

THE LITTLE WOMEN OF LOJA — GROWTH HORMONE-RECEPTOR DEFICIENCY IN AN INBRED POPULATION OF SOUTHERN ECUADOR

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Abstract Background and Methods. Laron-type dwarfism, which is characterized by the clinical appearance of isolated growth hormone deficiency with elevated serum levels of growth hormone and decreased serum levels of insulin-like growth factor I (IGF-I), has been described in approximately 50 patients. This condition is caused by a deficiency of the cellular receptor for growth hormone, and it is transmitted as an autosomal recessive trait, as indicated by an equal sex distribution and a high rate of consanguinity in affected families. We studied 20 patients (19 females and 1 male, 2 to 49 years of age), from an inbred Spanish population in southern Ecuador, who had the clinical features of Laron-type dwarfism.

Results. Seventeen patients were members of two large pedigrees. Among the 13 affected sibships, there were 19 affected and 24 unaffected female siblings and 1 affected and 21 unaffected male siblings. The patients' heights ranged from 10.0 to 6.7 SD below the normal mean height for age in the United States. In addition to the previously described features, 15 patients had limited elbow extensibility, all had blue scleras, affected adults had

relatively short extremities, and all four affected women over 30 years of age had hip degeneration. Basal serum concentrations of growth hormone were elevated in all affected children (30 to 160 μg per liter) and normal to moderately elevated in the adults. The serum level of growth hormone-binding protein ranged from 1 to 30 percent of normal; IGF-I concentrations were low — $\leq 7 \mu\text{g}$ per liter in the children and $\leq 66 \mu\text{g}$ per liter in the adults (normal for Ecuadorean women, 98 to 238). Serum levels of IGF-II and growth hormone-dependent IGF-binding protein-3 were also low.

Conclusions. We describe an inbred population with a high incidence of growth hormone-receptor deficiency resulting in a clinical picture resembling Laron-type dwarfism but differing principally in showing a marked predominance of affected females. This population, of Mediterranean origin, may be genetically related to other reported populations with Laron-type dwarfism, but with the genetic defect linked to a trait resulting in the early fetal death of most affected males. (N Engl J Med 1990; 323: 1367-74.)

LARON and colleagues in Israel were the first to describe short stature with the characteristic features of isolated growth hormone deficiency but with elevated serum levels of growth hormone in children and normal-to-slightly-increased levels in adults.^{1,2} They reported an equal sex distribution and a high rate of consanguinity in affected families, suggesting autosomal recessive inheritance. Their patients had small facies, with small mandibles, prominent foreheads, and saddle noses, giving the appearance of large heads. Hair growth was slow and sparse. Dentition was delayed, and teeth were discolored, fragile, and crowded. The patients' hands and feet were disproportionately small, their voices during childhood were high-pitched, and hypoglycemia was frequent in infancy and childhood. There was an excess of truncal fat, with light bones and small muscle mass.³ Mild intellectual retardation was typical.² Sexual maturation occurred normally, but it was delayed by several years in the boys; of a total of 30 patients, the final stature of the 18 adults ranged from 108 to 136 cm.⁴

In addition to those described in the reports from Israel, patients have been described in reports from

Lebanon (brother and sister),⁵ Mexico,⁶ Pakistan (two sisters),⁷ Saudi Arabia,⁸ Brazil,⁹ Spain (brother and sister),¹⁰ the United States,^{11,12} the Netherlands,¹³ Tunisia (four siblings),¹⁴ Italy (two sets of siblings),^{14,15} France,¹⁶ and Japan.⁹ The condition was originally thought to result from an abnormal growth hormone molecule,^{1,6} but subsequent studies have established that the low levels of insulin-like growth factor I (IGF-I) were caused by a defective growth hormone receptor. This defect was first demonstrated by experiments showing the absence of binding of growth hormone to hepatocytes from two patients.¹⁷ Later studies described a deficiency of serum growth hormone-binding protein, which is the solubilized extracellular domain of the growth hormone receptor.¹⁸⁻²⁰ The gene for this receptor, located on chromosome 5, has been characterized.¹² Attempts to demonstrate deletions in this gene in Laron-type dwarfism have been successful in only 5 of 12 patients studied, suggesting heterogeneity for this disorder.^{12,21} The deletion was limited to persons who traced their ancestry to Iraq or Iran.²¹ One of two Mediterranean families studied had a single nucleotide substitution in the extracellular domain of the growth hormone-receptor gene,¹⁴ but a synthetic mutant with this substitution was reported to have normal growth hormone-binding affinity.²²

We have identified 20 patients with short stature due to growth hormone-receptor deficiency among members of an inbred white population from the province of Loja in southern Ecuador. We observed a number of clinical and biochemical features that had not been described in Laron-type dwarfism, in particular a striking preponderance of female patients.

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Table 1. Anthropometric Characteristics of Patients with Growth Hormone–Receptor Deficiency and Family Members from Loja Province, Ecuador, as Compared with U.S. Reference Data.*

PATIENT No.†	AGE	HEIGHT				ARM SPAN MINUS HEIGHT (CM)	RATIO, UPPER TO LOWER SEGMENT	HAND LENGTH (CM)	FOOT LENGTH (CM)
		PATIENT	MEAN PARENTAL	MEAN SIBLING‡					
	yr, mo	cm	standard-deviation score			value for patient (normal value)			
1	9, 8	87.4	-7.2	-1.1	-0.4 (1)	-1.9 (-2.5)	1.13 (1.08)	10.0 (9.6)	13.2 (12.2)
2	7, 7	76.5	-8.1			-2.0 (-4.0)	1.12 (1.27)	9.5 (8.5)	11.7 (11.4)
3	25, 1	121.3	-7.2			-10.3 (1.2)	1.21 (1.01)	12.5 (12.8)	13.0 (17.6)
4	21, 0	119.0	-7.6	-2.4	-1.9 (4)	-9.0 (1.2)	1.25 (1.01)	11.5 (12.5)	14.2 (17.4)
5	2, 3	64.5	-8.2			-5.5 (-3.5)	1.44 (1.41)	6.0 (7.3)	9.5 (10.2)
6	27, 3	114.7	-8.4			-5.5 (1.2)	1.20 (1.01)	12.2 (12.0)	16.3 (16.8)
7	30, 2	119.7	-7.6	-2.0	-2.0 (5)	-4.9 (1.2)	1.13 (1.01)	12.7 (12.6)	16.0 (17.5)
8	21, 0	106.2	-10.0			-6.3 (1.2)	1.06 (1.01)	11.2 (11.4)	16.2 (15.4)
9	16, 1	108.2	-8.4			-7.7 (0)	1.04 (0.99)	11.7 (11.4)	16.2 (15.4)
10	6, 4	72.0	-8.8	-2.6	-0.3 (2)	-6.6 (-3.8)	1.29 (1.34)	7.8 (7.9)	10.8 (10.7)
11	32, 3	115.1	-8.3			5.5 (1.2)	1.11 (1.01)	13.4 (12.1)	16.5 (16.6)
12	26, 5	106.2	-10.0			-5.5 (1.2)	0.99 (1.01)	11.2 (11.3)	15.3 (15.2)
13	24, 10	112.3	-8.8	-1.9	-1.0 (4)	-5.3 (1.2)	1.22 (1.01)	11.1 (11.9)	14.4 (16.1)
14	5, 5	69.9	-10.0			-2.9 (-3.5)	1.22 (1.43)	8.0 (7.7)	10.5 (10.6)
15	1, 8	59.0	-8.2			-4.0 (3.0)	1.56 (1.60)	6.5 (6.5)	8.5 (10.2)
16	48, 7	107.2	-9.8	-3.0	-2.3 (5)	-2.7 (1.2)	1.26 (1.01)	11.9 (11.4)	16.5 (15.4)
17	40, 9	106.1	-10.0			-5.6 (1.2)	1.28 (1.01)	11.8 (11.4)	15.0 (15.4)
18	7, 10	86.0	-6.7			-1.0 (-4.0)	1.04 (1.22)	9.7 (9.6)	12.0 (12.2)
19	17, 10	121.8	-7.0	-2.0	-0.8 (2)	-6.8 (1.2)	1.11 (1.01)	13.1 (12.8)	16.1 (17.6)
20	8, 9	84.1	-8.8			-6.1 (-3.0)	1.26 (1.25)	9.2 (9.3)	12.4 (12.2)

*For children, normal values shown for arm span minus height and ratio of upper to lower segment are given according to bone age. Normal values shown for hand and foot length represent the 10th percentile of values for height age.

†All patients except Patient 20 were female.

‡Numbers in parentheses are numbers of siblings.

METHODS

Patients and Clinical Studies

Loja is one of the southern provinces of Ecuador bordering Peru, with a population of approximately 350,000 and an area of 10,000 km². The majority of the population is a racial mixture of Indian and white (mestizos), with smaller numbers of whites and Indians. The proportion of whites is thought to be considerably higher than elsewhere in the country, however, accounting for about one third of the population of the province. In addition, there has been an out-migration of Lojanians as a result of earthquakes, drought, or ambition; many left the province during a severe drought in the late 1960s. The migrants have tended to form Lojanian enclaves and to continue to intermarry.

The city of Loja, at an altitude of more than 2 km, is home to about a third of the province's population and to 9 of the 20 patients for whom data are shown in Table 1. The small Andean village of San Vicente del Rio, three to four hours' drive from Loja, has only white inhabitants and is the home of five patients (Patients 7, 8, 9, 12, and 13). La Toma, 40 km from Loja, at an altitude of 1.2 km, is the home of Patients 3 and 4. The rest of the patients are Lojanians living in and near Quito in the north central portion of Ecuador.

The initial patients (Patients 1 and 2) were Lojanian sisters living in Quito who were first studied by us in December 1988. Another child from Loja (Patient 5) was seen in June 1989, as were several women who came to Quito from Loja for consultations as a result of contact with one of the coauthors, who is a native of Loja. In December 1989 and June 1990, additional patients were seen in their home villages in Loja province or in Quito. Families, local medical personnel, and officials assisted in finding other patients; their efforts indicated that all but two possible patients had been identified. These were sisters in a remote village that can be reached only by a daylong journey on foot or by mule.

In Quito the heights of the patients and their siblings and parents were measured with a permanent wall-mounted device (Accustat). In the patients' homes in Loja and in the provincial hospital, we used a portable device (Raven Minimetre) that attached to the

wall. Standard-deviation scores for height were calculated as the number of standard deviations below the mean height of the U.S. population of the same age and sex.²³ These data from the U.S. National Center for Health Statistics were used because no Ecuadorian standards were available. The difference between arm span and height and the ratios of the length of the upper body segment to that of the lower body segment were compared with published normal U.S. standards for chronologic age and, in children, for bone age.²⁴ Hand length was measured across the palmar surface from the distal wrist crease to the tip of the middle finger, and foot length from the heel to the tip of the longest toe along the plantar surface. These measurements were compared with standards, with use of the patient's height age (age for which height is zero standard-deviation score) as a reference in an attempt to quantitate the subjective impressions of acral hypoplasia.²⁵ Weight was measured with a balance-beam scale, and weight age determined as the age for which weight was zero standard-deviation score with reference to the U.S. National Center for Health Statistics data. X-ray films of the prepubertal patients were interpreted according to the method of Greulich and Pyle to determine bone age.²⁶ A standard plastic goniometer was used to determine the degree of elbow-joint limitation if full extension was not possible.

Blood was obtained for biochemical and genetic studies from all patients, parents, and available siblings under a protocol approved by the institutional review board of the J. Hillis Miller Health Center of the University of Florida, and informed consent was obtained from all patients or their parents. In addition, a support organization was established (The Little People of Ecuador), with chapters in Quito and Loja, that serves as a vehicle for continuing education about growth hormone–receptor deficiency through meetings and periodic bulletins. Biochemical studies of relatives and genetic studies of the families will be reported separately.

Biochemical Studies

Serum samples were obtained from five Ecuadorian women 23 to 28 years of age, who were not of Loja origin but also lived in the

Andes, for comparison with serum samples from the 20 patients, 19 of whom were female, and with our normal reference serum pool of adult U.S. men with respect to growth hormone-receptor binding, levels of IGF-I and IGF-II, and content of IGF-binding protein.

Serum Growth Hormone Measurements

Some serum growth hormone determinations were performed at the Institute of Endocrinology, Metabolism, and Reproduction with a commercial radioimmunoassay (RIA) kit (Pharmacia), and others in the laboratories of Genentech (South San Francisco) with the Hybritech immunoradiometric assay.²⁷ Extensive growth hormone studies were done in Patient 19 at 7 and 27 months of age at the Children's Hospital of Los Angeles.

Analysis of Serum Growth Hormone-Binding Proteins

One-hundred-microliter serum samples were incubated with 5 μ l of ¹²⁵I-labeled recombinant human growth hormone at 37°C for one hour. The ¹²⁵I-labeled growth hormone-binding protein complexes were then separated according to their molecular weights on a 45-by-0.7-cm Sephadex G-100 column equilibrated with 20 mM HEPES, 10 mM calcium chloride, and 0.1 percent bovine serum albumin, pH 7.4 — an established method for measuring serum growth hormone-binding proteins.²⁸ The elution profile of the reference serum contained two peaks of radioactivity: a high-molecular-weight complex of growth hormone-binding protein with ¹²⁵I-labeled growth hormone, which accounted for about 18 percent of the total radioactivity present, and a free ¹²⁵I-labeled growth hormone peak. Nonspecific binding was determined by incubating the serum with ¹²⁵I-labeled growth hormone in the presence of an excess of unlabeled growth hormone (10 μ g). The total binding, total specific binding, and nonspecific binding of the reference serum were determined for each series of assays. Total binding and nonspecific binding were calculated by dividing the number of counts per minute in the high-molecular-weight peak by the total number of counts per minute in both that peak and the free (¹²⁵I-labeled growth hormone) peak. Total specific binding of the unmeasured serum was determined by subtracting the percentage of nonspecific binding from the percentage of total binding for each sample and expressing the result as the percentage of total specific binding in the reference serum. The addition of 50 ng of unlabeled growth hormone per milliliter to the reference serum did not alter the specific binding substantially.

Serum IGF-I and IGF-II Measurements

To separate insulin-like growth factors (IGFs) from their respective binding proteins, serum samples (500 μ l) were first separated by chromatography in 0.25 M formic acid on 0.9-by-100-cm columns of Sephadex G-50.²⁹ The fractions eluting between 50 and 67 ml, which contained 90 percent of the IGF activity and no binding-protein activity, were collected and lyophilized in 1.0 ml of 1 percent bovine serum albumin. The IGF-I concentrations were then determined by RIA with ¹²⁵I-labeled IGF-I and a polyclonal anti-IGF-I antiserum. This antiserum, a generous gift from Drs. L.E. Underwood and J.J. Van Wyk (University of North Carolina at Chapel Hill), is distributed through the Hormone Distribution Program of the National Institute for Diabetes and Digestive and Kidney Diseases to the National Hormone and Pituitary Program. IGF-II concentrations were also determined in the same fractions by RIA with use of recombinant ¹²⁵I-labeled IGF-II and a monoclonal antibody against rat IGF-II (Amano Laboratories, Troy, Va.).

Analysis of Serum IGF-Binding Proteins

Serum samples (2 μ l) were applied to a 4 percent stacking gel and separated by sodium dodecyl sulfate electrophoresis through a 10 percent polyacrylamide gel according to the method of Laemmli.³⁰ Prestained molecular-weight standards were run in parallel lanes. The gels were run overnight under nonreducing conditions at 50 V per sample. After electrophoresis, the gels were washed for 15 minutes in transfer buffer (0.025 M TRIS-base, 0.192 M glycine, and 20 percent methanol). The proteins were then transferred to nitrocellulose membranes by electroblotting with a semidry elec-

trophoresis-transfer unit.³¹ Ligand blotting of the filter-immobilized serum proteins was carried out according to the method of Hossenlopp et al.³² The nitrocellulose membranes were first washed in triethanolamine-buffered saline (TBS) (0.15 M sodium chloride and 0.01 M TRIS-hydrochloric acid) containing 3 percent Nonidet P-40 for 30 minutes at 4°C. Nonspecific ¹²⁵I-labeled IGF-II binding to the membranes was blocked by preincubation in TBS containing 1 percent bovine serum albumin for 2 hours and then in TBS containing 0.1 percent Tween 20 for 15 minutes at 4°C. The membranes were then incubated for 20 hours with 1.0×10^6 cpm of ¹²⁵I-labeled IGF-II in 20 ml of TBS containing 1 percent bovine serum albumin and 0.1 percent Tween. Finally, the membranes were washed with TBS, air-dried, and exposed to Kodak X-Omat AR film for three to five days at -70°C. After development, the autoradiographs were scanned with a laser densitometer, and the relative densities of the bands corresponding to each IGF-binding protein were expressed as arbitrary absorbency units per millimeter.

Statistical Analysis

The mean data were compared with use of Student's t-test. The results are expressed as means \pm SD.

RESULTS

Clinical Features

The 20 patients for whom anthropometric data are shown in Table 1 ranged in age from 1 year 8 months to 49 years. The 11 women over the age of 17 were all fully mature sexually and had regular menstrual cycles. Their menarche had occurred at a time normal for the family or after a delay of up to five years; in general, the delay was two to three years beyond the age expected for the family. At the age of 16, Patient 9 was prepubertal and had a bone age of 11 years; her affected sister (Patient 8) had her menarche at the age of 20. The heights of the 20 patients ranged from -10 to -6.7 standard-deviation scores. The heights of the adult patients ranged from 106 to 122 cm — approximately 40 cm below the heights expected on the basis of parental stature. The heights of the unaffected siblings generally reflected a trend toward taller stature than that of their parents. There was tight clustering of standard-deviation scores among the unaffected persons within sibships, providing no clinical evidence of partial expression of the defect in growth hormone action.

The three oldest patients (Patients 11, 16, and 17) had clinically evident hip degeneration. For approximately one year, all three had had a waddling gait associated with mild hip pain. Radiography demonstrated bilateral loss of the acetabulum, with dislocation of the hip and degeneration of the head of the femur, resulting in a telescoping of the femoral neck and a tilting of the pelvis. At the age of 30, the next-oldest woman (Patient 7) had pain in the left leg while walking and a normal gait; she had degeneration of the left femoral head and acetabulum but no dislocation. The other seven women had normal hip films with varying degrees of osteopenia.

Body proportions, expressed as arm span minus height or as the ratio of the length of the upper body segment to that of the lower segment, were normal for bone age in the affected children, with the possible exception of Patient 20, who may have had a reduced arm span. The patients over the age of 16,

including the prepubertal 16-year-old girl (Patient 9) and excluding only Patient 11, had substantially reduced arm spans, ranging from 4 to 11.5 cm less than expected. Similarly, the ratios of the upper to the lower segments of the body were childlike in all but Patient 12. In Patients 11, 16, and 17, all of whom had severe hip dysplasia with shortening of the femurs and dislocation of the hips, shortening of the lower extremities would have been expected to result in abnormal body proportions and reduce the discrepancy between span and height. Indeed, the two patients who had the least disproportionate arm spans (Patients 11 and 16) had severe hip dysplasia, although the affected sister of Patient 16, who had similar upper-to-lower-segment disproportion, had an arm span 6.8 cm less than expected; four of the women with normal hips (Patients 3, 4, 6, and 13) had upper-to-lower-segment disproportion comparable to that of Patients 16 and 17, who had hip dysplasia, and greater than that of Patient 11, who also had hip dysplasia.

Hand length was below the 10th percentile for height in 4 of 9 children (Patients 5, 10, 15, and 20) and in 5 of 11 adults (Patients 3, 4, 8, 12, and 13). Abnormally small feet were present in a comparable number of children and in more adults. Four children (Patients 5, 14, 15, and 18) had foot length below the 10th percentile for height, as did eight affected adults (Patients 3, 4, 6, 7, 11, 13, 17, and 19). Thus, as actually measured and in relation to stature, 16 of 20 patients had acral hypoplasia, and in 5 of them both the hands and the feet were involved. Laron et al.³ described acral hypoplasia subjectively in all their patients.

X-ray films to determine bone age in the nine prepubertal patients and in Patient 19 at the age of 27 months demonstrated markedly delayed osseous maturation. Bone age was advanced for height age, however, in the patients more than 1½ years old (Table 2).

All patients had prominence of the forehead and a depressed nasal bridge, giving the appearance of a large cranium (Fig. 1). Otherwise, the patients' facies and mandibles were not disproportionate. Sparse hair was present only in the four patients under the age of seven (Patients 5, 10, 14, and 15). All the patients had central adiposity, but the prepubertal patients (Patients 1, 2, 5, 9, 10, 14, 15, 18, and 20) had ratios of weight age to height age of 0.57 to 0.96. The adult patients had true obesity, however, with ratios of 1.37 to 2.20. Delayed or malerupted dentition was apparent in five patients (Patients 1, 2, 5, 6, and 15), whereas four had poorly formed, decayed teeth (Patients 1, 14, 18, and 20). All 12 patients 16 years of age or older had normal teeth, although in several the teeth were crowded. All the affected children had high-pitched voices, as did 10 of the 12 patients 16 years of age or older.

Blue scleras, a feature not previously described in this condition, were present in all 20 patients. Another feature not previously noted was limitation in the ex-

Table 2. Height Age and Bone Age in Prepubertal Patients with Growth Hormone-Receptor Deficiency.

PATIENT No.	AGE*	HEIGHT	HEIGHT AGE		BONE AGE
			UNCORRECTED	CORRECTED†	
			<i>yr, mo</i>	<i>cm</i>	
1	8, 2	82.7	1, 7	1, 9	5, 6
	9, 8	87.4	2, 0	2, 4	6, 9
2	6, 1	72.9	0, 11	1, 1	2, 6
	7, 9	76.5	1, 2	1, 5	3, 6
5	1, 4	58.0	0, 3	0, 3	0, 3
	2, 3	64.5	0, 6	0, 6	2, 0
9	16, 1	107.1	4, 11	6, 0	11, 0
10	6, 5	72.0	0, 10	1, 1	2, 6
14	5, 5	67.5	0, 7	0, 11	1, 9
15	1, 2	57.0	0, 2	0, 4	0, 6
18	7, 10	86.0	1, 11	2, 6	3, 3
19	2, 3	65.8	0, 6	0, 7	1, 6
20	8, 7	82.5	1, 6	2, 9	4, 0

*At the time of the determination of bone age.

†Computed with use of the mean standard-deviation score of the siblings as a reference value, or that of the parents if there were no siblings (as was the case for Patient 18).

tensibility of the elbow, a characteristic of four of the eight children and all but one patient over the age of 16; the extent of limitation ranged from 5 to 25 degrees of flexion (mean \pm SD, 18 ± 11).

None of these patients were intellectually retarded, although several had begun walking late, presumably as a result of the hypomuscularity apparent on examination and on x-ray films. Six patients had apparently had hypoglycemic episodes, but without long-term deleterious effects. All the adults had graduated from high school and had attained or exceeded the educational level appropriate for their families, although some patients were restricted socially by their small stature. At least two thirds of the 17 patients who had attended school had been very good or excellent students.

Family Analysis

Twelve patients (Patients 3, 4, 8 through 15, 18, and 20) were interrelated in a complex consanguineous pedigree. The parents of Patient 5 were first cousins, with the mother's sister (Patient 6) also affected; this family was related to the family of Patients 16 and 17, as well as to that of Patient 7. Thus, 17 of the patients can be encompassed within two pedigrees. The parents of Patient 19 were also first cousins. These families were all considered to be *blancos puros*, with no evidence of outbreeding with the Indian population.

The sex distribution of the affected and normal members of these sibships is shown in Table 3. Only 1 of the 20 patients (Patient 20) was male, and males constituted only a third of the sibships, with 22 living, 3 dead in childhood, and 2 stillborn. One of the deaths was that of an 18-month-old boy with dysentery who was thought to resemble his two affected sisters (Patients 14 and 15). Five female sib-



Figure 1. The Family of Patients 8, 9, and 10.

From left to right, this photograph shows a sister, 25 years old (height, 158.8 cm); a brother, 18 years old (164.7 cm); Patient 9; the father, 52 years old (165 cm); Patient 8; a brother, 12 years old (135.9 cm); a sister, 8½ years old (115.4 cm); and the mother, 46 years old (156.7 cm), holding Patient 10.

lings also died of childhood illnesses between one and six years of age; three of them were thought to be affected. There was a total of five spontaneous abortions. Thus, half the female members of these families were affected, and there were twice as many female siblings as males, the discrepancy being completely accounted for by the marked predominance of affected females.

Biochemical Findings

The biochemical characteristics of the patients are shown in Table 4.

Serum Growth Hormone Concentration

All the affected children had markedly elevated basal serum levels of growth hormone. In addition, several of the adults (Patients 3, 11, 12, 16, and 17) had elevated basal levels of serum growth hormone or dramatic increases in response to insulin-induced hypoglycemia.

Serum Growth Hormone–Binding Proteins

Gel filtration of the normal reference serum pool incubated with ^{125}I -labeled growth hormone resulted in two peaks of radioactivity. The first peak to be eluted from the column contained the complex of ^{125}I -labeled growth hormone and growth hormone–binding protein and accounted for approximately 18 percent of the total added radioactivity. The second peak contained free ^{125}I -labeled growth hormone and accounted for approximately 82 percent of the added radioactivity. The addition of excess unlabeled growth hormone to the incubation mixture resulted in

a decrease in the amount of radioactivity present in the first peak to approximately 3 percent of the total. The radioactivity remaining in the first peak was probably a combination of aggregated radioligand, nonspecifically bound radioligand, and a protein that binds growth hormone irreversibly.³¹ In the serum of normal Ecuadorean women, the mean activity in the first peak was 13 ± 3 percent of the specifically bound radioactivity. In the patients, the first peak contained decreased amounts of specifically bound radioactivity, ranging from 1 to 30 percent of the value in the normal reference serum pool (Table 4). Unlike the other biochemical measures, the levels of serum growth hormone–binding protein in the prepubertal patients were not more abnormal than those in the adult patients; the activity of the first peak was 10 ± 6 percent of normal, and in the adult patients it was 10 ± 7 percent of normal. There were no significant correlations between the activity of serum growth hormone–binding protein and the serum growth hormone or IGF-I concentrations in the patients.

Serum IGF Measurements

The serum IGF-I concentrations in the patients ranged from 1 to 66 μg per liter (mean, 15 ± 16), in contrast to 98 to 238 μg per liter (mean, 157 ± 55) in normal Ecuadorean women. The 9 prepubertal patients had markedly lower serum IGF-I concentrations of 7 μg per liter or less (mean, 4 ± 2) than did the 11 adults (mean, 31 ± 18 μg per liter; $P < 0.001$). The serum IGF-II concentrations in the patients were very low (17 to 160 μg per liter; mean, 93 ± 43) as compared with the values in normal Ecuadorean women (454 to 541 μg per liter; mean, 484 ± 33). Although serum IGF-II levels do not normally vary with age, as do IGF-I levels,³³ the prepubertal patients also had lower IGF-II levels than did the adult patients (59 ± 42 vs. 124 ± 52 μg per liter; $P < 0.01$). Thus, in the adult patients the levels of IGF-I and IGF-II averaged 20 and 25 percent of the values in normal Ecuadorean women, respectively. The similarity between affected children and adults with respect to variation in IGF-I and IGF-II values was also evident in the positive correlation between serum IGF-I and IGF-II values among the patients ($r = 0.67$, $P < 0.01$). The serum growth hormone concentrations and the IGF-I or IGF-II values were not correlated. Four pa-

Table 3. Sex Distribution of Patients with Growth Hormone–Receptor Deficiency and Normal Siblings in Affected Families.

	MALES	FEMALES	ALL
Affected			
Living	1	19	20
Dead	1*	3*	4*
Unaffected			
Living	21	24	45
Dead	4†	2	6
Total	27	48	75

*Reported by parents to have resembled affected probands.

†Includes two stillbirths.

tients for whom specimens were obtained six months apart or more had similar serum IGF values on both occasions (Patients 7, 11, 16, and 17).

Serum IGF-Binding Proteins

A representative Western ligand blot of serum IGF-binding proteins in the patients is shown in Figure 2. Pooled normal adult serum (lanes 1, 7, and 12) generally resolved into five bands: a doublet of 43-kd and 39-kd bands (IGF-binding protein-3), a 32-kd band (IGF-binding protein-2), a 28-kd band (IGF-binding protein-1), and a 24-kd band (the classification of which was uncertain). IGF-binding protein-3 is growth hormone-dependent, and as shown in Figure 2, it was found in markedly reduced quantities in serum from the patients. In some patients there appeared to be an increase in the band corresponding to IGF-binding protein-2. The average amount of IGF-binding protein-3 present in the samples from the patients, as compared with that in the reference serum pool, was only 4 ± 3 percent (range, 0 to 9 percent) in the nine prepubertal patients and 21 ± 18 percent (range, 4 to 58 percent) in the adult patients. The relative amount of IGF-binding protein-3 in the normal Ecuadorean women was 85 percent of normal for the U.S. adult-male reference pool (Table 4).

DISCUSSION

We identified a high frequency of growth hormone-receptor deficiency that affected approximately half the surviving female members (19 of 43) but only 1 of 22 male members of affected sibships in a highly inbred, Spanish population in Ecuador. In contrast to the patients studied by Laron et al.,³ these patients had a notable absence of subnormal intellectual function. All the adults in the Loja group achieved a level of education appropriate for their families, despite a degree of social isolation reflected in the unmarried status of all the adults. In addition, dental abnormalities other than a delay in dentition, which would be expected in patients with IGF-I deficiency, were less common in this population than among the Israeli patients, 16 of 26 of whom had dental problems.² Eighty percent of the patients had acral hypoplasia, defined as hand or foot length below the 10th percentile for height. Laron et al.³ noted high-pitched voices only in children, a feature that persisted in most of our adults. The timing of menarche, reported as normal for the family in the series of 11 women studied by Laron et al.,⁴ was moderately to markedly delayed in

Table 4. Biochemical Characteristics of Serum of the Patients with Growth Hormone-Receptor Deficiency.

PATIENT NO.	GROWTH HORMONE LEVEL			IGF-I*	IGF-II*	GROWTH HORMONE-BINDING PROTEIN	IGF-BINDING PROTEIN-3
	BASAL	CLONIDINE†	INSULIN‡				
	micrograms per liter					% of normal§	
1	>50¶/14			7	123	13	0
2	>50¶/14			3	59	22	2
3	13/12		26	33	154	4	4
4	<3.5			58	160	14	7
5	99¶/24	100¶		6	24	10	0
6	7¶	3¶		66	111	8	33
7	2¶/4	13¶	6	36/47	100/107	10	45
8	<3.5			37	121	15	15
9	9.5/17		43	3	46	1	4
10	32¶			7	128	8	6
11	4¶	3¶	128¶	23/5	46/86	4	10
12	<3.5/10		80	15	112	10	19
13	<3.5			12	82	8	20
14	30¶/24	50¶		2	51	8	9
15	36¶/17	40¶		1	83	5	4
16	4¶/5		64¶	13/10	66/120	7	6
17	8¶/15		200¶	16/12	100/87	4	11
18	36/7	56		3	53	8	4
19	160¶			43	228	30	58
20	49¶		49¶	4	46	18	3

*Duplicate values, indicated with a slash, were from specimens obtained at least six months apart.

†Values obtained 60 minutes after the oral administration of clonidine.

‡Peak values obtained 30 or 60 minutes after the intravenous administration of insulin (0.05 or 0.075 U per kilogram of body weight).

§Expressed as a percentage of the U.S. adult-male control serum pool assayed concurrently. For comparison, the binding in serum samples from five normal Ecuadorean women was 78 to 90 percent of the growth hormone-binding protein content of this pool and 77 to 92 percent of the IGF-binding protein-3 content of the pool.

¶Determination made in Ecuador; all other serum growth hormone determinations were performed at Genentech.²⁴ Duplicate basal values (indicated with a slash) were measurements in serum specimens obtained 6 to 18 months apart.

||Studied at Children's Hospital of Los Angeles at 27 months of age.

5 of the 12 women more than 16 years old in the Loja population. The features of blue scleras, limited elbow extensibility, relatively short extremities in adults, and the development of hip dysplasia in older patients have not to our knowledge been described in other patients with growth hormone-receptor deficiency.

In the prepubertal patients, bone age was well ahead of statural age — an observation confirmed by a review of the data of Laron et al.⁴ The explanation for this discrepancy is not apparent. In patients with a deficiency of thyroid-stimulating hormone and growth hormone, thyroid hormone replacement alone can advance osseous maturation beyond linear growth.^{34,35} The presence of normal thyroid secretion in the absence of growth hormone action in the Loja patients does not provide a convincing explanation, however, since this phenomenon does not occur in patients with isolated growth hormone deficiency.

Laron et al. have noted that growth hormone-receptor deficiency is clinically indistinguishable from isolated growth hormone deficiency.^{1-4,9} However, acral hypoplasia, which was present in all the patients of Laron et al. and in 80 percent of ours, has not been considered a characteristic of growth hormone deficiency, nor have blue scleras, limited elbow extensibility, short extremities, and late-onset hip degeneration. The advanced osseous maturation relative to height age in childhood is also atypical. Thus, patients with growth hormone-receptor deficiency appear to have a

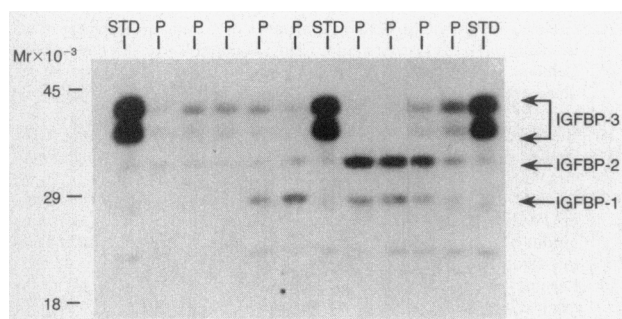


Figure 2. Autoradiograph of Serum IGF-Binding Proteins.

Each lane represents a serum sample from a patient (P) or a pool of serum from normal U.S. men (STD). The IGF-binding proteins (IGFBP) were separated by sodium dodecyl sulfate–polyacrylamide-gel electrophoresis on a 10 percent gel, transferred to nitrocellulose, ligand blotted with ^{125}I -labeled IGF-II, and visualized by autoradiography. The numbers at the left represent molecular-weight standards estimated as molecular retention ($\text{Mr} \times 10^{-3}$).

The identity of the individual IGF-binding proteins is shown at the right.

distinctive clinical phenotype that is similar but not identical to that of growth hormone deficiency. There is, however, a close resemblance to some patients with congenital growth hormone deficiency resulting from the deletion of the growth hormone gene.³⁶ This similarity suggests that some of these features result from a severe fetal and neonatal deficiency of growth hormone or its receptor.

All the patients we studied had markedly reduced serum IGF-I levels. Serum IGF-I levels have been described as normal in some affected patients,^{7,37} probably because of failure to separate IGF-I from IGF-binding proteins before assay.²⁸ Patients 1 and 2 previously had normal serum IGF-I levels when measured in such an assay.

Serum IGF-II levels have been reported to be approximately 40 percent of normal in persons with Laron-type dwarfism.³⁸ The mean value in our patients, 93 μg per liter, was only 20 percent of that in normal Ecuadorian women (484 μg per liter), and the patients' levels of IGF-II correlated with those of IGF-I. Thus, in this population, the generation of IGF-II appears to be highly dependent on the presence of an intact growth hormone receptor. In addition, levels of the growth hormone–dependent IGF-binding protein-3 were reduced by approximately 90 percent in these patients.

Growth hormone-binding protein deficiency, reflecting the relative lack of cell-surface growth hormone receptors, did not vary between children and adults. However, biochemical abnormalities due to the presumed deficiency of growth hormone receptors, including elevated serum growth hormone levels with low concentrations of IGF-I, IGF-II, and growth hormone–dependent IGF-binding protein-3, were more severe in the prepubertal patients. These results indicate that sexual maturation is associated with some activation of IGF synthesis in these patients, presumably an effect of sex steroids.³⁹

The most striking difference between the Loja patients and those previously described is the remarkable paucity of affected males. Equally remarkable is the distorted sex ratio in the sibships, with twice as many females as males (both affected and unaffected persons). We were unable to elicit any history of frequent abortion in these families, and infant and child mortality was not sex-specific or unusual. The genetic basis for this finding is being explored in molecular genetic studies of the families.

The likelihood is great that growth hormone–receptor deficiency is a homogeneous condition throughout this population, and this possibility is reinforced by the inclusion of most of the patients in two pedigrees and the high frequency of consanguinity. Studies to date in other patients with Laron-type dwarfism have suggested heterogeneity for the molecular defect in the growth hormone receptor.^{12,14,21} Since the Ecuadorian patients are of Mediterranean origin, as are the Israeli patients, they may share one such defect that in Ecuador may have become linked to a trait resulting in the early fetal death of most affected males. We anticipate that the concentration of affected persons in the Loja population will permit the identification of a common mutation in the growth hormone–receptor gene, allowing the accurate identification of heterozygotes and a solution to the conundrum presented by the remarkable predominance of females in this population.

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