

Brief Report

**INTRAUTERINE GROWTH
RETARDATION AND POSTNATAL
GROWTH FAILURE ASSOCIATED WITH
DELETION OF THE INSULIN-LIKE
GROWTH FACTOR I GENE**

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INSULIN-LIKE growth factor I (IGF-I) mediates the majority of the growth-promoting effects of growth hormone (GH) after birth.¹ In the prenatal period, GH does not appear to have a major influence on fetal growth, whereas IGF-I does. Infants with congenital GH deficiency and defects in the GH-receptor gene have only mild retardation of growth at birth,²⁻⁴ whereas transgenic mice with a homozygous defect of the *IGF-I* gene (IGF-I knockout mice) have profound embryonic and postnatal growth retardation.⁵⁻⁷ Although there is no direct evidence that IGF-I has a prominent role in human fetal growth, fetal tissues express IGF-I from an early stage, and fetal and cord serum IGF-I concentrations are correlated with fetal size.⁸⁻¹¹

IGF-I knockout mice also have defects in neurologic development, indicating that IGF-I may have specific roles in axonal growth and myelination.¹² In addition, neonatal mortality is substantial, suggesting that this defect may be lethal in humans.

In this report, we describe a 15-year-old boy with severe prenatal and postnatal growth failure, sensorineural deafness, and mental retardation who had a homozygous partial deletion of the *IGF-I* gene.

CASE REPORT

The patient had been born at 37 weeks' gestation by cesarean section. The operation was performed because of poor fetal growth, first noted the preceding week. The pregnancy had until then been uneventful. At birth, the infant had symmetric growth retardation, with a weight of 1.4 kg (3.9 SD below the mean in normal subjects), a length of 37.8 cm (5.4 SD below the mean),

and a head circumference of 27 cm (4.9 SD below the mean).¹³ The placental weight was 350 g (1.3 SD below the mean).¹⁴ The infant required nasogastric-tube feeding for three weeks. The highest serum bilirubin concentration was 12.9 mg per deciliter (221 μ mol per liter), and the lowest plasma glucose concentration was 40 mg per deciliter (2.2 mmol per liter). At four weeks, he was well and was sent home.

Throughout infancy and childhood, severe growth failure continued (Fig. 1). Poor responses to sound were noted, and audiograms showed profound bilateral sensorineural deafness (auditory threshold, 90 dB). The patient had moderately delayed motor development and behavioral difficulties, with hyperactivity and a short attention span.

Studies performed at the age of eight years showed a normal male karyotype, normal thyroid function, a basal serum GH concentration of 18 ng per milliliter, and a peak serum GH concentration of 94 ng per milliliter after the administration of clonidine (both values are elevated). From the age of 11 to 12.7 years, the patient was treated with recombinant human GH in a dose of 22 U per square meter of body-surface area per week, which had no effect on his growth rate (height velocity before and during treatment, 2.5 and 2.2 cm per year, respectively) (Fig. 1). At the age of 14 years, his serum IGF-I concentration was 0.05 U per milliliter (normal range for his age, 0.48 to 2.8). He was referred to our unit with a possible diagnosis of GH insensitivity.

At 15.8 years, the boy's height was 119.1 cm (6.9 SD below the mean) and his weight was 23.0 kg (6.5 SD below the mean). The body-mass index (the weight in kilograms divided by the square of the height in meters) was 16.2 (1.9 SD below the mean), the ratio of the upper to the lower segment was 1.07 (normal mean value at 15 years, 0.98) and the triceps skin-fold thickness was 6.0 cm (0.9 SD below the mean); the subscapular skin-fold thickness was normal (7.6 cm). He had microcephaly (head circumference, 47 cm) and mild dysmorphism, with micrognathia, bilateral ptosis, and a low hairline (Fig. 1). There was bilateral clinodactyly (an incurved fifth finger) and a single palmar crease in the left hand. A neurologic examination showed severe bilateral hearing loss and mild myopia. He had normal-size genitalia and was in early puberty, with stage 2 genitalia and stage 1 pubic hair; the testicular volume was 4 ml bilaterally. Cardiovascular, respiratory, and abdominal examinations were normal.

The boy's parents were first cousins once removed. His mother was 154 cm tall (1.4 SD below the mean), and his father was 163 cm (1.8 SD below the mean). He had one sibling, a 10-year-old sister, who was 130 cm tall (1.0 SD below the mean). There was no family history of miscarriage or neonatal death.

METHODS

We performed clinical and molecular studies after obtaining the approval of the hospital ethics committee and the informed consent of the boy's parents.

Endocrine Studies

Bone age was determined with the use of the Tanner-Whitehouse-2 RUS (radius, ulna, and small bones) method,¹⁵ and the bone mineral density at the lumbar spine was determined by dual-energy x-ray absorptiometry. An IGF-I generation test and other endocrine tests were performed as previously described.^{4,16}

Assays

Serum GH and insulin-like growth factor-binding protein 3 (IGFBP-3) were measured by immunoradiometric assay. Serum IGF-I was measured by radioimmunoassay,¹⁷ and undetectable values were confirmed by immunoradiometric assay (Diagnostic Systems Laboratories, Webster, Tex.). Serum IGF-II was measured by radioimmunoassay.¹⁸ Serum IGF-binding proteins were characterized by Western blotting,¹⁹ and the acid-labile subunit was determined by immunoblotting with the use of a polyclonal antibody (kindly provided by Dr. P. Lee, Diagnostic Systems Laboratories).

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Serum GH-binding protein was measured by high-performance liquid chromatography.²⁰ The mean (\pm SE) degree of specific binding of GH to the binding protein of the reference serum sample was 22.9 ± 2.1 percent (11 determinations). All other hormones were measured by standard radioimmunoassay procedures.

Molecular Studies

DNA was obtained from peripheral-blood lymphocytes from the patient and his family. The segregation of the polymorphic dinucleotide-repeat marker D12S346 (located approximately 8 cM from the locus of the *IGF-I* gene²¹) was determined in the patient and his family with the use of the polymerase chain reaction (PCR) and end labeling of one primer.

Exons 3, 4, 5, and 6 of the *IGF-I* gene from the patient were individually amplified by PCR. A skin-biopsy specimen was obtained from the patient, and fibroblasts were grown in culture. RNA was isolated from the patient's fibroblasts, his parents' peripheral-blood lymphocytes, and normal human liver by a single-step method (RNAzol B, Biogenesis, Bournemouth, United Kingdom).²² Complementary DNA (cDNA) from the patient was obtained by reverse transcription, and *IGF-I* exons 3, 4, 5, and 6 were amplified with the use of PCR as previously described,²² subcloned into pGEM-T (Promega, Southampton, United Kingdom), and then sequenced (Amersham, Amersham, United King-

dom). The sequences of the oligonucleotide primers are available elsewhere.*

RESULTS

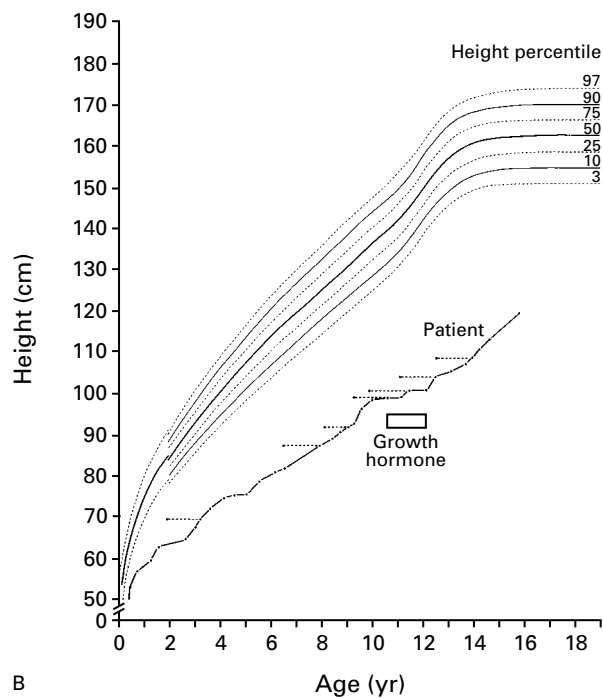
Endocrine Studies

The patient's bone age was 13.5 years (delay in growth, 1.8 years). There was severe osteopenia of the lumbar region (L2–L4 bone mineral density, 4.78 SD below the mean value for age-matched normal subjects). The results of laboratory tests are shown in Table 1. Serum GH concentrations, measured every 20 minutes from 8 p.m. to 8 a.m., ranged from 2.2 to 171 ng per milliliter (peak value in nor-

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A



B

Figure 1. Retarded Growth of a Patient with a Deletion of the *IGF-I* Gene.

Panel A shows microcephaly and micrognathia. The growth chart (Panel B) shows poor growth throughout infancy and childhood. There was no response to therapy with human growth hormone, which was administered from the age of 11 to 12.7 years (bar). The horizontal dotted lines indicate the degree of delay in bone age.

mal subjects, >10 ng per milliliter) (Fig. 2). The values for the serum acid-labile subunit and IGF-binding protein were normal (data not shown). Serum IGF-I, IGF-II, and IGFBP-3 values in the parents are shown in Table 2.

Neurologic Studies

Magnetic resonance imaging of the brain showed slightly enlarged occipital horns of the lateral ventricles but no other abnormalities, with an adult pattern of myelination. Electrophysiologic studies showed normal conduction times for visual evoked, somatosensory, and motor potentials.

Molecular Studies

The patient was homozygous for the D12S346 polymorphism, whereas his parents and sister were heterozygous at this locus — findings consistent with the role of the *IGF-I* gene in the patient's condition. Exons 4 and 5 of the patient's *IGF-I* gene were not amplified in repeated PCR studies (Fig. 3), which is consistent with the deletion of these exons. Skin fibroblasts from the patient had a reverse-transcriptase PCR product that was 181 bp shorter than in normal subjects (Fig. 4). Sequencing of this product showed that *IGF-I* exon 3 continued directly into exon 6, confirming the absence of exons 4 and 5 from the *IGF-I* cDNA (data not shown). This deletion would result in a mature IGF-I peptide truncated from 70 to 25 amino acids, followed by an additional out-of-frame nonsense sequence of eight residues and a premature stop codon. Reverse-transcriptase PCR of cDNA from both parents yielded both the abnormal product and a product of the expected size.

DISCUSSION

Our patient had a homozygous partial deletion of the *IGF-I* gene. The main manifestation of the severe IGF-I deficiency was extreme growth failure in utero that persisted after birth. The patient also had profound sensorineural deafness and mental retardation.

Defects in the *GH* gene, the gene for the pituitary transcription factor Pit-1, and the gene for the GH-releasing-hormone receptor have all been reported to cause severe congenital GH deficiency associated with predominantly postnatal growth failure.^{2,4,23} In contrast, this patient had severe intrauterine growth retardation, providing direct evidence that IGF-I plays a critical part in human fetal growth, independently of GH. Transgenic mice with a homozygous deletion of the *IGF-I* gene have prenatal growth failure (noted from embryonic day 13.5) and a birth weight that is 60 percent of normal.^{5,6} After birth, the patient grew poorly, as do the transgenic mice, confirming the central role of IGF-I in postnatal growth, as suggested in the somatomedin hypothesis, which proposes that many of the actions of GH are derived from hepatic IGF-I.¹

TABLE 1. RESULTS OF LABORATORY STUDIES IN A PATIENT WITH A PARTIAL DELETION OF THE *IGF-I* GENE.*

VARIABLE	VALUE	NORMAL RANGE
Serum glucose in the fasting state (mg/dl)	86	70–110
Serum thyrotropin (μ U/ml)	2.5	0.3–5.0
Serum prolactin (ng/ml)	19	<20
Serum cortisol at 9 a.m. (μ g/dl)	14.1	4–19
Serum dehydroepiandrosterone sulfate (ng/ml)	2400	Before puberty, <100–600; after puberty, 2000–3350
Serum testosterone (ng/dl)†		
Basal	120	Before puberty, <60;
On day 3	300	after puberty, 400–
On day 5	380	800
Serum luteinizing hormone (mU/ml)‡		
Basal	2.2	After puberty, 3–25
At 20 min	27.4	(basal)
At 60 min	24.1	
Serum follicle-stimulating hormone (mU/ml)‡		
Basal	2.6	After puberty, 1–10
At 20 min	6.3	(basal)
At 60 min	4.7	
Serum growth hormone (ng/ml)§		
Basal	2.2	>10 (stimulated)
Peak	61 (at 60 min)	
Serum cortisol (μ g/dl)§		
Basal	5.4	>20 (stimulated)
Peak	35.7 (at 120 min)	
Serum IGF-I (ng/ml)¶		
Basal	Undetectable (<2)	After puberty, 100–494
On day 5	Undetectable (<2)	(basal)
Serum IGFBP-3 (mg/liter)¶		
Basal	3.3	Age 13–15 yr, 2.3–5.2
On day 5	3.3	(basal)
Basal serum IGF-II (μ g/liter)	1430	1010 (in pooled serum)
Basal serum GHBP (%)	18.7	14–42

*IGF denotes insulin-like growth factor, IGFBP-3 insulin-like growth factor-binding protein 3, and GHBP growth hormone-binding protein. To convert the values for glucose to millimoles per liter, multiply by 0.05551; to convert the values for cortisol to nanomoles per liter, multiply by 27.59; to convert the values for dehydroepiandrosterone sulfate to micromoles per liter, multiply by 0.002714; and to convert the values for testosterone to nanomoles per liter, multiply by 346.7.

†Serum testosterone was measured after 2000 U of chorionic gonadotropin had been administered intramuscularly. Normal values are for male subjects.

‡Serum luteinizing and follicle-stimulating hormones were measured after 100 μ g of gonadotropin-releasing hormone had been administered intravenously.

§Serum growth hormone and cortisol were measured after 0.15 U of insulin per kilogram of body weight had been administered intravenously.

¶Serum IGF-I and IGFBP-3 were measured after the subcutaneous administration of 0.1 U of growth hormone per kilogram daily for four days.

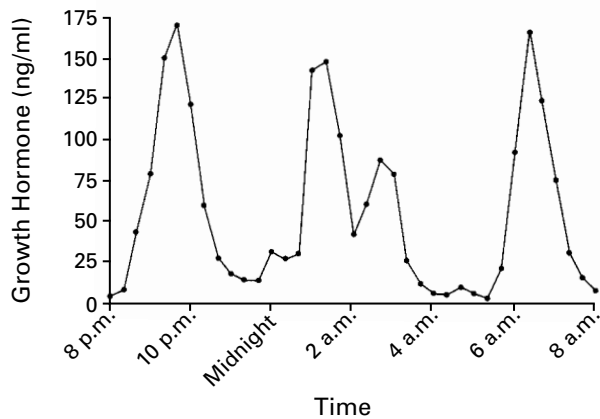


Figure 2. Serum Growth Hormone Concentrations in the Patient from 8 p.m. to 8 a.m., Showing Abnormally High Peaks and an Absence of Undetectable Values between Peaks.

TABLE 2. HEIGHT AND LABORATORY VALUES IN MEMBERS OF THE PATIENT'S FAMILY AND IN NORMAL SUBJECTS.*

SUBJECT	HEIGHT†	SERUM IGF-I	SERUM IGF-II	SERUM IGFBP-3
	SD	ng/ml	μg/liter	mg/liter
Family member				
Father	-1.8	118	1200	3.0
Mother	-1.4	101	1112	3.3
Sister	-1.0	129	1198	3.9
Normal subjects		Adults, 126-369; chil- dren (9-11 yr), 50-280	1010 (in pooled serum)	Adults, 1.7-5.6; chil- dren (9-11 yr), 2.1-3.0

*IGF denotes insulin-like growth factor, and IGFBP-3 insulin-like growth factor-binding protein 3.

†Values are numbers of standard deviations of the mean for normal subjects.

Although the pattern of growth failure in this patient appears to be very similar to that in the IGF-I knockout mice, there are a number of phenotypic differences. At the patient's birth, the weight of the placenta was below average, whereas the placental size is normal with the IGF-I knockout mice. Furthermore, the IGF-I knockout mice have very small reproductive organs and are infertile in adulthood, whereas our patient has normal-size genitalia and is progressing through puberty, albeit rather late.

A comparison of our patient with patients who have GH insensitivity (Laron dwarfism), who also have high serum GH concentrations and low serum IGF-I concentrations, provides valuable information on the effects of GH that are not mediated by IGF-I. The patient's serum IGFBP-3 and acid-labile subunit concentrations were normal — findings in

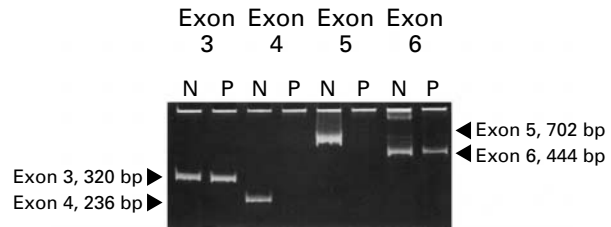


Figure 3. PCR Analysis of Exons 3, 4, 5, and 6 of the *IGF-I* Gene in the Patient (P) and a Normal Subject (N), Showing an Absence of Amplification of Exons 4 and 5 in the Patient.

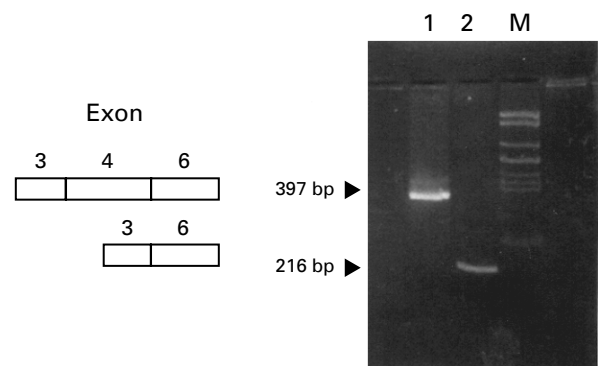


Figure 4. Amplification of *IGF-I* cDNA in the Patient, Showing the Absence of Exons 4 and 5.

IGF-I cDNA from the patient's skin fibroblasts and normal liver was amplified by PCR. Normally, exon 5 or exon 6 is included in the *IGF-I* RNA transcript. We examined the exon 3-4-6 splice variant, using one primer spanning the junction of exons 2 and 3 and another in exon 6. The results demonstrate the expected product with 397 bp in the normal tissue (lane 1) and a shorter product (216 bp) in the fibroblasts from the patient (lane 2). M denotes molecular-weight marker.

support of recent studies showing that these peptides are controlled independently of IGF-I in humans.²⁴⁻²⁷ Patients with GH insensitivity are sensitive to insulin and have episodes of hypoglycemia, especially during infancy.²⁸ Our patient has not had hypoglycemia, perhaps because of a resistance to insulin resulting from excess GH secretion. In addition, his bone age has been only minimally delayed, supporting the hypothesis that GH stimulates bone maturation directly.²⁹

Deafness, mental retardation, and microcephaly are not features of congenital GH deficiency or insensitivity.²⁸ Studies in both IGF-I knockout mice and mice with an overexpression of the *IGF-I* gene suggest that IGF-I has a role in the central nervous system.^{7,30} IGF-I knockout mice have small brains, hypomyelination, and loss of certain subtypes of neurons.⁷

Our patient appeared to have normal myelination, but his neurologic abnormalities may indicate that prenatal IGF-I is important in other aspects of central nervous system development in humans.

An intriguing finding was the short stature of the patient's parents and their borderline low serum IGF-I concentrations. The finding of a heterozygous effect of the *IGF-I* gene would indicate that this genotype could be prevalent in the population of children with idiopathic short stature, as has been suggested for heterozygous defects of the GH receptor.³¹

In summary, we have described a child with a homozygous defect of the *IGF-I* gene. This defect was associated with growth failure before and after birth, indicating that IGF-I is critical for prenatal as well as postnatal growth. In addition, the patient's neurologic development was abnormal, suggesting the role of IGF-I in the development of the central nervous system.

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