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Phenotype and Genetic Analysis of a Syndrome Caused by an Inactivating Mutation in the Growth Hormone-Releasing Hormone Receptor: Dwarfism of Sindh*

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ABSTRACT

We report, in detail, a new form of familial dwarfism, including its phenotypic features, hormonal profile, and molecular basis. Following a newspaper report of severe dwarfism in two villages in the province of Sindh, Pakistan, we organized an expedition to study its clinical, genetic, and molecular characteristics. We identified 18 dwarfs (15 male, 3 female), all members of a consanguineous kindred, ranging in age from newborn to 28 yr. Mean height was 7.2 SD below the norm, with mean adult heights of 130 cm for males and 113.5 cm for females. Body proportions and habitus were normal; but head circumference was 4.1 SD, and blood pressure approximately 3 SD below the norm. There was no dysmorphism, no microphallus, and no history of hypoglycemia. Serum GH did not respond to provocative stimuli (GHRH, L-dopa, or clonidine). Insulin-like growth factor I (IGF-I) and IGF-binding protein 3 were low (5.2 ± 2.0 ng/mL and 0.42 ± 0.13 μg/mL, respectively; mean ± SD) but rose normally with GH treatment. One affected, dwarfed couple had a son, demonstrating fertility in both sexes. Clinical and endocrinological evidence suggested isolated GH deficiency with a recessive inheritance pattern. The GH-N gene was found to be intact. Linkage analysis of microsatellite chromosomal markers near other candidate genes yielded a high LOD score (6.26) for the GHRH receptor (GHRH-R) locus. DNA sequencing revealed a nonsense mutation (Glu^{50}→Stop) in the extracellular domain of the GHRH-R. This mutation predicts a severely truncated GHRH-R; it is identical to that recently reported in four patients from two other families. Inheritance is autosomal recessive (chromosome 7p) with a high degree of penetrance. Relatives heterozygous for the mutation had moderately decreased IGF-I levels and slightly blunted GH responses to GHRH and L-dopa, but they showed only minimal or no height deficit.

This syndrome represents the human homologue of the little (*lit/lit*) mouse and closely resembles its phenotype. It demonstrates the absolute requirement of GHRH signaling for pituitary GH secretion and postnatal growth in humans, and its relatively minor (but discernible) biological importance in extrapituitary sites. The syndrome is distinct from other forms of GH deficiency with respect to microcephaly, asymptomatic hypotension, and absence of features such as facial dysplasia, significant truncal obesity, microphallus, or hypoglycemia. Its discovery raises the possibility of milder mutations in the GHRH-R gene as potential causes for partial GH insufficiency and idiopathic short stature. (*J Clin Endocrinol Metab* **83:** 4065–4074, 1998)

EXTREMES of body size, such as dwarfism and gigantism, have fascinated mankind for millenia. More recently, body stature has become a public interest because of the perceived social advantage conferred by being tall (1, 2) and because short stature, even if not caused by recognized disease, has become amenable to treatment with GH. Dwarfism, when severe, represents a handicap in a society that is largely adapted to normative dimensions. Genetic, nutritional, and possibly other factors interact in determining stature. However, the biochemical mediators responsible for genetic height achievement are poorly understood, and the cause of idiopathic short stature is known only in a small minority of cases. Familial or genetic syndromes of short or tall stature can potentially shed light on what factors are important in the genetic determination of height. Known genetic causes of proportionate dwarfism are mutations in the GH-N gene (3), the Pit-1 gene (4), the Prop-1 gene (5), or the GH receptor gene (6). The former three manifest themselves in GH or combined pituitary hormone deficiency, the latter in GH resistance. A recently recognized cause of familial short stature is a mutation in the GHRH receptor (GHRH-R) gene (7–9). Because of the strong but mechanistically poorly understood genetic component in height variation, detailed studies of genetic forms of short (or tall) stature are particularly important. We report here the full genetic and phenotypic characterization of a large kindred affected with an inactivating mutation in the GHRH-R—a syndrome known as "Dwarfism of Sindh." A preliminary report has appeared as part of a symposium proceedings (8).

In 1994, an article appeared in Dawn (a Karachi, Pakistan, newspaper), describing the existence of a cluster of familial severe dwarfism in two remote villages in the lower Indus valley (10). We reasoned that this syndrome may reveal new information on the genetic regulation of growth, and we organized an expedition to investigate the nature and cause of this growth disorder.

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Subjects and Methods

Clinical studies

A preliminary visit by one author (H. G. Maheshwari) identified 13 dwarfs in 2 neighboring villages (Umar Burro and Fakirabad), approximately 30 km from Sukkur, in the Province of Sindh, Pakistan. Photographs documented the severity of growth retardation and suggested that dwarfism was proportionate. Two of us (H. G. Maheshwari and G. Baumann) then traveled to Sukkur to obtain precise population, ecological, nutritional, pedigree, clinical, biochemical, and genetic information. A field laboratory was set up in the district hospital. The study plan was approved by the Northwestern University Institutional Review Board, and subjects participating in the study gave informed consent or parental assent.

We conducted extensive interviews to establish family relationships and medical histories. Height was measured using a wall-mounted ruler and a draftsman's triangle, and weight was determined using a portable scale. Height and weight were compared with the NCHS/CDC reference population (11); head circumference with the standards published by Nellhaus (12); body proportions with those of Wilkins (13), Roche and Malina (14), and Forbes (15); and blood pressure with those of Drizd *et al.* (16) and Blumenthal *et al.* (17). Age-adjusted data are expressed as SD scores (SDS). Bone age was determined on wrist radiographs, according to the TW2 method (18). Nutritional status was assessed by nutritional histories and inspection of sc fat deposits. Intellectual function was estimated during conversations with patients and relatives and by judging the appropriateness of school year for age.

Baseline blood samples were obtained after a 12-h fast. The following pituitary function tests were performed: GHRH-1–44 1 μg/kg iv; TRH 7 μg/kg iv; L-dopa 250 mg (9–25 mg/kg) po; clonidine 125 or 250 μg (3.9–6.2 μg/kg) po. An insulin-like growth factor I (IGF-I) generation test was performed by injecting recombinant human GH (hGH; 0.05 mg/kg sc) once daily for 5 days. GHRH and TRH were purchased from Ferring (Lübeck, Germany, and Suffern, NY, respectively). hGH was kindly provided by Eli Lilly & Co., Indianapolis, IN.

Biochemical and hormone assays

Blood was centrifuged and plasma immediately frozen until transported to Chicago on dry ice. Serum SMAC-20 and immunoglobulins were determined by standard procedures; T_4, cortisol, aldosterone, testosterone, dehydroepiandrosterone sulfate (DHEA-S), GH, and PRL by RIA; and TSH by immunoradiometric assay. GH was also measured by ultrasensitive chemiluminescent assay (19). Measurements of extracted IGF-I, IGF-II, and IGF-binding proteins 2 and 3 (IGFBP2 and IGFBP3) were performed by Corning-Nichols Laboratories, San Juan Capistrano, CA. GH-binding protein (GHBP) was determined by GH-binding assay (20). Statistical analysis was performed by *t* test or ANOVA, as appropriate.

Nucleic acid extraction, amplification, and cloning

Genomic DNA and RNA were extracted from peripheral leukocytes in whole blood according to standard procedures. PCR was used to amplify genomic DNA or reversely transcribed complementary DNA of candidate genes. Generally, 30–35 thermal cycles were used, followed by agarose gel purification of amplimers and either direct DNA sequencing or subcloning into pCR II vector.

Linkage analysis

Candidate genes (see below) were probed for linkage to the dwarf phenotype using amplification and size determination of polymorphic chromosomal markers (microsatellites) in the vicinity of the candidate genes, using fluorescent primers and an ABI 373A semiautomatic DNA sequencer with the aid of ABI GeneScan software. Two- and three-point linkage analysis was performed using version 5.0 of the LINKAGE program (21) and version 3.0 of the FASTLINK subroutines (22, 23), as well as by analysis of homozygosity by descent. A fully penetrant autosomal recessive mode of inheritance was assumed, with a disease allele frequency of 0.0001. The allele frequencies of the microsatellites were set to 1/n, where n is the number of alleles observed. However, the robustness of the LOD scores was verified by setting the frequencies to the published values, when available. For the three-point analysis, two of the inbreeding loops had to be omitted because of computational constraints.

DNA sequencing

DNA sequencing was performed using cycle sequencing with AmpliTaq FS, fluorescent dideoxy chain terminators and analysis in a semiautomatic DNA sequencer. Generally, PCR products were directly sequenced; in the case of cloned DNA, at least 6 clones were sequenced to guard against *Taq* polymerase errors.

Results

General health and nutritional status

The affected population lives in two neighboring villages in the semiarid, but irrigated lower Indus valley. The population is native Sindhi and Muslim. Occupation consists primarily of farming (dates, rice, livestock) and brick manufacture. There is neither shortage of food nor evidence of malnutrition. We found no infectious, gastrointestinal, or other general medical reasons for growth retardation. Consanguineous marriages are common. Dwarfism emerged recently, with the oldest patient being 28 yr old. Parents of affected subjects reported that pregnancy, birth size, and breast feeding were normal and that growth retardation became apparent during early childhood. There were no unusual illnesses or deaths among affected children. In particular, no symptoms of hypoglycemia could be elicited. Intelligence appeared normal. Puberty in males was reported to occur at about age 16 (a delay of 2–3 yr for the local population standard); in females this information could not be obtained.

Pedigree (Fig. 1)

The pedigree of the affected kindred shows 7 consanguineous unions (6 first cousin and 1 uncle/niece). Because of the complex intergenerational marriage patterns, we were unable to assign generations. Subjects are therefore numbered consecutively throughout the pedigree. Based on the physical phenotype, we found 16 clearly dwarfed subjects (13 male, 3 female, age range 7–28 yr; *black symbols)*. We were uncertain how to classify 6 subjects (no. 6, 12, 27, 28, 38, and 43) based on phenotype alone, because of their intermediate stature (-2.5 to -4.5 height SDS). Of note is subject no. 12, who is the offspring of two dwarfed parents. He was 25 days old at the time of examination; having been delivered at or near term by cesarean section. His mother had sufficient milk to satisfy his needs.

Physical phenotype and anthropometrics

Affected patients were short but exhibited normal body proportions (Fig. 2). There were no dysmorphic features or deformities. In some (but not all) patients, there was minimal facial hypoplasia, some had abducted ears, and two (subjects no. 45 and 46) had a slightly protruberant abdomen. None of the patients had microphallus. Tanner staging in adolescents confirmed a pubertal delay of 2–3 yr. Adult male patients had a distinctive, raspy, high-pitched voice. Anthropometric data are summarized in Table 1. Height, weight, head circumference, bone age, and blood pressure were significantly below normal, and waist/hip ratio was above American norms (14, 15). Adult height was 130 $\pm$ 10.6 cm for men and 113.5 $\pm$ 0.7 cm for women (mean $\pm$ SD). Blood pressure was low, even if allowance is made for measurements with an adult-size cuff. The newborn boy born of two dwarfed parents (subject no. 12) showed evidence of intrauterine growth retardation (hypoplas-

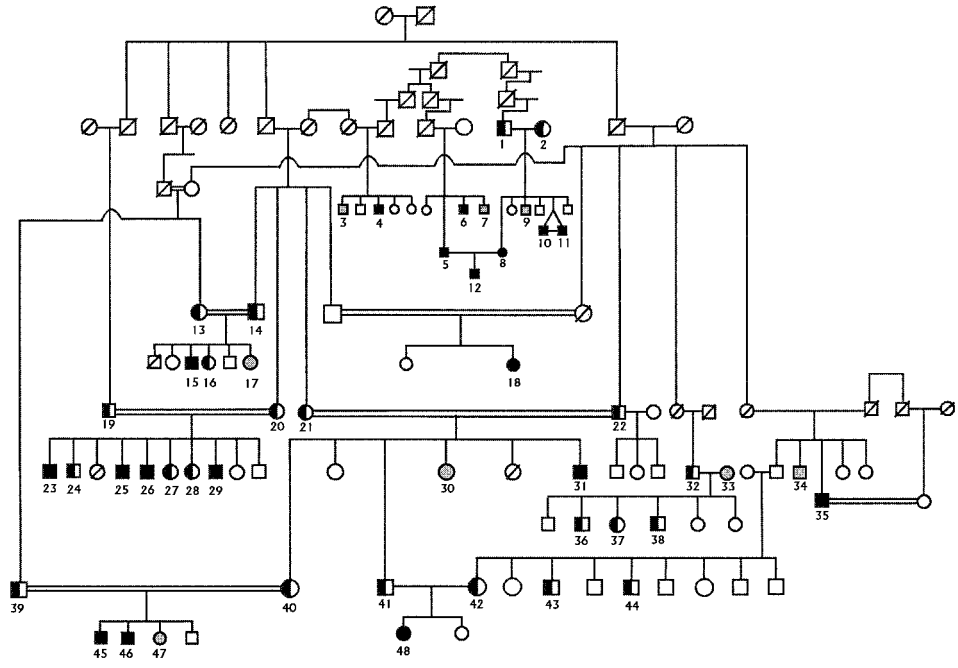

FIG. 1. Pedigree of kindred affected by dwarfism of Sindh. *Squares*, Males; *circles*, females; *double lines*, second-degree consanguinity; *filled black symbols*, affected subjects homozygous for the mutation; *half-filled symbols*, heterozygous subjects; *shaded symbols*, subjects homozygous for the wild-type allele; *open symbols*, phenotypically normal subjects who were not genotyped.

tic adipose tissue and skeletal muscle, but both testes normal in size and descended). This is in contrast to the reported normal birth size of affected subjects born of normal statured parents.

Parents and siblings of affected persons had no abnormality in height or body habitus. There was no correlation between mean parental height SDS and height SDS in the affected persons ($P = 0.28$).

Biochemical phenotype

Patients and relatives had normal routine blood chemistry and immunoglobulin levels. Baseline endocrine parameters are shown in Table 2. The patients were euthyroid and had normal PRL, cortisol, aldosterone, DHEA-S, and testosterone levels. Abnormalities were confined to the GH-IGF-I axis and included low IGF-I, IGF-II, and IGFBP3, and elevated IGFBP2 levels. Basal plasma GH was undetectable in a conventional RIA (<0.3 ng/mL) but measurable in a chemiluminescent assay. GHBP was in the normal range.

There was no significant increase in plasma GH in response to GHRH, L-dopa, or clonidine (Fig. 3A). GH remained undetectable by RIA (<0.3 ng/mL); when measured by the chemiluminescent assay, GH rose slightly from 0.070 ± 0.054 to 0.107 ± 0.053 ng/mL (mean ± SD) after GHRH, from 0.150 ± 0.175 to 0.255 ± 0.193 ng/mL after L-dopa, and from 0.073 ± 0.052 ng/mL to 0.374 ± 0.406 ng/mL after clonidine. None of these responses are statistically or clinically significant. The responses of TSH and PRL to TRH (Fig. 3B) were present but somewhat blunted [normal range for TSH response, 10–17 μU/mL; for PRL response, 15–30 ng/mL (Ref. 24 and references cited therein)]. Exogenous GH treatment resulted in a significant rise of serum IGF-I and IGFBP3 (Fig. 3C), indicating normal tissue responsiveness to GH.

Structure of the GH-N gene

Based on the pedigree, clinical and biochemical phenotype, the patients were diagnosed as having isolated GH

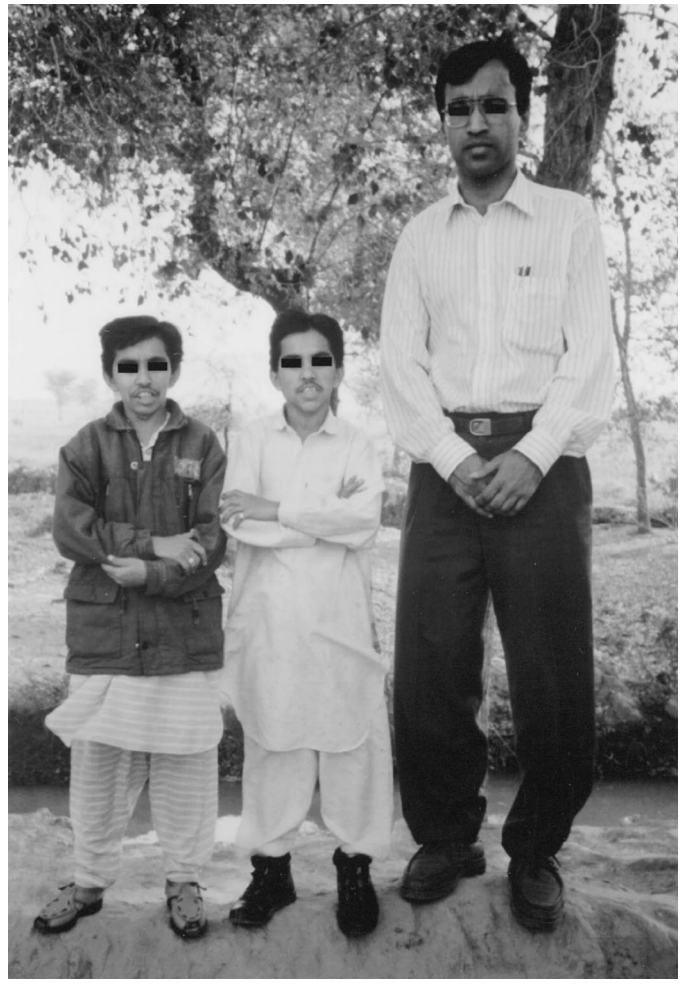

FIG. 2. Affected adult twins (no. 10 and 11; height, 118 and 119 cm) shown with a normal adult from the region (height, 168 cm).

TABLE 1. Anthropometric data

Subject no.[a]	Age (yr)	Sex	Height (cm)	Height SDS	Weight (kg)	Weight SDS	Upper/lower ratio	Arm span/height ratio	Waist/hip ratio	Head circumference (cm)	Head circumference SDS	Delay in bone age (yr)	Blood pressure (mm Hg)[b]	Blood pressure SDS[c]
8	28	F	113	−8.4	26	−4.2								
35	27	M	136	−6.3	30	−4.3	0.94	1.02	0.89	50	−4.0	adult	60/30	<−3.0
5	25	M	134	−6.5	28	−4.5	0.97	0.94	0.85	51	−3.7	adult	80/40	−2.3
18	25	F	114	−8.3	16	−5.4								
23	25	M	124	−8.0	21	−5.3	0.91	1.0	0.87	50	−4.0	adult	70/30	<−3.0
6	24	M	148	−4.5										
10	21	M	119	−8.8	17	−5.8	0.95	1.0	0.88	47	−6.0		60/30	<−3.0
11	21	M	118	−8.9	20	−5.4	0.97	1.0	0.92	49	−4.6		75/30	<−3.0
4	20	M	131	−7.0	27	−4.7	0.94	0.99	0.93	50	−4.0	−3	105/40	−1.3
25	15	M	103	−8.2	10	−5.6	0.95	0.96	0.89	46	−6.0	−7.9	65/40	<−3.0
31	15	M	110	−7.3	16	−4.9	0.93	0.95	0.91	51	−2.7		60/35	<−3.0
15	13	M	104	−6.3	13	−4.4	0.89	0.98	0.92	48	−4.0	−4.2	70/30	<−3.0
26	13	M	110	−5.6	14	−4.2	0.97	0.98	0.88	47	−4.5		80/30	<−3.0
45	9.5	M	91	−7.4	11	−4.4	1.08	0.95	0.98	49	−2.6	−4.2	60/30	−3.0
46	7.5	M	89	−6.8	11	−4.6	1.23	0.95	1.04	48	−2.7	−4.6	55/30	−3.0
29	7	M	87.5	−6.7	9.5	−5.1	1.08	0.93	0.98	47	−4.7	−4.1	60/30	−3.0
48	3	F	66	−7.4	6	−5.5								
12	NB[d]	M	44	−4.3	2.0	−3.4				34	−2.0			
Mean				−7.2[e]		−4.9[e]	0.99	0.97	0.92[f]	49[f]	−4.1[f]	−4.7[e]	69/33[f]	<−2.8[f]
SD				1.18		0.54	0.09	0.03	0.05	1.7	1.1	1.7	14/5	0.5
SEM				0.29		0.134	0.03	0.01	0.01	0.5	0.3	0.7	4/1	0.14

[a] Numbers refer to subject numbers in pedigree (Fig. 1).
[b] Blood pressure was measured with an adult-size cuff, which underestimates values by 10–15 mm Hg.
[c] For statistical comparison with normative data, blood pressure readings were corrected upwards by 12 mm Hg to minimize the impact of artifactually low readings.
[d] NB denotes newborn (25 days of age). The newborn data are excluded from the statistics because they are nonrepresentative (see text).
[e] Statistically significant difference from normal subjects ($P < 0.0001$).
[f] Statistically significant difference from normal subjects ($P < 0.01$).

deficiency on a genetic basis. We first analyzed the GH gene locus by restriction mapping but found no major rearrangement of the GH and placental lactogen genes. Sequencing of the GH-N gene in its entirety showed no mutation, excluding a defective structural GH gene as the basis for the disease.

Linkage analysis for candidate genes

We then considered other candidate genes, which included the promoter region of the GH-N gene, genes for GHRH, the GHRH-R, Bruton's tyrosine kinase (25), Pit-1 (26), and several other less likely candidates coding for factors involved in the regulation of GH secretion. The first three candidate genes (deemed most likely to be at fault) were examined for linkage between dwarfism and chromosomal markers near the candidate gene. Markers D17S795, D17S784 (near GH gene), D20S95, D20S27, D20S115, D20S470 (near GHRH gene), D7S493, and D7S1830 (near GHRH-R gene) were not linked to dwarfism, but D7S1808 and D7S817 (also near the GHRH-R gene) yielded LOD scores of 3.44 and 6.26 at Θ of 0.106 and 0.042, respectively, indicating linkage of the GHRH-R locus to the dwarf phenotype. Analysis of homozygosity by descent was consistent with linkage to that locus, though not statistically proven ($P \sim 0.02$ and ~ 0.06 for the two closest markers).

Amplification and sequencing of the GHRH-R gene

Segments of the GHRH-R gene were PCR-amplified from genomic DNA in a stepwise fashion based on the known complementary DNA structure (27, 28). We found a G-to-T transversion (GAG→TAG) in codon 72 (numbered from the AUG start codon), corresponding to amino acid residue 50 in the mature GHRH-R protein (Fig. 4A). This mutation predicts a translational stop (Glu→Stop), which truncates the GHRH-R in its extracellular domain (Fig. 5). The resulting GHRH-R protein, if extant, is predicted to be severely dysfunctional, bearing a disrupted binding site and lacking all transmembrane helices and intracellular segments.

The mutation creates a new B*fa*I restriction site, which permitted rapid genotyping of the whole kindred (Fig. 4B). In addition to restriction analysis with B*fa*I, genotypes were confirmed by DNA sequencing. The homozygous mutation segregated 100% with the dwarf phenotype, and all obligate heterozygotes were heterozygous for the mutation (Fig. 1). Thus, the disorder is transmitted in an autosomal recessive fashion with a high degree of penetrance. The subjects who were difficult to classify phenotypically included two homozygous subjects [one adult (no. 6) and one newborn (no. 12)] and four heterozygotes (no. 27, 28, 38, 43; aged 9–14).

Heterozygous phenotype

In an effort to determine whether there is a gene dosage effect in this disease, we carefully examined heterozygotes (n = 22) for subtle abnormalities (data are given as mean ± SD). Adults had no obvious short stature (height SDS, −1.5 ± 0.7) or other distinctive physical characteristics, but children and adolescents were relatively short (height SDS, −2.73 ± 0.94). The average age for male puberty in the affected kindred (with a high prevalence of heterozygous subjects) was reported as age 13; our Tanner staging of 2 heterozygous ad-

TABLE 2. Biochemical data (fasting basal serum or plasma)

Subject no.[a]	Age (yr)	Sex	Basal GH (ng/mL)	IGF-I (ng/mL)	IGF-II (ng/mL)	IGFBP3 (μg/mL)	IGFBP2 (ng/mL)	GHBP (nM)	PRL (ng/mL)	T_4 (μg/dl)	TSH (μU/mL)	Cortisol 9 AM (μg/dl)	Aldosterone (pg/mL)	DHEA-S (μg/dL)[b]	Testosterone (ng/dL)[b]
8	28	F	<0.3	4	82	0.4	458	1.3		9.1	1.2	5.7	15	49	
35	27	M	0.018	9	122	0.6	295	2.5	3.8	12.5	1.3	13.0	212	277	
5	25	M	0.069	8	89	0.5	356	1.7	2.5	7.2	1.5	11.3	155	178	625
18	25	F	<0.3	3	76	0.4	530	1.1		10.3	0.5	11.3	7	49	
23	25	M	0.036	8	117	0.6	463	1.1	3.1	9.0	1.2	10.8	76	139	1045
10	21	M	0.047						7.2			7.8	85	131	
11	21	M	0.046	6					3.9			6.5	48	124	
4	20	M	0.026	7	98	0.5	478	1.2	2.4	8.4	3.4	7.5	43	197	941
25	15	M	<0.3	4	68	0.3	398	1.2	0.7	11.1	0.9	9.3	12	11	11
31	15	M	0.093	3	51	0.3	315	1.8	3.2	6.4	0.5	9.2	421	29	10
15	13	M	0.072	4	65	0.3	376	0.9	6.1	11.8	2.2	8.5	104	28	
26	13	M	0.207	5	98	0.5	676	0.9	2.4	10.5	0.6	11.6	46	15	11
45	9.5	M	<0.3	3	63	0.2	331	1.3	2.9	7.9	1.8				
46	7.5	M	<0.3	5	75	0.5	381	0.7	3.7	7.3	2.4				11
29	7	M	0.081	4	110	0.3	666	1.0	1.5	6.0	0.8				
Mean			0.070	5.2[c]	86[c]	0.42[c]	440[c]	1.29	3.3	9.0	1.4	9.4	102	143	871
SD			0.054	2.0	22	0.13	123	0.48	1.7	2.1	0.9	2.3	117	76	219
SEM			0.016	0.6	6	0.04	34	0.13	0.5	0.6	0.2	0.7	34	27	127
Range			0.02–0.21	3–9	51–122	0.2–0.6	295–676	0.7–2.5	0.7–7.2	6–12.5	0.5–3.4	5.7–13	7–421	49–277	625–1045
Normal range[d]			0.02–5	90–840	250–740	2.0–6.0	50–325	0.5–2.5	3–20	5–13	0.04–4.0	5–20	40–310	35–560	300–1200

[a] Numbers refer to subject numbers in pedigree (Fig. 1).
[b] Only adult DHEA-S and testosterone values are included in the statistic. Pediatric, as adult values, are in the normal range.
[c] Statistically significant differences from normal subjects ($P < 0.0001$).
[d] Some of the normal ranges are wide because they encompass both adult and pediatric ranges.

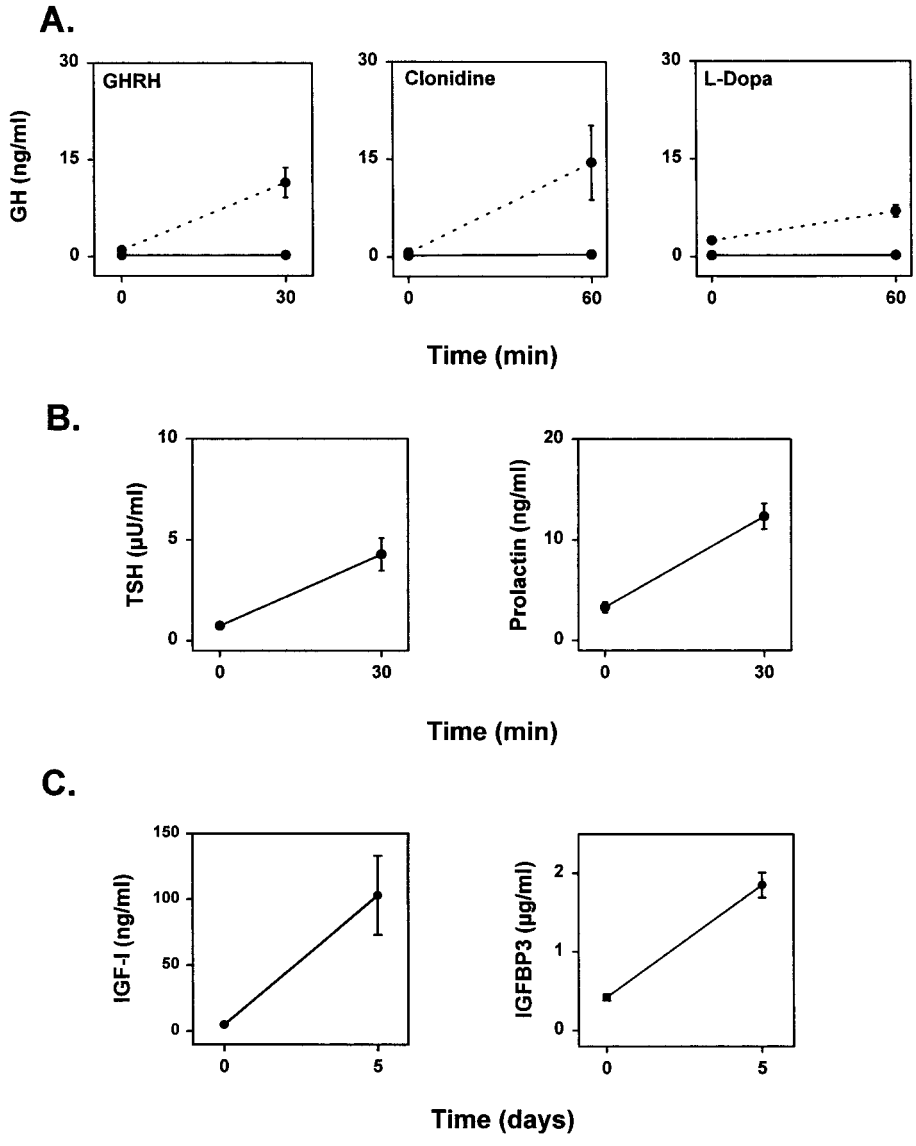

FIG. 3. A, GH stimulation tests (n = 9–12 patients and 2–5 heterozygous subjects); B, TRH stimulation test (n = 12 patients); C, IGF generation test (exogenous GH for 5 days) (n = 12 patients). *Solid lines*, affected patients; *dashed lines*, heterozygous subjects; *error bars*, SEM.

olescents was consistent with that timing. When all heterozygous subjects were pooled, their height SDS was −1.89 ± 1.1, whereas that of 5 subjects homozygous for the wild-type allele was −1.51 ± 1.8 (difference not significant). The average height of randomly selected men without known genotype was 172.8 ± 6 cm, which corresponds to a height SDS of −0.6 ± 0.9 [based on recent reports, the growth data of adequately nourished Pakistani children are indistinguishable from American NCHS data (29); this is consistent with our own observations about adult height in the affected region.] Our measurements suggest, but do not prove, a slight growth delay and, perhaps, adult height deficit in the heterozygous state.

In adult heterozygotes, serum IGF-I was 103 ± 53 ng/mL, IGF-II was 395 ± 110 ng/mL, IGFBP3 was 2.8 ± 1.7 μg/mL, IGFBP2 was 389 ± 106 ng/mL, and GHBP was 1.36 ± 0.58 nM (insufficient data are available in wild-type homozygotes for comparison). The mean GH responses to GHRH, L-dopa, and clonidine showed peak levels of 7.1 ± 2.0, 6.6 ± 1.5, and 10.5 ± 5.4 ng/mL, respectively, all except clonidine slightly below the normal range [normal for GHRH, 8–40 ng/mL; for L-dopa, 11–20 ng/mL; and for clonidine, 6.5–10 ng/mL (Ref. 24 and references cited therein)]. The overall data are consistent with a partially blunted GH-IGF axis in heterozygotes.

Chromosomal location of the GHRH-R gene

Multipoint linkage analysis permitted a more precise localization of the GHRH-R gene than was previously available (30, 31). The gene is located on the short arm of chromosome 7 (7p14), approximately 2.6 cM telomeric from microsatellite D7S510 (Fig. 6).

Discussion

This study describes, in detail, a newly identified syndrome (7–9) of inherited dwarfism caused by an inactivating mutation (E50X)[1] in the GHRH-R gene. The mutated

[1] Amino acid residue no. 50 refers to the mature protein; it corresponds to residue 72 when the signal peptide is included.

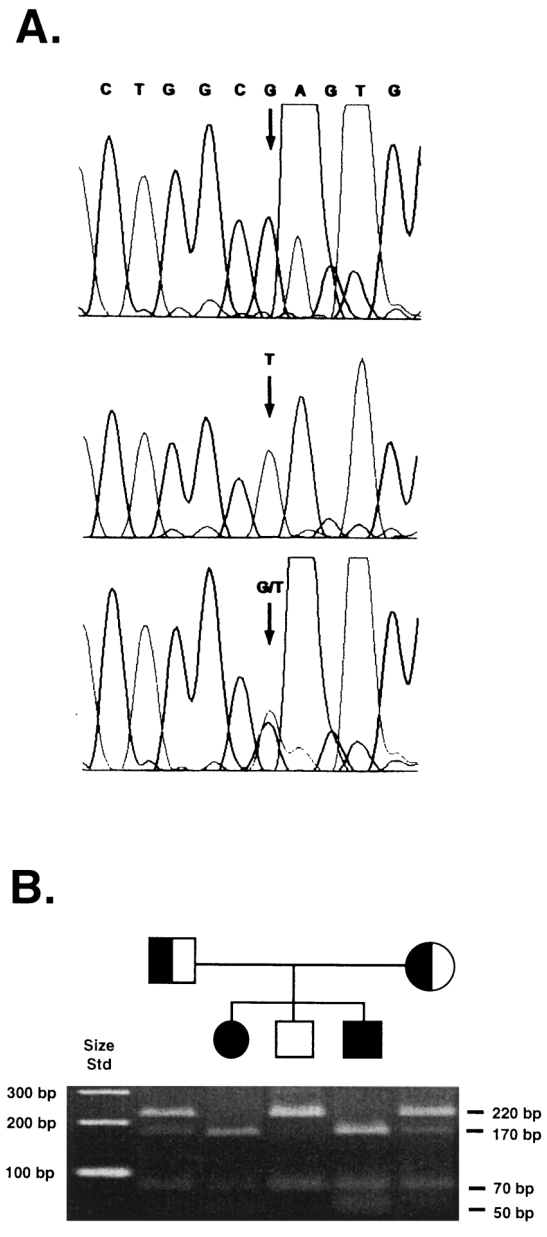

FIG. 4. A, Representative DNA sequencing profiles of the relevant segment of the GHRH-R gene. Profiles from a homozygous wild-type (*top*), homozygous affected (*middle*), and heterozygous (*bottom*) subject are shown. The *arrow* points to the mutated base. B, Restriction patterns of a relevant GHRH-R gene fragment containing the mutation (bp 148–313; Ref. 27) with B*fa*I [a 126-bp intron (Ref. 7) is included in the fragment]. A partial pedigree is shown on *top*. The wild-type gene yields two bands (220 and 70 bp in size), resulting from a restriction site in the intron. The mutation generates a new B*fa*I site, converting the 220-bp band to fragments of 170 and 50 bp.

GHRH-R causes isolated GH deficiency due to GHRH resistance. The large number and age span of affected, untreated subjects in our kindred, together with the four pediatric-age patients described in two other families (7, 9), permits a fairly comprehensive definition of this syndrome. Its salient features are as follows. Inheritance is autosomal recessive with high penetrance. There is an unexplained male preponderance in our kindred; of the other four reported patients, three are male. Clinical features include severe postnatal growth failure, proportionate dwarfism, decreased cranial size, minimal or no facial hypoplasia, normal intelligence, no known hypoglycemia, no microphallus, delayed puberty, and asymptomatic hypotension. Truncal adiposity was likely increased, though not clinically evident, as suggested by the high waist/hip ratios in this relatively lean population. However, interpretation of this finding should be tempered by the known tendency of South Asians towards high waist/hip ratios (32); unfortunately, there are no age-appropriate normative Pakistani or Indian data available to permit formal statistical comparison. Nevertheless, we believe that a mild degree of truncal/visceral obesity was present—a finding consistent with GH deficiency. The biochemical phenotype includes severe isolated GH deficiency, with low levels of IGF-I, IGF-II, and IGFBP3, and elevated levels of IGFBP2. Pituitary hypoplasia has been documented by magnetic resonance imaging in two patients (9). Heterozygotes have a blunted GH-IGF axis; they may show a slightly delayed growth pattern but are only minimally affected with respect to adult height.

Dwarfism of Sindh represents the human homologue of the little (*lit/lit*) mouse, first described in 1976 (33). This mouse harbors a missense mutation in the GHRH-R (34, 35), which abolishes ligand binding, causing GHRH resistance, pituitary hypoplasia, isolated GH deficiency, and a dwarf phenotype. Brain weight is decreased by 15–20% (36), a finding consistent with the small head circumference in our patients. Despite the existence of this mouse model, human GHRH resistance was not identified until 1996 (4, 37).

This syndrome demonstrates that in humans, as in mice, GHRH signaling is absolutely required for normal GH production and somatic growth. Because of the complex regulation of GH secretion, which involves GHRH, SRIF, and probably the putative endogenous ligand for the GH-releasing peptide (GHRP) receptor (38), it is difficult to discern their relative contributions to GH secretion in the intact subject. Dwarfism of Sindh clearly identifies GHRH as a critical factor because of its importance for somatotroph development (39, 40), GH synthesis (41), and GH release (42). Nonetheless, a minuscule amount of GH seems to be released with secretagogues, in agreement with data in the little mouse (43, 44).

Several aspects of this syndrome are of interest, and some differ from other forms of GH deficiency. Although growth retardation is severe, there is significant variability in height. In particular, one patient (no. 6) is relatively tall. Head size is significantly smaller than that observed in conventional GH deficiency (45–47), and there is no facial hypoplasia or frontal bossing. This contributes to the "normal, but miniature adult" aspect of these patients. The asymptomatic low blood pressure is a unique finding. We did not observe significant truncal obesity or microphallus, nor could we elicit a history of hypoglycemia, all well-recognized features of classical GH deficiency (or GH resistance). Absence of these features was also noted by Netchine *et al.* (9), though Wajnrajch *et al.* reported frontal bossing and obesity in their patients (7). The blunted PRL and TSH responses [the former also reported by Netchine *et al.* (9)] are unexpected in classical isolated GH deficiency. They may be related to pituitary hypoplasia or to a specific role of GHRH in maximizing PRL and TSH production.

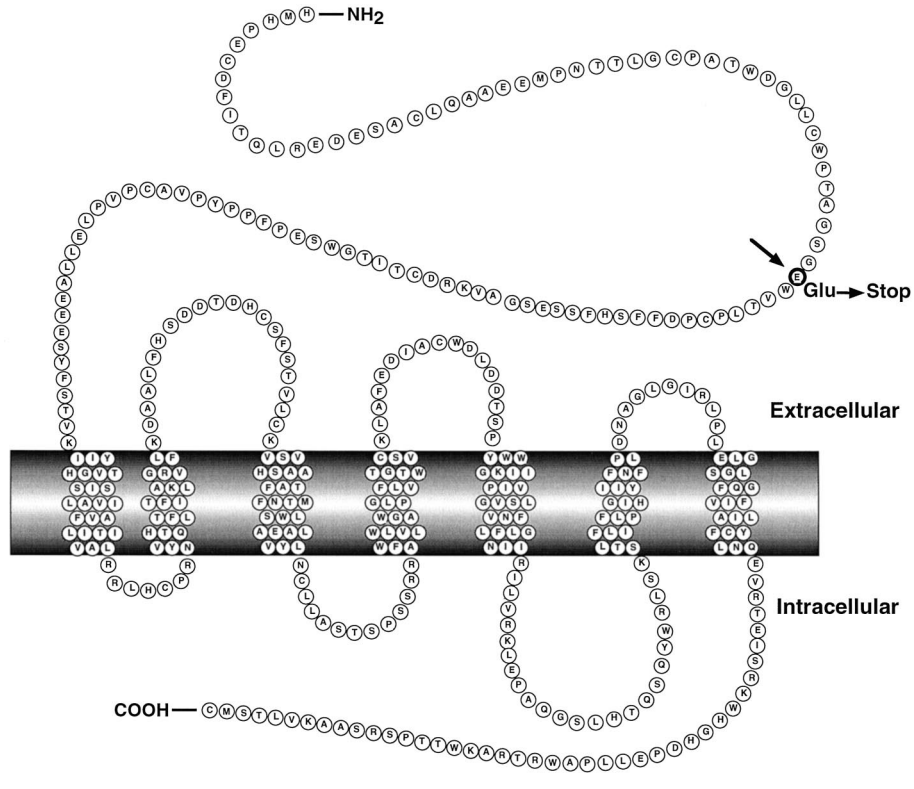

FIG. 5. Model of the mature human GHRH receptor protein in its plasma membrane environment. The *arrow* shows the amino acid residue affected by the mutation, with resulting truncation of most of the receptor.

Neither PRL nor TSH insufficiency is clinically apparent in the patients, but the little mouse has some difficulty with lactation (33). It is tempting to attribute these deviations from classical GH deficiency to the absence of direct GHRH effects. Although direct extrapituitary GHRH effects remain poorly characterized, they are plausible in view of the widespread expression of GHRH and the GHRH-R (48). An alternative explanation for unexpected findings, such as hypotension, is consanguinity, affecting loci unrelated to the GHRH-R gene.

Pubertal delay is common in GH deficiency and was also seen in our patients and in a 16-yr-old male reported by Wajnrajch *et al.* (7). In contrast, the two male patients of Netchine *et al.* (9) started puberty at age 13, but as pointed out by the authors, they had been treated with GH for several years. It seems likely that pubertal delay is a result of GH deficiency rather than a direct consequence of abnormal GHRH-R function.

Reports of the identical mutation in two other families originating on the Indian subcontinent (7, 9) raises the question of a founder effect. However, there is no known recent connection among the three populations. The Sindhi population that gave rise to our patients has lived in the same area for at least 200 yr; they are not recent immigrants from India. Conversely, the patients reported by Wajnrajch *et al.* (7) originate from the Bombay area (J.M. Gertner, personal communication), without known ancestry in the Indus valley. The patients reported by Netchine *et al.* (9) are Tamouleans from Delft, an island between India and Sri Lanka. In addition to their geographic separation, ethnic and linguistic evidence does not suggest a recent relationship among the three families. A historically remote connection, though probable, fails to fully explain why this syndrome was not observed earlier (see below). Therefore, the possibility of an independent occurrence of the same mutation cannot be dismissed, possibly implying a mutational hot spot. This issue may be resolved by haplotype analysis.

A related puzzle is the very recent emergence of this syndrome. Given the long-standing custom of consanguineous unions in this population, the distinctive phenotype, high penetrance, apparent lack of lethality, limited gene pool, and large size of families, it would be expected that the homozygous dwarf phenotype would emerge soon after the occurrence of the mutation. One possible explanation for the dwarfism being limited to the youngest generation is that the mutation is of recent origin. We presently have no evidence to confirm or exclude this hypothesis. Other possibilities include: 1) lack of recognition of dwarfism in earlier generations, which may have been of shorter stature because of nutritional deficiency; 2) mortality of the affected (perhaps of hypoglycemia), in previous generations, during early childhood, at an age when dwarfism is not yet obvious; and 3) suboptimal health and nutrition, leading to fetal death of the affected in earlier generations. None of these explications is entirely satisfactory. Future haplotype analysis, with closely spaced markers surrounding the GHRH-R gene, may provide the answer to when the mutation occurred.

The offspring of two affected parents provides some unique insights. First, the child proves that fertility is possible, in both sexes, in the absence of a GHRH-R. GHRH and its receptor are expressed in testis and ovary (49, 50), but their role in gonadal function is not clear. Our observation shows that the GHRH-R is not critical for fertility (though subfertility cannot be excluded). This is consistent with fertility in the little mouse (33, 34). It has been proposed that the reproductively important GHRH gene product in testis is a peptide distinct from GHRH (the GHRH-related peptide)

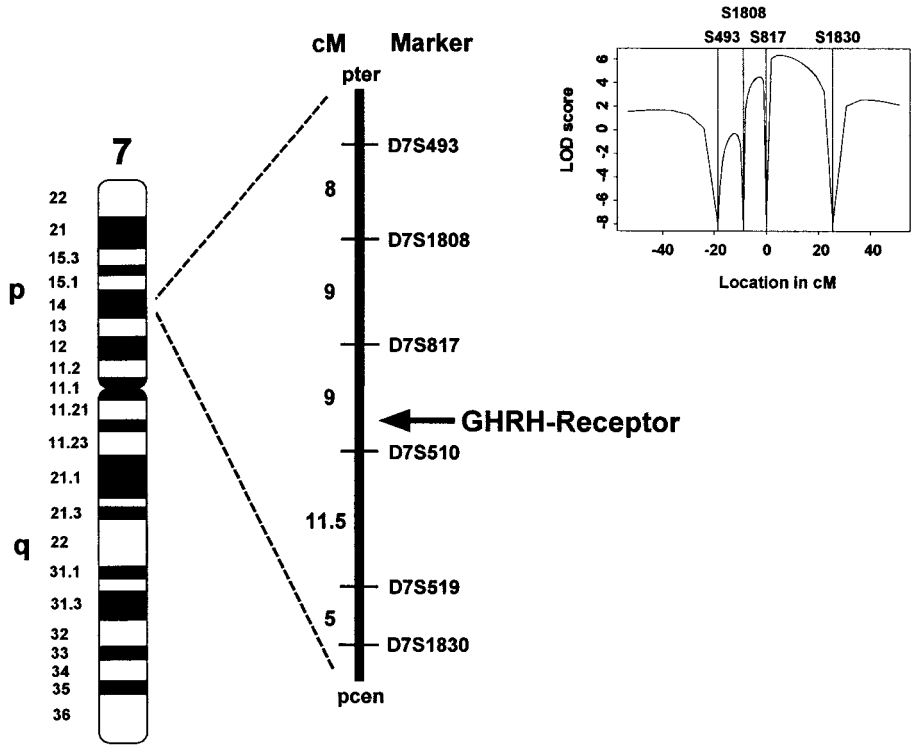

FIG. 6. Localization of the GHRH-R gene on the short arm of human chromosome 7. The gene is located 2.6 cM telomeric from marker D7S510. *Inset*, Multipoint linkage analysis results.

and that this peptide signals through a receptor distinct from the GHRH-R (51). The second insight results from the phenotype of the newborn (no. 12), which resembles intrauterine growth retardation. This may be a result of maternal size, the conceptus' genotype, or both. A mismatch between (dwarfed) maternal and (relatively normal) fetal size may have resulted in placental insufficiency. Dwarfed mothers have an increased rate of fetal loss for unknown reasons (52). Alternatively, GHRH-R deficiency in the fetoplacental unit may have played a role, although little is known about expression or function of the GHRH-R in human placenta.

Treatment of this syndrome with GH results in normalization of growth (7, 9), without evidence of significant anti-GH antibody production. This suggests that the small amount of GH produced is sufficient to induce immune tolerance to GH, similar to what has been postulated for isolated GH deficiency type IB (3). A potential alternative for therapy is GHRP, but it is unknown whether GHRP can release GH in the absence of a functional GHRH system. GHRH is known to potentiate GHRP effects (53), but the mechanistic basis of this phenomenon is poorly understood. Therefore, testing GHRH-R-deficient patients with one of the GHRPs will be important for both heuristic and therapeutic reasons. Pituitary (somatotroph) hypoplasia will have to be taken into account in interpreting the response to GHRP.

As a byproduct of linkage analysis, we have refined the localization of the GHRH-R gene on the short arm of chromosome 7. This should facilitate the search for disorders potentially linked to the GHRH-R gene (*e.g.* short stature, pituitary tumors, and acromegaly). In a recent listing of human genetic diseases, a locus associated with GH-deficient dwarfism is identified on the short arm of chromosome 7 (54). It is likely that the gene in question encodes the GHRH-R.

In summary, we describe, in detail, a cluster of severe familial dwarfism caused by a nonsense mutation in the GHRH-R gene, which, in its homozygous form, causes isolated GH deficiency with some unusual features. This discovery should facilitate the search for milder mutations in the GHRH-R as a potential cause of short stature and should help in the identification of factors responsible for genetic height determination.

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Note Added in Proof

Since submission of this manuscript, we have had the opportunity to re-examine four adult male patients (no. 4, 5, 23, 35) and the offspring of the two affected parents (no. 12). Height in the adults has not changed in the intervening three and a half years, but subjects no. 5, 23, and 35 had gained 4, 3.2, and 6.8 kg, respectively, with significant increases in waist circumference (11, 7.5, and 12 cm) and hip circumference (6, 9.5, and 7 cm). Waist/hip ratios changed by +0.08, −0.01 and +0.08, respectively. In contrast, subject no. 4 had lost 0.8 kg. Blood pressures, measured with a pediatric cuff, were 105/75, 115/80, 90/58, and 88/60 mm Hg, respectively. The newborn (no. 12, now 3.5 yr old) was 75 cm tall (−5.9 SDS) and weighed 8.75 kg (−3.9 SDS). His physical phenotype is

proportionate and eumorphic. Except for his size, physical and mental development was unremarkable; he had no history of symptomatic hypoglycemia or seizures. Patient no. 35 had fathered a boy with a first degree, unaffected cousin whose genotype is unknown. We were unable to examine the child, but he was said to be developing normally. Thus there are now at least two instances of male fertility among the affected patients. GHRH levels in peripheral blood (collected with protease inhibitors) were normal in the four adults (22, 16, 25, and 26 pg/mL; normal range 0–50 pg/mL). Plasma atrial natriuretic peptide (ANP) levels were 55, 58, 129, and 77 pg/mL, respectively (normal range 25–77 pg/mL). GHRH and ANP were measured at Quest-Nichols Laboratories.

References

1. **Cassidy CM.** 1991 The good body: when big is better. Med Anthropol. 13:181–213.
2. **Lee E.** 1994 The high and the mighty among man and beast: how universal is the relationship between height (or body size) and social status? In: Lee E, ed. Social stratification and socioeconomic inequality. Reproductive and interpersonal aspects of dominance and status. Vol 2. Westport, CT: Praeger; 93–111.
3. **Phillips III JA, Cogan JD.** 1994 Genetic basis of endocrine disease 6: molecular basis of familial growth hormone deficiency. J Clin Endocrinol Metab. 78:11–16.
4. **Cohen LE, Wondisford FE, Radovick S.** 1996 Role of Pit-1 in the gene expression of growth hormone, prolactin, and thyrotropin. Endocrinol Metab Clin N Am. 25:523–540.
5. **Wu W, Cogan JD, Pfäffle RW, et al.** 1998 Mutations in PROP1 cause familial combined pituitary hormone deficiency. Nat Genet. 18:147–149.
6. **Rosenfeld RG, Rosenbloom AL, Guevara-Aguirre J.** 1994 Growth hormone (GH) insensitivity due to primary GH receptor deficiency. Endocr Rev. 15:369–390.
7. **Wajnrajch MP, Gertner JM, Harbison MD, Chua Jr SC, Leibel RL.** 1996 Nonsense mutation in the human growth hormone-releasing hormone receptor causes growth failure analogous to the little (lit) mouse. Nat Genet. 12:88–90.
8. **Baumann G, Maheshwari H.** 1997 The dwarfs of Sindh: severe growth hormone (GH) deficiency caused by a mutation in the GH-releasing hormone receptor gene. Acta Paediatr Suppl. 423:33–38.
9. **Netchine I, Talon P, Dastot F, Vitaux F, Goossens M, Amselem S.** 1998 Extensive phenotypic analysis of a family with growth hormone (GH) deficiency caused by a mutation in the GH-releasing hormone receptor gene. J Clin Endocrinol Metab. 83:432–436.
10. **Anonymous.** 1994 The dwarfs of Sindh. Dawn (Karachi, Pakistan, newspaper), February 21: p. 7.
11. **National Center for Health Statistics Growth Curves for Children: Birth-18 Years.** 1988 US Department of Health, Education and Welfare Publication No. (PHS) 78–1650, Series 11 (165), Government Printing Office.
12. **Nellhaus G.** 1968 Head circumference from birth to eighteen years. Practical composite international and interracial graphs. Pediatrics. 41:106–114.
13. **Wilkins L.** 1966 The diagnosis and treatment of endocrine disorders in childhood and adolescence. 3rd ed. Springfield, IL: CG Thomas: 33.
14. **Roche AF, Malina RM.** 1983 Manual of physical status and performance in childhood. New York: Plenum Press; 976–981.
15. **Forbes GB.** 1990 The abdomen:hip ratio. Normative data and observations on selected patients. Int J Obes. 14:149–157.
16. **Drizd T, Dannenberg AL, Engle A.** 1986 Blood pressure levels in persons 18–74 years of age in 1976–80, and trends in blood pressure from 1960 to 1980 in the United States. Vital Health Stat [11]. DHHS Pub. No. (PHS). 86–1684.
17. **Blumenthal S, Epps RP, Heavenrich R, et al.** 1977 Report of the task force on blood pressure control in children. Pediatrics. [Suppl]59:797–820.
18. **Tanner JM, Whitehouse RH, Cameron N, Marshall WA, Healy MJR, Goldstein H.** 1983 Assessment of skeletal maturity and prediction of adult height (TW2 Method). London: Academic Press.
19. **Chapman IM, Hartman ML, Straume M, Johnson ML, Veldhuis JD, Thorner MO.** 1994 Enhanced sensitivity growth hormone (GH) chemiluminescence assay reveals lower postglucose nadir GH concentrations in men than women. J Clin Endocrinol Metab. 78:1312–1319.
20. **Baumann G, Shaw MA, Amburn K.** 1989 Regulation of plasma growth hormone-binding proteins in health and disease. Metabolism. 38:683–689.
21. **Lathrop GM, Lalouel JM, Julier C, Ott J.** 1984 Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA. 81:3443–3446.
22. **Cottingham Jr RW, Idury RM, Schaffer AA.** 1993 Faster sequential genetic linkage computations. Am J Hum Genet. 53:252–263.
23. **Schaffer AA, Gupta SK, Shriram K, Cottingham Jr RW.** 1994 Avoiding recomputation in linkage analysis. Hum Hered. 44:225–237.
24. **Dufour DR, Jubiz WA.** 1995 Dynamic procedures in endocrinology. In: Becker KL, ed. Principles and practice of endocrinology and metabolism. 2nd ed. Philadelphia: J. B. Lippincott; 2013–2033.
25. **Fleisher TA, White RM, Broder S, et al.** 1980 X-linked hypogammaglobulinemia and isolated growth hormone deficiency. N Engl J Med. 302: 1429–1434.
26. **Tatsumi K, Miyai K, Notomi T, et al.** 1992 Cretinism with combined hormone deficiency caused by a mutation in the PIT1 gene. Nat Genet. 1:56–58.
27. **Mayo KE.** 1992 Molecular cloning and expression of a pituitary-specific receptor for growth hormone-releasing hormone. Mol Endocrinol. 6:1734–1744.
28. **Gaylinn BD, Harrison JK, Zysk JR, Lyons CE, Lynch KR, Thorner MO.** 1993 Molecular cloning and expression of a human anterior pituitary receptor for growth hormone-releasing hormone. Mol Endocrinol. 7:77–84.
29. **Akram DS, Agboatwala M.** 1991 Growth parameters of Pakistani children. Indian J Pediatr. 58:825–832.
30. **Gaylinn BD, von Kap-Herr C, Golden WL, Thorner MO.** 1994 Assignment of the human growth hormone-releasing hormone receptor gene (GHRHR) to 7p14 by *in situ* hybridization. Genomics. 19:193–195.
31. **Vamvakopoulos NC, Kunz J, Olberding U, et al.** 1994 Mapping the human growth hormone-releasing hormone receptor (GHRHR) gene to the short arm of chromosome 7 (7p13–p21) near the epidermal growth factor receptor (EGFR) gene. Genomics. 20:338–340.
32. **Ramachandran A, Snehalatha C, Dharmaraj D, Viswanathan M.** 1992 Prevalence of glucose intolerance in Asian Indians. Urban-rural difference and significance of upper body adiposity. Diabetes Care. 15:1348–1355.
33. **Eicher EM, Beamer WG.** 1976 Inherited ateliotic dwarfism in mice. Characteristics of the mutation, little, on chromosome 6. J Hered. 67:87–91.
34. **Godfrey P, Rahal JO, Beamer WG, Copeland NG, Jenkins NA, Mayo KE.** 1993 GHRH receptor of little mice contains a missense mutation in the extracellular domain that disrupts receptor function. Nat Genet. 4:227–232.
35. **Lin SC, Lin CR, Gukovsky I, Lusis AJ, Sawchenko PE, Rosenfeld MG.** 1993 Molecular basis of the little mouse phenotype and implications for cell type-specific growth. Nature. 364:208–213.
36. **Noguchi T.** 1991 Retarded cerebral growth of hormone-deficient mice. Comp Biochem Physiol [C]: 98:239–248.
37. **Maheshwari H, Dupuis J, Silverman BL, Baumann G.** Dwarfism of Sindh: a novel form of familial isolated GH deficiency linked to the locus for the GH releasing hormone receptor. Proc of the 10th Annual Meeting of The International Congress of Endocrinology, San Francisco, CA, 1996, p 709.
38. **Howard AD, Feighner SD, Cully DF, et al.** 1996 A receptor in pituitary and hypothalamus that functions in growth hormone release. Science. 273:974–977.
39. **Billestrup N, Swanson LW, Vale W.** 1986 Growth hormone-releasing factor stimulates proliferation of somatotrophs *in vitro*. Proc Natl Acad Sci USA. 83:6854–6857.
40. **Lin SC, Lin CR, Gukovsky I, Lusis AJ, Sawchenko PE, Rosenfeld MG.** 1993 Molecular basis of the little mouse phenotype and implications for cell type-specific growth. Nature. 364:208–213.
41. **Barinaga M, Yamamoto G, Rivier C, Vale W, Evans R, Rosenfeld MG.** 1983 Transcriptional regulation of growth hormone gene expression by growth hormone releasing factor. Nature. 306:84–85.
42. **Ocampo-Lim B, Guo W, DeMott-Friberg R, Barkan AL, Jaffe CA.** 1996 Nocturnal growth hormone (GH) secretion is eliminated by infusion of GH-releasing hormone antagonist. J Clin Endocrinol Metab. 81:4396–4399.
43. **Clark RG, Robinson ICAF.** 1985 Effects of a fragment of human growth hormone-releasing factor in normal and Little mice. J Endocrinol. 106:1–5.
44. **Cheng TC, Beamer WG, Phillips III JA, Bartke A, Mallone RL, Dowling C.** 1983 Etiology of growth hormone deficiency in Little, Ames, and Snell dwarf mice. Endocrinology. 113:1669–1678.
45. **Goodman HG, Grumbach MM, Kaplan SL.** 1968 Growth and growth hormone. II. A comparison of isolated growth-hormone deficiency and multiple pituitary-hormone deficiencies in 35 patients with idiopathic hypopituitary dwarfism. N Engl J Med. 278:55–68.
46. **Burt L, Kulin HE.** 1977 Head circumference in children with short stature secondary to primary hypothyroidism. Pediatrics. 59:628–630.
47. **Laron Z, Roitman A, Kauli R.** 1979 Effect of human growth hormone therapy on head circumference in children with hypopituitarism. Clin Endocrinol (Oxf). 10:393–399.
48. **Matsubara S, Sato M, Mizobuchi M, Niimi M, Takahara J.** 1995 Differential gene expression of growth hormone (GH)-releasing hormone (GRH) and GRH receptor in various rat tissues. Endocrinology. 136:4147–4150.
49. **Berry SA, Srivastava CH, Rubin LR, Phipps WR, Pescovitz OH.** 1992 Growth hormone releasing hormone-like messenger ribonucleic acid and immunoreactive peptide are present in human testis and placenta. J Clin Endocrinol Metab. 75:281–284.
50. **Bagnato A, Moretti C, Ohnishi J, Frajese G, Catt KJ.** 1992 Expression of the growth hormone releasing hormone gene and its peptide product in the rat ovary. Endocrinology. 130:1097–1102.
51. **Breyer PR, Rothrock JK, Beaudry N, Pescovitz OH.** 1996 A novel peptide from the growth hormone releasing hormone gene stimulates Sertoli cell activity. Endocrinology. 137:2159–2161.
52. **Tyson JE, Barnes AC, McKusick VA, Scott CI, Jones GS.** 1970 Obstetric and gynecologic considerations of dwarfism. Am J Obstet Gynecol. 108:688–704.
53. **Bowers CY, Reynolds GA, Durham D, Barrera CM, Pezzoli SS, Thorner MO.** 1990 Growth hormone (GH)-releasing peptide stimulates GH release in normal men and acts synergistically with GH-releasing hormone. J Clin Endocrinol Metab. 70:975–982.
54. **Anonymous.** 1994 Human genetic disorders. J NIH Res. 6(8):115–134.