Partial Primary Deficiency of Insulin-Like Growth Factor (IGF)-I Activity Associated with *IGF1* Mutation Demonstrates Its Critical Role in Growth and Brain Development

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Context: IGF-I is essential for fetal and postnatal development. Only three *IGF1* defects leading to dramatic loss of binding to its type 1 receptor, IGF-1R, have been reported.

Patient: We describe a very lean boy who has intrauterine growth restriction and progressive postnatal growth failure associated with normal hearing, microcephaly, and mild intellectual impairment. He had markedly reduced concentrations of IGF-I, with IGFBP-3 and ALS serum levels in the upper normal range or above. IGF-I serum concentrations differed according to the immunoassay used. A higher than average GH dose was required for catch-up growth. Given the mismatch between IGF-I and IGFBP-3 levels, we sequenced his *IGF1* gene.

Result: We identified a homozygous missense *IGF1* mutation. This causes the replacement of a highly conserved amino acid (arginine 36) by a glutamine (R36Q) in the C domain of the predicted peptide. We showed that the abnormal IGF-I peptide has reduced mitogenic activity and partial loss of binding to its receptor IGF-1R. The patient's IGF-I level was undetectable in a highly specific monoclonal assay but elevated in a polyclonal assay.

Conclusion: This first report of mild deficiency of IGF-I activity demonstrates that the integrity of IGF-I signaling is important for normal growth and brain development. Molecular defects leading to partial loss of IGF-I activity may not be uncommon in patients born small for gestational age. The characterization of this complex phenotype and identification of such molecular defects have therapeutic implications, particularly now that, in addition to GH, recombinant IGF-I is available for clinical use. **(*J Clin Endocrinol Metab* 94: 3913–3921, 2009)**

Fetal growth is a complex process involving maternal, placental, and fetal factors (1). The etiology of fetal growth retardation remains unknown in many cases (2). In mammals, the IGF system plays a crucial role in growth and development. The IGF type 1 receptor (IGF-1R) is responsible for IGF signal transduction. IGF-I is essential for both fetal and postnatal growth and development (3). IGF-I forms a large, high molecular weight complex with IGF binding protein 3 (IGFBP-3) and acid labile subunit (ALS), thereby increasing its half-life in the circulation and its bioavailability. During postnatal life, these three components are GH-dependent. Only a few molecular defects

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Abbreviations: ALS, Acid labile subunit; IGFBP-3, IGF binding protein 3; IGF-1R, IGF type 1 receptor; IRS-1, insulin receptor substrate-1; rh, recombinant human; SDS, SD score; SGA, small for gestational age; sIGF-1R, soluble IGF-1R; wt, wild type.

in the IGF system have been identified in patients born small for gestational age. These include defects in *IGF-1R*, *ALS* (4), and epigenetic defects of the *IGF2* locus (5, 6). Only three *IGF1* defects leading to complete (7) or very severe (8, 9) loss of binding to its receptor, IGF-1R, have been identified so far. These three patients all had intrauterine and postnatal growth retardation associated with sensorineural deafness and severe intellectual deficit. Additional clinical features included microcephaly, adiposity, and insulin resistance, with partial gonadal dysfunction and osteoporosis present in at least one case (9). Until recently, the only treatment for patients with short stature was GH. Recombinant human IGF-I is now also available to treat short children with growth retardation caused by primary IGF-I deficiency (10).

We investigated the cause of growth retardation in an extensive analysis of the IGF system in a patient born to consanguineous parents and displaying severe intrauterine growth restriction, whose growth retardation became more severe during postnatal life.

Patient and Methods

For more details on the methods, see Supplemental Data (published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Measurement of hormones

Blood samples were collected in the morning after one night of fasting, and serum samples were stored at −20 C until assayed. Serum IGF-I concentrations were measured with three different assays [IRMA, Immunotech, Marseille, France; CIS Bio International, Gif-sur-Yvette, France; and an in-house assay (11)]. Serum IGF-II concentration was determined using a specific immunoradiometric assay (9100; Diagnostic Systems Laboratories, Webster, TX), serum IGFBP-3 and ALS using a Diagnostic Systems Laboratories assay, and GH with an Immunotech assay. Patient results were compared with controls matched for age and pubertal status. There was no cross-reactivity observed in IGF-I, IGF-II, or insulin assays.

Molecular studies

Informed consent for genetic analysis was obtained from the patient and all family members analyzed in compliance with national ethics regulation. Genomic DNA was isolated from blood samples from the patient, his parents, and his grandparents using standard techniques. PCR was used to amplify the five *IGF1* coding exons (exons 2, 3, 4, 5, and 6) and their intron-exon boundaries, as previously described (12). Total RNA was isolated from lymphoblastoid cells with RNAble solution (Eurobio, Courtaboeuf, France), and reverse transcribed into cDNA with superScript II RNase H^{-} Reverse Transcriptase kit (Invitrogen, Cergy Pontoise, France). *IGF1* cDNA encompassing the entire coding sequence was amplified by PCR using the following primer pair: forward, 5′-GCTTCATTATTCCTGCTAAC-3′; reverse, 5′-AACTCGTGAGAGCAAAGGATC-3′.

Sequencing

Sequences of the five coding exons and intron-exon junctions of the *IGF1* gene and its cDNA were determined on both strands using the same primers as used for PCR, the ABI PRISM BigDye terminator v1.1 cycle sequencing kit and the ABI 3100 Genetic Analyzer (Applied Biosystems, Courtaboeuf, France).

Production of recombinant IGF-Is in the baculovirus/insect cell system

Wild-type IGF-I (wt-IGF-I), R36Q-IGF-I (predicted IGF-I protein from patient V1) and V44M-IGF-I (previously reported in Ref. 9) recombinant proteins were produced in the baculovirus/insect cell system as previously described (13) using a wt-IGF-I cDNA template (14) mutated by oligonucleotide-driven site-directed mutagenesis (15).

Recombinant IGF-Is binding to IGFBP-3

The binding of wt-IGF-I, R36Q-IGF-I, and V44M-IGF-I to IGFBP-3 was characterized using ELISA.

Surface plasmon resonance analysis

Sensor chip preparation

Recombinant human IGFBP-3 (rhIGFBP-3; produced in the same bacculovirus/insect system) was coupled to the CM5 BIAsensor chip (BIAcore, Uppsala, Sweden) via amine group linkage using standard coupling procedures as previously reported (16, 17).

Binding kinetic studies

Kinetic analysis by surface plasmon resonance was performed on a BIAcore 2000 biosensor (BIAcore, Uppsala, Sweden) at 25 C in PBS at a flow rate of 35 μl/min. The kinetic parameters were then calculated.

Cell culture and ^{3}H-thymidine incorporation

The mitogenic action of wt and mutated IGF-I was studied in mouse fibroblast cells (BP-A31)—a serum-dependent transformed cell line—as previously described (18).

Competitive binding studies using sIGF-1R

The binding affinity of wt and mutated recombinant IGF-I to IGF-1R was assessed using a soluble form of the IGF-1R (sIGF-1R) encompassing the entire IGF-1R extracellular domain (R&D Systems, Abingdon, UK). IGF-I was labeled with Na^{125}I (Amersham. Aylesbury, UK) using the chloramine-T method, modified to a specific activity of 350–450 mCi/mg, and purified by gel filtration.

Phosphorylation of IGF-1R and insulin receptor substrate-1 (IRS-1)

Phosphorylation of IGF-1R and of IRS-1 was assessed in MCF-7 cells with or without different concentrations of wt-IGF-I, R36Q-IGF-I, or V44M-IGF-I.

Results

Case report

The patient (V1; Fig. 1), born to consanguineous parents, had a healthy brother (V2) with normal birth parameters. His father (IV2) had a birth weight and length of 2900 g [−1 SD score (SDS)] and 47 cm (−2 SDS), respectively. His mother

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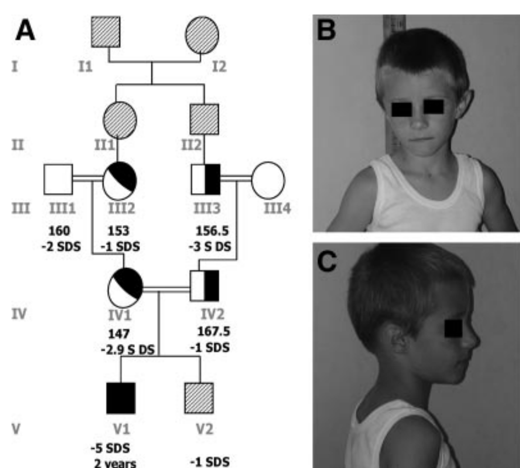

FIG. 1. Pedigree (A) and patient (B and C) at the age of 8.5 yr. Parents gave their informed consent for the publication of pictures. The patient's parents and two of his grandparents were heterozygous for the R36Q (*black square*) mutation in the *IGF1* gene; two grandparents did not carry this mutation (*white square and circle*). The *hatched squares and circles* represent individuals for whom DNA was not available. Final heights are indicated in panel A as the number of SD values below the mean based on French reference values (19). Patient V1 showed no signs of dysmorphia at age 8.5 yr (B and C).

(IV1) had a birth weight and length of 2710 g (−1.5 SDS) and 47 cm (−2 SDS), respectively. Their final heights were 167.5 cm (IV2, −1 SDS) and 147 cm (IV1, −2.9 SDS) (19) (Fig. 1A). The patient's target height was 163.9 cm (−1.9 SDS). The pregnancy was spontaneous, with an insufficient weight gain of 3 kg during the first 7 months, at which time intrauterine growth retardation of the fetus was diagnosed without known cause. The patient was born small for gestational age (SGA) at 40 wk, with a birth length of 44 cm (−3.7 SDS) and birth weight of 2350 g (−2.4 SDS). He had microcephaly with a head circumference of 32 cm (−2.5 SDS), according to Usher and McLean curves as a reference (20).

He was referred to our pediatric-endocrinology unit at 11 months for failure to thrive and deterioration of his growth parameters: height, 64 cm (−3.7 SDS); weight, 5360 g (−5 SDS); head circumference, 43 cm (−2.5 SDS), according to the French reference growth curves (19) (Fig. 2A). He was nondysmorphic, with anorexia leading to insufficient food intake (630 kcal/d) and very poorly developed adipose tissue. He had normal male external genitalia with palpable testis of volume 3 ml. Clinodactyly of the fifth finger was observed. The extensive work-up performed at this age was normal, including renal, thyroid function, cortisol level at 0800h, small intestine biopsy, sweat test, and karyotype. The patient showed no inflammatory abnormality. Cerebral magnetic resonance imaging excluded a third ventricle tumor. Nutritional support by enteral feeding was offered, but was refused by the parents. At the age of 2.8 yr, bone age was 18 months, height was 76 cm (−4.9 SDS), weight was 7 kg (−7 SDS), and head circumference was 44.5 cm (−4 SDS).

GH stimulation tests performed at 2.8 yr showed basal serum GH concentrations of 0.7 ng/ml after an overnight fast, with a peak concentration of 26 ng/ml after stimulation with ornithine (14.5 g/m^2; normal value, >7). Serum IGF-I at 2.7 yr, measured with an Immunotech kit, was extremely low (11 ng/ml) (Table 1). GH treatment was initiated at 3.2 yr, at a dose of 0.4 mg/kg · wk for 3 yr, according to French treatment guidelines for SGA. This led to an initial increase in growth rate. Treatment was reduced to 0.2 mg/kg · wk after 3 yr of treatment, as recommended. Growth velocity, which had returned to normal, decreased during treatment at the reduced dose. GH therapy was subsequently withdrawn for 14 months. During the first 12 months, there was no difference between growth velocity observed during the 0.2 mg/kg · wk GH treatment period and that observed when the patient was no longer receiving GH therapy; the patient's growth curve was consistently at −2.5 SDS, with or without GH therapy (Fig. 2, A and B). He therefore appeared to be partially GH resistant. During this period of GH withdrawal, spontaneous secretion of GH was studied in the morning and showed peaks of 7 and 10 ng/ml, and a 24-h urinary GH was 7 ng for a normal range of 1 to 7 ng. GH binding protein was normal (550 pmol/liter, for a normal range of 300 to 1400 pmol/liter). During the last 2 months without GH therapy, his growth velocity dropped under −2 SDS. GH was started again at 8.5 yr at its initial dose (0.4 mg/kg · wk), and an acceleration in growth was again observed, which then tapered over time (Fig. 2B). Body mass index and food intake remained insufficient, even during GH treatment periods (Fig. 2C). Bone age was at least 1 yr below normal even during GH therapy (Fig. 2A). At restart of the GH treatment (8.5 yr), an IGF-I generation test (GH injected at a dose of 0.057 mg/kg · d for 5 consecutive days, with serum IGF-I levels measured after an overnight fast before the first injection and 24 h after the fifth injection) was performed. The patient had a basal IGF-I level of 51 ng/ml, reaching only 82 ng/ml on d 5, showing an increase of only 1.6 when measured with the Immunotech kit (Table 1). Growth velocity increased, however, when treated with 0.4 mg/kg · wk GH. Patient serum was subjected to Western ligand-blot with recombinant labeled IGF-I and IGF-II as described previously (21). Results were normal, ruling out an abnormal IGFBP-3 binding capacity or an increase in IGFBP-3 proteolysis, excluding thereby an enhancement of IGF-I clearance (data not shown). IGF-I levels were monitored regularly during treatment, using the Immunotech kit. IGF-I levels remained low in contrast to IGFBP-3 and ALS levels, which were in the upper normal range or above. We therefore investigated a potential *IGF1* defect. Moreover, IGF-I levels varied between different immunoassays. Increased levels were observed at 10.6 yr using an immunoassay with a polyclonal antibody, with even higher levels observed during a

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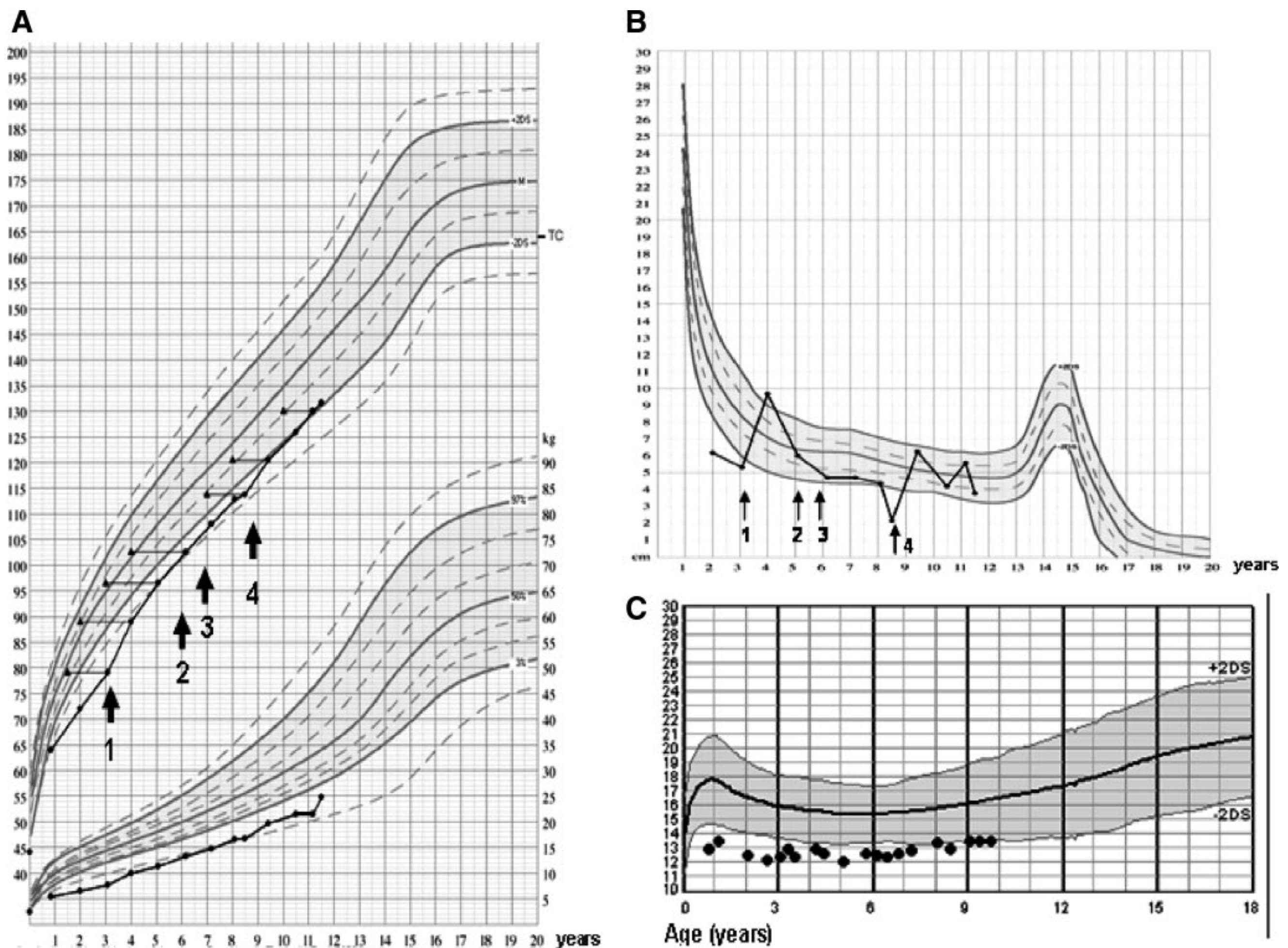

FIG. 2. Growth [weight and height (A)], growth velocity (B), and body mass index (C) curves between the ages of 1 and 11.5 yr. Growth curves and growth velocity are plotted on the Sempé reference charts (19) and body mass index on Roland Cacherat charts (22). The patient received three courses of GH therapy, as indicated by the *arrows*. *Arrow 1* indicates the start of GH treatment at 0.4 mg/kg · wk, *arrow 2* indicates the beginning of treatment with a reduced dose of GH (0.2 mg/kg · wk), *arrow 3* indicates the arrest of GH treatment, and *arrow 4* indicates the period during which GH treatment was returned to 0.4 mg/kg · wk.

period of increased food intake (Table 1). His body mass index increased from −2.3 SDS to −2 SDS at 8 yr, according to Rolland-Cachera *et al.* (22) (Fig. 2C). However, he remained very lean with very poorly developed fat tissue and had no biological signs of insulin resistance (Fig. 2 and Table 1). During the follow-up, he did not have any sign of pubertal development.

A hearing test at 9 yr was normal (audiogram including pure-tone audiometry, hearing and listening test, speech audiogram, ottoacoustic emissions, and acoustic impedance). His head circumference was 47.5 cm (−4.8 SDS), showing persistent microcephaly without dysmorphia (Fig. 1, B and C). Cerebral magnetic resonance imaging was repeated and showed normal myelinization and normal hippocampus. Blood phosphorus and calcium levels were normal, as were skeletal x-rays. Bone densitometry was normal for his height.

Patient psychomotor evaluation showed mild developmental delay. At 9 yr, he was not reading fluently and behaved like a 6 yr old. Wechsler Intelligence Scale for Children III testing showed a 70–75 development quotient.

Identification of a missense mutation in the C domain of IGF-I

Sequencing of the five *IGF1* coding exons and its cDNA in patient V1 revealed a previously unidentified homozygous mutation G>A in exon 4 (Fig. 3, A and B). This mutation was not identified in 50 controls. The predicted protein product would replace an arginine in position 36 of the C domain by a glutamine (R36Q-IGF-I). Intrafamilial segregation analysis for this mutation suggests autosomal recessive transmission (Fig. 1A). The affected amino acid is highly conserved, not only between species but also between IGF-I and IGF-II (Fig. 3C). The software program PolyPhen (http://genetics.bwh.harvard.edu/pph) predicted the R36Q mutation to be possibly damaging, suggesting that this substitution may affect protein function or structure.

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TABLE 1. Measurement of hormones and GH treatment

	Age (yr)	GH treatment (mg/kg · wk)	IGF-I kit Immunotech monoclonal (ng/ml)[a]	IGF-I, Cis Bio monoclonal (ng/ml)[b]	IGF-I, in-house polyclonal (ng/ml)[c]	IGFBP-3, DSL (μg/ml)[d]	ALS, DSL (μg/ml)[e]	IGF-II, DSL (ng/ml)[f]	Gly/INS (mmol/liter · μU/ml)[g]	Leptine (ng/ml)[h]
Start GH treatment at 3.2 yr	2.7	0	11		22					
	3.3	0.4	61							
	5.6	0.2	54			5.98	27.8		5/5.5	
	6.4	0.2	47		231		28.3		4/6.4	
Stop GH treatment at 7.2 yr	7.2	0.2	34		204	4.1	30.8	1042	Nd/4	
	7.5	0	12			3.7	23.2	917	3.5/1.2	0.7
	8.1	0	32		173	4.3				
Restart GH treatment at 8.5 yr:	8.5	0	51		175	3.3	15.5		3.5/4.5	1.3
On d 6 after 5 GH injections	8.5	0.4	82		286	4.1	22		4.6/5.9	
	8.75	0.4	166		275	4.1			4.1/6.7	
	9.14	0.4		Undetectable		3.2			3.8/4.6	1.8
	10.2	0.4		Undetectable	501	6.9			4.2/5.3	1.8
	10.6	0.4		Undetectable	619	5				
	10.8	0.4		Undetectable	509	6.7			4.8/7.8	1.6
	11.6	0.4			408	7.2			4.6/4.6	1.8

Gly, Glycemia; INS, insulin; DSL, Diagnostic Systems Laboratories; Nd, not determined.

[a] Normal values, 60–168 for 2–5 yr; 68–200 for 6–11 yr.

[b] Normal values for the age, 107–392.

[c] Normal values, 65–265 for 2–5 yr; 100–340 for 6–11 yr.

[d] Normal values, 1.1–3.7 for 2–5 yr; 1.65–4.44 for 6–11 yr.

[e] Normal values, 8.1–19.3 for 2–5 yr; 9–26 for 6–11 yr.

[f] Normal values for the age, 400–1100.

[g] Normal fasting values for insulin, <10.

[h] Leptine levels are low.

Affinity of R36Q-IGF-I for rhIGFBP-3

The affinity of R36Q-IGF-I for rhIGFBP-3 was first analyzed in a competition ELISA test using SeAP-IGF-I as a tracer. We did not find any differences between the binding affinities of wt-IGF-I, V44M-IGF-I, and R36Q-IGF-I for rhIGFBP-3 (Fig. 4A). A second set of experiments based on BIAcore was carried out, allowing apparent K_D values of 0.2 and 0.55 nM to be calculated for the R36Q-IGF-I and V44M-IGF-I mutants, respectively (Fig. 4B). Thus, the affinity of R36Q-IGF-I for IGFBP-3 is consistent with a recent study of wt-IGF-I (K_D = 0.23 nM) (23), and that of V44M-IGF-I is similar to that reported by Denley *et al.* (24) (K_D = 0.75 nM) for this IGF-I mutant.

Cell proliferation assays

We then assessed the ability of the R36Q-IGF-I mutant to induce cell proliferation in serum-starved BP-A31 cells by monitoring the incorporation of ^{3}H-thymidine after stimulation with varying concentrations of either wt or mutated IGF-I peptides (Fig. 5A). Whereas wt-IGF-I concentrations as low as 0.1 nM were sufficient to stimulate ^{3}H-thymidine incorporation, concentrations of R36Q-IGF-I two times higher are required to produce the same effect. However, the ineffective IGF-I mutant V44M-IGF-I, used as a control (24), required more than 30-fold higher concentrations (30 nM) to induce a similar response (Fig. 5A).

Binding to sIGF-1R

To test the binding of R36Q-IGF-I to IGF-1R, we used sIGF-1R. We first measured binding affinities for sIGF-1R using ^{125}I-IGF-I as a tracer and wt or mutant IGF-I as competitors. Figure 5B shows the competition binding curves of the three recombinant IGF-I peptides. As expected, the V44M mutation significantly impairs IGF-I binding to this receptor (86 times lower), whereas the R36Q-IGF-I mutant peptide has only a moderately lower affinity for the receptor than wt (3.9 times). The kinetic parameters calculated with Scatchard plot gave apparent K_D values of 2.8 and 62.5 nM for the R36Q-IGF-I and V44M-IGF-I mutants, respectively (Fig. 5C).

Phosphorylation of IGF-1R and IRS-1 by R36Q-IGF-I

We analyzed the effect of the R36Q mutation on IGF-I signal transduction by monitoring the phosphorylation of IGF-1R and IRS-1 in MCF-7 cells. Activation of the receptor can be seen after a 10-min stimulation with increasing doses of wt and R36Q-IGF-I. However, R36Q-IGF-I was less effective than wt-IGF-I in phosphorylating the receptor and IRS-1, suggesting that signal transduction by the mutant is impaired. As expected, V44M-IGF-I was drastically less efficient to induce phosphorylation of IGF-1R and IRS-1 (Fig. 5, D–F).

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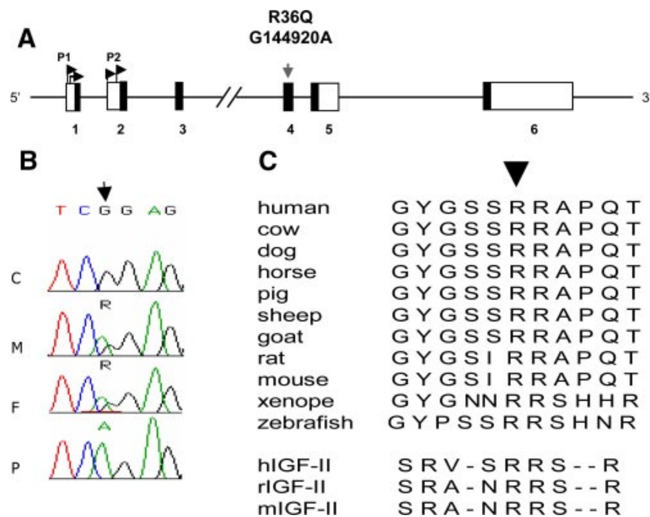


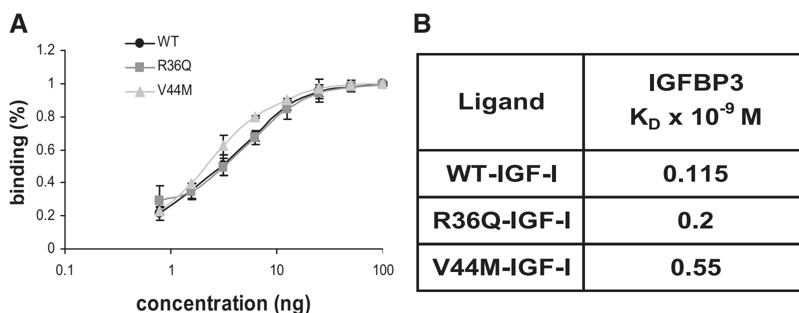
FIG. 3. A, Schematic representation of the *IGF1* gene. The *squares* indicate the six exons; *black squares* denote the coding sequence, and the *arrows* indicate the transcription start sites of the P1 and P2 promoters. The mutation G144920A (GenBank accession no. AC010202) is indicated by the *black arrow*. B, Sequence electrophoregram of *IGF1* exon 4 showing the mutation G>A (*arrow*), heterozygous in the parents (M, mother; F, father) and homozygous in the patient (P); C is control. C, Alignment of IGF-I and IGF-II C-peptides showing the conserved R36 (shown by *arrow*) between the two IGF peptides and between species; *dashes* are used for alignment of sequences with different numbers of amino acids.

Discussion

We describe a patient born to consanguineous parents with a homozygous mutation in the *IGF1* gene, resulting in partial primary deficiency of IGF-I activity. The patient had intrauterine growth restriction and progressive postnatal growth failure associated with normal hearing, microcephaly, and mild intellectual impairment. He had anorexia and was very lean. His IGF-I serum levels were markedly reduced, but, by contrast, two other GH-dependent components, IGFBP-3 and ALS, were in the upper normal range or above, and GH treatment led to an initial increase in growth rate. However, IGF-I serum concentrations differed depending upon the immunoassay used. Low IGF-I serum concentrations using a monoclonal antibody assay were found to be normal in a polyclonal antibody assay. Moreover, increased levels were observed when the polyclonal antibody assay was used to measure IGF-I during a period when the patient was receiving GH therapy and had improved food intake. GH deficiency was ruled out by dynamic tests and spontaneous peaks of GH. Twenty-four-hour urinary GH was in the upper range for the age. The patient's GH binding protein level was in the normal range. Serum samples were subjected to Western ligand-blot with recombinant-labeled IGFs. Results were normal, reflecting normal binding activity of patient's IGFBP-3 to wt-IGF-I, ruling out thereby an enhancement of IGF-I clearance. GH therapy was initiated for SGA. Growth rate was improved only by a high dose of GH therapy.

To date, three patients have been described with an *IGF1* molecular defect leading to complete or very severe IGF-I deficiency. The same combination of intrauterine and postnatal growth retardation associated with sensorineural deafness and severe intellectual deficit was reported for all three patients (7–9). The first two cases reported had undetectable or very low IGF-I serum levels (7, 8). The third patient had elevated IGF-I levels; this patient was carrying a homozygous missense mutation of the *IGF1* gene, resulting in bioinactive IGF-I (V44M-IGF-I), which binds normally to IGFBP-3 but has a significantly reduced affinity for its receptor, IGF-1R (9, 24). IGF-I signaling plays a key role in intrauterine development and postnatal growth and metabolism (25–27). *In utero*, IGF-I is necessary for normal intrauterine development of the brain and inner ear (28, 29). Knockout models have shown that the secretion of IGF-I *in utero* is not dependent on GH (30). However, during the postnatal period, IGF-I, IGFBP-3, and ALS secretion seem to be mainly controlled by GH. Secretion of these peptides also depends on nutritional status, explaining some of the variation in these components in our patient (31).

The discrepancy between the reduced IGF-I levels and IGFBP-3 and ALS serum levels in the upper normal range, measured using a monoclonal antibody assay, was compatible with an *IGF1* defect in our patient. We indeed identified an *IGF1* homozygous missense mutation in exon 4 of the gene encoding the C domain of IGF-I. The IGF-I C domain is highly conserved and is necessary for binding to the IGF-1R. Arg^{36} is conserved not only among species but also between IGF-I and IGF-II. The PolyPhen software predicts that the R36Q mutation may affect protein function or structure. Previous studies using site-directed mutagenesis have specifically identified residues



Ligand	IGFBP3 $K_D \times 10^{-9}$ M
WT-IGF-I	0.115
R36Q-IGF-I	0.2
V44M-IGF-I	0.55

FIG. 4. The affinity of R36Q-IGF-I for rhIGFBP-3. A, Competition curves for rhIGFBP-3 binding for SeAP IGF-I and wt-IGF-I (*black circle*), R36Q-IGF-I (*dark gray square*), and V44M-IGF-I (*light gray triangle*). rhIGFBP-3 was incubated with a constant concentration of SeAP IGF-I and increasing concentrations of each IGF-I. This experiment was repeated four times with similar results. B, Affinities of each IGF-I peptide as calculated by BIAcore.

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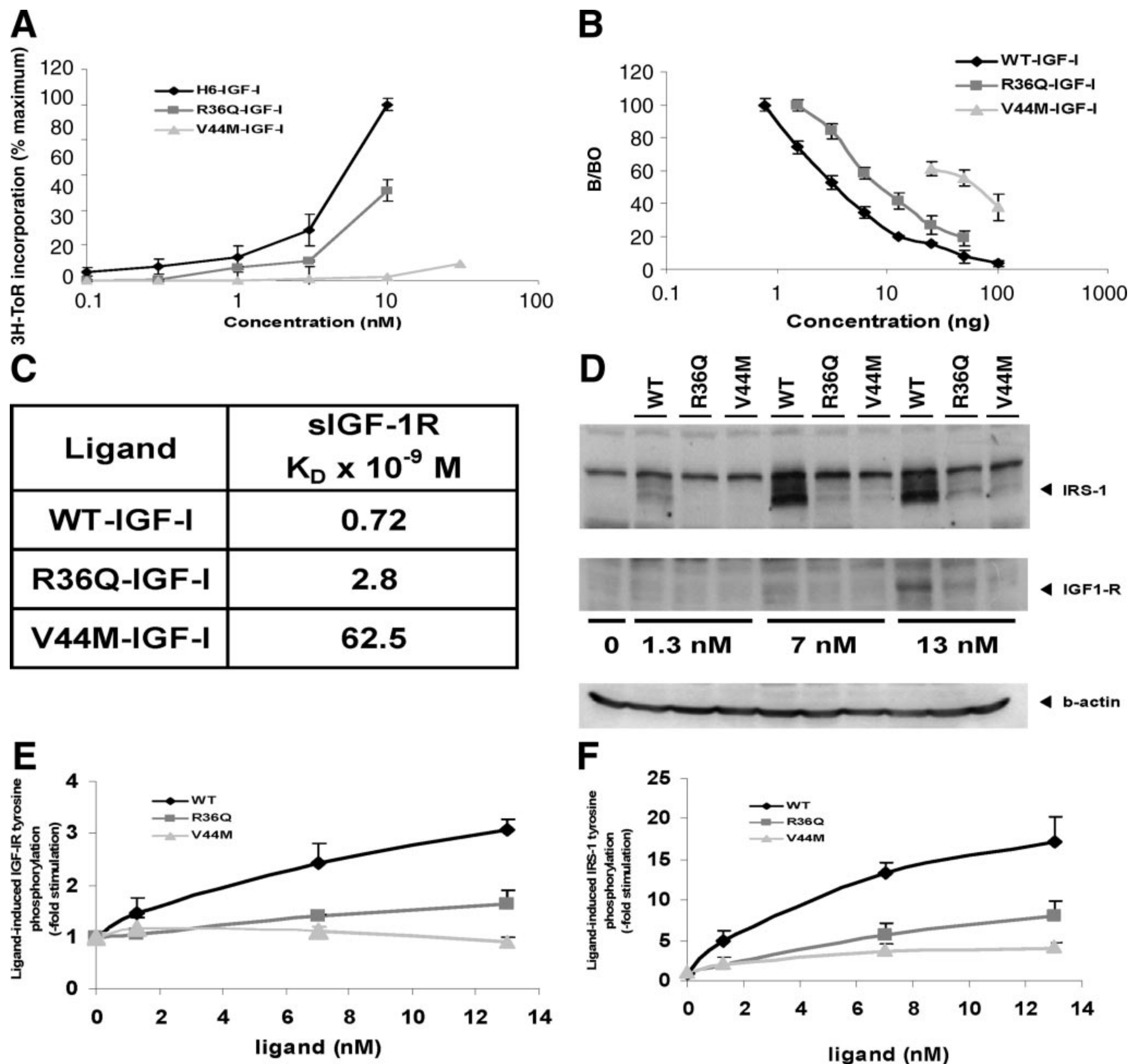

FIG. 5. The mitogenicity and affinity of R36Q-IGF-I for IGF-1R. A, Induction of cell growth by R36Q-IGF-I. The incorporation of ^{3}H-thymidine by murine BP-A31 fibroblast cells was measured after incubation with increasing amounts of wt-IGF-I (*black circle*), R36Q-IGF-I (*dark gray square*), and V44M-IGF-I (*light gray triangle*). Induction of cell growth by wt-IGF-I is observed at 0.1 nM, whereas a higher concentration is required for R36Q-IGF-I and an even higher concentration for V44M-IGF-I (30 nM). Similar data were obtained in three separate experiments; R36Q-IGF-I is therefore less efficient (2-fold) than wt-IGF-I in inducing cell growth; however, its activity is not as profoundly affected as that of the V44M-IGF-I (>30 fold). B, The affinity of R36Q-IGF-I for IGF-1R soluble receptor. Competition curves for wt-IGF-I (*black circle*), R36Q-IGF-I (*dark gray square*), and V44M-IGF-I (*light gray triangle*) binding to the sIGF-1R in the presence of ^{125}I-IGF-I. Different concentrations of the three recombinant IGF-I peptides were incubated with 1.25 μg sIGF-1R in 0.1 M phosphate buffer in the presence of 3000 cpm of ^{125}I-IGF-I, added as tracer. Similar data were obtained in four separate experiments. C, Binding kinetics were determined using Scatchard plot. V44M-IGF-I binding to sIGF-1R (62.5 nM) was more severely impaired than R36Q-IGF-I (2.8 nM), which in turn had a lower binding affinity than wt-IGF-I (0.72 nM). Thus, in order of decreasing binding affinity for sIGF-1R, wt-IGF-I > R36Q-IGF-I > V44M-IGF-I. D, Activation of the IGF-I signaling pathway by R36Q-IGF-I. Cells were incubated with or without different concentrations of wt-IGF-I, R36Q-IGF-I, or V44M-IGF-I for 10 min. Proteins from lysed cells were separated by SDS-PAGE, transferred to polyvinylidene difluoride sheets, and immunoblotted with antiphosphotyrosine antibody as described in Supplemental Data. β-Actin was used as a loading control. The results shown represent a typical experiment (n = 3); the curves represent quantitative analysis of the relative ligand-induced IGF-IR (E) and IRS-1 (F) tyrosine phosphorylation, corrected for background and corresponding to the mean ± SEM for three independent experiments. R36Q-IGF-I phosphorylates the receptor and IRS-1 less efficiently than wt-IGF-I (1.8- and 2-fold, respectively), suggesting that the mutant-induced signal transduction is impaired. As expected, V44M-IGF-I was drastically less efficient to induce phosphorylation of IGF-1R and IRS-1.

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arginine 36 and 37 in the IGF-I C domain as being important for IGF-1R binding. By contrast, deletion of the entire IGF-I C domain and replacement with four glycines, as well as alanine scanning mutagenesis across the C domain, have not shown a role for the C domain in IGFBP binding (32–34). The C region was predicted to encompass two β-turns between residues 27 and 32. Moreover, Arg^{36} in this region may form an ion-pair interaction with Asp^{45}. As a result of the β-turns and the Arg^{36}-Asp^{45} interaction, the side chain of Tyr^{31} is brought close to the Phe^{23}-Tyr^{24}-Phe^{25} region. This is important for binding to the IGF-1R (35). A change from arginine to glutamine at position 36 is therefore predicted to be disruptive, affecting the functionality of the C domain. This could thus distort the epitopes recognized by certain monoclonal antibodies, explaining the discrepancy in IGF-I serum levels between assays using different monoclonal or polyclonal antibodies. The substitution of arginine residues 36 and 37 with alanine in the IGF-I C domain decreases the affinity for IGF-1R by a factor of 5 to 15, depending on the assay used (36, 37). Here we showed that the substitution of only arginine 36 by a glutamine in a modified recombinant IGF-I peptide decreases IGF-1R affinity by a factor of about 2 in the different assays performed. By contrast, substitution of the valine in position 44 of the A domain by a methionine in control experiments substantially impaired IGF-1R binding, consistent with previous findings. R36Q-IGF-I also induces cell growth less efficiently than wt-IGF-I; however, its activity is not as profoundly affected as that of V44M-IGF-I. Both recombinant modified IGF-I peptides had normal binding to IGFBP-3, as expected. This may also be the case for other IGFBPs.

This report demonstrates that even partial IGF-I deficiency has marked effects on fetal and postnatal growth, brain development, and cognitive functions. However, consistent with the phenotype of patients with IGF-1R mutations (38), partial deficiency of IGF-I signaling is sufficient for normal development of the inner ear. This patient's growth failure is due to partial primary deficiency of IGF-I activity leading to partial secondary GH insensitivity. The complexity of this case illustrates the importance of elucidating the clinical spectrum of the different causes of growth failure, for GH therapy to be adapted to individual cases, potentially with the introduction of, or replacement by, IGF-I therapy. Recombinant GH has been used for over 30 yr. However, depending on the underlying cause of growth failure, the doses required for effective treatment can vary. Recombinant IGF-I has recently become available for treatment of primary IGF-I deficiency. Patients with different molecular defects may thus benefit from more effective treatment with either GH or IGF-I (39). Although our patient had an IGF-I primary molecular defect, his growth velocity increased under GH therapy when treated with a sufficient dose. To date, no signs of insulin resistance have been observed in our patient, even at a relatively high dose of GH. However, the replacement of GH therapy by IGF-I treatment, or a combination of the two treatments, may improve growth velocity. Such treatment has not yet been prescribed for our patient because of the risk of hypoglycemia in this very lean boy with anorexia. Partial IGF-I loss of activity leads to a milder phenotype than complete IGF-I deficiency. Therefore, such molecular defects may be not uncommon in patients born SGA with microcephaly who respond poorly to standard doses of GH. IGF-I serum levels should thus be measured in these patients using at least two different immunoassays, one with a monoclonal and another with a polyclonal antibody. Similarly, the measurement of IGFBP-3 and, if possible, ALS serum levels could help to detect such patients. Nutritional status can also significantly affect the results of IGF-I assays. Indeed, even when using an assay with a polyclonal antibody, IGF-I serum levels in our patient were only significantly elevated when his body mass index and food intake had improved. However, even during his most anorectic period during GH treatment, there was a discrepancy between the IGF-I serum levels and IGFBP-3 and ALS serum levels in the upper normal range. This demonstrates the benefit of an extensive analysis of the IGF system to detect such patients and broaden the phenotypic spectrum associated with *IGF1* mutations.

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