IGF-I Receptor Mutations Resulting in Intrauterine and Postnatal Growth Retardation

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BACKGROUND
Approximately 10 percent of infants with intrauterine growth retardation remain small, and the causes of their growth deficits are often unclear. We postulated that mutations in the gene for the insulin-like growth factor I receptor (IGF-IR) might underlie some cases of prenatal and postnatal growth failure.

METHODS
We screened two groups of children for abnormalities in the IGF-IR gene. In one group of 42 patients with unexplained intrauterine growth retardation and subsequent short stature, we used single-strand conformation polymorphism analysis, followed by direct DNA sequencing of any abnormalities found. A second cohort consisted of 50 children with short stature who had elevated circulating IGF-I concentrations. Complete sequencing of the IGF-IR gene was performed with DNA from nine children. We also studied a control group of 43 children with normal birth weights.

RESULTS
In the first cohort, we identified one girl who was a compound heterozygote for point mutations in exon 2 of the IGF-IR gene that altered the amino acid sequence to Arg108Gln in one allele and Lys115Asn in the other. Fibroblasts cultured from the patient had decreased IGF-I–receptor function, as compared with that in control fibroblasts. No such mutations were found in the 43 controls. In the second group, we identified one boy with a nonsense mutation (Arg59stop) that reduced the number of IGF-I receptors on fibroblasts. Both children had intrauterine growth retardation and poor postnatal growth.

CONCLUSIONS
Mutations in the IGF-IR gene that lead to abnormalities in the function or number of IGF-I receptors may also retard intrauterine and subsequent growth in humans.
Insulin-like growth factors 1 (IGF-I) and II (IGF-II) are major regulators of somatic growth and cellular proliferation and act through a common receptor, the type I IGF receptor. The gene for the IGF-I receptor (IGF-IR) is homologous to the insulin receptor gene in terms of both exon and intron organization and its amino acid sequence (more than 50 percent identical). Both encode precursor proteins that undergo post-translational modification to yield receptors composed of two α and two β subunits. The α subunits are extracellular, containing ligand-binding domains. The β subunits contain intracellular tyrosine kinase domains.

More than 50 mutations of the human insulin receptor gene have been described, and the loss of cellular insulin signaling caused by these mutations results in various degrees of carbohydrate intolerance. By contrast, defects in the closely related IGF-I gene have been suggested only from indirect evidence. Since deletion of the murine IGF-IR gene causes marked prenatal growth failure (birth weight, 45 percent of normal weight), with the affected neonates dying from respiratory depression, the complete absence of IGF-I receptors in humans would be expected to cause severe disease and perhaps be lethal. However, less severe perturbations might attenuate the phenotype, as do naturally occurring missense mutations in the insulin receptor gene that cause moderate insulin resistance. We describe two children with fetal and postnatal growth failure caused by defects in the IGF-IR gene.

**Methods**

**Study Population**

Between 1998 and 2002, we evaluated two groups of children with short stature for abnormalities of the IGF-IR gene. The first group consisted of 42 children from the United States who had unexplained intrauterine growth retardation (birth weight more than 2 SD below the mean for gestational age) and persistent short stature (height more than 2 SD below the mean for age after 18 months of age). The children were recruited from pediatric endocrine clinics at participating centers. Children with circulating concentrations of IGF-I and insulin-like growth factor–binding protein 3 (IGFBP-3) that were below the normal reference range for age were excluded, as were children born to mothers with toxemia, severe hypertension, or cardiovascular disease and children with underlying chronic disease. Control subjects were 43 healthy children residing in Cincinnati whose birth weights were within 1 SD of the average for gestational age.

A second cohort of children with potential resistance to IGF was selected from the CrescNetR registry, which includes data on the growth of 150,000 children in Germany, 3000 of whom had a height that was more than 2.5 SD below the average for age. In this group, 50 children were found to have serum IGF-I concentrations that were more than 2 SD above the means for age and sex; DNA was obtained from 9 of these children, who were arbitrarily selected on the basis of their availability, for direct sequencing of the IGF-IR gene.

The study was conducted independently at the Division of Endocrinology, Cincinnati Children’s Hospital Medical Center, Cincinnati, and at the Hospital for Children and Adolescents, University of Leipzig, Leipzig, Germany. The study was approved by the institutional review board at each institution. Written informed consent was obtained from all those who underwent research procedures or from their parents or guardians.

**Single-Strand Conformation Polymorphism Analysis**

Exons 2 to 6 and 16 to 20 of the IGF-IR gene were selected for initial single-strand conformation polymorphism (SSCP) analysis because they encode most of the binding and catalytic domains of the IGF-I receptor. DNA was extracted from whole blood with the use of the Puregene DNA isolation kit (Gentra Systems). In a few cases, microwaved serum served as the DNA source. Samples were electrophoresed under two conditions (10 percent glycerol and 20 mM HEPEs) to improve sensitivity (described in Supplementary Appendix 1, available with the full text of this article at www.nejm.org).

**Cell Culture and IGF-I–Binding Studies**

Fibroblast cultures were initiated from skin-biopsy specimens obtained from the two children who were found to have IGF-IR mutations. Cells were routinely cultured at 37°C in Dulbecco’s modified essential medium with penicillin, streptomycin, and 10 percent fetal-calf serum. To overcome the effects of cell-surface–associated IGF-I-binding proteins, IGF-I binding was quantified in the presence of a high concentration of an IGF-I analogue that is bound by IGF-binding proteins but not by the IGF-I receptor (Ala36–Leu690-IGF-I, GroPep). The addition of this analogue saturates the IGF-binding proteins but leaves the IGF-I receptor free to interact with native IGF-I. Binding studies were per-
formed in triplicate on intact cells grown for five to seven days in 24-well plates (Falcon) without changing the medium, as previously described. After being washed with serum-free medium, the cells were incubated at 15°C with 30,000 cpm of [125I]IGF-I, 250 ng of Ala31Leu60-IGF-I per milliliter, and graded amounts of native IGF-I in 250 µl of HEPES binding buffer (100 mM HEPES, pH 7.8; 0.5 percent fatty-acid–free bovine serum albumin; 120 mM sodium chloride; 1.2 mM magnesium sulfate; 5 mM potassium chloride; 15 mM sodium acetate; and 10 mM dextrose). After 18 hours, the cells were washed and then solubilized in 1 N sodium hydroxide. Cell-associated radioactivity was measured with a gamma counter.

**PHOSPHORYLATION OF IGF-I RECEPTORS**

Serum-starved fibroblasts were exposed to graded amounts of IGF-I (Groppe), and the resultant receptor kinase activity was assessed by quantifying the phosphorylation of the IGF-I receptor β subunit according to previously described methods. Cell lysates were subjected to sodium dodecyl sulfate–polyacrylamide-gel electrophoresis and then to immunoblotting with an antibody for the phosphorylation of the IGF-I receptor at Tyr1131 (PY1158, BioSource International). Specific protein bands were identified with a chemiluminescent method (ECL, Perkin Elmer).

**ANALYSIS OF IGF-I RECEPTOR RNA**

A single-step method (RANzol, Invitrogen) was used to isolate total RNA from monolayers of skin fibroblasts from Patient 2 and from fibroblasts from three controls matched for age and sex (GM 05565, GM 00498, and GM 05381, Human Genetic Mutant Cell Repository, Coriell Institute of Medical Research, Camden, N.J.), and cultured under identical conditions. RNA was also obtained from peripheral-blood lymphocytes from the mother and a half sibling of Patient 2. IGF-IR complementary DNA (cDNA) was prepared by reverse transcription and amplified by means of the polymerase chain reaction (PCR) with use of the 5‘TCGACATCCGC-AACGATATC3’ forward primer and the 5‘CGAA-GATGACGAGGGCTAG3’ reverse primer. PCR products were cloned into pCR II-TOPO (Invitrogen), and inserts were confirmed by sequencing.

For restriction-site analysis, genomic DNA and cDNA, as well as the cloned mutant and wild-type alleles, were amplified by PCR with the use of the primers mentioned above. PCR products were digested with Ddel (New England Biolabs), and the resulting fragments were characterized by agarose-gel electrophoresis and staining with ethidium bromide.

**FLOW-CYTOMETRIC MEASUREMENT OF IGF-I RECEPTORS**

Fibroblasts from six-well plates were suspended in 0.5 mM EDTA buffer without trypsin, washed three times with phosphate-buffered saline containing 0.2 percent bovine serum albumin, stained with monoclonal phycoerythrin-conjugated antibody against the human IGF-I receptor (R&D Systems), and analyzed by means of flow cytometry (Epics XL, Coulter).

**RESULTS**

Two patients with mutations of the IGF-IR gene were identified among the 51 children with short stature who provided DNA for analysis (the U.S. and German cohorts combined).

**PATIENT 1**

**Case Report**

Patient 1 was the product of a nonconsanguineous union, born to a gravida 1, para 1 woman after 38 weeks of pregnancy complicated only by poor fetal growth. Her birth weight was 1.4 kg (3.5 SD below the mean for gestational age). Although she ingested up to 124 kcal per kilogram of body weight per day of high-calorie formulas, there was no catch-up in linear growth. Acquisition of gross and fine motor skills was mildly delayed. Her first tooth erupted at 14 months (average, 6 to 8). Her growth remained markedly retarded (Fig. 1), and at the age of 4½ years, she was enrolled in a research study evaluating growth hormone therapy in patients with short stature associated with intrauterine growth retardation. At that time, her serum IGF-I concentration was normal for her age (63 ng per milliliter) and her bone age was three years (2.2 SD below the mean for age, according to Greulich and Pyle18). In response to clonidine (0.15 mg per square meter of body-surface area), the serum concentration of growth hormone peaked at 51 ng per milliliter (7.5 SD above the normal mean).19 The mean overnight growth hormone concentration was 11.4 ng per milliliter (2.8 SD above the normal mean).19

The growth hormone concentration was decreased to 2.9 ng per milliliter with a single oral dose of diazepam (5 mg) and increased to 28.2 ng per milliliter with three doses of clonidine (0.15 mg per square meter of body-surface area), the response to clonidine (0.15 mg per square meter of body-surface area). The mean overnight growth hormone concentration was 11.4 ng per milliliter (2.8 SD above the normal mean).19

The child was treated with growth hormone according to the research protocol (0.375 mg per kilogram of body weight per week, given as three injections per week), and her growth rate increased (from 5.2 cm per year at base line to 7.2 cm per year...
during the first year), but at the age of six years and nine months, therapy was discontinued for two years in order to reassess her basal growth rate. Her average height velocity during this period was 3.6 cm per year (below the third percentile for her age), and the serum concentration of IGF-I at this time was 891 ng per milliliter (normal range, 123 to 330). Growth hormone therapy was resumed at a daily dose of 0.375 mg per kilogram per week, and her height velocity increased to 6.5 cm per year (75th percentile for her age). She had normal concentrations of 25- and 1,25-dihydroxyvitamin D (13 and 59.7 pg per milliliter, respectively). Her lumbar-spine bone mineral density (1.5 SD below average for the age of 12 years on dual-energy x-ray absorptiometry) was considered to be normal in relation to her stature.

The results of a physical examination at the age of 11 years were normal except for severe short stature. The patient’s arms and legs were symmetric and proportional. Her verbal IQ was 134, but her performance IQ was 89. She had rapid, pressured speech; an anxious affect; and psychomotor agitation. A psychiatric evaluation reported signs of a nonverbal learning disorder, with obsessive tendencies, excessive fantasy role playing, and social phobias. The rate of acquisition of secondary sexual characteristics was normal, with pubarche at the age of 10 years and 2 months, thelarche at the age of 10 years and 9 months, and menarche at the age of 12 years and 6 months. The patient reached a mature height of 134.1 cm (4.8 SD below the normal mean) at the age of 14 years. Her target height of 152 cm was calculated from the heights of her parents, each of whom was of below-average height (Fig. 1).

Characterization of the IGF-IR Gene

Patient 1 had an abnormal SSCP-band pattern for exon 2. DNA sequencing showed that this abnor-
ormality was due to two distinct single-base-pair substitutions in the codon for amino acid 108 (CGG→CAG) and the codon for amino acid 115 (AAA→AAC) of the mature protein (see Supplementary Appendix 1, available with the full text of this article at www.nejm.org). Analysis of parental DNA indicated that the mutation at position 108 was inherited from her father, and the mutation at position 115 from her mother. Thus, Patient 1 was a compound heterozygote for mutations in exon 2 of the IGF-IR gene. Exon 2 was normal on SSCP analysis in the other 41 children with intrauterine growth retardation and in the 43 controls in the U.S. cohort (data not shown).

The point mutations identified alter the amino acid sequence of the receptor, changing arginine to glutamine at position 108 and lysine to asparagine at position 115. Because of the heterotetrameric structure of the receptor, each of the two α subunits in the patient’s mature receptor will contain one of these mutations in various combinations (i.e., 108/108, 108/115, or 115/115).

**Effect of Mutations on the Function of IGF-I Receptors**

The amino acid substitutions found in the extracellular domain of the IGF-IR gene suggested a resultant functional deficit. Specific binding of IGF-I to IGF-I receptors on fibroblasts was reduced in Patient 1 as compared with the controls (P<0.05 by the Mann–Whitney U test) (Fig. 2A). Detailed binding studies involving graded amounts of IGF-I and Scatchard analysis with the use of a two-site model indicated that the IGF-I binding affinity was one third of that of the controls (Fig. 2B).

Receptor phosphorylation in response to IGF-I was assessed in order to determine the effect of reduced hormone binding on receptor signaling. IGF-I stimulated receptor tyrosine phosphorylation in a dose-dependent manner in fibroblasts from Patient 1 and control fibroblasts, but the patient’s fibroblasts had a marked decrease in sensitivity relative to that of controls (Fig. 3). In control fibroblasts, receptor phosphorylation was evident at an IGF-I concentration of 10^-9 M, with maximal phosphorylation at an IGF-I concentration of 10^-8 M. By contrast, stimulation was barely detectable at a concentration of 3×10^-9 M in fibroblasts from Patient 1 and increased substantially at concentrations above 10^-8 M (Fig. 3C). In fibroblasts from two control subjects, half-maximal phosphorylation occurred at IGF-I concentrations of approximately 1 and 3 nM, respectively, whereas half-maximal phosphorylation in cells from the patient required 80 nM of IGF-I (P<0.01 by Student’s t-test).

**IGF–IGFBP Axis**

The discovery of the defects in the IGF-IR gene led to a more detailed examination of IGF and IGF-binding proteins in Patient 1 (Table 1). Her basal circulating IGF-I concentration was high and increased during growth hormone therapy. Serum concentrations of IGFBP-3 and the acid-labile subunit were also elevated. Serum IGFBP-2 concentrations were low, and IGF-II concentrations were normal. The changes in IGF and IGF-binding protein concent-
Trations reflect supranormal secretion of growth hormone. Neither of the patient’s parents had increased concentrations of IGF-I or IGFBP-3.

**Patient 2**

**Case Report**

Patient 2 was born at term after an uneventful pregnancy. At birth, his weight was 2000 g (3.5 SD below the mean for gestational age), his length was 40 cm (5.8 SD below the mean for gestational age), and he had microcephaly (head circumference, 31.0 cm; 4.6 SD below the mean for gestational age). He did not have catch-up growth (Fig. 4), although he had a normal caloric intake and no evidence of gastrointestinal dysfunction. He was referred to the Hospital for Children and Adolescents, University of Leipzig, at the age of 14 months with extremely short stature (height, 3.8 SD below the mean for age). The microcephaly had persisted, and he had mild retardation of motor development and speech. His growth was monitored, and he was tested for growth hormone deficiency, but no specific treatment was given. Physical examination when the boy was five years of age showed, in addition to severe short stature, a receding hairline, bushy eyebrows, a broad nasal bridge, a broad and rounded nasal tip, a long and smooth philtrum, a thin upper lip, and a broad, everted, and fleshy lower lip (Fig. 4, inset). He also had short fingers (especially the thumbs), clinodactyly, wide-set nipples, and pectus excavatum.

Because of the child’s short stature and reduced growth rate, stimulated and spontaneous growth hormone secretion were assessed. The peak growth hormone responses after an infusion of arginine (0.5 g per kilogram) and insulin-induced hypoglycemia were 6.0 and 5.7 ng per milliliter, respectively (normal value, >7.0 for each), whereas the response of growth hormone to an infusion of growth hormone–releasing hormone (1 µg per kilogram) was 21.2 ng per milliliter (within the normal range) when he was three years old. The mean overnight growth hormone concentration was 1.9 and 2.5 ng per milliliter on two separate occasions (at the ages of three and five years). Serum IGF-I concentrations, measured on several occasions, ranged from 121 to 222 µg per liter (from 1.1 to 2.3 SD above the mean for age), whereas IGFBP-3 concentrations remained within normal limits (2.1 to 3.7 mg per liter; from 0.7 SD below to 1.8 SD above the mean for age). The boy’s radiographic bone age was retarded by 1 to 1.5 years. Secretory responses of thyrotropin, corticotropin, and prolactin to stimulation...
were normal, as were the results of a chromosome analysis, blood counts, electrolyte measurements, and tests of liver and kidney function.

**Characterization of the IGF-IR Gene**

Analysis of the IGF-IR gene revealed that Patient 2 was heterozygous for the point mutation CGA to TGA (Arg59stop) in exon 2 (see Supplementary Appendix 2, available with the full text of this article at www.nejm.org). His mother and a half sibling were also heterozygous for this mutation and were small for gestational age at birth (Fig. 4). The mother was short (a height that was 2.6 SD below the normal mean) as an adult but not dysmorphic. No other relatives were tested for the mutation. Sequencing of all exons encoding the IGF-I receptor showed no other mutations, ruling out the possibility of compound heterozygous mutations involving both alleles. Exon 2 is the first exon to encode a substantial portion of the mature receptor; therefore, no viable receptor protein would be expected as a result of the mutant allele. No IGF-IR mutations were found in DNA from the other eight German children with elevated IGF-I concentrations who were evaluated.

**Effect of Mutations in IGF-I Receptors**

Normal transcription of the mutant IGF-IR allele was anticipated and verified by restriction-endonuclease analysis of IGF-IR cDNA from Patient 2 and his mother (Fig. 5A). However, flow-cytometric data indicated that the number of IGF-I receptors per fibroblast was lower in the patient than in the control subjects. Intact cells labeled with a monoclonal antibody against human IGF-I receptor were quantified by flow-cytometric analysis. As compared with three control subjects, Patient 2 had significantly fewer specifically labeled, cultured fibroblasts and lower median fluorescence intensity (P<0.001) (Fig. 5B).

**DISCUSSION**

We identified two children with biologically significant mutations of the IGF-IR gene. Patient 1, a compound heterozygote for missense mutations within the highly conserved, ligand-binding domain of the IGF-IR gene, had biochemical features of IGF-I resistance, with high circulating concentrations of IGF-I and growth hormone. The missense mutations R108Q and K115N are located at the edge of the putative ligand-binding pocket of the IGF-IR extracellular domain in the L1 region (Fig. 6) and involve changes in the amino acid charge (from basic to neutral), which may alter the binding-domain conformation and thereby reduce ligand binding. Studies of cultured fibroblasts supported this possibility by showing reduced affinity of the patient’s fibroblasts for IGF-I binding and a rightward shift of the dose–response curve for the activation of IGF-I receptors. However, further studies will be required to determine whether the decrease in binding alone explains these observations. Indeed, a defect in the homologous domain of the insulin receptor (I119M) (Fig. 6) appears to disrupt intracellular transport and processing and decrease the abundance of cell-surface insulin receptors.

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**Table 1. Circulating Concentrations of Insulin-like Growth Factors and Binding Proteins in Patient 1 and Her Parents.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patient 1</th>
<th>Father</th>
<th>Mother</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin-like growth factor</td>
<td>Basal Concentration</td>
<td>During Growth Hormone Therapy</td>
<td></td>
</tr>
<tr>
<td>I (µg/liter)</td>
<td>1130 (191–462)</td>
<td>1770 (191–462)</td>
<td>198 (131–374)</td>
</tr>
<tr>
<td>II (ng/ml)</td>
<td>352 (245–737)</td>
<td>382 (245–737)</td>
<td></td>
</tr>
<tr>
<td>Insulin-like growth factor–binding protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (mg/liter)</td>
<td>5.6 (2.1–6.2)</td>
<td>8.2 (2.1–6.2)</td>
<td>3.2 (1.9–3.6)</td>
</tr>
<tr>
<td>2 (ng/ml)</td>
<td>164 (200–470)</td>
<td>156 (200–470)</td>
<td></td>
</tr>
<tr>
<td>Acid-labile subunit (mg/liter)</td>
<td>28 (5.6–16.0)</td>
<td>24 (5.6–16.0)</td>
<td></td>
</tr>
</tbody>
</table>

*Patient 1 was studied at the age of 12 years, her father at the age of 48 years, and her mother at the age of 47 years. Normal values for age and sex are in parentheses. Endocrine Sciences (Calabasas Hills, Calif.) performed the assays.
more straightforward. These three all had nonsense mutations, which, as predicted, reduced the expression of IGF-I receptors on fibroblasts.

IGF-I resistance due to a reduction in the number of IGF-I receptors has been proposed to explain the growth phenotypes of the African Efe Pygmies and patients with partial deletions of chromosome 15. However, Pygmies have low circulating IGF-I concentrations, and the finding of an apparent reduction in the number of IGF-I receptors is based entirely on studies in transformed lymphocyte lines. Thus, it is not clear whether a defect in the IGF-IR gene explains the lack of growth of the Pygmy. Patients who are haploinsufficient for the IGF-IR gene because of aneuploidy of chromosome 15 typically have dysmorphism and mental as well as growth retardation. The extent to which these features reflect the loss of contiguous genes on chromosome 15 is uncertain. There was a clear gene-dosage effect on somatic growth in the family of Patient 2, suggesting that the growth retardation associated with chromosome 15 aberrations is due to IGF-I resistance. Furthermore, an apparent effect of heterozygosity was suggested in the parents of Patient 1, each of whom had marginal growth retardation at birth and whose adult height was substantially below the population mean.

IGF-I has diverse effects in multiple tissues. In addition to stimulating skeletal growth, this protein affects carbohydrate homeostasis, brain growth, and vitamin D metabolism and serves as a negative-feedback regulator of growth hormone secretion. Both of our patients had several traits typically associated with IGF-I resistance, including intrauterine and postnatal growth failure, delayed bone maturation, and increased concentrations of IGF-I.

Figure 4. Pedigree (Panel A) and Growth Curve (Panel B) for Patient 2.
Affected family members were heterozygous for a point mutation resulting in a stop codon on the insulin-like growth factor I receptor (IGF-IR) gene, as indicated by the black shading. Birth weights and lengths and final and current heights are indicated in Panel A, as are the number of standard deviations above or below the means for these values. The heights of the boy’s grandparents (Subjects I-1 and I-2) were reported, but they were not available for genotyping (as indicated by the question marks). Circles denote female family members, and squares male family members. The inset in Panel B shows the patient at the age of five years. Normative data are from the Zurich Longitudinal Study of growth and development.
Figure 5. Restriction-Endonuclease Analysis of Genomic and Complementary DNA (cDNA) of the Gene for the Insulin-like Growth Factor I (IGF-I) Receptor in Patient 2 and His Mother (Panel A) and Expression of IGF-I Receptors on Fibroblasts (Panel B).

Panel A shows the susceptibility to DdeI of polymerase-chain-reaction (PCR) products generated from genomic DNA and cDNA from Patient 2 and his mother. The uncut DNAs migrate as a single band in an agarose gel stained with ethidium bromide, as indicated by the minus signs. The mutation introduces a restriction site for DdeI, resulting in two new fragments with sizes of 97 bp and 155 bp when the PCR products are exposed to DdeI (lanes marked with plus signs). There are similar amounts of mutant- and normal-allele cDNA in samples from the patient (lanes 1 and 3) and his mother (lanes 5 and 7), indicating that both alleles are transcribed. Lanes 9, 10, 11, and 12 show the results of an analysis of cloned cDNA fragments with the mutation (lanes 11 and 12) or the wild-type DNA sequence (lanes 9 and 10), confirming that there was complete digestion of the PCR products. Lane 13 shows a 50-bp ladder (MBI Fermentas).

In Panel B, the expression of IGF-I receptors on intact skin fibroblasts from Patient 2 and three controls was assessed by flow-cytometric analysis (Epics model XL, Coulter) with the use of a monoclonal antibody against the human IGF-I receptor conjugated with phycoerythrin (R&D Systems). Specific staining for phycoerythrin was defined as a fluorescence intensity exceeding the nonspecific fluorescence intensity of an isotype control (mouse IgG1). The fluorescence intensity of the specifically labeled IGF-I receptors on fibroblasts from the patient was significantly lower than that of the control fibroblast cell lines. The plotted data are mean (±SD) values of four replicates and represent one of three identical experiments. The Mann–Whitney U test was used for the statistical analysis (Prism 3.0 software).
However, there were also unexpected findings. Bone age was less delayed than is typical in patients with growth hormone deficiency, and height velocity nearly doubled in Patient 1 in response to exogenous growth hormone, perhaps because the direct actions of growth hormone were unimpeded or because the mutant IGF-I receptors still had residual function. In addition, her bone mineral status appeared to be adequate, as did brain growth and intelligence.

It is noteworthy that these patients with defects in the IGF-IR gene were not phenotypically identical, nor did they share all the clinical characteristics of the patient described by Woods et al., who lacked the IGF-I gene. For instance, head size was decreased only in Patient 2 and the patient of Woods et al., measures of growth hormone secretion were increased only in Patient 1 and the patient of Woods et al., and only Patient 2 and the patient of Woods et al. had dysmorphic features. The reasons for these differences are not evident but could reflect differences in the intensity of IGF-I signaling among these patients, since the IGF-IR mutations blunt but do not abrogate IGF-I signaling. The phenotypic differences may also be explained by tissue-specific imprinting of the expression of the IGF-IR alleles. For example, if expression were monoallelic in certain tissues, Patient 2 might have a complete deficiency of IGF-I receptors in those tissues, whereas the same tissues in Patient 1 would have residual signaling of IGF-I receptors because of the mutant IGF-IR. Although tissue-specific imprinting has been

**Figure 6. The Position of Mutated Amino Acids in the Insulin-like Growth Factor I Receptor in Patient 1.**
Panel A shows the location of the mutated amino acids in the crystallographic structure (modified from Garrett et al.23). Panel B shows the homology between this receptor and the insulin receptor in the L1 domain. Conserved amino acids are enclosed in boxes. The mutations identified in Patient 1 are shown above the sequence. Indicated below the insulin-receptor sequence is the location of a naturally occurring mutation at position 119 that causes a mild form of leprechaunism.24 The numbering of the amino acid sequences differs slightly for the two receptors.
observed with other growth-regulating genes, studies of the IGF-IR in the limited tissues examined to date have not shown imprinting.

We have established that molecular defects in the IGF-1 receptor lead to a clinical phenotype dominated by reduced growth. Experimental evidence that the IGFs are necessary for normal brain development and function suggest that these mutations may also affect brain growth and neurologic development; the constellation of psychiatric anomalies found in Patient 1 may even reflect the state of partial resistance to IGF-I.

Our findings suggest that IGF-IR mutations are uncommon causes of intrauterine and postnatal growth failure, although the frequency of such mutations in different populations remains to be defined. A better understanding of the nature of such mutations and their associated phenotypes may provide insights into both growth retardation and the IGF system in humans.

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APPENDIX

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