Genetic disorders in the growth hormone-IGF-I axis

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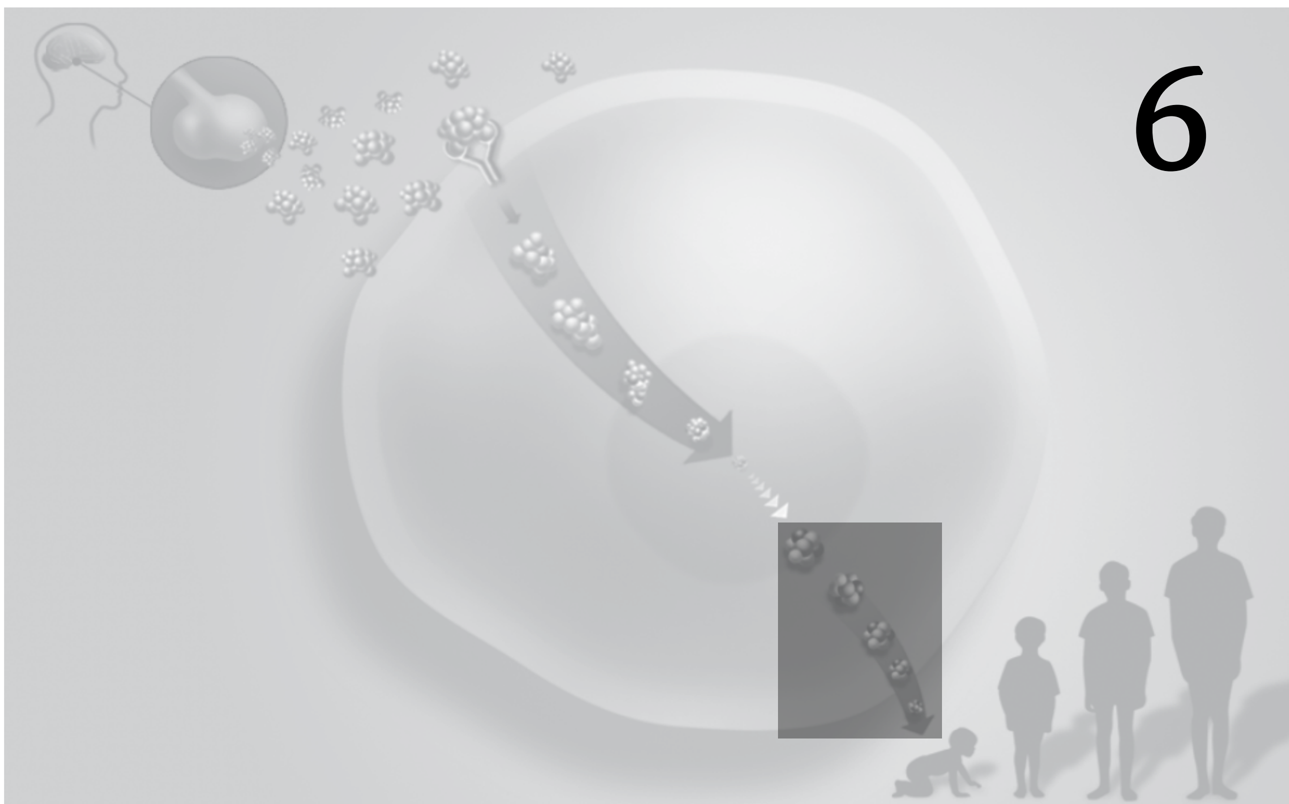
Homozygous and heterozygous expression of a novel IGF-I mutation



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Abstract

IGF-I is a key factor in intrauterine development and postnatal growth and metabolism. The secretion of IGF-I *in utero* is not dependent on GH, whereas in childhood and adult life IGF-I secretion seems to be mainly controlled by GH, as revealed from studies on patients with GHRH-R and GH-receptor mutations.
In a 55-yr-old male, the first child of consanguineous parents, presenting with severe intrauterine and postnatal growth retardation, microcephaly, and sensorineural deafness we found a homozygous G to A nucleotide substitution in the IGF-I gene changing valine 44 into methione (V44M). The inactivating nature of the mutation was proven by functional analysis demonstrating a 90-fold reduced affinity of recombinantly produced V44M for the IGF-I receptor. Additional investigations revealed osteoporosis, a partial gonadal dysfunction, and a relatively well preserved cardiac function. Nine of the 24 relatives studied carried the mutation. They had a significantly lower birth weight, final height and head circumference than noncarriers.

In conclusion, the phenotype of our patient consists of severe intrauterine growth retardation, deafness, and mental retardation, reflecting the GH-independent secretion of IGF-I *in utero*. The postnatal growth pattern, similar to growth of untreated GH deficient or GH-insensitive children, is in agreement with the hypothesis that IGF-I secretion in childhood is mainly GH-dependent. Remarkably, IGF-I deficiency is relatively well tolerated during the subsequent four decades of adulthood. IGF-I haploinsufficiency results in subtle inhibition of intrauterine and postnatal growth.

Introduction

In mice, the GH-IGF-I system plays a key role in intrauterine development and postnatal growth and metabolism (1-3). Knockout models of the GH receptor (GHR) and IGF-I have indicated that *in utero* IGF-I, but not GH, is required for normal fetal growth (1, 3, 4). Postnatally, growth is mediated predominantly via GH-dependent IGF-I secretion. In mice the effects on bone accretion before puberty are mediated predominantly via mechanisms independent of GH whereas the pubertal and postpubertal changes are predominantly mediated via GH-dependent mechanisms (5).

So far in humans only one adolescent male has been reported with a total IGF-I deficiency, resulting from a homozygous deletion of exons 4 and 5 of the IGF-I gene (6). This patient exhibited severe intrauterine growth retardation (birth weight –3.9 SDS), severe postnatal growth failure (height in childhood –6.9 SDS), sensorineural deafness, and mental retardation. Recently two patients with mutations of the IGF-I receptor (IGF1R) were described (one compound heterozygote and one heterozygote) (7). Both had decreased intrauterine growth (birth weight -3.5 SDS), comparable with the birth weight of the IGF-I deficient boy, but postnatal growth failure was less severe (a final height of -4.8 SDS and a height in childhood of -2.6 SDS, respectively). The patient who was compound heterozygous for missense mutations had signs of a nonverbal learning disorder and psychiatric anomalies. The patient with the heterozygous nonsense mutation had dysmorphic features, microcephaly, and mild retardation of motor development and speech. The heterozygous parents had heights of -1.6, -2.8 and -2.6 SDS.

The severely compromised intrauterine growth of the patients with IGF-I deficiency and (partial) IGF-I insensitivity is in contrast with the slightly diminished intrauterine growth of patients with either congenital complete GH deficiency (GHD) (8) or GH resistance (9-11) suggesting that, as observed in mice, *in utero* the production of IGF-I is largely independent of GH. Postnatally, IGF-I insufficiency leads to a persistent and severe growth retardation, of approximately equal magnitude as observed in complete GHD or GH resistance, implying that postnatal growth is the result of GH dependent IGF-I production

We describe a 55-yr-old patient with severe pre-and postnatal growth retardation, deafness, and mental retardation. He and his younger brother were described in 1969 as a familial syndrome of prenatal dwarfism, associated with elevated GH levels and end-organ unresponsiveness (12). We show that this clinical picture is caused by a homozygous missense mutation in the IGF-I gene, resulting in an IGF-I polypeptide with strongly decreased binding affinity to the IGF-I receptor *in vitro* and a lack of bioactive IGF-I *in vivo*. This unique patient enables us to study the effects of nearly complete primary IGF-I deficiency in adulthood. Investigations in 24 family members, of whom nine proved heterozygous for the mutation, have also enabled us to characterize for the first time the phenotype of IGF-I haploinsufficiency in detail.

Methods

The guardian of the index case, and all relatives who underwent investigations, provided written informed consent.

Clinical measurements and auxology

Height and sitting height were determined with a Harpenden stadiometer, and head circumference was assessed with a tape measure. Height was expressed as standard deviation score (SDS) based on Dutch references (13), after correction for shrinking (age) and secular trend (birth cohort) (14). Sitting height, sitting height/height ratio and head circumference were also expressed as SDS for the Dutch population (13, 15). Blood pressure was measured with a Dynamap and testicular volume with the Prader orchidometer.

Radiological and sonographic measurements

Bone mineral density (BMD) was measured by dual energy X-ray absorptiometry (QDR 4500; Hologic Inc., Bedford, MA) at the lumbar spine and femoral neck. Bone mineral apparent density (BMAD) of the lumbar spine was calculated using the formula BMAD=BMD x [4 / (π x width)]. The validity of this model has been tested using volumetric data obtained from magnetic resonance imaging of lumbar vertebrae (16). Cardiac ultrasound was performed (GE System 7, Vingmed, Milwaukee, WI) and routine images (for assessment of left ventricular

systolic function) and color Doppler data (to detect valvular abnormalities) were obtained. In addition, tissue Doppler imaging was performed for detailed evaluation of diastolic function (17).

Biochemical measurements

Plasma GH was measured with time-resolved immunofluorometric assay (Wallac/PE, Turku, Finland). Spontaneous GH secretion was assessed after an overnight fast by sampling every 20 min from 0900 to 1300 h. A fasting combined GHRH (1 μg/kg iv at 0 min) and arginine test (0.5 g/kg iv over 30 min, from 0 to +30 min) was performed.

Plasma IGF-I, IGF-II, IGF-binding-protein (IGFBP)-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5, and IGFBP-6 were determined by specific RIAs (18-22). Acid-labile subunit (ALS) was measured by a commercially available ELISA (Diagnostics Systems Laboratories, Inc., Webster, TX) (23). With the exception of IGFBP-1 and -5, for all parameters smoothed references were available, based on the LMS method (24), allowing conversion of patient data to SDS values. Plasma IGFBP-1 concentration after an overnight fast was compared with a reference group of six healthy adult controls. We compared IGFBP-5 with a reference population (5). The proportion of intact IGFBP-3 was determined by a ligand immunofunctional assay (25). The different molecular-size classes of endogenous IGF-IGFBP complexes in plasma were determined by neutral gel filtration (26). IGF-I bioactivity was assessed by an IGF-I kinase receptor activation assay based on cells transfected with the human IGF1R gene (KIRA) (27).

Molecular studies

A skin biopsy was taken, and a culture of dermal fibroblasts was established. These cells were used for a thymidine incorporation assay in response to IGF-I (28). Total RNA was isolated and reversed transcribed into cDNA. Full length IGF-I cDNA was isolated by PCR using the following primer combination (forward 5'-GCTTCATTATTCCTGCTAAC-3'; reverse 5'-AACTCGTGAGAGCAAAGGATC-3'), cloned, and subjected to direct sequencing, using routine procedures. The V44M mutation was introduced into *Escherichia coli* codon optimised human IGF-I cDNA by QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). Expression and purification of recombinant V44M IGF-I was performed as previously described (29). Folding patterns of V44M IGF-I and native IGF-I were very similar. Purified

V44M IGF-I was shown to be more than 95% pure by N-terminal sequencing and of the correct mass by mass spectroscopy. IGF1R binding affinity was determined using an europium-labeled IGF-I binding assay, essentially as described previously for epidermal growth factor binding to the epidermal growth factor receptor (30) but using IGF1R from lysed P6 cells. 3T3 cells overexpressing IGF1R (gift from Professor Renato Baserga, Thomas Jefferson University, Philadelphia, PA) captured with antibody 24-31 (a gift from Professor Ken Siddle, University of Cambridge, UK). Europium labeled IGF-I (DELFIA Eu-labeling kit, Perkin Elmer, Norton, OH) was added with competing IGF-I or V44M IGF-I and the amount of labeled IGF-I bound was measured by time-resolved fluorescence.

IGF-I binding to bovine placenta membrane fractions was performed using acid Sep-Pak C18 extracts of the sera. Sep-Pak C18 extraction is a well-established technique that effectively removes interfering IGF-binding proteins from both normal and chronic renal failure patient sera or plasma (the latter contain elevated levels of various IGF-binding proteins) as we have verified previously by [^{125}I] IGF-I or [^{125}I] IGF-II probed Western ligand blots of chromatographed materials (31-33). In addition, experiments were performed in which ^{125}I radiolabeled (30,000 cpm) wild-type IGF-I was incubated (overnight at 4 °C) with an aliquot (200 µl) of pooled normal serum. In a similar way, other 200 µl-aliquots of pooled normal serum were incubated with the same amount of ^{125}I radiolabeled wild-type IGF-I in the presence of either 200 ng recombinant wild-type IGF-I or 200 ng recombinant V44M IGF-I. The distribution of [^{125}I]IGF-I among the various molecular-size classes (*i.e.* 150 kD, 40-50 kD, and free IGF-I) in each aliquot of serum was determined after separation by S200 gel filtration and counting of ^{125}I activity in each fraction.

Statistical analysis

Data were analyzed with SPSS for Windows (version 11.0; SPSS Inc., Chicago, IL). Independent-samples *t* tests were used to compare the data of the carriers with the non-carriers. $P < 0.05$ was considered significant.

Results

Subjects

The index case, RV, was born in 1947 as the first of five children of a consanguineous marriage (Fig. 1). His grandfathers were brothers. At birth after 8 months' gestation, weight was 1420 g (-3.9 SDS) and length 39 cm (-4.3 SDS) (34). Postnatally there was a persistent progressive growth failure (Fig. 2) with normal proportions, retarded skeletal maturation, microcephaly, deaf-mutism, and severe mental retardation. At 11.4 yr, a 6-month trial with a testosterone depot preparation (75 mg im once a month) was given, which caused a slight acceleration of growth velocity, but also a clear advance in skeletal maturation. At 15 yr of age he had to be institutionalized because of severe mental retardation (IQ < 40) and motor unrest. Pubic hair and testicular growth occurred at the age of nearly 20 yr. Studies performed at the age of 21 yr (1968) revealed elevated basal [13 ng/ml (20 mU/liter)] and stimulated [127 ng/ml (191 mU/liter)] plasma GH levels in response to insulin-induced hypoglycemia (12). Adrenal and thyroid function was normal. He had a history of a right radial fracture following a minor trauma.

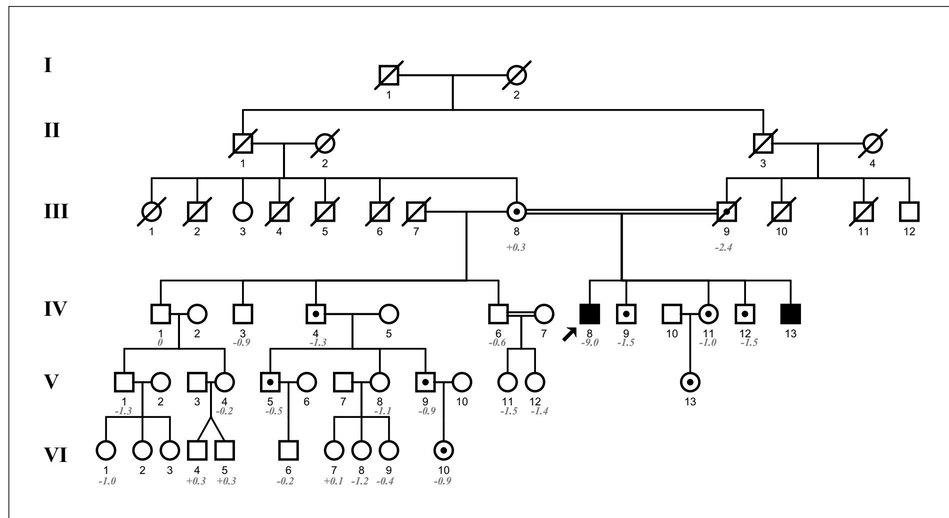

Figure 1. Pedigree of the family. The *arrow* points at the index case. Height SDS of the familymembers is added in *italic*. Heterozygotes are marked with a *central dot*.

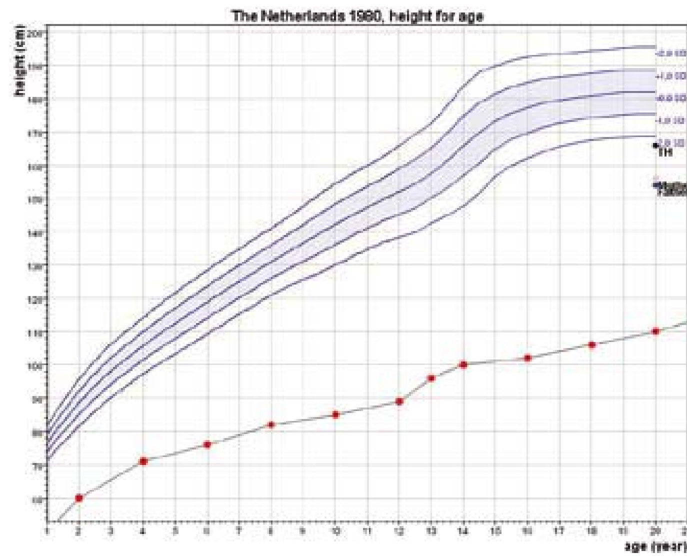

Figure 2. Growth chart of RV. Target height (TH) corrected for secular trend is calculated as: [height father + height mother + 13 cm] /2 + 4.5 cm.

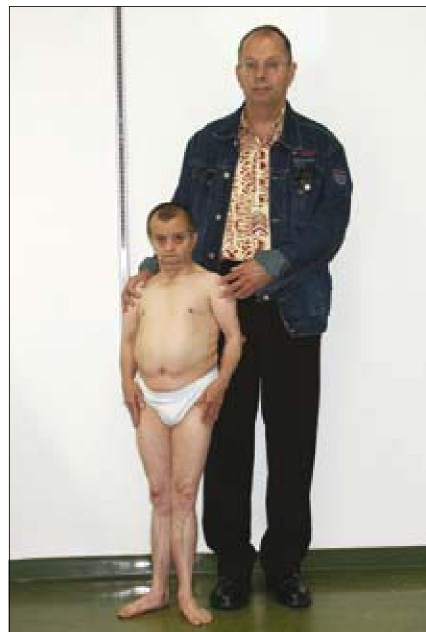

Figure 3. RV at 55 *yr* of age, together with one of his brothers (168.2 cm).

At 55 yr of age he came again to our attention because of a request for genetic counseling by one of his healthy younger brothers (Fig. 3). The auxological findings are summarized in Table 1. Abdominal fat mass was increased. There were several dysmorphic features, including deep-set eyes, flat occiput, a columella extending beyond the alae nasi and a striking micrognathia. His extremities showed broad end phalanges and convex nails. There was hypermobility of the interphalangeal joints but not of the other joints. The mobility of both elbows was restricted. Blood pressure was 172/93 mm Hg. Neurological examination was normal. Audiological analysis demonstrated severe bilateral hearing loss confirmed by absent brainstem evoked potentials. Testicular volume was 7 (left) and 1 ml (right). The stretched penile length was 8 cm (P10-90: 10.2-16.4 cm) (35). Bilateral inguinal scars were seen. In 1975 an operation for a bilateral inguinal hernia with ileus had been performed. Cardiovascular, respiratory, and abdominal examinations were normal. His IQ was less than 40. Ophthalmological examination revealed cataracts of both eyes and shallow anterior chambers. Vision was 0.63.

His youngest brother, AV, was born at term with a birth weight of 1900 g (-4.5 SDS) (34). His clinical phenotype strongly resembled that of RV. He too showed deaf-mutism and severe retardation (12). At the age of 12 yr (1968) endocrine investigations were performed. At baseline GH was 3 ng/ml and after insulin-induced hypoglycemia, plasma GH exhibited an exaggerated response [maximum 206 ng/ml (309 mU/liter)] (36). An oral glucose tolerance test showed an elevated baseline plasma insulin (15 mU/liter) (37), an insulin peak of 46 mU/liter with a maximum glucose of 125 mg/dl (6.9 mmol/l) [normal according to the American Diabetes Association (38)], and a normal suppression of plasma GH (<1 ng/ml). Prolonged fasting resulted in a physiological rise of GH (up to 56 ng/ml) and free fatty acids. Human pituitary GH was administered im for 4 d in a dose of 5 mg (≈ 4 IU) once daily, which did not result in a reduction of urinary excretion of nitrogen, potassium, and phosphate or an increase of hydroxyl-proline excretion (12). He died at 32 yr of age of aspiration pneumonia and severe gastritis with reflux oesophagitis after orthopedic surgery (triple athrodesis of left ankle).

Radiological features

BMD at the right femoral neck was 0.57 gr/cm^2 (-3.7 SDS) and 0.51 gr/cm^2 at the left femoral neck (-4.3 SDS). Lumbar spine BMD (L2-L4) was 0.52 gr/cm^2

Table 1. Auxological and biochemical characteristics of RV at 55 yr of age

Variable	**Value**	**SDS**
Height (cm)	117.8	- 8.5[a]
BMI (kg/m^2)	17.9	- 2.0[b]
Height/sitting height ratio	0.51	- 0.3[b]
Head circumference (cm)	44.2	- 8.0[b]
GH maximum (ng/ml) after GHRH/arginine stimulation	91.9	
GH maximum (ng/ml) in 3-h GH profile	1.8	
Total IGF-I (ng/ml)	606	+ 7.3
IGF-II (ng/ml)	454	+ 0.5
ALS (μg/ml)	28.9	+ 3.4
IGFBP-1 (ng/ml)	12	Low[c]
IGFBP-2 (ng/ml)	106.3	- 1.0
IGFBP-3 (μg/ml)	1.98	+ 0.1
IGFBP-4 (ng/ml)	145.6	+ 0.6
IGFBP-5 (ng/ml)	347.7	- 0.4
IGFBP-6 (ng/ml)	121.8	- 1.5
		Normal value
Testosterone (ng/dl)	271	>230
Inhibin B (ng/liter)	64	>150
LH (U/liter)	3.5	2-8
FSH (U/liter)	19.2	2-10

To convert GH to milliunits per liter, multiply by 2.6; to convert IGF-I to nanomoles per liter, multiply by 0.131; to convert IGF-II to nanomoles per liter, multiply by 0.134; to convert ALS to nanomoles per liter, multiply by 15.8; to convert IGFBP-1 to nanograms per milliliter, multiply by 0.033; to convert IGFBP-2 to nanamoles per liter, multiply by 0.032; to convert IGFBP-3 to nanomoles per liter, multiply by 33.3; to convert IGFBP-4 to nanamoles per liter, multiply by 0.038; to convert IGFBP-5 to nanomoles per liter, multiply by 0.035; to convert IGFBP-6 to nanomoles per liter, multiply by 0.034; to convert testosterone to nanomoles per liter, multiply by 0.035

[a] See Ref. 60

[b] See Ref. 13

[c] Normal range for nonfasting subjects: 24 - 58 ng IGFBP-1 per liter. After overnight fasting there is an average 5-fold rise in normal individuals.

(-5.2 SDS). BMAD was 0.2 gr/cm^3 (-3.5 SDS) (16). Cardiac ultrasound showed a left ventricular ejection fraction of 60%, without regional wall motion abnormalities. Left ventricular mass was normal. Diastolic function was impaired (abnormal relaxation), with an E (early flow) to A (late flow) ratio of 0.53, and the E' (early diastolic myocardial velocity assessed by tissue Doppler imaging) being 4.5 cm/sec (normal value >12 cm/sec).

Biochemical features

The results of the biochemical analysis are shown in Table 1. Maximum GH concentration after stimulation was in the upper normal range (36). Serum IGF-I was markedly elevated. After an overnight fast, the IGFBP-1 level remained low. Circulating levels of IGFBP-2, -3, -4, -5, and -6 were within the normal range whereas ALS was increased. The proportion of intact IGFBP-3 did not differ from that encountered for age- and gender-matched normal controls. As with normal plasma, in the patient's plasma, most of IGF-I (> 90%) (Fig. 4A) and IGF-II (>70%) (Fig. 4B) was associated with the 150 kD complex. The distribution of IGFBP-3 and IGFBP-5 over the various molecular weight classes in serum appeared normal as did IGFBP profiles analyzed by Western ligand blotting (data not shown).

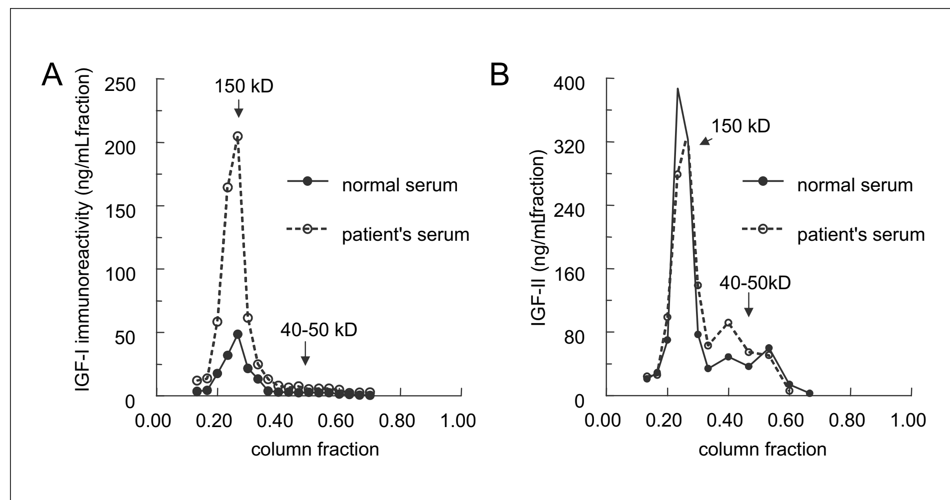

Figure 4. IGF-I (A) and IGF-II (B) in serum of RV are normally distributed over the various molecular weight classes. The majority of IGF-I and -II is present in the 150-kDa complex with IGFBP-3 and

Mutational analysis

Considering the increased level of IGF-I in our patient, we reasoned that the clinical symptoms could be caused by either an inactivating mutation in the IGF1R or in IGF-I itself. The wild-type (wt) IGF-I induced stimulation of ^{3}H-thymidine incorporation by cultured fibroblasts of the patient was comparable with the average response observed in cells obtained from 10 healthy control subjects. Considering the variation observed in IGF-I responses using dermal fibroblast cultures (28), the presence of an inactive IGF1R appeared unlikely (Fig. 5A). Subsequently, IGF-I cDNA was isolated by RT-PCR from fibroblasts. Sequence analysis identified a homozygous G>A nucleotide substitution at position 274, changing valine at position 44 of the mature IGF-I protein to methionine (Fig. 5B). The same nucleotide substitution was also present in the genomic DNA but not in a control panel of 87 individuals. V44 is located in the first α helix of the A domain of wt IGF-I and is exposed at the surface of the polypeptide (39). A normal male karyogram was found (46 XY).

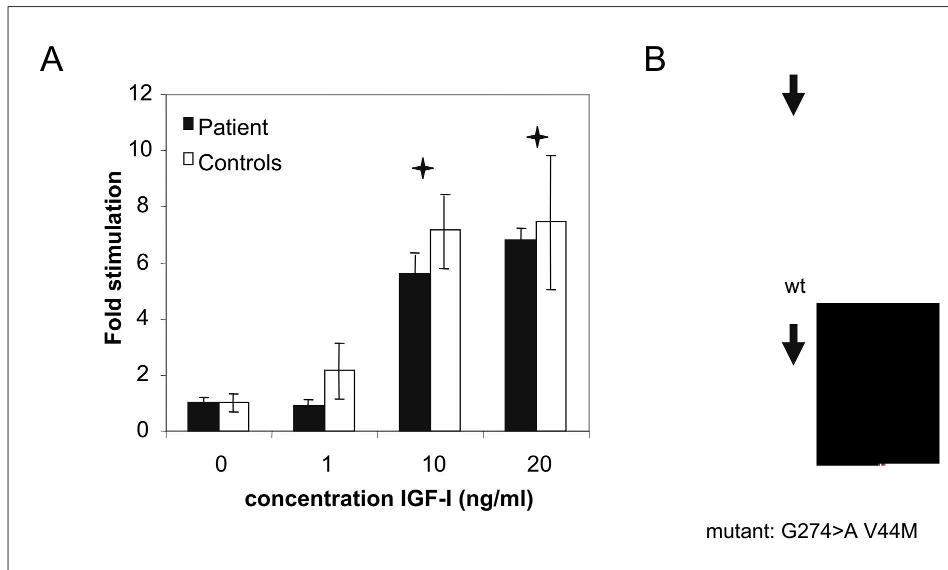

Figure 5.

A. Fibroblasts of RV respond normally to wt IGF-I in a thymidine incorporation assay. An *asterisk* indicates a statistical difference from unstimulated cells ($P < 0.01$).

B. Sequence analysis of wt IGF-I (*upper panel*) and IGF-I of RV (*lower panel*). *Arrow* indicates the homozygous G>A nucleotide alteration.

Functional analysis

We subsequently used the mutant cDNA for the synthesis of mutant IGF-I (V44M). V44M had an approximately 90-fold lower affinity for the IGF1R compared with wt IGF-I measured in both europium (Fig. 6A) and ^{125}I-labeled IGF-I competition receptor binding assays (Fig. 6B). Competition binding studies with ^{125}I-labeled IGF-I and serum of our patient or an age-and sex-matched control revealed that serum of the patient displaced ^{125}I-wt IGF-I with an ED_{50} of 11.3 µl serum equivalents (Fig. 6B). This volume of serum contained 5.3 ng IGF-II (serum value of 454 ng/ml, Table 1) that also competed for binding to the receptor. This is close to the deduced ED_{50} value of purified human IGF-II (4.3 ng) in this assay. Thus, these calculations demonstrate that the displacement of ^{125}I-wt IGF-I in the bovine placenta membrane assay can be explained by the presence of IGF-II and that V44M in serum did not significantly contribute to this process, in agreement with the data obtained for recombinant V44M providing further support for the inactivating nature of the mutation. In addition, a competition experiment was performed in which ^{125}I-wt IGF-I was incubated with normal serum in the absence or presence of unlabeled wt and V44M IGF-I. As shown in Fig. 6C wt IGF-I and V44M IGF-I competed with equal affinity for 150 kD and 40-50 kD complex forming with radiolabeled ligand. This suggests that the V44M mutation has only minor effect on binding to IGFBPs in marked contrast to binding to the IGF1R.

Carriers of the IGF-I mutation

We investigated a total of 24 relatives of the index patient. Nine of them carried the heterozygous V44M IGF-I mutation. Their birth weight and head circumference were lower in comparison with noncarriers (Table 2). Their height SDS was lower as shown in Fig. 7. Testicular volume was normal.

Except for one diabetic individual and the 87-yr-old mother, fasting glucose levels were normal. Fasting insulin levels were significantly higher in carriers (Table 2). Serum LH, FSH, testosterone, and estradiol were normal (data not shown). Basal GH levels (a single fasting sample) were within the normal range. Carriers had higher total IGF-I levels (Fig. 7) and lower IGF-I bioactivity than noncarriers (Table 2).

We measured BMD at the femoral neck and lumbar spine in all carriers. They had values within the normal range for their age and sex. In 21 relatives (three young children were excluded), we performed audiometry, revealing hearing abnormalities in seven individuals. However, no statistically significant association with carriership could be detected.

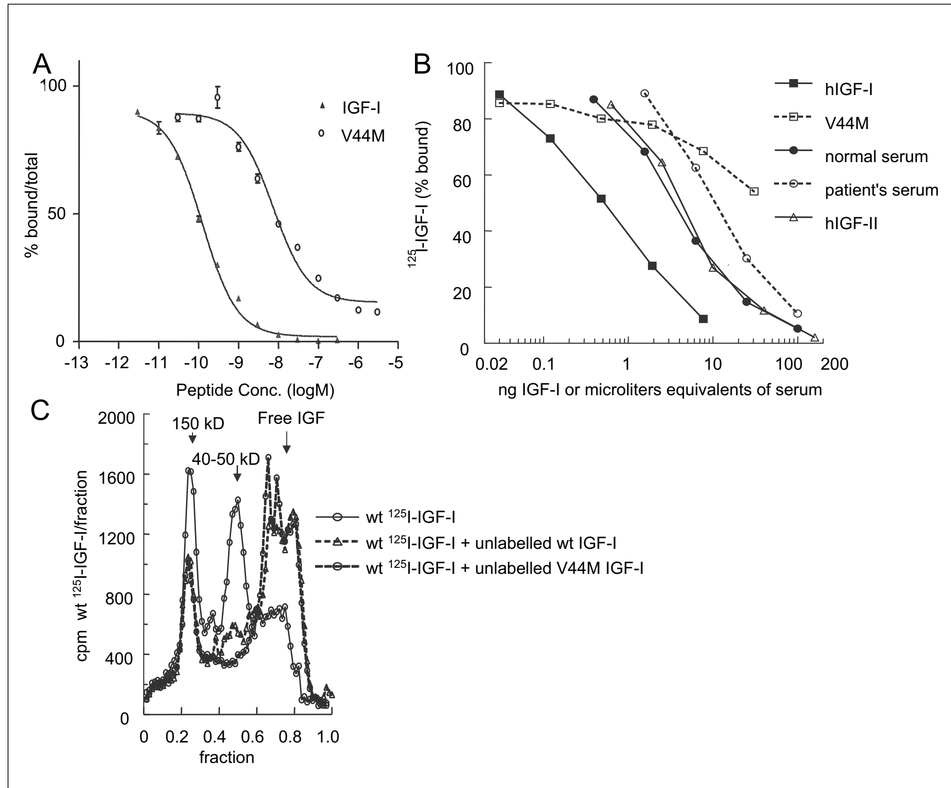

Figure 6.

A Receptor binding assay showing 90-fold reduced binding affinity of V44M IGF-I to the IGF-I receptor.

B. Competition binding assay using ^{125}I-IGF-I as tracer and IGF1R-expressing membranes of bovine placenta. Competition is performed with wt hIGF-I, wt hIGF-II, V44M, serum of the patient, or an age- and sex-matched control. The ED_{50} of a Sep-Pak C18 extract of RV is 11.3 µl, which corresponds to 5.3 ng IGF-II, being close to the deduced ED_{50} of IGF-II (4.3 ng). The values of IGF-I and IGF-II of the age- and sex-matched control were 185 ng/ml and 386 ng/ml.

C. ^{125}I radiolabeled wt IGF-I was incubated with an aliquot of pooled normal serum, in either the absence or presence of 200 ng recombinant wt IGF-I or 200 ng recombinant V44M IGF-I. wt IGF-I and mutant IGF-I efficiently competed for 150 kD and 40-50 kD complex forming with iodinated IGF-I.

Table 2. Auxological and biochemical characteristics of the carriers of the heterozygous IGF-I mutation (n = 9) *vs*. noncarriers (n = 15).

Variable	Carrier	Noncarrier	P value
Age (yr)	44.2 ± 7.8	27 ± 5.7	0.08
Male/female	5/4	8/7	
Height (SDS)[a]	-1.0 ± 0.2	-0.4 ± 0.2	0.04
Head circumference (SDS)	-1.0 ± 0.3	0.5 ± 0.3	< 0.01
BMI (SDS)[b]	0.5 ± 0.3	0.7 ± 0.3	0.62
Birth weight (g)[c]	3048 ± 101	3358 ± 85	0.04
Fasting insulin (mU/liter)[d]	16.5 ± 1.9	12.1 ± 0.7	0.03
Total IGF-I (SDS)	0.6 ± 0.3	-0.3 ± 0.2	0.04
Bioactive IGF-I (ng/ml)	0.9 ± 0.09	1.6 ± 0.16	< 0.01

Data are expressed as mean values ± SEM.

To convert insulin to picomoles per liter, multiply by 7.175; to convert bioactive IGF-I to nanomoles per liter, multiply by 0.131.

[a] For the calculation of mean height SDS, height of the father of the index cases was included (n=10).

[b] For the analysis of BMI, one noncarrier (obese and type 2 diabetes) was excluded.

[c] Birth weight data were available from eight carriers and 15 noncarriers.

[d] For the analysis of fasting insulin, one noncarrier (obese and type 2 diabetes) was excluded.

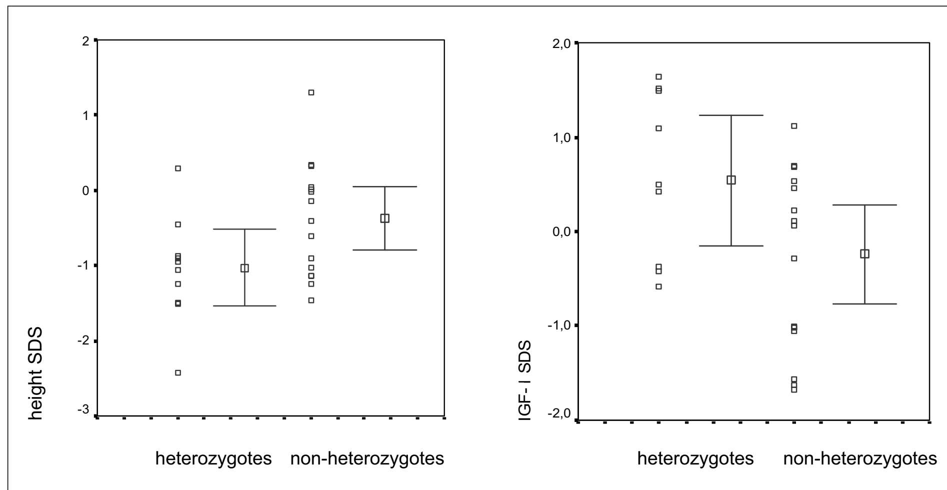

Figure 7: Height SDS and IGF-I SDS of heterozygous and nonheterozygous family members (*dots*) and 95% confidential interval (*bars*).

Discussion

There are two principal, novel findings in our study. It is the first report on a homozygous missense mutation in the human IGF-I gene resulting in an IGF-I protein that is hardly capable of interacting with the IGF1R but with relatively unaffected binding capacity for IGFBPs. This leads to severe effects on growth and development *in utero* and during childhood. It provides an opportunity to evaluate the effects of primary IGF-I insufficiency in adulthood, in comparison with the secondary IGF-I deficiency present in severe GH resistance or deficiency. Second, we have shown for the first time that IGF-I haploinsufficiency results in a subtle, but statistically significant, inhibition of intrauterine and postnatal statural and cranial growth and increased fasting insulin concentrations.

From observations on analogous peptides in the human as well as in other species, one can predict that a change in V44 should lead to a severe loss of function. V44 is not conserved only in IGF-I of all species except one (40) available in the National Center for Biotechnical Information database, but also in the two structurally related proteins IGF-II and insulin. In insulin, amino acid V^{A3}, structurally homologous to V44 in IGF-I, is involved in binding to the insulin receptor (41). Interestingly, a germline mutation of V^{A3} to leucine (insulin Wakayama) resulting in a variant that poorly binds to the insulin receptor has been associated with diabetes mellitus (42).

Proof for the inactivating nature of V44M was provided by demonstrating a 90-fold lower binding affinity for the IGF-I receptor in receptor binding assays using recombinantly produced protein. In addition, total receptor binding activity of the patient's serum was equivalent to a level that would be achieved by the same concentration of IGF-II found in his serum. This observation indicated that V44M did not contribute significantly to receptor binding despite the increased circulating levels of V44M in serum. Thus, the phenotype of our patient is caused by a complete lack of bioactive IGF-I. Furthermore IGF-II, although in the upper normal range, cannot compensate for IGF-I deficiency *in utero*, in childhood and neither in adulthood, in agreement with earlier reports (1-3, 6, 7).

Remarkably, gel filtration experiments revealed that the relative distribution of V44M over the various molecular weight classes in serum is not different from wt IGF-I in normal serum. This suggests normal binding of V44M to IGFBPs and is indicative of a relatively small impact on overall structure of V44M. It also implies that V44M can compete normally with IGF-II in the formation of complexes with the binding proteins and ALS. This was supported by our observation that V44M efficiently competed with wt IGF-I for 150- and 40-50 kD complex formation in serum, and was recently confirmed by BIAcore analysis showing normal binding of V44M to human (h)IGFBP-2, hIGFBP-3 or hIGFBP-6 biosensor surfaces. In addition, nuclear magnetic resonance analysis demonstrated only subtle changes in overall structure of V44M (43).

Considering the respective roles of IGF-I and GH *in utero* and in childhood, the clinical presentation of these two sibs with a dysfunctional IGF-I generally confirm the observations made in the single previously reported case of the IGF-I-deficient adolescent (6) and the two patients with partial IGF-I insensitivity (7). *In utero*, biologically active IGF-I is necessary for normal intrauterine growth, brain development, development of the inner ear, and mandibular growth, similarly to the phenotype of IGF-I knockout mice (44, 45). The functional effects of a partial IGF-I insensitivity on the brain are much milder, but the head circumference at birth of the heterozygote was severely decreased (-4.6 SDS). No effects were seen on inner ear development. This suggests that the heterozygous and compound heterozygous mutations in the IGF1R do not completely preclude IGF-I signaling. The variation in dysmorphic features in the different cases of IGF-I deficiency and IGF-I insensitivity may be partially due to a tissue-specific expression of IGF1R alleles (7). The absence of intrauterine growth retardation, mental retardation, hearing problems, and micrognathia in GH-deficient and GH-insensitive infants implies that in the fetal stage IGF-I secretion is GH-independent.

In childhood and adolescence, a complete lack of IGF-I function seems to lead to a qualitatively similar postnatal growth pattern as seen in a complete lack of GH effect: severe proportionate growth retardation accompanied by retarded skeletal maturation and delayed pubertal development. In our case and the case with IGF-I deletion growth was extremely retarded, and led in our male patients to a final height of 118 cm (about 8.5 SD below the mean). In the two patients with partial

IGF-I insensitivity short stature was less extreme (final height -4.8 SDS and a height in childhood of -2.6 SDS, respectively). Patients with GH insensitivity due to a GHR mutation reach an average height varying from –5.3 to -12 SDS (46-48). Mean height of patients with GHRH-R mutations is –7.2 SDS. So the effect of primary or secondary IGF-I insufficiency on postnatal growth is similar.

Whereas IGF-I deficiency leads to specific detrimental consequences for growth and development of various organs *in utero* and during childhood, IGF-I deficiency appears to be tolerated relatively well during the subsequent four decades. We found a low BMD and decreased testicular function in our patient, but both findings might be explained by other factors than IGF-I deficiency. The severe osteoporosis in our 55-yr-old patient could be explained by hypomuscularity and a low level of physical activity. Because our patient had a normal serum testosterone level, it is unlikely that his osteoporosis is due to hypogonadism. Our findings are in contrast with the absence of osteoporosis in the adolescent with the IGF-I deletion, and in the 12-yr-old girl with IGF1R mutation. They are also in contrast with the normal BMAD values observed in patients with Laron syndrome (GH insensitivity) and in patients with untreated GHD due to GHRH receptor mutations (49-51). Similar observations in future cases with primary IGF-I deficiency are needed before it can be assumed that maintenance of normal bone density is dependent on GH-independent IGF-I secretion.

At 55 yr of age, secondary sex characteristics and plasma testosterone level were normal, but FSH was elevated and inhibin was low in our patient. This suggests that mainly Sertoli cell function and spermatogenesis are impaired, probably resulting in infertility. The significance of the partial gonadal failure that we observed in our patient is most probably the result of testicular damage due to the bilateral inguinal hernia operation. This speculation is strengthened by the observation that the adolescent with IGF-I deletion has normal basal and GnRH-stimulated LH and FSH values. Furthermore, in patients with a secondary IGF-I deficiency due to a GHR or GHRH receptor mutation, fertility is normal.

IGF-I deficiency appears noncritical for adult cardiac function because only diastolic function was mildly impaired, a nonspecific finding observed frequently in individuals of 50 yr and older. The hypertension we found is in line with the

findings in IGF-I deficient mice. IGF-I has growth-promoting effects on smooth vascular muscle and has potent vasodilator effects, suggesting a possible role for IGF-I in the regulation of vascular tone (52). In our patient, with probably life-long exposure to increased GH levels, the absence of cardiac abnormalities is striking, compared to the findings in patients with untreated acromegaly who are exposed to GH and IGF-I excess for years before diagnosis. In these patients the development of both ventricular hypertrophy and valvular abnormalities are strongly dependent on the duration of exposure to increased GH production (53, 54). We speculate that the cardiomyopathic changes found in patients with acromegaly are caused by increased levels of IGF-I due to GH-excess.

One of the clinical characteristics of our patient is a restricted mobility of both elbows. This feature was also described in patients with GHR deficiency and GH deficiency caused by Prophet of Pit-1 (PROP 1) gene mutation (55, 56). This suggests that the effect of GH on the development of a normal joint mobility is mediated through IGF-I.

With regard to the IGFBPs, the normal IGFBP-3 level in RV and the patient with the IGF-I deletion supports the notion that the production of IGFBP-3 is controlled independently of IGF-I in humans (57). ALS is increased, which suggests that ALS is primarily GH dependent, and in this case may predominantly circulate in an unbound form. Also in the case of IGF-I deficiency, an elevated plasma ALS was reported, which decreased after IGF-I therapy (57).

In contrast to patients with GHD and GH insensitivity, in whom IGFBP-1 is usually elevated, IGF-I deficiency is associated with a low fasting IGFBP-1. IGFBP-1 has an inhibitory effect on the growth-promoting and anabolic effects of IGF-I. It is primarily regulated by insulin, but also IGF-I, IGF-II, and GH have specific inhibitory effects on IGFBP-1 expression (58). We speculate that the persistently low IGFBP-1 levels are caused by the direct suppressive effect of high GH levels (59).

Finally, it is of interest that heterozygous carriers of the mutation have a lower birth weight, height, and head circumference than noncarriers. Although height SDS was significantly lower than in the noncarriers, it was still within the normal range. The clinical findings are in line with the elevated IGF-I, and lower IGF-I bio-

activity. We hypothesize that a gene-dose effect may explain this mild effect on growth. The carriers had higher fasting insulin levels than the noncarriers supporting the role of IGF-I bioactivity in insulin sensitivity (57). Fertility in heterozygous carriers appears normal, considering the offspring of the obligate heterozygous parents of the index cases and the fact that four other adult heterozygotes had children. This observation is supported by normal testosterone, estradiol, LH, and FSH levels in carriers.

In conclusion, this homozygous missense mutation in the human IGF-I gene results in an abnormal IGF-I molecule with low receptor binding. In contrast to the severe effects on pre- and postnatal growth and development, IGF-I insufficiency is well tolerated in adulthood. IGF-I haploinsufficiency results in decreased IGF-I bioactivity, a subtle, but statistically significant, inhibition of intrauterine and postnatal statural and cranial growth, and elevated fasting insulin levels.

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