. [a]Peak GH serum levels after GH stimulation test; [b]Change in IGF-I and ALS serum levels after IGF-I generation test.
AS/H, armspan divided by height ratio; CA-BA, calendar age minus bone age; HC, head circumference; SH/H, sitting height divided by height ratio.

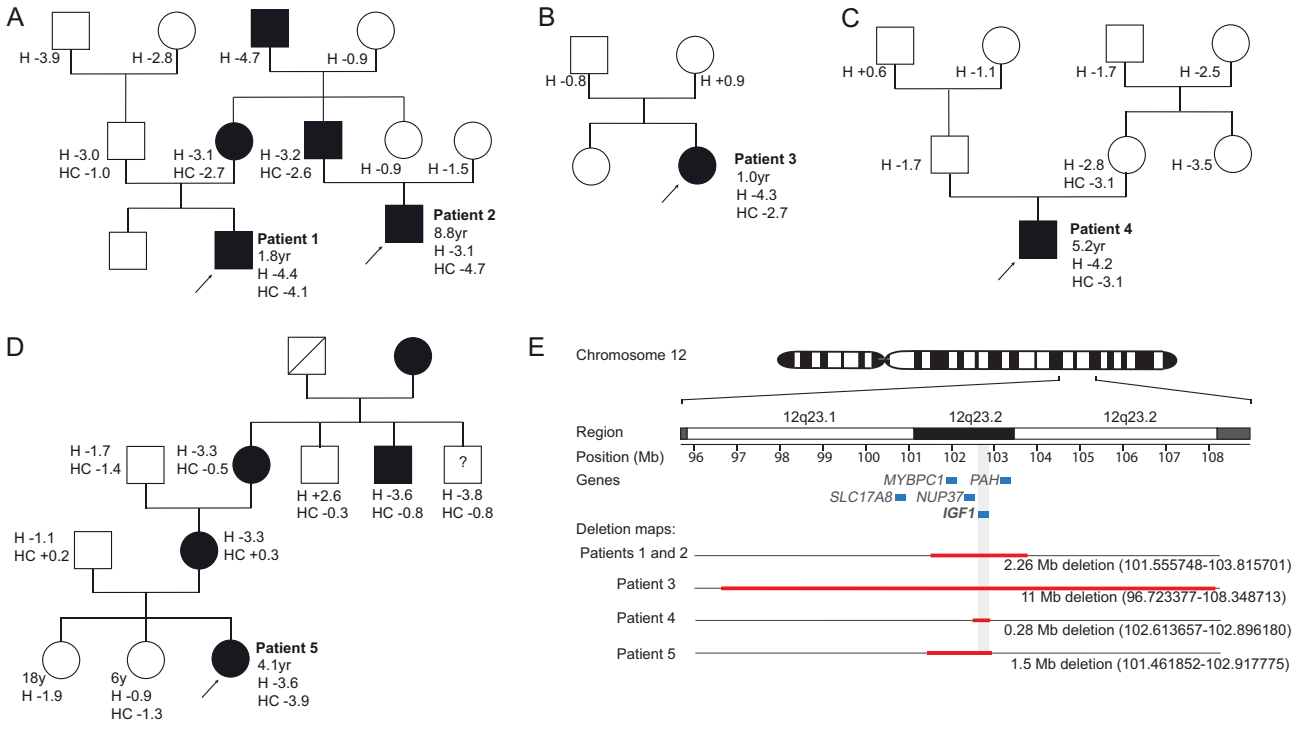

Figure 1

Pedigrees and gene defects of the probands with *IGF1* haploinsufficiency. (A, B, C and D) Pedigree charts show auxological data for the patients 1–5 at age of presentation and their relatives. Arrow indicates proband. (E) Schematic representation of the gene defects of patients 1–5. H, height SDS; HC, head circumference SDS. A full color version of this figure is available at https://doi.org/10.1530/EJE-20-1463.

the father was normal but the proband's mother carried the same deletion as did his maternal grandfather (−4.5 height SDS), and maternal uncle (−3.0 height SDS). Patient 1 received GHT (initially 0.025 mg/kg/day gradually increased to 0.050 mg/kg/day) from the age of 2 years and 3 months onwards. At the start of GHT, his height was at −4.4 SDS, after 1 year it was at −3.7 SDS (gain of +0.7 SDS), after 2 years at −3.2 SDS (gain of +1.2 SDS) and after 2.7 years at −3.1 SDS (gain of +1.3 SDS) (Fig. 2A). IGF-I levels rose from −2.2 SDS at baseline to −0.8 SDS after 1.5 years, and to −0.4 SDS after 2 years of GHT (Fig. 2B). Bone age delay (CA-BA) was 1.1 years at baseline, 1.6 years after 1 year GHT and 1.4 years after 2 year GHT, so that the bone age progression was 1.5 years in 2 years (Fig. 2C).

Patient 2

Patient 2 is a 11-year-old boy. He is a first cousin of patient 1 and presented independently in a different clinic. He was born by vaginal delivery after an uncomplicated pregnancy at 38 weeks of gestation. The boy was born SGA with a BW at −3.2 SDS, BL at −4.0 SDS, and BHC at −2.4 SDS. He stayed briefly in the NICU during the first days of life for monitoring in view of his low birth weight. His parents are of Belgo-Sicilian origin and non-consanguineous. The father's height is −3.1 SDS and his HC −2.6 SDS and the mother's height is −1.4 SDS. Target height is −2.3 SDS. Patient 2 was referred to a pediatric endocrinology unit at the age of 8 years and 9 months for short stature. At physical examination, his height was −3.1 SDS, weight −3.3 SDS, and BMI −1.3 SDS. He had a microcephaly with HC at −4.7 SDS and clinodactyly of the fifth finger. His SH/H was at −1.0 SDS and AS/H at −0.2 SDS. No GH stimulation tests were done. The boy had learning difficulties and attended specialized education. Hearing tests were normal. His IGF-I serum level was in the low normal range at −1.8 SDS (Table 1). No abnormalities in the *SHOX* gene region were found by copy number variation (CNV) analysis and Sanger sequencing. CGH array provided the diagnosis of *IGF1* gene haploinsufficiency at the age of 9 years and 6 months by showing a 12q23.1q23.3 deletion similar to patient 1, which comprises the *IGF1* gene (Fig. 1A and E). This deletion was also found in the father, the paternal grandfather (−3.1 height SDS), and the paternal aunt (−3.1 height SDS). Patient 2 received GHT (initially

0.035 mg/kg/day gradually increased to 0.050 mg/kg/day) from the age of 8 years and 9 months onwards. At the start of GHT, his height was at −3.2 SDS, after 1 year at −2.7 SDS (gain of +0.5 SDS), after 2 years at −2.6 SDS (gain of +0.6 SDS), after 3 years at −2.5 SDS (gain of +0.7 SDS), and after 3.5 years at −2.1 SDS (gain of +1.1 SDS) (Fig. 2A and Table 1). Of note, the latter accelerated gain in height SDS (from 3 to 3.5 years of treatment) is also due to the pubertal growth spurt. Serum IGF-I levels rose from −1.8 SDS at baseline to +0.4 SDS after 10 months, +0.7 SDS after 28 months of GHT and +0.9 SDS after 42 months (Fig. 2B). While at baseline, he had a bone age delay of 1.8 year, at 3 years GHT his BA was 0.62 years ahead on his CA (Fig. 2C and Table 1).

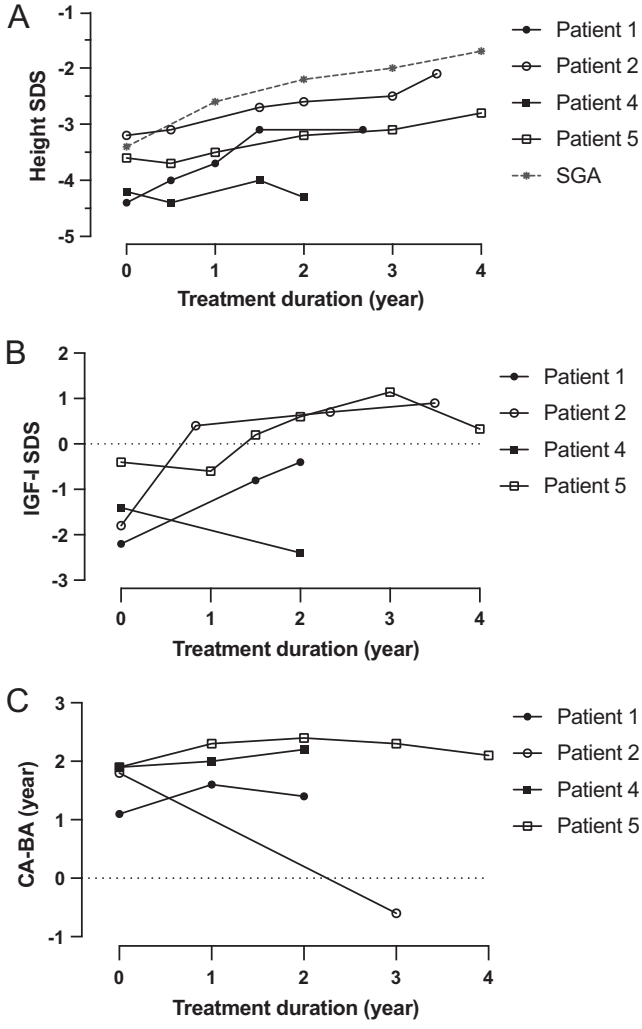

Figure 2

Evolution of growth, serum IGF-I levels and bone age progression in response to growth hormone treatment (GHT). (A) Height SDS, (B) serum IGF-I SDS levels, and (C) bone age delay (chronological age minus bone age) during GHT. For patients 1, 4 and 5, IGF-I levels at diagnosis, just before start of GHT; for patient 2, baseline IGF-I level dates of ~1.5 years prior to diagnosis and start of GHT. The dotted line in panel A corresponds to the growth response in short children born SGA as reported by de Zegher *et al.* CA-BA, chronological age minus bone age; SGA, small for gestational age.

Patient 3

Patient 3 is a girl of 6 years and 7 months. She was born by vaginal delivery after an uncomplicated pregnancy at 41 weeks of gestation. The girl was born SGA with a BW at −2.8 SDS, BL at −2.7 SDS, and BHC at −2.0 SDS. She stayed briefly in the NICU during the first days of life due to a congenital cytomegalovirus infection. In this context, a brain MRI was performed and was normal. Her parents are of Belgian and Togolese origin and non-consanguineous. The father's height is −0.8 SDS and the mother's +1.0 SDS. Target height is +0.2 SDS. Her 8-year-old sister is healthy and has a normal height.

Patient 3 was referred to a pediatric endocrinology unit at the age of 1 year for short stature. At physical examination, the height was −4.3 SDS, the weight −5.2 SDS, the BMI −2.7 SDS, and a microcephaly with HC at −2.7 SDS was noted. GH response upon glucagon stimulation was normal (GH peak 7.9 µg/L) and her IGF-I serum level was low-normal at −1.6 SDS (Table 1).

CGH array provided the diagnosis of *IGF1* gene haploinsufficiency by showing a de novo 11Mb microdeletion of the 12q23.1q23.3 region, which comprises the *IGF1* gene (Fig. 1B and E), which was not carried by the parents. Patient 3 did not receive GHT.

At 6 years and 7 months of age, she is at −2.3 height SDS (gain of +2.0 SDS), +0.3 BMI SDS (gain of +3.0 SDS), and −2.0 HC SDS (Table 1). No BA readings are available. She had learning difficulties and received multidisciplinary support (speech therapist, occupational therapy and psychomotricity sessions). Hearing tests were normal.

Patient 4

Patient 4 is a 10-year-old boy. He was born after an uncomplicated pregnancy at 38 weeks of gestation. The boy's BW SDS was −1.4, BL SDS −0.9, and BHC SDS −3.0. He developed no neonatal complications. His parents are of Belgo-Spanish origin and non-consanguineous. The father's height is −1.6 SDS and the mother's −2.8 SDS with her HC SDS at −3.1. Target height is −2.1 SDS. At presentation to the pediatric endocrine clinic, he was 5 years and 2 months old, height SDS was −4.2, weight

SDS −4.5, and BMI SDS −1.0 SDS. A microcephaly with HC at −4.0 SDS and clinodactyly of the fifth finger were noted. His psychomotor development was normal as were his hearing tests. He had a normal GH response upon glucagon stimulation (GH peak 15.1 µg/L) and his IGF-I serum level was in the low normal range at −1.4 SDS. His IGFPB-3 serum level was normal at +0.9 SDS. An IGF-I generation test showed an increase of IGF-I after 7 days of GHT to +0.3 SDS (gain of + 1.7 SDS) (Table 1). At start of the IGF-I generation test ALS levels were slightly elevated (1499 U/L, reference: 528–1440 U/L) and at the end of the test significantly elevated levels were measured (2335 U/L) (Table 1). CGH array provided the diagnosis of *IGF1* gene haploinsufficiency at the age of 5 years by showing a 12q23.2 deletion, which comprises the *IGF1* gene (Fig. 1C and E). The same deletion was not found in the mother and the father could not be tested. Patient 4 received GHT (0.035 mg/kg/day) from the age of 5 year and 5 months onwards; however, poor compliance and irregular visits do not allow correct evaluation of the response to treatment (Fig. 2A). In line with the reported poor therapeutic compliance, IGF-I levels remained low after 24 months of GHT (−1.4 SDS at start and −2.4 SDS at last evaluation) (Fig. 2B). Bone age delay was 1.9 years at baseline, 2.0 years after 1 year GHT, and 2.2 year after 2 year GHT, with the BA (2 years) – BA (baseline) at 1.5 years (Table 1 and Fig. 2C).

Patient 5

Patient 5 is 7-year-old girl. She was born after an uncomplicated pregnancy at 40 weeks and 1 day of gestation. Her BW SDS was –3.4, BL SDS −2.5, and BHC SDS −0.7 SDS. She stayed in the neonatal intensive care unit during the first 3 weeks of life for monitoring in view of her SGA. Her parents are Belgian in origin and non-consanguineous. Her father's height is at −1.0 SDS and HC at +0.2 SDS and her mother's height at –3.3 SDS and HC at +0.3 SDS. Target height is −2.1 SDS. Patient 5 presented to the pediatric endocrine clinic for short stature at 4 years and 1 month of age. At physical examination, her height SDS was −3.6, weight SDS −3.9 SDS, and BMI SDS −1.6. A microcephaly with HC SDS at −3.9 and bilateral fifth finger clinodactyly were noted. Her SH/H SDS was at +0.4 SDS and AS/H ratio at −0.5 SDS. Patient 5 attended normal education, but she had learning difficulties for which she received additional support, including speech therapy, ergotherapy, and psychomotricity training. Hearing tests were normal. No GH stimulation tests were done. Her IGFPB-3 serum level was normal at +0.9 SDS and her IGF-I level at −0.4 SDS was normal (Table 1). At the start of the IGF-I generation test, the ALS level was elevated (2023 U/L, reference: 705–1818 U/L) and remained elevated at the end of the test (2372 U/L) (Table 1). These features indicated a diagnosis of SGA without catch-up growth. No abnormalities in the *SHOX* gene region were found by CNV analysis and Sanger sequencing. However, CGH array documented an *IGF1* gene haploinsufficiency at the age of 4 years by showing a 1.5Mb deletion of the 12q23.1q23.2 region, which comprises the *IGF1* gene. The same deletion was found in the mother, the maternal grandmother (−3.3 height SDS), a maternal aunt (−1.6 height SDS), and the maternal great-grandmother (no height measurements available) (Fig. 1D and E). An IGF-I generation test in patient 5 showed an increase in IGF-I levels to +0.4 SDS (gain of +0.8 SDS) (Table 1). She received GHT (0.05 mg/kg/day) from the age of 4 years and 10 months onwards. At the start of GHT her height was at −3.6 SDS, after 1 year at −3.5 SDS (gain of +0.1 SDS), after 2 years at -3.2 SDS (gain of +0.4 SDS), after 3 years at −3.1 SDS (gain of +0.5 SDS), and 4 years at −2.8 SDS (gain of +0.7 SDS) (Fig. 2A). IGF-I levels rose from –0.4 SDS to +0.2 SDS after 1.5 years, and to +0.6 SDS after 2 years of GHT (Fig. 2B). Bone age delay was 1.9 years at baseline, 2.3 after 1 year GHT, 2.4 years after 2 years, 2.3 years after 3 years, and 2.1 years after 4 years, with the BA (4 years) – BA (baseline) at 3.5 years (Table 1 and Fig. 2C).

Discussion

In this case series, we describe five patients with *IGF1* haploinsufficiency that presented with a similar phenotype with features including severe short stature (−4.4 to −3.1 height SDS), microcephaly (−4.7 to −2.7 HC SDS), and a mild bone age delay. At birth, four out of five were born SGA (−3.4 to −2.8 BW SDS) and already had a microcephaly (−3.0 to −2.0 BHC SDS). In children presenting with short stature, the underlying cause often cannot be pinpointed and a diagnosis of familial or idiopathic short stature by exclusion provides an unsatisfactory label. In recent years, the lowered threshold to perform genetic testing in such cases has revealed several genetic defects in the GH/IGF-I pathway responsible for the growth failure. Genetic diagnoses were first established in case of severe growth failure and now increasingly unravel the causes of mild growth failure. Patients with severe IGF-I insufficiency as seen in biallelic loss-of-function mutations have a severe prenatal and postnatal growth failure, developmental delay, microcephaly, and sensorineural deafness (OMIM #608747) (5, 21, 22). Heterozygous variants in the *IGF1*

gene have also been found in three patients with severe short stature (23, 24). While *IGF1* gene defects are very rare and an association of the genetic variant with short stature was found in these families, a causal link could not be directly established. In case of such heterozygous variants, a dominant negative effect of a mutated protein might explain the severity of the growth failure in the presence of an unaffected allele. Haploinsufficiency allows a clearer evaluation of the implication of having just a single WT copy of the *IGF1* gene on growth. A patient with mild short stature (−2.7 height SDS) and *IGF1* haploinsufficiency was previously reported (10), but the absence of a large pedigree in which genotype-phenotype correlation could be documented made conclusions regarding causality very difficult. In the current case series, we provide such evidence by reporting five different kindreds with a genotype–phenotype correlation of the *IGF1* insufficiency with short stature in up to four generations.

The patients reported here had a phenotype with mostly growth-related features and few other organ systems were affected. We observed no sensorineural hearing loss as reported in previous cases of homozygous *IGF1* mutations (5, 21, 22). In addition, the developmental delay which was present in three out of five patients was mild. Clinodactyly of the fifth finger was noted in four out of five patients reported here as well as in the previously published case study of *IGF1* haploinsufficiency (10) but whether this is coincidental, related to intrauterine growth retardation, or to the genetic *IGF1* defect remains unclear. Of note, one patient that was not born SGA still presented with clinodactyly. Phenotypical differences related to paternal or maternal inheritance of the gene defect were not evident (patients 1 and 5 inherited the deletion from their mother, patient 2 from his father, patient 3 had a de novo deletion, and for patient 4 parental inheritance could not be established). Without genetic testing, all patients would have received a descriptive diagnosis: in four out of five patients SGA without spontaneous catch-up growth and in 1 patient familial short stature. All patients were found to harbor heterozygous 12q23.2 overlapping deletions of variable size, from 0.28 Mb to 11 Mb (Fig. 1E), encompassing the entire *IGF1* gene.

Genetic testing is becoming more readily available, and its diagnostic indications broaden. This implicates that children with short stature with a milder phenotype undergo such tests more frequently, even in the presence of classically sufficient descriptive diagnoses that are considered valid indications for GHT, as was the case for four out of five patients in this study (SGA without catch-up growth). While all five patients harbor heterozygous 12q23.2 overlapping deletions encompassing the entire *IGF1* gene, the size of the deletions is variable (0.28–11 Mb), implying the deletion of other genes and non-coding genomic regions that might contribute to the phenotype and more specifically to the cognitive impairment or learning difficulties. Within the deleted contiguous genes, however, none is reported as a causal gene for autosomal dominant intellectual disability. Nevertheless, we cannot exclude that contiguous deleted genes of which the function is poorly understood or loss of regulatory elements in the non-coding DNA, act as modifying factors that increase the risk of intellectual disability, especially in patient 3 with the largest deletion. The *PAH* gene, responsible for autosomal recessive phenylketonuria, classically associated with intellectual disability and microcephaly, is deleted in all patients. However, neonatal screening showed normal phenylalanine levels in the patients, suggesting the absence of a pathogenic mutation on the second allele of the *PAH* gene. In all patients except patient 4, the *NUP37* gene is deleted. This gene is known to cause autosomal recessive primary microcephaly syndrome, but heterozygous carriers are not reported to be symptomatic. A link between the haploinsufficiency of this gene and the microcephaly observed in our patients is, therefore, unlikely (25). Finally, the *MYBPC1* gene is also deleted in all patients except patient 4. Missense mutations in this gene were described in association with autosomal dominant distal arthrogryposis type 1B and with congenital myopathy with tremor (26, 27, 28). The absence of distal contractures and congenital hypotonia in our patients is an argument to attribute a dominant negative effect to the reported *MYBPC1* missense mutations rather than a pathological mechanism based on haploinsufficiency. In patient 3, long term follow-up is recommended to screen for progressive hearing loss as the *SLC17A8* gene, which is involved in late-onset autosomal dominant hearing loss, is located within the deleted region (29, 30). One out of the 5 patients, who had the smallest deletion (0.28 Mb), was not born SGA but had a similar birth weight (−1.4 BW SDS) as the previously reported patient (−1.5 BW SDS) (10). Indeed, the microdeletion in both patients is very similar and situated between position 102.6 Mb and 102.8 Mb. This suggests that the SGA phenotype could be due to a contiguous deleted gene and not to the *IGF1* haploinsufficiency as such. The postnatal growth failure and microcephaly at clinical presentation, however, were as pronounced in these patients as in the other four patients we report here, which suggest that these features are strictly due to the *IGF1* haploinsufficiency.

Serum IGF-I levels were in the lower half of the normal range in all patients and two out of three patients who underwent an IGF-I generation test had a normal increase in IGF-I. The limited response in one patient is possibly related to his severe underweight and young age. The finding of low normal IGF-I levels is in line with the previous case report of *IGF1* haploinsufficiency (10) but in that patient no IGF-I generation test was reported. A normal response to an IGF-I generation test was, however, reported in a patient with a heterozygous duplication in the *IGF1* gene resulting in a frameshift and a premature stop codon (24). Nonetheless, these results indicate that a single *IGF1* copy suffices to maintain low normal serum IGF-I levels and to raise these in response to GH stimulation, but it does not suffice for normal linear growth. The poor growth in these patients might be explained by low IGF-I bioavailability due to normal IGFBP-3 levels and a subsequently decreased IGF-I/IGFBP-3 ratio. Basal ALS levels in one out of the three tested patients were elevated, and by the end of an IGF-I generation test, stimulated ALS levels were elevated in two out of three patients. The normal or increased ALS response can be interpreted as normal hepatic GH sensitivity in the tested patients. The borderline GH secretion in two patients is rather unexpected in the context of normal IGFBP-3 and high-normal ALS levels, which would suggest increased GH secretion. As discussed in this context by Batey *et al.* (10), we tend to agree that these cases illustrate that sometimes genetic testing is more useful than relying on circulating serum levels of GH-dependent growth factors to diagnose pathology in the GH/IGF-I axis. In patients with homozygous *IGF1* mutations, low IGF-I levels and high IGFBP-3 and ALS levels are reported and thought to be related to an increased GH secretion.

GHT is commonly used to support linear growth both in GH-deficient and non-GH-deficient disorders (31). Four out of five patients reported in this study received GHT and three out of four had good therapeutic compliance. The gain in height SDS (+0.8 to +1.3) following 2–4 years of regular GHT can be considered as a modest response when interpreted in the context of the SGA without catch-up growth indication that was used for reimbursement purposes (17, 32). In the previous report of a patient with *IGF1* haploinsufficiency GHT was not pursued (10). Reports of the growth response to GHT in patients with heterozygous *IGF1* mutations are variable with a poor response in 1 patient after 9 months of treatment (gain of +0.39 height SDS) (24) and a good response in two patients after 2 years of treatment (gain of +1.0 to +1.5 height SDS) (23). The latter good responders had an accompanying significant increase in serum IGF-I levels (gain of +2.7 and +3.2 height SDS after 2 years on GHT at 1.4 mg/m^2/day). Regarding our case series, the IGF-I levels in patient 1 rise under GHT and the first-year growth response is reasonable but between 1.5 and 2.5 years of GHT, he remains at −3.1 height SDS. This stagnation may be related to another gene defect possibly from the paternal side; however, we did not pursue further genetic analyses to evaluate this hypothesis. GHT in patient 2 led to a limited height SDS gain in the first year (+0.5 SDS), followed by a stagnation at −2.5 SDS and a recent accelerated gain related to his pubertal growth spurt – as reflected in the bone age progression (CA-BA shifted from 1.8 to −0.6). Intriguingly, patient 3 who did not receive GHT, nonetheless, had a spontaneous height SDS increase, possibly related to a firm increase in BMI. This patient harbors the largest deletion, including several genes (*ANKS1B*, *SLC17A8*, *RN1H4*, *PMCH*, *ASCL1*) whose SNPs were associated with body height and/or weight by genome-wide association (GWA) studies (33, 34, 35). However, the current scientific knowledge does not allow to extrapolate the results of these GWA studies to predict the consequences of haploinsufficiency of these genes on final weight or height. Patient 4 did not comply well to GHT, as reflected in his unchanged, low IGF-I levels after 2 years and his growth response was poor. Patient 5 had no growth response in the first year of GHT (gain of +0.1 height SDS) but her IGF-I levels also remained low. A limited gain in height SDS has been obtained after 4 years of GHT (+0.8 SDS). For conclusions on the efficiency of GHT in patients with *IGF1* haploinsufficiency, we will have to wait until final height is reached, but the modest short-term response is unlikely to translate into a satisfactory gain in adult height.

In summary, we report on five patients with complete heterozygous deletion of the *IGF1* gene. The documented genetic defects unveil the true phenotype of *IGF1* haploinsufficiency – unbiased by heterozygous mutations with possible dominant negative effects on IGF-I function. Their clinical characteristics include SGA without catch-up growth, failure to thrive with severe short stature and microcephaly, clinodactyly of the fifth finger, and a variable degree of cognitive impairment. The triad of SGA, short stature, and microcephaly, in particular, should prompt genetic screening for *IGF1* defects. Serum IGF-I levels tend to be in the low normal range, while IGFBP-3 levels are normal, and ALS levels normal to high-normal. GHT with supra-physiological doses seems to be modestly effective in the short term (2–4 years), but long-term effectiveness cannot be concluded until these children have reached their final height.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement
W S and N A wrote the original draft. D B supervised the writing of the manuscript. W S, O C, C H, D B treated patients. All authors helped on revising the article critically for important intellectual content and have given final approval.

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