Genetic causes of growth disorders

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Two short children born small for gestational age with insulin-like growth factor 1 receptor haploinsufficiency illustrate the heterogeneity of its phenotype

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Abstract

Context: Small for gestational age (SGA)-born children comprise a heterogeneous group in which only few genetic causes have been identified.

Objective: To determine copy number variations in 18 growth-related genes in 100 SGA children with persistent short stature.

Methods: Copy number variations in 18 growth-related genes (*SHOX, GH1, GHR, IGF1, IGF1R, IGF2, IGFBP1-6, NSD1, GRB10, STAT5B, ALS, SOCS2,* and *SOCS3*) were determined by an "in house" multiplex ligation-dependent probe amplification kit. The deletions were further characterized by single-nucleotide polymorphism array analysis.

Results: Two heterozygous *de novo* insulin-like growth factor 1 receptor (IGF1R) deletions were found: a deletion of the complete *IGF1R* gene (15q26.3, exons 1-21), including distally flanking sequences, and a deletion comprising exons 3-21, extending further into the telomeric region. In one case, serum IGF-I was low (–2.78 SD Score), probably because of a coexisting growth hormone (GH) deficiency. Both children increased their height during GH treatment (1 mg/m^2 per day). Functional studies in skin fibroblast cultures demonstrated similar levels of IGF1R autophosphorylation and a reduced activation of protein kinase B/Akt upon a challenge with IGF-I in comparison with controls.

Conclusions: *IGF1R* haploinsufficiency was present in 2 of 100 short SGA children. GH therapy resulted in moderate catch-up growth in our patients. A review of the literature shows that small birth size, short stature, small head size, relatively high IGF-I levels, developmental delay, and micrognathia are the main predictors for an *IGF1R* deletion.

Introduction

Children born with a low birth weight and/or birth length corrected for gestational age (small for gestational age, SGA) comprise a heterogeneous group with a broad spectrum of clinical characteristics. Reduced size at birth may result from fetal, maternal, placental, and/or genetic factors. Although many children born SGA achieve sufficient growth to normalize their stature by 2 yr of age, approximately 15% maintain a height below –2 SD score and continue to be short throughout adolescence and adulthood (1). Short SGA children have a reduced lean body mass, fat mass, skinfolds, and body mass index (BMI) (2-4), as well as a lower caloric, fat, and carbohydrate intake (5). SGA children with a persistent short stature and/or a small head size have a higher risk of subnormal intellectual and psychological performance (6-9).

Genetic causes have only been found in a small proportion of short SGA children, including point mutations and deletions in the *IGF1* (10-12) and insulin-like growth factor 1 receptor (*IGF1R*) genes (13-26). The availability of the complete sequence of the human genome and the introduction of high-throughput DNA-scanning techniques provide novel tools to investigate the genetic basis of short stature. In this study, we used multiplex ligation-dependent probe amplification (MLPA) to investigate rapidly whether copy number variations in growth-related genes (*SHOX, GH1, GHR, IGF1, IGF1R, IGF2, IGFBP1-6, NSD1, GRB10, STAT5B, ALS, SOCS2,* and *SOCS3*) were present in a group of 100 children born SGA with persistent short stature. The extent of the two deletions that were found was determined with single-nucleotide polymorphism (SNP) array analysis. Functional studies on dermal fibroblasts were performed to investigate the IGF1R signal transduction pathway in the two patients and age- and sex-matched healthy controls.

Subjects and Methods

Study population

The first hundred short SGA children participating in four prospective cohort trials evaluating the effect of GH treatment (3, 27) were included in the study. SGA was defined as a birth length and/or weight ≤–2 SD score for their gestational age (28), and only children were included who remained short in postnatal life [at age 3: height ≤–2.00 SD score: short SGA (29)]. All children were Caucasian and had an uncomplicated postnatal period. Severe chronic illness or endocrine disorders, chromosomal or genetic abnormalities, positive endomysial or transglutaminase antibodies, skeletal abnormalities and psychosocial dwarfism were reasons to exclude children from the study. The study protocol was approved by the Medical Ethics Committee of the Erasmus Medical Center (Rotterdam), and written informed consent was obtained.

Clinical and biochemical measurements

Birth and growth data before the start of GH treatment were retrieved from records of hospitals, community health services, and general practitioners. Children were systematically measured at the start of and during the GH trials (3, 27). Height and head circumference were expressed as SD scores (29). Body mass index was calculated (weight in kg/height in meters^2) and expressed as SD scores for age and sex (30). Body proportion was assessed by the sitting height/height ratio and expressed in SD scores (31). GH production was assessed by arginine and clonidine GH provocation tests, and GH was measured by AutoDelphia (Perkin-Elmer) and standardized according to World Health Organization 80/505 guidelines. Serum IGF-I and IGFBP-3 levels were measured in the SGA subjects as described previously (32, 33), and values were transformed to SD scores by adjusting for sex and age (33). Bone age was determined according to Greulich and Pyle (34). Dysmorphological examination was performed by an experienced clinical geneticist (L.C.P.G.).

Genetic analysis

Genomic DNA was extracted from peripheral blood samples (35). DNA from control samples was isolated from leukocytes using Puregene nucleic acid purification chemistries for the Autopure LS Instrument (Gentra Systems, Minneapolis, MN).

The "in house" probe kit was designed according to the criteria described in White *et al.* (36). The kit contained 34 probes (supplementary Table 1) in 18 different growth-related genes (*SHOX, GH1, GHR, IGF1, IGF1R, IGF2, IGFBP1-6, NSD1, GRB10, STAT5B, ALS, SOCS2,* and *SOCS3*). Reactions were performed as described by Walenkamp *et al.* (25). MLPA of all 21 *IGF1R* exons was performed using the MRC Holland P217 MLPA kit according to the manufacturer's instructions (MRC Holland, Amsterdam, The Netherlands).

All MLPA kits that were used were validated with DNA from patients with a deletion in that particular gene, that have been diagnosed with other molecular techniques. As a positive control, a patient that was previously published (25) was used. A number of normal individuals, including their parents, and a blank (no DNA) were used as negative controls.

The Affymetrix GeneChip Human Mapping 262K *NspI* array was used according to the instructions provided in the Affymetrix GeneChip Human Mapping 500K Manual (http://www.affymetrix.com). SNP copy number was assessed using CNAG (Copy Number Analyser for GeneChip) version 2.0 (37).

IGF1 gene sequencing of all four exons and flanking intron-exon boundaries was performed according to standard procedures (primers and conditions available upon request).

Functional studies

Skin biopsies were taken from patients A and B, and a culture of dermal fibroblasts was established (38). For Western blotting, fibroblasts were stimulated for 10 minutes with 5, 10, and 20 ng/ml IGF-I (PeproTech, Inc., Rocky Hill, NJ). Blots were probed with an antiphosphoprotein kinase B (PKB)/Akt (Ser^{473}), total PKB/Akt, total IGF1Rβ (Cell Signaling Technology, Beverly, MA), and an antiphospho-IGF1R (Biosource International, Camarillo, CA) antibody as described previously (39).

Results

All 100 short SGA children were investigated with the "in house" MLPA growth kit, and two patients were identified with a deletion of the *IGF1R* gene. No copy number variants (CNVs) in the other growth-related genes were detected.

Patient A

Clinical description

Patient A was a girl who was born spontaneously after 40 weeks of gestation as the third child of nonconsanguineous parents. Because maternal age was 36 yr, her mother chose for amniocentesis showing a 46,XX karyotype. The pregnancy was complicated by vaginal bleeding and limited fetal movements. Her birth weight was 2890 g (–1.28 SD score) and birth length 47 cm (–2.21 SD score). The height of her father was 184.7 cm (0.40 SD score) and of her mother 176.6 cm (1.34 SD score), resulting in a (secular trend-corrected) target height of 178.8 cm (1.25 SD score). She had bilateral hip dysplasia and clubfeet, for which she had hip casting from 4 to 12 months of age. Her bilateral hearing loss improved by tympanostomy tubes, which were implanted at 3 yr of age. Psychomotor development was delayed. At 2.3 yr of age, her height was 78.9 cm (–3.46 SD score), weight 10.3 kg (–2.13 SD score weight for height), and head circumference 47.2 cm (–0.82 SD score). Arginine and clonidine stimulation tests were performed at age 2 with a maximal GH response of 19.1 mU/liter and 14.1 mU/liter, respectively. She had a delayed dentition, starting at age 2. At age 3 her bone age was 2 yr. Her IGF-I level was 46 ng/ml (–1.61 SD score) and IGFBP-3 level 1.17 mg/liter (–1.63 SD score). Cardiovascular, respiratory, and abdominal examinations were all normal. On magnetic resonance imaging of the hypothalamic and pituitary region, no abnormalities were seen.

From age 4 onward, GH treatment was initiated at a dose of 1 mg/m^2 per day (Fig. 1). At start of GH treatment, her height was 90.6 cm (–3.42 SD score), BMI 14.3 kg/m^2 (–1.02 SD

score), sitting height/height ratio 0.60 (0.00 SD score), and head circumference 48.6 cm (–0.95 SD score). Her serum total IGF-I level was 34 ng/ml (–2.78 SD score), IGFBP-3 level 1.35 mg/liter (–1.33 SD score), and her bone age was 1 yr behind. After 1 yr of GH treatment, height had increased by 1.02 SD and head circumference by 0.58 SD. Her serum total IGF-I level was 197 ng/ml (1.51 SD score), and IGFBP-3 level was 2.62 mg/liter (0.92 SD score). After 4 yr of GH treatment, she had an increase in height of +1.65 SD score and an increase in IGF-I level of +5.75 SD. Currently, at age 8.3, her height is 123.3 cm (–1.68 SD score). Cardiac ultrasound showed an undulating shape of the left ventricular wall which could not be further specified.

Dysmorphological examination showed hypertelorism, hypocanthal folds, medial flaring of the eyebrows, broad nasal bridge, and thick hair (Fig. 2). She had a triangular face, large mouth, short and pigmented upper lip, and low-placed, posterior rotated ears. Her abdomen was protruded. She had bilateral clinodactyly, short fingers, and proximally placed, broad thumbs. She had a bilateral sandal gap and broad forefeet with pes planus. There was hyperlaxity of the joints, especially of the elbows and knees (Beighton score 7/9). Dimples were present at the right flank and in the lumbar region. These dysmorphic features were absent in her parents. She goes to a regular primary school. Testing of her verbal and performance intelligence quotient showed an average intellectual level, with a score of 93 and 121 points, respectively. These scores indicate a disharmonic intellectual profile although being in the normal range. Her two brothers were born after 40 weeks gestation, had a normal birth size, and grew normally (data not shown).

Genetic analysis

Patient A had a deletion of all three probes in the *IGF1R* gene on the MLPA growth kit. Confirmation of this result with the MRC Holland P217 MLPA kit showed a heterozygous deletion of all probes in the *IGF1R* gene (exon 1-21), including the two telomeric control probes which were located at 2.0 and 2.8 Mb downstream of the *IGF1R* gene. Her parents and both brothers did not carry this deletion. SNP array analysis showed a terminal deletion from location rs12912857, the first deleted SNP probe located at 95.883.282 bp until rs7169385, the last deleted SNP probe located at 100.192.115 bp (Ensembl release 49), comprising a 4.5 Mb region on chromosome 15 (Fig. 3). No other pathogenic CNV was observed with SNP array analysis. Additional sequence analysis of the coding region of the *IGF1* gene, to exclude a mutation as a cause of the low IGF-I level revealed no mutation.

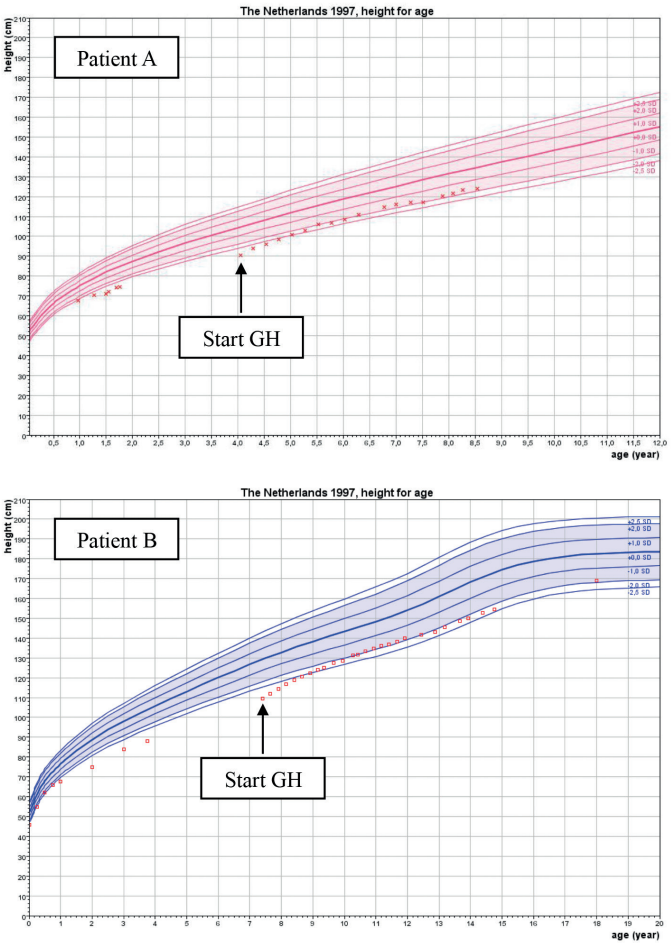

Figure 1 Growth charts of the two children with a *IGF1R* deletion.

Patient B

Clinical description

Patient B was a boy born spontaneously after 40 weeks of gestation as the first child of nonconsanguineous parents. A decrease in fetal growth was observed during the last trimester by ultrasound. His birth weight was 2600 g (–1.91 SD score) and birth length 47 cm (–2.21 SD score). The height of his father and mother was 187.6 cm (0.84 SD score) and 165.2 cm (–0.50 SD score), respectively. His secular trend-corrected target height was 187.4 cm (0.49 SD score). Bone age was 2 yr delayed at the chronological age of 4 yr. At age 2,

a rotation deformity of both tibial bones was observed, and tympanostomy tubes were implanted because of recurrent ear infections. At 3.0 yr of age, his height was 84.0 cm (–3.84 SD score), and his weight was 11.2 kg (–2.65 SD score). A clonidine stimulation test was performed at age 5 with a maximal GH response of 69.7 mU/liter. Cardiovascular, respiratory, and abdominal examinations were all normal.

From age 7 onward, he started GH treatment in a dose of 1 mg/m^2 per day (Fig. 1). At start of GH treatment, his height was 109.4 cm (–3.57 SD score), BMI 16.5 kg/m^2 (0.49 SD score), sitting height/height ratio of 0.55 (–1.86 SD score), and head circumference 51.3 cm (–0.53 SD score). IGF-I was 208 ng/ml (1.25 SD score) and IGFBP-3 level 2.99 mg/liter (1.24 SD score). After 1 yr of GH treatment, his height had increased by 0.83 SD and head circumference by 0.29 SD. His serum total IGF-I level was 356 ng/ml (2.28 SD score), and IGFBP-3 level was 1.99 mg/liter (–0.40 SD score). After 4 yr of GH treatment, he had an increase in height of +1.69 SD and an increase in IGF-I of +0.39 SD. Currently, at age 17, his height is 168.9 cm (–1.89 SD score).

From age 11 onward, he used methylfenidate (10/5/5 mg) because of attention-deficit hyperactivity disorder. After primary school, he started a secondary school for children with hearing and speech difficulties. Currently, he receives training for becoming a baker. Cardiac evaluation showed no abnormalities.

Dysmorphological examination showed hypertelorism, upward slant, thin upper lip, bilateral extra nipple, proximal implanted thumbs, and broad feet (Fig. 2). These dysmorphic features were absent in his parents and brother. His brother has a height within the normal range.

Genetic analysis

MLPA analysis showed a deletion of two of three *IGF1R* probes (exons 8 and 18). The MRC Holland P217 MLPA kit showed a heterozygous deletion of exons 3-21 of the *IGF1R* gene comprising also both telomeric control probes located at 2.0 and 2.8 Mb downstream of the *IGF1R* gene. His parents and brother did not carry the deletion. SNP array analysis showed a terminal deletion of 3.1 Mb on chromosome 15, ranging from rs11857366, first deleted SNP probe at 97.081.324 bp until rs7169385, last deleted SNP probe at 100.192.115 bp, containing 282 SNP probes (Fig. 3). No other pathogenic CNVs were observed in the SNP array analysis.

Patient A

Patient B

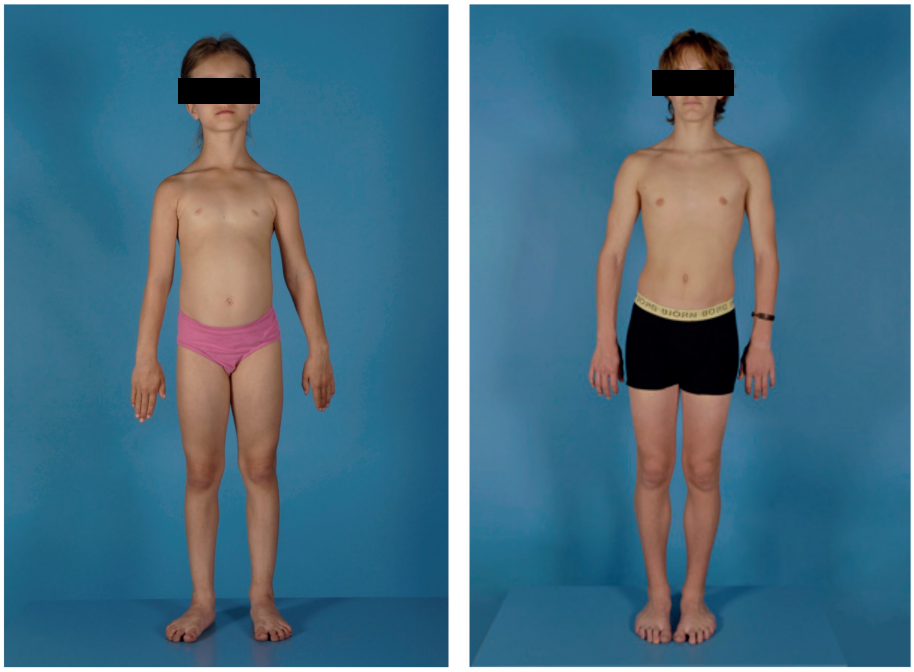

Figure 2 Physical characteristics of Patient A, at age 9, and of Patient B at 18 yr of age.

Functional analysis

In previous reports it was shown that a patient with a heterozygous *IGF1R* mutation (E1050K) demonstrated a decreased activation of IGF1R intracellular signaling upon challenging with IGF-I (26), and in a patient with a heterozygous *IGF1R* deletion the activation of the IGF1R and PKB/Akt tended to be lower, but this did not reach significance (25).

In our two patients Western blots from cultured skin fibroblasts demonstrated a slightly lower autophosphorylation of the IGF1R in patient B in comparison with controls (Fig. 4A), but on average autophosphorylation of the IGF1R in the two patients was similar to that in controls (Fig. 4B). There was a significantly reduced activation of PKB/Akt upon a challenge with 10 ng/ml IGF-I for 10 minutes compared with healthy controls. The levels of total PKB/Akt and IGF1R protein expression were similar to controls, although total IGF1R protein expression appeared lower in patient B (Fig. 4A). Stimulation of the fibroblasts with a dose range of IGF-I for 10 minutes (Fig. 4, C and D) demonstrated reduced phosphorylation of PKB/Akt in the patients compared with controls. Total PKB/Akt and IGF1R autophosporylation were similar in the patients and controls. Total IGF1R protein expression tended to be lower in the patients compared with controls.

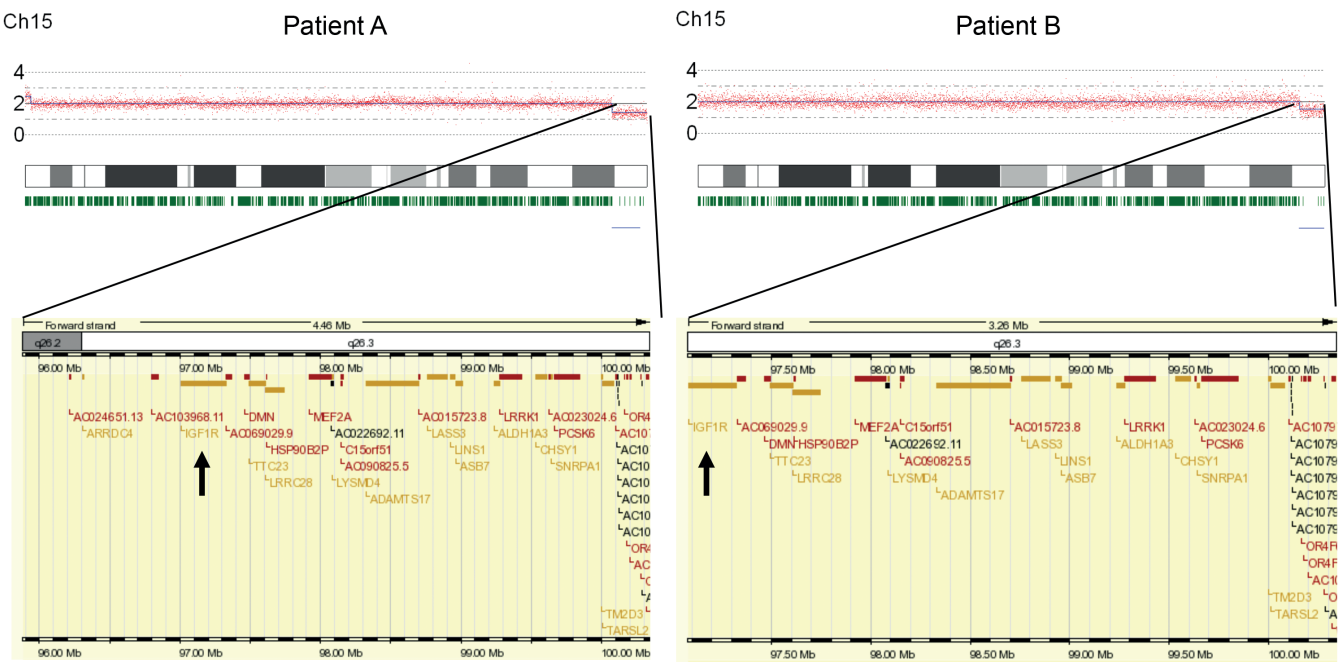

Figure 3 Array analysis of chromosome 15 of Patient A showed a heterozygous deletion of 15q26.2 to 15qter, from rs12912857 to rs7169385, comprising the whole *IGF1R* gene. Patient B showed a heterozygous deletion of 15q26.3 to 15qter, from rs11857366 to rs7169385. This deletion starts within intron 2 of the *IGF1R* gene.

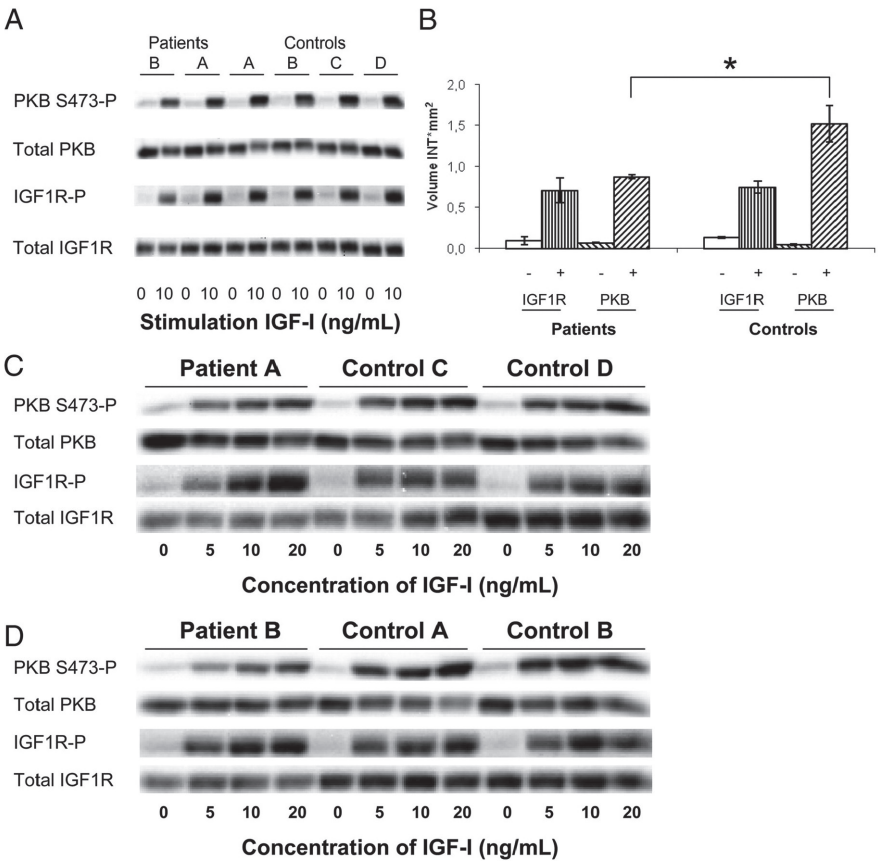

Figure 4 Functional analysis. A, Dermal fibroblasts of the patients and four age- and sex-matched controls were stimulated with 10 ng/ml IGF-I for 10 minutes. Protein lysates were collected, and 25 µg of protein was used for Western blotting with phosphospecific IGF1R and PKB/Akt (Ser^{473}) antibodies and total IGF1R and PKB/Akt antibodies. B, Densitometric quantification of the Western blot shown in A. Data are expressed as a ratio of phosphospecific IGF1R or PKB/Akt and total IGF1R or PKB/Akt, respectively. The activation of PKB/Akt was significantly lower in the patients compared with the controls. Autophosporylation and total protein expression of the IGF1R tended to be lower in patient B, although this did not reach significance. C and D, Activation of PKB/Akt by phosphorylation on Ser^{473} as well as autophosphorylation of the IGF1R in fibroblasts of Patients A and B and four age- and sex-matched controls were determined by Western blotting after a challenge with a dose range of IGF-I. Protein lysates were collected after a 10-minutes stimulation. Total PKB/Akt was used to check for equal loading.

Discussion

This study shows that *IGF1R* haploinsufficiency was detected in 2 of 100 short SGA children and that no other copy number variants were found in 18 other growth-associated genes. Because dominant *de novo* deletions are a well-recognized sign of pathogenicity, it is very likely that the observed deletions explain the short stature of both patients. In addition, according to the database of genomic variants (http://projects.tcag.ca/variation/) no deletion of the *IGF1R* gene region has been reported yet in the general population. This is further supported by previous clinical studies reporting subjects with *IGF1R* point mutations and deletions (13-15, 17-24, 26), including functional analysis of patients with a missense mutation and one with a complete deletion of the *IGF1R* gene (16, 25, 26). *In vitro* functional analysis of our patients showed almost similar IGF1R autophosporylation, a tendency toward reduced total IGF1R protein expression, and reduced intracellular PKB/Akt activation compared with healthy controls. Our results are in line with the findings we reported earlier on another patient with *IGF1R* haploinsufficiency (25) and suggest that this condition is characterized by a lower number of IGF1 receptors on the cell surface, resulting in less signal transduction. In the family with a missense mutation in the intracellular kinase domain of the *IGF1R*, we observed a stronger decrease in activation of downstream signaling (26). Previously we have hypothesized (25) that the discrepancy between the results in *IGF1R* deletions and mutations *in vitro* may be explained by a dominant-negative effect of the mutation, which would decrease the number of fully functional receptors to 25%, whereas haploinsufficiency would theoretically lead to a reduction of 50%.

In our fibroblast model, the expression of the IGF1R tended to be lower in the patient's cells but was not reduced with 50%. Downstream signaling as assessed by measuring phosphorylation of PKB/Akt was, however, reduced in the patient's fibroblasts. These observations are in line with previously reported results obtained in fibroblasts of a patient with a terminal 15q deletion, which also demonstrated a nonsignificant reduction in IGF1R expression but a much stronger and significant reduction in activation of downstream signaling (25). However, the observation that the effect on growth is similar in patients with *IGF1R* mutations and deletions suggests a similar biological effect on growth. Thus, the consequences of *IGF1R* haploinsufficiency may be cell type-dependent, with possibly a relatively strong effect in growth plate chondrocytes, which are responsible for longitudinal growth, whereas the differences in the fibroblast model are less pronounced.

Both children responded to GH treatment, increasing their height by 1.02 SD and 0.83 SD, respectively, after 1 yr of GH treatment. This growth response was comparable with the mean 1-yr growth response in short SGA children (approximately 0.8 SD in prepubertal short SGA children on the same GH dosage) (40).

This positive growth response can be explained by a combination of the direct effect of GH on the epiphyseal chondrocytes which is independent of the biological actions of serum IGF (41), and of elevated serum IGF-I levels, which may partially overcome the diminished sensitivity.

When previous case reports of children with an *IGF1R* deletion or mutation were reviewed, only six other children had received GH treatment (Table 1). The children who received a GH dose of 1 mg/m^2 per day increased their height with ~1.0 SD per year of GH treatment. Their serum IGF-I level increased with ~1.0 SD except for Patient A (discussed in the next paragraph). The other children received a higher GH dose but showed a similar variable response: a similar response in two of them, and no apparent response in two other patients (13, 16). Thus, six of eight children showed a beneficial effect of GH treatment.

An unexpected finding in Patient A was the low serum IGF-I level, in contrast to a high or normal serum IGF-I level usually observed in patients with an *IGF1R* mutation or deletion. Because no abnormalities in the *IGF1* gene were found, we hypothesize that this may be explained by a partial GH deficiency. The marginal response of GH in the provocation test and the observation that IGF-I and IGFBP-3 strongly rose by +4.3 SD and +2.2 SD after 1 yr of GH treatment support our hypothesis. In the child with an *IGF1R* missense mutation who presented with severe failure to thrive, serum IGF-I was initially not elevated (+1.3 SD score; van der Kamp, H.J., personal communication) (26), but after realimentation by a gastrostoma her IGF-I levels increased up to +2.9 SD score.

The children we identified had a relatively mild phenotype compared with the other patients with a terminal *IGF1R* deletion who had lung hypoplasia, atrial and/or ventricular septal defects, hypoplastic left atrial or ventricular heart, dextrocardia, and diaphragmatic hernia (Table 2) (19-25). We believe that these clinical signs are primarily linked to other genes in the area, as in children with an *IGF1R* mutation a much smaller number of additional characteristics has been observed. Poot *et al*. indicated *IGF1R* flanking genes that might be responsible for several characteristics of the variation in the phenotypes of children with an *IGF1R* deletion (20).

Both of our patients had hearing problems, showed mild developmental delay, and had proximal implanted thumbs. Hearing problems and developmental delay have been described in previous case reports of mutations in the *IGF1* and *IGF1R* gene and might also be caused by their tissue-specific expression in the auditory and central nervous system (42, 43).

To our knowledge, there are no diagnostic criteria for children with an *IGF1R* mutation or deletion. In Table 3 we have summarized clinical features of children with an *IGF1R* mutation or deletion according to their organ system. We identified major criteria that are predominantly related to growth restriction and minor criteria that are based on dysmorphic

features and signs of joint hypermobility. Based on the information summarized in Table 2, we propose the combinations of major and minor criteria which indicate a high likelihood of an *IGF1R* mutation or deletion. Interestingly, joint hypermobility is related to the fibrillin (*FBN1*) gene, which is located upstream of the *IGF1R* gene on 15q21. *FBN1* gene mutations are responsible for Marfan's syndrome, which is associated with tall stature. Because both clinical syndromes display joint hypermobility, we speculate that the *IGF1R* and *FBN1* genes together might play a role in variations in height and hypermobility in short SGA patients.

The remaining 98 short SGA children had no CNVs in the 18 growth-associated genes we selected, but we did not exclude mutations in these genes. Future studies are needed to investigate whether deletions or mutations in other genes, or combinations of several gene defects, are associated with the short SGA phenotype.

In summary, this study has shown that *IGF1R* haploinsufficiency was present in 2 of 100 short SGA children. This study illustrates that the combination of a small birth size, short stature, small head size, relatively high IGF-I level, developmental delay, and micrognathia is suggestive for children with an *IGF1R* deletion. Because GH therapy leads to a moderate catch-up growth of ~1.0 SD in the first year, we recommend that the SGA children with a persistent short stature are tested for an *IGF1R* deletion, particularly if some of the major or minor criteria are present. The MLPA has shown to be a valuable tool in rapidly identifying these relatively large deletions in short SGA children.

Acknowledgments

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Table 1 Literature overview of short stature and/or SGA patients with a heterozygous *IGF1R* mutation or terminal chromosome 15q deletion who received GH treatment

			Abuzzahab	Abuzzahab	Inagaki	Walenkamp	Siebler	Walenkamp
	Patient A	Patient B	(13)	(13, 44)	(16)	(26, 45)	(23)	(25)
Molecular characterization	del(15)(q26.2)	del(15)(q26.3)	*IGF1R* R108Q/K115N	*IGF1R* R59X	*IGF1R* R481Q	*IGF1R* E1050K	del(15)(q26.1)	del(15)(q26.2)
Birth								
GA (wk)	40	40	38	At term	41	39	39	39
Length (SD score)	–2.21	–2.74	NA	–5.89	–5.63	–2.94	NA	–1.95
Weight (SD score)	–1.28	–1.91	–4.33	–3.22	–3.06	–6.13	–3.05	–2.93
At start GH								
Age (yr)	4.0	7.0	4.5	6.4	13.6	1.6	3.5	5.3
Height (SD score)	–3.42	–3.57	~ –4.0[a]	–2.51	–5.0	–2.3	~ –5.0[a]	–3.5
IGF-I (SD score)	–2.78	1.25	"Normal"[b]	+1.20	↑↑[c]	2.1	NA[b]	2.5
GH dose (mg/m² per day)	1.0	1.0	1.6	0.9	2.1	1.4	1.6[d]	1.0
After 1 yr GH								
Height (SD score)	–2.40	–2.74	~ –4.0[a]	–1.96	–5.0[e]	–1.5[e]	~ –4.0[a]	–2.0[f]
IGF-I (SD score)	1.51	2.28	↑↑[b]	+1.77	NA	3.6	NA	3.5[f]
Change ht (SD)	*1.02*	*0.83*	*0*	*0.55*	*0*	*0.8*	*~1.0*	*1.5*
Change IGF-I (SD)	*3.53*	*1.03*	↑↑[b]	*0.57*	*NA*	*1.5*	*NA*	*1.0*

All birth weight and birth lengths are presented as standard deviation scores according to the Usher growth charts. NA, Not available.
[a] Estimated from the growth chart.
[b] No SD score is provided.
[c] IGF-I SD score not available, but level was above the normal range and unchanged after GH treatment.
[d] Three different GH doses were used, of which this dose was maintained for the longest period (age 8–10 yr).
[e] Measured after 6 months of GH treatment.
[f] Measurements are provided when height and IGF-I level stabilized.

Table 2 Overview of phenotypic characteristics in short SGA patients with a heterozygous *IGF1R* mutation or terminal chromosome 15q deletion

Genotype		Phenotype										
Mutation or deletion	Study	Birth weight ≤ –2.0 SDS	Birth length ≤ –2.0 SDS	Birth HC ≤ –2.0 SDS and/or microcephaly	Postnatal height ≤–2.0 SDS	IGF-I level > +1.0 SDS	Developmental delay and/or MR	Micrognathia/ triangular facies	Proximal placed dig I	Cardiac disorders	Total	Extra diagnoses
15q26.2→qter	Patient A	–	+	–	+	–	+	+	+	±	6	Clubfeet, hip dysplasia, aberrant left ventricular wall
15q26.3→qter	Patient B	–	+	–	+	+	+	–	+	–	5	
IGF1R exon 2	R108Q/ K115N (13)	+	NA	–	+	+	+	–	–	–	4	
IGF1R exon 2	R59X (13)	+	+	+	+	+	+	–	–	–	6	
IGF1R exon 7	R481Q (16)	+	+	NA	+	+	–	+	–	–	5	
IGF1R exon 11	R709Q (17)	–	–	NA	+	+	+	–	–	–	3	
IGF1R exon 11	R709Q (17)	+	NA	NA	+	–	NA	NA	NA	–	2	
IGF1R exon 16	E1050K (26)	+	–	+	+	+	–	–	–	–	4	
IGF1R exon 16	E1050K (26)	+	+	+	+	+	–	+	–	–	6	Oligohydramnios
15q26.1→qter	(14)	+	+	+	NA	NA	NA	–	–	+	4	Right-sided aorta, VSD, tethered spinal cord, death at 5 months

15q26.1→qter	(15)	–	NA	NA	NA	NA	NA	NA	+	+	2	Diaphragmatic hernia, bicuspid aortic valve, aortic coarctation
15q26.1→qter	(23)	+	+	NA	[a]	[a]	[a]	+	–	+	4	Lung hypoplasia, diaphragmatic hernia
15q26.1→qter	(23)	+	NA	NA	+	NA	+	+	+	–	5	Cubitus valgus
15q26.1→qter	(21)	+	+	+	+	NA	+	+	–	–	6	Oligohydramnios, hypoplastic lungs
15q26.1	(24)	+	+	+	+	NA	+	+	–	+	7	Gastroesophagal reflux
15q26.2→qter	(19)	+	+	+	+	+	+	+	+	–	8	Seizures, gastroesophagal reflux
15q26.2→qter	(20)	+	+	+	+	–	+	+	–	+	7	Oculocutaneous albinism, subluxation radial heads
15q26.2→qter	(25)	+	–	+	+	+	–	–	–	–	4	Severe myopia
15q26.2	(22)	+	NA	+	+	NA	+	+	–	–	5	Clubfeet, genu recurvatum
15q26.2	(18)	+	NA	–	+	NA	–	+	–	+	4	
Total (number)		16	11	10	17	9	11	11	5	7		
Total (%)		80	79	71	100	75	69	61	26	35		

IGF1R is located at 15q25-26 (OMIM *147370). All birth weight and lengths were calculated as standard deviation scores (28).
HC, Head circumference ; MR, mental retardation; NA, not available.
[a] Died directly postpartum.

Table 3 Suggested clinical indicators for a heterozygous *IGF1R* mutation or terminal chromosome 15q deletion based on published cases

Anatomical system	Major	Minor
1. Skeletal	Height ≤ –2.00[a] Birth length ≤ –2.00 Birth weight ≤ –2.00 Head circumference ≤ –2.00 IGF-I level SDS > 1.00	Triangular face/micrognathia Proximal placed thumb
2. Nervous system	Developmental delay Mental retardation	Tethered spinal cord Seizures
3. Cardiac	Right sided aorta, bicuspid aortic valve, aortic coarctation VSD Aberrant left ventricular wall	
4. Gastro-intestinal		Diaphragmatic hernia Gastroesophagal reflux
5. Connective tissue		Clubfeet Hip dysplasia Cubitus valgus Genu recurvatum Subluxation radial heads Severe myopia
6. Skin		Oculocutaneous albinism

[a] SD score. VSD, Ventricular septal defect.

chapter
5

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Supplemental Data

Table 1 Probe sequences of the "in house" MLPA kit.

Gene name	exon	Upstream (U)/ Downstream (D)*	Sequence	Total fragment length (bp)	Label
IGF1R	2	U	GATGTGTGAGAAGACCACCATCAACA	92	HEX
		D	ATGAGTACAACTACCGCTGCTGGACCACAA		
	8	U	CTACATGGGCTGAAGCCCTGGACTCAG	94	HEX
		D	TACGCCGTTTACGTCAAGGCTGTGACCCTCA		
	18	U	CAGTCCTAGCACCTCCAAGCCTGAGCA	90	HEX
		D	AGATGATTCAGATGGCCGGAGAGATTG		
IGF1	3	U	GAGTGCTGCTTCCGGAGCTGTGATCT	88	HEX
		D	AAGGAGGCTGGAGATGTATTGCGCAC		
	4	U	GTACATTTGAAGAACGCAAGTAGAGGGAGT	98	HEX
		D	GCAGGAAACAAGAACTACAGGATGTAGGAAGA		
GH1	4	U	CAACAGCCTGGTGTACGGCGCCTCTGACAGCAACGTCTATGA	116	FAM
		D	CCTCCTAAAGGACCTAGAGGAAGGCATCCAAA		
GHR	4	U	GCCACTGGACAGATGAGGTTCATCATGGTACAAAGAACC	112	FAM
		D	TAGGACCCATACAGCTGTTCTATACCAGAAG		
	10	U	GGTACTTTGGTGGCCACATAAGCCATTATTCACTAGTATGA	110	HEX
		D	CTAGTTGTGTCTGGCAGTTTATATTTAACTCTC		
SHOX	3	U	GGATTTATGAATGCAAAGAGAAGCGCGAGGACG	106	HEX
		D	TGAAGTCGGAGGACGAGGACGGGCAGACCAAGCTGAA		
	8	U	CCCTGAGTTTCTCTGGTGACGCCCTCATTCTCCTAA	108	HEX
		D	CGTTCAATAATCTCAATGTTGAGTTGCAGCAACAGA		
IGFBP1	1	U	CAAACTTATTTTGAACACTCAGCTCCTAGCGTGCG	102	HEX
		D	GCGCTGCCAATCATTAACCTCCTGGTGCAAG		
	4	U	GTGAGACATCCATGGATGGAGAGGCGGGACTCTGCT	104	HEX
		D	GGTGCGTCTACCCTTGGAATGGGAAGAGGATC		
IGFBP2	2	U	CACTCAGAAGGAGGCCTGGTGGAGAACCACGT	100	FAM
		D	GGACAGCACCATGAACATGTTGGGCG		
	4	U	CAAGATGTCTCTGAACGGGCAGCGTGGGGAGTGCT	108	FAM
		D	GGTGTGTGAACCCCAACACCGGGAAGCTGAT		
IGFBP3	2	U	GCTAAAGACAGCCAGCGCTACAAAGTTGACTACG	100	HEX
		D	AGTCTCAGAGCACAGATACCCAGAACTTCT		
	5	U	CTGGAGCTCACAGCCTTCTGTGGTGTCATT	96	HEX
		D	TCTGAAACAAGGGCGTGGATCCCTCAACCA		
IGFBP4	3	U	GACCTCTACATCATCCCCATCCC	84	HEX
		D	CAACTGCGACCGCAACGGCAACTTC		

	4	U	CAGAGTCAGAGGAGAAGAGACATGTACCTTGACCATCGTCC	114	HEX
		D	TTCCTCTCAAGCTAGCCAGAGGGTGGGAGCCTAAGGA		
IGFBP5	2	U	GATCTTCCGGCCCAAACACACCCGCATCTCCGAGCTGAAGGCTGAAG	120	HEX
		D	CAGTGAAGAAGGACCGCAGAAAGAAGCTGACCCAGTC		
	3	U	GTGCTGTGTACCTGCCCAATTGT	86	HEX
		D	GACCGCAAAGGATTCTACAAGAGAAAG		
IGFBP6	2	U	CCACAGGATGTGAACCGCAGAGA	92	FAM
		D	CCAACAGAGGAATCCAGGCACCTCTAC		
	3	U	CAAACACTCTACGTGCCCAATTGTG	90	FAM
		D	ACCATCGAGGCTTCTACCGGAAG		
ALS	2	U	GAACCTCTCTGGGAACTGTCTCCGG	88	FAM
		D	AACCTTCCGGAGCAGGTGTTC		
STAT5B	2	U	GATACAAGCTCAGCAGCTCCAAGGAGAAGCCCT	102	FAM
		D	TCATCAGATGCAAGCGTTATATGGCCA		
	14	U	GAGAATTTACCAGGACGGAATTACACTTTCTG	104	FAM
		D	GCAATGGTTTGACGGTGTGATGGAAGTGTT		
SOCS3	2	U	CTTCAGCATCTCTGTCGGAAGACCGTCAACG	98	FAM
		D	GCCACCTGGACTCCTATGAGAAAGT		
GRB10	3	U	GATGTGGACCTGGAAGCCCTGGTGAACGAT	96	FAM
		D	ATGAATGCATCCCTGGAGAGCCTG		
	10	U	CAGAGGACGAGCAAACCAGGACGTGCT	94	FAM
		D	GGATGACAGCGTTCAGACTCCTCAA		
NSD1	3	U	GAGATCTCATCTGGGCAAAATTCAAGAGACGCCCATGGTGGCCCT	116	HEX
		D	GCAGGATTTGTTCTGATCCGTTGATTAACACACAT		
	13	U	CACTGTTATGCAGAACAAGGGCTTCCGGTGCTCCCTCCACATCTGTA	118	HEX
		D	TAACCTGTCATGCTGCTAATCCAGCCAATGTTTCT		
	20	U	CTCGGTTCATGAATCATTGCTGCCAGCCCAACTGTGAAACACAGAAG	120	FAM
		D	TGGTCTGTGAATGGAGATACCCGTGTAGGCC		
IGF2	2	U	GAATCCCAATGGGGAAGTCGAT	86	FAM
(11p15)		D	GCTGGTGCTTCTCACCTTCTTG		
	4	U	CATCCTGCAGCCTCCTCCTGACCACGGACGTTTCCATCAGGTT	114	FAM
		D	CCATCCCGAAAATCTCTCGGTTCCACGTC		
SOCS2	1	U	GCCGCGGCCTCAACTAAAAGTGGCCATTGACCTTTCAA	110	FAM
		D	GCTTTCGAGCAGTGATGCAATAGAATAGAA		

* The Upstream hybridizing sequence (U) was extended with a labeled primer 5'-GGGTTCCCTAAGGGTTGGA-3'; the downstream hybridizing sequence was extended with an unlabeled primer 5'-GTGCCAGCAAGATCCAATCTAGA-3'.