



Universiteit
Leiden
The Netherlands

Genetic causes of growth disorders

Duyvenvoorde, H.A. van

Citation

Duyvenvoorde, H. A. van. (2013, June 25). *Genetic causes of growth disorders*. Retrieved from <https://hdl.handle.net/1887/21013>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/21013>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/21013> holds various files of this Leiden University dissertation.

Author: Duyvenvoorde, Hermine van

Title: Genetic causes of growth disorders

Issue Date: 2013-06-25

5



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

Wietske A. Ester¹, Hermine A. van Duyvenvoorde^{2,3,4},
Caroline C. de Wit², Alexander J. Broekman⁴, Claudia A.L. Ruivenkamp⁴,
Lutgarde C.P. Govaerts⁵, Jan M. Wit², Anita C.S. Hokken-Koelega¹, and
Monique Losekoot⁴

¹Department of Pediatrics, Subdivision of Endocrinology, Erasmus Medical Center Sophia Children's Hospital, Rotterdam, The Netherlands

²Department of Pediatrics, ³Department of Endocrinology and Metabolic Diseases,

⁴Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands

⁵Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands

The Journal of Clinical Endocrinology & Metabolism 2009;94(12):4717–4727

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/m² 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 meters²) 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 (Ser⁴⁷³), total PKB/Akt, total IGF1R β (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/m² per day (Fig. 1). At start of GH treatment, her height was 90.6 cm (−3.42 SD score), BMI 14.3 kg/m² (−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.

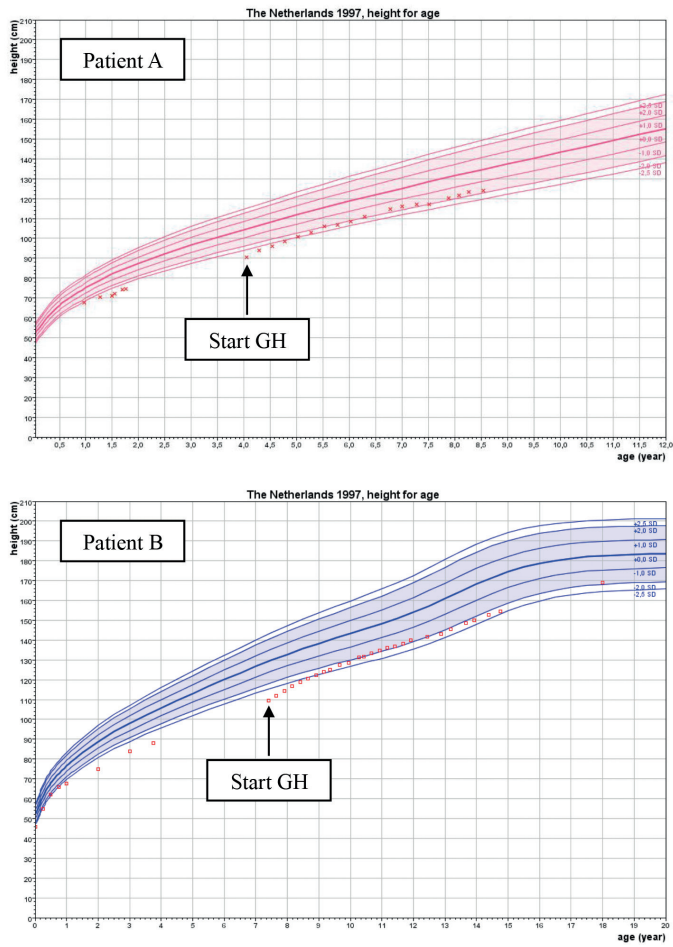


Figure 1 Growth charts of the two children with a *IGF1R* deletion.

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/m² per day (Fig. 1). At start of GH treatment, his height was 109.4 cm (-3.57 SD score), BMI 16.5 kg/m² (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 methylphenidate (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 autophosphorylation were similar in the patients and controls. Total IGF1R protein expression tended to be lower in the patients compared with controls.

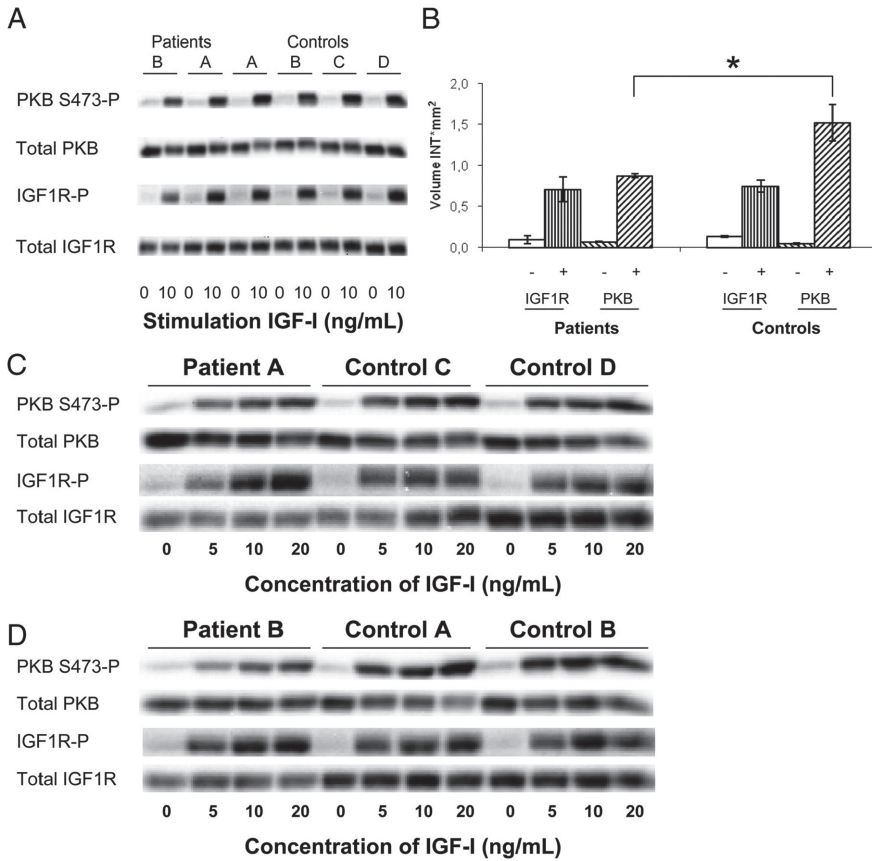


Figure 4 Functional analysis. A, Dermal fibroblasts of the patients and four age- and sex-matched controls were stimulated with 10 ng/ml IGF-I for 10 minutes. Protein lysates were collected, and 25 µg of protein was used for Western blotting with phosphospecific IGF1R and PKB/Akt (Ser⁴⁷³) antibodies 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. Autophosphorylation 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 Ser⁴⁷³ 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 autophosphorylation, 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/m² 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

We thank the children and their families who took part in this study, and we thank Ms. J. van Nieuwkastele, research nurse, and Ms. J. Vergeer, technician, for help with data collection and technical assistance. We acknowledge Drs. M. van Dijk, S. de Kort, N. Arends, V. Boonstra, and R. Willemsen for data collection. We thank Ms. A. Gijsbers for help with the SNP array and Ms. E. Peters and Dr. M. Karperien for assistance in the functional studies. We acknowledge Dr. M.J.E. Walenkamp for her careful reading and comments on the manuscript.

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

	Patient A	Patient B	Abuzzahab	Abuzzahab	Abuzzahab	Inagaki	Walenkamp	Siebler	Walenkamp
Molecular characterization	de(15)(q26.2)	de(15)(q26.3)	(13)	<i>IGF1R</i> R108Q/K115N	(13,44)	(16)	(26, 45)	(23)	(25)
Birth									
GA (wk)	40	40	38	At term	41	39	39	39	39
Length (SD score)	-2.21	-2.74	NA	-5.89	-5.63	NA	-2.94	NA	-1.95
Weight (SD score)	-1.28	-1.91	-4.33	-3.22	-3.06	-3.05	-6.13	-3.05	-2.93
At start GH									
Age (yr)	4.0	7.0	4.5	6.4	13.6	3.5	1.6	3.5	5.3
Height (SD score)	-3.42	-3.57	~ -4.0 ^a	-2.51	-5.0	~ -5.0 ^a	-2.3	~ -5.0 ^a	-3.5
IGF-I (SD score)	-2.78	1.25	"Normal" ^b	+1.20	↑↑	NA ^b	2.1	NA ^b	2.5
GH dose (mg/m ² per day)	1.0	1.0	1.6	0.9	2.1	1.6 ^d	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	~ -4.0 ^a	-1.5 ^e	~ -4.0 ^a	-2.0 ^f
IGF-I (SD score)	1.51	2.28	↑↑	+1.77	NA	NA	3.6	NA	3.5 ^f
Change ht (SD)	1.02	0.83	0	0.55	0	~1.0	0.8	~1.0	1.5
Change IGF-I (SD)	3.53	1.03	↑↑	0.57	NA	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										Extra diagnoses
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	
15q26.2→qter	Patient A	-	+	-	+	-	+	+	+	±	6	Clubfeet, hip dysplasia, aberrant left ventricular wall
15q26.3→qter	Patient B	-	+	-	+	+	+	-	+	-	5	
<i>IGF1R</i> exon 2	R108Q/ K115N (13)	+	NA	-	+	+	+	-	-	-	4	
<i>IGF1R</i> exon 2	R59X (13)	+	+	+	+	+	+	-	-	-	6	
<i>IGF1R</i> exon 7	R487Q (16)	+	+	NA	+	+	-	+	-	-	5	
<i>IGF1R</i> exon 11	R709Q (17)	-	-	NA	+	+	+	-	-	-	3	
<i>IGF1R</i> exon 11	R709Q (17)	+	NA	NA	+	-	NA	NA	NA	-	2	
<i>IGF1R</i> exon 16	E1050K (26)	+	-	+	+	+	-	-	-	-	4	
<i>IGF1R</i> 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	NA	NA	NA	NA	+	+	2	Diaphragmatic hernia, bicuspid aortic valve, aortic coarctation
15q26.1→qter	(23)	+	+	NA	a	a	a	a	a	a	a	+	-	+	4	Lung hypoplasia, diaphragmatic hernia
15q26.1→qter	(23)	+	NA	NA	+	+	+	+	+	+	+	+	+	-	5	Cubitus valgus
15q26.1→qter	(21)	+	+	+	+	+	+	+	+	+	+	+	-	-	6	Oligohydramnios, hypoplastic lungs
15q26.1	(24)	+	+	+	+	+	+	+	+	+	+	+	-	+	7	Gastroesophageal reflux
15q26.2→qter	(19)	+	+	+	+	+	+	+	+	+	+	+	+	-	8	Seizures, gastroesophageal reflux
15q26.2→qter	(20)	+	+	+	+	+	+	+	+	+	+	+	-	+	7	Oculocutaneous albinism, subluxation radial heads
15q26.2→qter	(25)	+	-	+	+	+	+	+	+	+	+	-	-	-	4	Severe myopia
15q26.2	(22)	+	NA	+	+	+	+	+	+	+	+	+	-	-	5	Clubfeet, genu recurvatum
15q26.2	(18)	+	NA	-	+	+	+	+	+	+	+	+	-	+	4	
Total (number)		16	11	10	17	9	11	11	11	11	11	11	5	7		
Total (%)		80	79	71	100	75	69	61	61	61	61	26	35			

IGFR 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 $\leq -2.00^a$ 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 Gastroesophageal 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

Reference List

1. **Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL** 1995 Children born small for gestational age: do they catch up? *Pediatr Res* 38:267-271
2. **Arends NJ, Boonstra VH, Duivenvoorden HJ, Hofman PL, Cutfield WS, Hokken-Koelega AC** 2005 Reduced insulin sensitivity and the presence of cardiovascular risk factors in short prepubertal children born small for gestational age (SGA). *Clin Endocrinol (Oxf)* 62:44-50
3. **Arends NJ, Boonstra VH, Hokken-Koelega AC** 2004 Head circumference and body proportions before and during growth hormone treatment in short children who were born small for gestational age. *Pediatrics* 114:683-690
4. **Leger J, Garel C, Fjellestad-Paulsen A, Hassan M, Czernichow P** 1998 Human growth hormone treatment of short-stature children born small for gestational age: effect on muscle and adipose tissue mass during a 3-year treatment period and after 1 year's withdrawal. *J Clin Endocrinol Metab* 83:3512-3516
5. **Boonstra VH, Arends NJ, Stijnen T, Blum WF, Akkerman O, Hokken-Koelega AC** 2006 Food intake of children with short stature born small for gestational age before and during a randomized GH trial. *Horm Res* 65:23-30
6. **Lundgren EM, Cnattingius S, Jonsson B, Tuvemo T** 2001 Intellectual and psychological performance in males born small for gestational age with and without catch-up growth. *Pediatr Res* 50:91-96
7. **Brandt I, Sticker EJ, Lentze MJ** 2003 Catch-up growth of head circumference of very low birth weight, small for gestational age preterm infants and mental development to adulthood. *J Pediatr* 142:463-468
8. **Gale CR, O'Callaghan FJ, Godfrey KM, Law CM, Martyn CN** 2004 Critical periods of brain growth and cognitive function in children. *Brain* 127:321-329
9. **van Pareren YK, Duivenvoorden HJ, Slijper FS, Koot HM, Hokken-Koelega AC** 2004 Intelligence and psychosocial functioning during long-term growth hormone therapy in children born small for gestational age. *J Clin Endocrinol Metab* 89:5295-5302

10. Woods KA, Camacho-Hubner C, Savage MO, Clark AJ 1996 Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *N Engl J Med* 335:1363-1367
11. Bonapace G, Concolino D, Formicola S, Strisciuglio P 2003 A novel mutation in a patient with insulin-like growth factor 1 (IGF1) deficiency. *J Med Genet* 40:913-917
12. Walenkamp MJ, Karperien M, Pereira AM, Hilhorst-Hofstee Y, van Doorn J, Chen JW, Mohan S, Denley A, Forbes B, van Duyvenvoorde HA, van Thiel SW, Sluimers CA, Bax JJ, de Laat JA, Breuning MB, Romijn JA, Wit JM 2005 Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. *J Clin Endocrinol Metab* 90:2855-2864
13. Abuzzahab MJ, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E, Kiess W, Klammt J, Kratzsch J, Osgood D, Pfaffle R, Raile K, Seidel B, Smith RJ, Chernausek SD 2003 IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med* 349:2211-2222
14. Bhakta KY, Marlin SJ, Shen JJ, Fernandes CJ 2005 Terminal deletion of chromosome 15q26.1: case report and brief literature review. *J Perinatol* 25:429-432
15. Biggio Jr JR, Descartes MD, Carroll AJ, Holt RL 2004 Congenital diaphragmatic hernia: is 15q26.1-26.2 a candidate locus? *Am J Med Genet A* 126A:183-185
16. Inagaki K, Tiulpakov A, Rubtsov P, Sverdlova P, Peterkova V, Yakar S, Terekhov S, LeRoith D 2007 A familial insulin-like growth factor-I receptor mutant leads to short stature: clinical and biochemical characterization. *J Clin Endocrinol Metab* 92:1542-1548
17. Kawashima Y, Kanzaki S, Yang F, Kinoshita T, Hanaki K, Nagaishi J, Ohtsuka Y, Hisatome I, Ninomoya H, Nanba E, Fukushima T, Takahashi S 2005 Mutation at cleavage site of insulin-like growth factor receptor in a short-stature child born with intrauterine growth retardation. *J Clin Endocrinol Metab* 90:4679-4687
18. Okubo Y, Siddle K, Firth H, O'Rahilly S, Wilson LC, Willatt L, Fukushima T, Takahashi S, Petry CJ, Saukkonen T, Stanhope R, Dunger DB 2003 Cell proliferation activities on skin fibroblasts from a short child with absence of one copy of the type 1 insulin-like growth factor receptor (IGF1R) gene and a tall child with three copies of the IGF1R gene. *J Clin Endocrinol Metab* 88:5981-5988

19. **Pinson L, Perrin A, Plouzennec C, Parent P, Metz C, Collet M, Le Bris MJ, Douet-Guilbert N, Morel F, De Braekeleer M** 2005 Detection of an unexpected subtelomeric 15q26.2 --> qter deletion in a little girl: clinical and cytogenetic studies. *Am J Med Genet A* 138:160-165
20. **Poot M, Eleveld MJ, van 't Slot R, van Genderen MM, Verrijn Stuart AA, Hochstenbach R, Beemer FA** 2007 Proportional growth failure and oculocutaneous albinism in a girl with a 6.87 Mb deletion of region 15q26.2-->qter. *Eur J Med Genet* 50:432-440
21. **Roback EW, Barakat AJ, Dev VG, Mbikay M, Chretien M, Butler MG** 1991 An infant with deletion of the distal long arm of chromosome 15 (q26.1----qter) and loss of insulin-like growth factor 1 receptor gene. *Am J Med Genet* 38:74-79
22. **Rujirabanjerd S, Suwannarat W, Sripo T, Dissaneevate P, Permsirivanich W, Limprasert P** 2007 De novo subtelomeric deletion of 15q associated with satellite translocation in a child with developmental delay and severe growth retardation. *Am J Med Genet A* 143:271-276
23. **Siebler T, Lopaczynski W, Terry CL, Casella SJ, Munson P, De Leon DD, Phang L, Blakemore KJ, McEvoy RC, Kelley RI**, 1995 Insulin-like growth factor I receptor expression and function in fibroblasts from two patients with deletion of the distal long arm of chromosome 15. *J Clin Endocrinol Metab* 80:3447-3457
24. **Tonnies H, Schulze I, Hennies H, Neumann LM, Keitzer R, Neitzel H** 2001 De novo terminal deletion of chromosome 15q26.1 characterised by comparative genomic hybridisation and FISH with locus specific probes. *J Med Genet* 38:617-621
25. **Walenkamp MJ, de Muinck Keizer-Schrama SM, de Mos M, Kalf ME, van Duyvenvoorde HA, Boot AM, Kant SG, White SJ, Losekoot M, Den Dunnen JT, Karperien M, Wit JM** 2008 Successful Long-Term Growth Hormone Therapy in a Girl with Haploinsufficiency of the Insulin-Like Growth Factor-I Receptor due to a Terminal 15q26.2->qter Deletion Detected by Multiplex Ligation Probe Amplification. *J Clin Endocrinol Metab* 93:2421-2425
26. **Walenkamp MJ, van der Kamp HJ, Pereira AM, Kant SG, van Duyvenvoorde HA, Kruihof MF, Breuning MH, Romijn JA, Karperien M, Wit JM** 2006 A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor I receptor. *J Clin Endocrinol Metab* 91:3062-3070

27. **Sas TC, Gerver WJ, De Bruin R, Mulder PG, Cole TJ, De Waal W, Hokken-Koelega AC** 2000 Body proportions during 6 years of GH treatment in children with short stature born small for gestational age participating in a randomised, double-blind, dose-response trial. *Clin Endocrinol (Oxf)* 53:675-681
28. **Usher R, McLean F** 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr* 74:901-910
29. **Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM** 2000 Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res* 47:316-323
30. **Fredriks AM, van Buuren S, Wit JM, Verloove-Vanhorick SP** 2000 Body index measurements in 1996-7 compared with 1980. *Arch Dis Child* 82:107-112
31. **Gerver WJ, de Bruin R** 2001 *Paediatric morphometrics, a reference manual*. 2nd ed. Maastricht: University press Maastricht
32. **Hokken-Koelega AC, Hackeng WH, Stijnen T, Wit JM, de Muinck Keizer-Schrama SM, Drop SL** 1990 Twenty-four-hour plasma growth hormone (GH) profiles, urinary GH excretion, and plasma insulin-like growth factor-I and -II levels in prepubertal children with chronic renal insufficiency and severe growth retardation. *J Clin Endocrinol Metab* 71:688-695
33. **Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM** 1998 Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. *Horm Res* 50:166-176
34. **Greulich WW, Pyle SI** 1959 *Radiographic Atlas of Skeletal Development of the Hand and Wrist*. 2nd ed. Stanford, CA: Stanford University Press
35. **Miller SA, Dykes DD, Polesky HF** 1988 A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16:1215
36. **White SJ, Vink GR, Kriek M, Wuyts W, Schouten J, Bakker B, Breuning MH, den Dunnen JT** 2004 Two-color multiplex ligation-dependent probe amplification: detecting genomic rearrangements in hereditary multiple exostoses. *Hum Mutat* 24:86-92

37. Nannya Y, Sanada M, Nakazaki K, Hosoya N, Wang L, Hangaishi A, Kurokawa M, Chiba S, Bailey DK, Kennedy GC, Ogawa S 2005 A robust algorithm for copy number detection using high-density oligonucleotide single nucleotide polymorphism genotyping arrays. *Cancer Res* 65:6071-6079
38. Kamp GA, Ouwens DM, Hoogerbrugge CM, Zwinderman AH, Maassen JA, Wit JM 2002 Skin fibroblasts of children with idiopathic short stature show an increased mitogenic response to IGF-I and secrete more IGFBP-3. *Clin Endocrinol (Oxf)* 56:439-447
39. Ouwens DM, van der Zon GC, Pronk GJ, Bos JL, Moller W, Cheatham B, Kahn CR, Maassen JA 1994 A mutant insulin receptor induces formation of a Shc-growth factor receptor bound protein 2 (Grb2) complex and p21ras-GTP without detectable interaction of insulin receptor substrate 1 (IRS1) with Grb2. Evidence for IRS1-independent p21ras-GTP formation. *J Biol Chem* 269:33116-33122
40. Sas T, de Waal W, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A 1999 Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab* 84:3064-3070
41. van der Eerden BC, Karperien M, Wit JM 2003 Systemic and local regulation of the growth plate. *Endocr Rev* 24:782-801
42. Beck KD, Powell-Braxton L, Widmer HR, Valverde J, Hefti F 1995 Igf1 gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal granule and striatal parvalbumin-containing neurons. *Neuron* 14:717-730
43. Hawkins RD, Bashiardes S, Powder KE, Sajan SA, Bhonagiri V, Alvarado DM, Speck J, Warchol ME, Lovett M 2007 Large scale gene expression profiles of regenerating inner ear sensory epithelia. *PLoS ONE* 2:e525
44. Raile K, Klammt J, Schneider A, Keller A, Laue S, Smith R, Pfaffle R, Kratzsch J, Keller E, Kiess W 2006 Clinical and functional characteristics of the human Arg59Ter insulin-like growth factor I receptor (IGF1R) mutation: implications for a gene dosage effect of the human IGF1R. *J Clin Endocrinol Metab* 91:2264-2271

45. **van der Kamp HJ, Walenkamp MJE, Wit JM** 2007 Growth hormone treatment is efficacious in short stature due to a hetrozygous missense mutation in the tyrosine kinase domain of the IGF-I receptor. *Hormone Research* 68 (suppl 1):170

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	
<i>IGF1R</i>	2	U	GATGTGTGAGAAGACCACCATCAACA	92	HEX	
		D	ATGAGTACAACCTACCGTCTGGACCACAA			
	8	U	CTACATGGGCTGAAGCCCTGGACTCAG	94	HEX	
		D	TACGCCGTTTACGTCAAGGCTGTGACCCTCA			
	18	U	CAGTCTAGCACCTCCAAGCCTGAGCA	90	HEX	
		D	AGATGATTGAGATGGCCGGAGAGATTG			
<i>IGF1</i>	3	U	GAGTGTCTCTCCGGAGCTGTGATCT	88	HEX	
		D	AAGGAGGCTGGAGATGTATTGCCAC			
	4	U	GTACATTTGAAGAACGCAAGTAGAGGGAGT	98	HEX	
		D	GCAGGAAACAAGAACTACAGGATGTAGGAAGA			
	4	U	CAACAGCCTGGTGTACGGCCCTCTGACAGCAACGTCTATGA	116	FAM	
		D	CCTCTAAAGGACCTAGAGGAAGGCATCCAAA			
<i>GHR</i>	4	U	GCCACTGGACAGATGAGGTTTCATCATGTACAAGAACC	112	FAM	
		D	TAGGACCCATACAGCTGTTCTATACCAGAAG			
	10	U	GGTACTTTGGTGGCCACATAAGCCATTATCTACTAGTATGA	110	HEX	
		D	CTAGTTGTGTCTGGCAGTTTATATTTAACTCTC			
	<i>SHOX</i>	3	U	GGATTTATGAATGCAAAGAGAAGCGGAGGACG	106	HEX
			D	TGAAGTCGGAGGACGAGGACGGGCAGACCAAGCTGAA		
8		U	CCCTGAGTTTCTCTGGTGACGCCCTCATTCTCTAA	108	HEX	
		D	CGTTCAATAATCTCAATGTTGAGTTGCAGCAACAGA			
<i>IGFBP1</i>		1	U	CAAACCTATTTTGAACACTCAGCTCCTAGCGTGCG	102	HEX
			D	GCGCTGCCAATCATTAACTCTGGTGCAAG		
	4	U	GTGAGACATCCATGGATGGAGGCGGGACTCTGCT	104	HEX	
		D	GGTGCCTTACCCTTGAATGGGAAGAGGATC			
	<i>IGFBP2</i>	2	U	CACTCAGAAGGAGGCTGGTGGAGAACCAGT	100	FAM
			D	GGACAGCACCATGAACATGTTGGGCG		
4		U	CAAGATGTCTCTGAACGGGACGCTGGGGAGTGCT	108	FAM	
		D	GGTGTGTGAACCCCAACACCGGAAGCTGAT			
<i>IGFBP3</i>		2	U	GCTAAAGACAGCCAGCGCTACAAAGTTGACTACG	100	HEX
			D	AGTCTCAGAGCACAGATACCCAGAACTTCT		
	5	U	CTGGAGCTCACAGCCTTCTGTGGTGTCATT	96	HEX	
		D	TCTGAAACAAGGGCTGGATCCCTCAACCA			
	<i>IGFBP4</i>	3	U	GACCTCTACATCATCCCCATCCC	84	HEX
			D	CAACTGCGACCGCAACGGCAACTTC		

	4	U	CAGAGTCAGAGGAGAAGACATGTACCTTGACCATCGTCC	114	HEX
		D	TTCTCTCAAGCTAGCCAGAGGGTGGGAGCCTAAGGA		
<i>IGFBP5</i>	2	U	GATCTCCGGCCCAACACACCCGCATCTCCGAGCTGAAGGCTGAAG	120	HEX
		D	CAGTGAAGAAGACCCGAGAAAGAAGCTGACCCAGTC		
	3	U	GTGCTGTGTACCTGCCAATTGT	86	HEX
		D	GACCCGAAAGGATTCTACAAGAGAAAG		
<i>IGFBP6</i>	2	U	CCACAGGATGTGAACCCGAGAGA	92	FAM
		D	CCAACAGAGGAATCCAGGCACCTCTAC		
	3	U	CAAACACTTACGTGCCCAATTGT	90	FAM
		D	ACCATCGAGGCTTCTACCGAAG		
<i>ALS</i>	2	U	GAACCTCTCTGGGAAGTGTCTCCGG	88	FAM
		D	AACCTTCCGGAGCAGGTGTTT		
<i>STAT5B</i>	2	U	GATACAAGCTCAGCAGCTCCAAGGAGAAGCCCT	102	FAM
		D	TCATCAGATGCAAGCGTTATATGGCCA		
	14	U	GAGAATTTACCAGGACGGAATTACACTTTCTG	104	FAM
		D	GCAATGGTTTGACGGTGTGATGGAAGTGT		
<i>SOCS3</i>	2	U	CTTCAGCATCTGTGCGAAGACCGTCAACG	98	FAM
		D	GCCACCTGGACTCCTATGAGAAAGT		
<i>GRB10</i>	3	U	GATGTGGACCTGGAAGCCCTGGTGAACGAT	96	FAM
		D	ATGAATGCATCCCTGGAGAGCCTG		
	10	U	CAGAGGACGAGCAAACCCAGGACGTGCT	94	FAM
		D	GGATGACAGCGTTCAGACTCCTCAA		
<i>NSD1</i>	3	U	GAGATCTCATCTGGGCAAAATCAAGAGACGCCCATGGTGGCCCT	116	HEX
		D	GCAGGATTTGTTCTGATCCGTTGATTAACACACAT		
	13	U	CACTGTTATGCAGAACAAAGGGTCTCCGGTCTCCCTCCACATCTGTA	118	HEX
		D	TAACCTGTCATGCTGCTAATCCAGCCAATGTTTCT		
	20	U	CTCGTTTCATGAATCATGTGTCAGCCCAACTGTGAAACACAGAAG	120	FAM
		D	TGGTCTGTGAATGGAGATACCCGTGTAGGCC		
<i>IGF2</i>	2	U	GAATCCCAATGGGGAAGTCGAT	86	FAM
(<i>11p15</i>)		D	GCTGGTGTCTTCTCACCTTCTTG		
	4	U	CATCTGCAGCCTCCTCTGACCACGGACGTTTCCATCAGGTT	114	FAM
		D	CCATCCCGAAAATCTCTCGGTCCACGTC		
<i>SOCS2</i>	1	U	GCCGCGCCTCAACTAAAAGTGCCATTGACCTTTCAA	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'.