Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 8083–8087, October 1989
Medical Sciences

Characterization of the human growth hormone receptor gene and demonstration of a partial gene deletion in two patients with Laron-type dwarfism

(exon structure/DNA sequence/gene defects)

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Communicated by William H. Daughaday, July 24, 1989 (received for review June 8, 1989)

ABSTRACT **Laron-type dwarfism is an autosomal recessive genetic disorder that is characterized by high levels of growth hormone and low levels of insulin-like growth factor I in the circulation. Several lines of evidence suggest that this disease is caused by a defect in the growth hormone receptor. In order to analyze the receptor gene in patients with Laron-type dwarfism and with other growth disorders, we have first determined the gene structure in normal individuals. There are nine exons that encode the receptor and several additional exons in the 5′ untranslated region. The coding exons span at least 87 kilobase pairs of chromosome 5. Characterization of the growth hormone receptor gene from nine patients with Laron-type dwarfism shows that two individuals have a deletion of a large portion of the extracellular, hormone binding domain of the receptor gene. Interestingly, this deletion includes nonconsecutive exons, suggesting that an unusual rearrangement may have occurred. Thus, we provide direct evidence that Laron-type dwarfism can result from a defect in the structural gene for the growth hormone receptor.**

Growth hormone (GH) is secreted from the pituitary and has direct and indirect actions on various tissues, causing effects on growth and metabolism (1, 2). Though high-affinity binding sites for GH have been identified in a number of tissues (1, 3), the highest concentration of GH receptors is found in the liver (4) where GH induces the expression and secretion of insulin-like growth factor I (IGF-I) (3). The GH receptor recently has been purified and characterized from rabbit liver (5), and clones for the rabbit and human GH receptor have been isolated from liver cDNA libraries (6). These clones encode a protein of 620 amino acids with a single, centrally located transmembrane domain. Comparison of the primary sequences of the GH receptor and of the related prolactin receptor (7) suggests that they may be members of a new family of membrane-bound receptors.

A high-affinity GH binding protein has been demonstrated in the plasma of a number of mammals, including man (8–10). Characterization of this binding protein (11, 12), including direct amino acid sequence data (5, 6), shows that the GH binding protein is the extracellular, hormone binding domain of the GH receptor. The role of this protein in the regulation of growth is not known.

Laron-type dwarfism (LTD) is a rare, autosomal recessive disorder that is characterized by high circulating levels of biologically active GH accompanied by low levels of IGF-I (13–16) and a failure to respond to GH therapy (15). Some individuals with LTD have been shown to lack GH binding in liver biopsy samples (17), to lack GH binding activity in their serum (18–20), or to lack an IGF-I response in transformed T lymphoblasts (21). These studies suggest that LTD results from a defect in the GH receptor. In theory, LTD could result from defects in the gene that encodes the GH receptor itself as well as in other genes required for its expression or function.

In order to define the role of the GH receptor in human growth disorders, we present here the characterization of the gene for the human GH receptor.‖ We use these data to demonstrate the deletion of a large portion of the receptor gene in two LTD patients. A portion of this work has been presented previously.**

HUMAN SUBJECTS

Patient 147 is a male with LTD born to related parents of Jewish Iraqi origin. Birth length (49 cm) and weight (3.0 kg) were normal. He showed slow physical and mental development and had clinical features of GH deficiency but with high GH levels (>40 ng/ml) and low levels of IGF-I. At age 35 his height was 128.3 cm (7.3 SD below expected) and his weight was 41 kg. No serum GH binding protein activity was detected. No ^{125}I-labeled GH binding was detected in liver microsomal membranes from this patient after liver biopsy [case 2 (17)].

Patient D1 is a girl with LTD born to related parents of Jewish Iraqi origin. Birth length (45 cm) and weight (2.85 kg) were normal. At the age of 6 months she showed typical features of GH deficiency but with high plasma levels of GH (20–100 ng/ml). After 5 days of GH therapy, there was no increase in the plasma IGF-I level. At age 8 years, 10 months, her length was 104 cm (4.2 SD below expected) and her weight was 22.5 kg. No serum GH binding protein activity was detected (20).

Patient 1 is a boy with LTD born to unrelated parents in the United States. He lacks serum GH binding protein activity [see patient 1 (18) for a full clinical description]. DNA was obtained from fibroblasts.

The other six LTD patients, who had no alteration in their genomic DNA pattern, include three of oriental Jewish background, two from the United States, and one from

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Abbreviations: GH, growth hormone; IGF-I, insulin-like growth factor I; LTD, Laron-type dwarfism.

‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. M26401).

**Meacham, L. R., Parks, J. S., McKean, M. C., Keret, R. & Laron, Z., 71st Annual Meeting of the Endocrine Society, Seattle, June 21–24, 1989, abstr. 1651.

Poland (fibroblasts provided by Maria Malinowska and Thomasz Romer, Child Health Center, Warsaw, Poland). The three oriental Jewish patients [described previously as patients 8, 9, and 10 (14)] are from one clan and have clinical features of GH deficiency but with high plasma levels of GH, low levels of IGF-I, and undetectable GH serum binding protein activity (20). All three showed no response to GH therapy. The two U.S. patients lack serum binding protein activity and showed no response to GH therapy [described previously as patients 2 and 3 (18) or as patients 6 and 8, respectively (22)].

METHODS

Genomic clones for the GH receptor were isolated from λ libraries either described previously, λ4X (23) (λGG.9, -19, and -20), or kindly provided by John McLean (Genentech) (λGG.33, -47, and -48). The libraries were screened with ^{32}P-labeled (24) cDNA probes from the human GH receptor (6). The hybridization was performed at 42°C in 50% formamide/0.75 M NaCl/0.075 M trisodium citrate, and the filters were washed at 42°C in 0.03 M NaCl/3 mM trisodium citrate/0.1% SDS. DNA sequencing was performed by the dideoxy chain-termination method (25) using genomic fragments subcloned into plasmid vectors (26).

Genomic DNA was isolated from normal or LTD blood or from fibroblasts. Six micrograms of DNA was digested with *Hin*dIII or *Eco*RI, electrophoresed on a 0.7% agarose gel, transferred to nitrocellulose, hybridized as above, and washed at 50°C as above (24). Probes for the genomic hybridization were labeled with ^{32}P (24). The full-length probe was a 1928-base-pair (bp) *Xho* I to *Sna*BI fragment (bp −11 to +1917) of the human GH receptor cDNA (6). To generate the exon-specific probes, pairs of 60-mer oligonucleotides were synthesized; each pair contains a 10-bp complementary region at the 3′ end. Complementary oligonucleotides were annealed and extended with the large fragment of DNA polymerase I in the presence of ^{32}P-labeled nucleotides (24). The marker lanes contained ^{32}P-labeled λ *Hin*dIII DNA (24).

RESULTS

Characterization of the GH Receptor Gene. In order to analyze human gene defects in the GH receptor, we first characterized the structure of the normal gene. This analysis also would allow us to determine whether the multiple bands observed in genomic blots of the receptor (see below) were due to a family of closely related genes or to the several exons of a single-copy receptor gene. Six clones were isolated from human genomic libraries by screening with the cloned human GH receptor cDNA (Fig. 1). These clones were mapped, and the hybridizing regions were sequenced in order to determine precisely the extent of each exon (Fig. 2). With the exception of a single base difference in exon 10 (Fig. 2, legend), the sequence of the coding region determined for the genomic clones matched that determined previously from cDNA clones (6).

The coding and 3′ untranslated regions of the receptor are encoded by 9 exons, numbered 2–10 (Fig. 1). Exons 2–9 range in size from 66 to 179 bp; exon 10, which encodes nearly all of the cytoplasmic domain as well as the 3′ untranslated region, is about 3400 bp. Exons 2 and 8 are nearly coincident with the putative secretion signal sequence and the transmembrane domain, respectively. The extracellular, GH binding region of the receptor is encoded by exons 3–7. Most of the 5′ untranslated region appears to be present on a series of alternatively spliced exons that have not yet been localized (see below). The gene for the GH receptor has previously been localized to chromosome 5p13.1-p12 (27) and spans at least 87 kbp. Gaps of unknown length occur between the genomic clones in two places. Thus, the 2/3 and 6/7 introns are at least 14 and 24 kbp; the 3/4 intron is probably 27 kbp (Fig. 1). We have used the genomic map and hybridization data (see below) to assign exons 2–10 to the bands observed in genomic blots probed for the receptor (Fig. 3).

Analysis of LTD Patient DNA. The GH receptor gene was analyzed in DNA samples from patients with LTD. A cDNA fragment encompassing the complete protein coding region of the receptor was used to probe genomic blots of DNA from control and nine LTD individuals. No obvious alterations in the receptor gene were observed in six LTD DNA samples (data not shown). Patient 1 was shown to be heterozygous for a 200-bp insertion in the 3′ untranslated region (data not shown). Since it is unclear whether this insertion would affect the expression of the GH receptor gene, we have not analyzed it further. It is possible that this insertion is due to the expansion of the *Alu* repeat that is in the 3′ untranslated region of the gene (Fig. 1).

The restriction pattern of *Hin*dIII-digested DNA from two patients, 147 and D1, appeared abnormal, with the absence of the 16-kbp band assigned to exons 4 and 5 and the 3.5-kbp band for exon 6 (Fig. 3). In addition, the *Eco*RI band assigned

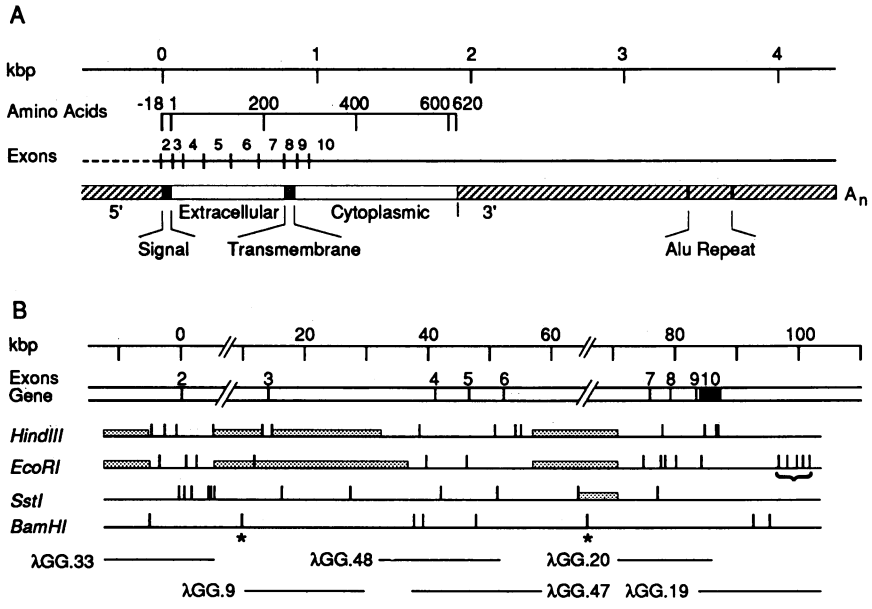

FIG. 1. (*A*) Schematic representation of the GH receptor mRNA and protein. Shown are scales of nucleotides and amino acids, the location of the exon boundaries, and the major features of the protein and mRNA. (*B*) Map of the GH hormone receptor gene. Shown are a scale of nucleotides, location of the exons, restriction map for four enzymes, and the location of six genomic clones. Two gaps in the map are shown at their minimum length. The length of the 3/4 intron is established only by the coincidence of genomic *Sst* I fragments in the region and thus could be greater. The *Bam*HI sites indicated by an asterisk may represent more than one site separated by an unknown distance. The order of the bracketed *Eco*RI sites is unknown. The restriction map is unknown in the shaded regions.

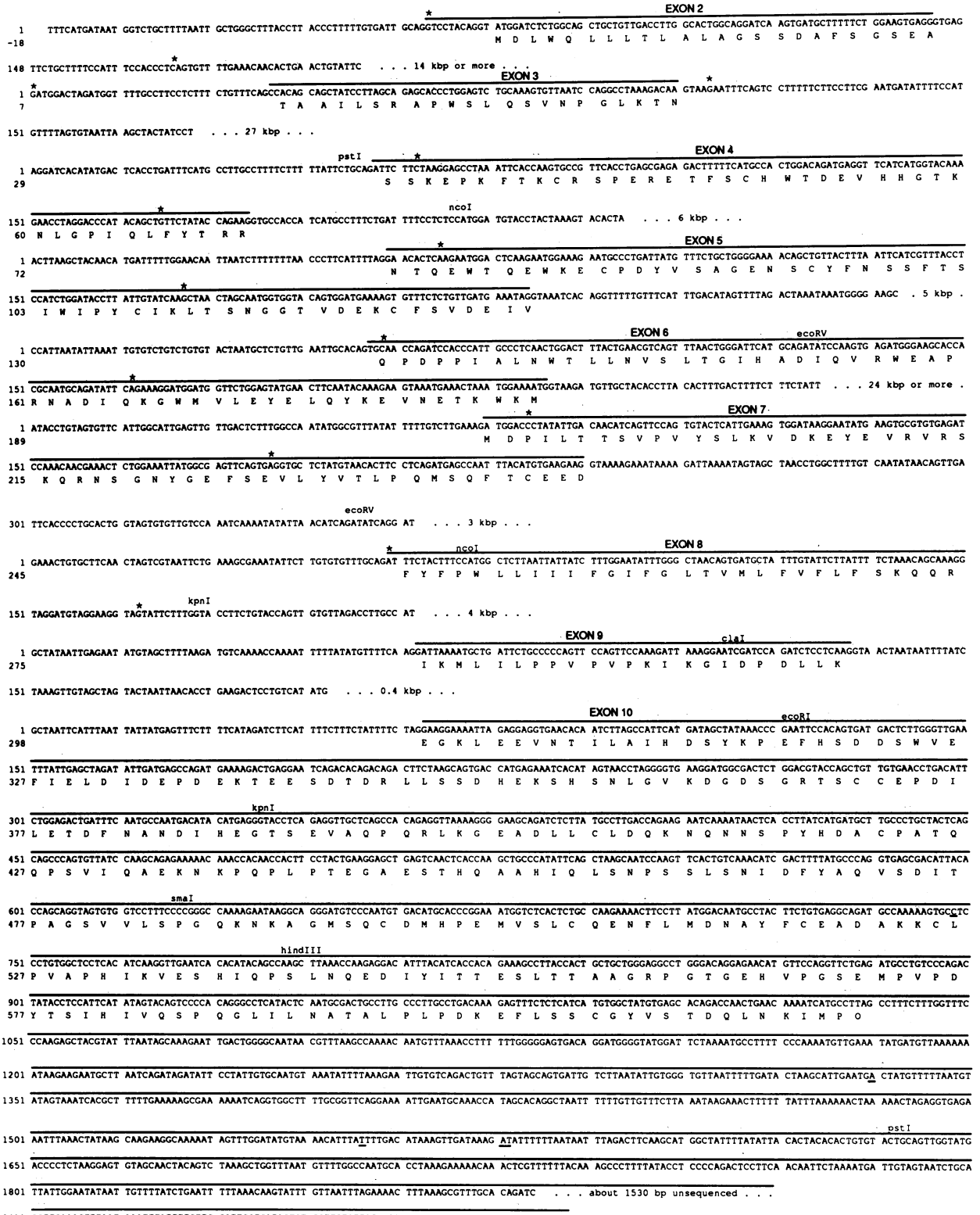

FIG. 2. DNA sequence of the GH receptor genomic clones. Exons 2–10 are overlined, and the encoded protein sequence is shown. Asterisks indicate the extent of the exon-specific oligonucleotide probes. Nucleotides that differ from the cDNA sequence (6) are underlined.

to exon 4 is absent. New bands not found in these and other control DNAs are observed for patient 147 (*Hin*dIII, 4.4 and 1.8 kbp; *Eco*RI, 4.8 and 1.3 kbp) (Fig. 3). The two large-sized *Hin*dIII bands for patient 147 (of about 20 and 11 kbp) appear to be a polymorphic pattern found in other control DNAs of Israeli origin (data not shown). The integrity of the remaining bands is difficult to judge because of the complex restriction pattern, but clearly much of the receptor gene is present. This result suggests that either the two patients contain a partial deletion in the GH receptor gene or, alternatively, they have an unusual polymorphism.

In order to reduce the complexity of the analysis and to examine individual exons, replicate blots containing DNA from the two LTD patients were hybridized with synthetic

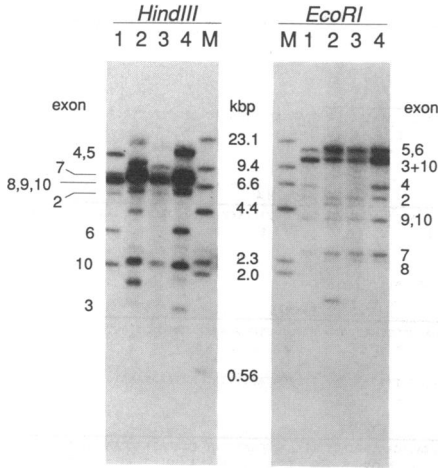

FIG. 3. Genomic blot hybridized with the full-length GH receptor cDNA probe. Blots contained DNA from two normal individuals (adult male, United States) (lanes 1 and 4) or DNA from LTD patient 147 (lanes 2) or patient D1 (lanes 3). The sizes of the marker DNAs (lanes M) are shown between the panels. Assignments of the GH receptor exons to the *Hin*dIII or *Eco*RI bands are shown on the left and right of the panels, respectively.

probes specific for exons 2–7. A normal pattern of hybridization was observed with the exon 2 and 7 probes (Fig. 4). DNAs from the two LTD patients failed to hybridize with the exon 3, 5, and 6 probes. The failure to detect hybridization was not due to the lack of DNA in the LTD lanes or to an artifact of the transfer since similar results were seen in multiple experiments and since these same filters produced normal hybridization signals when stripped and rehybridized with full-length or other exon-specific probes. Interestingly, an abnormal size band was found with the exon 4 probe in digests from both patients (Fig. 4). These results show that a substantial portion of the GH receptor gene is missing from both alleles of these two patients yet indicate that more than a simple deletion has occurred. Blots hybridized with an exon 10-specific probe show that for both patients two *Eco*RI bands of 11 and 16 kbp are found (data not shown). The larger band obscures the lack of the exon 5/6 *Eco*RI band for the two patients (Fig. 3).

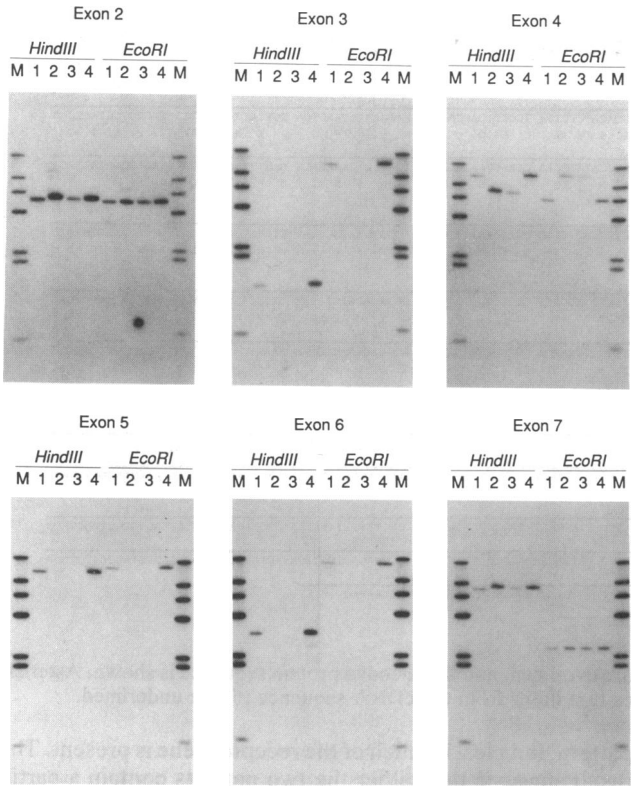

FIG. 4. Genomic blots hybridized with exon-specific probes. Blots containing DNA from two normal individuals (lanes 1 and 4) or LTD patient 147 (lanes 2) or patient D1 (lanes 3) were hybridized with exon-specific probes as indicated. Marker DNA (lanes M) is as in Fig. 3.

DISCUSSION

We have determined the exon structure of the human GH receptor gene, and, using this information, we have examined the receptor gene in nine LTD patients. The data show that two patients have a deletion in both receptor alleles that removes a large part of the coding region for the GH binding domain. With such a large deletion, we consider it very unlikely that the receptor would retain its normal function. Thus, these data provide direct evidence that LTD can result from defects in the structural gene for the GH receptor. These findings also provide convincing genetic data showing that the gene identified by GH binding (1, 5) and cloned as a putative GH receptor (6) is, in fact, required for proper growth.

No obvious alterations were observed in the restriction pattern of seven other LTD individuals, clearly demonstrating that LTD is caused by a heterogeneity of gene defects. Based on the wide collection of gene defects found in other inherited diseases (28–31), we expect that point mutations in the GH receptor gene also may account for many LTD cases. The detection of these defects will require a more detailed analysis than studies employing genomic blots as described here. In addition, mutations in other genes, such as those required for the expression of the receptor or for transduction of the GH signal, also might result in a similar or identical phenotype.

One surprising finding is that noncontiguous exons have been deleted in the two LTD patients. Such a mutation could result from two independent deletion events or from a complex deletion and rearrangement. A more detailed analysis of the genome structure of these two patients may suggest which mutational event is more likely. Both patients are from families with consanguinity and, at the level of resolution determined here, are homozygous for the mutation. Although both patients are from the same ethnic background, their hybridization patterns with the full-length probe are not identical (Fig. 3), showing that their GH receptor genes do have polymorphic differences.

Assignment of the GH receptor exons to the multiple bands found on genomic blots (Fig. 3) shows that the complex pattern observed results from the multiple exons of a single-copy gene rather than from a gene family. However, the many bands could obscure other cross-hybridizating genes, and our data do not exclude the existence of other genes closely related to the GH receptor. Under our hybridization conditions, we do not detect the distantly related prolactin receptor gene (7).

When cDNA clones for the human and rabbit GH receptor were isolated, nearly all of these clones diverged 12 bp 5′ of the initiating methionine codon (6). We speculated that these clones resulted from multiple splicing 5′ of the coding region (6) and perhaps from transcription initiated from different promoters. The current sequence data show that the point of 5′ divergence corresponds to the beginning of exon 2. Thus, there is a diverse splicing pattern in the 5′ untranslated region of the GH receptor gene. Localization of these 5′ exons and characterization of the promoter(s) and their possible tissue-specific regulation will await further studies.

Human and rabbit GH receptor cDNA clones also have been isolated that diverge within the coding region (6). Most (six in all) of these points of divergence coincide with the exon boundaries determined here (data not shown). These clones represent a series of unspliced or differentially spliced mRNAs, including two clones, ghr.244 and ghr.438, that have exon 3 or 4 missing, respectively. The biological significance of these observations awaits further analysis.

Recently, the two prominent GH receptor mRNAs found in mouse liver have been cloned (32). These mRNAs encode two forms of the mouse receptor, a high molecular weight, membrane-bound form and a low molecular weight form that diverges prior to the transmembrane domain and appears likely to encode the secreted GH binding protein (33). The point of divergence of these two clones matches the exon 7/8 boundary found here, supporting the proposal that alternative splicing of the GH receptor mRNA generates the two receptor species (32).

cDNA clones encoding the prolactin receptor also have been isolated from rat, rabbit, and mouse libraries (7, 34, 35). Some of these clones encode long and short forms of the prolactin receptor. The points of divergence of these clones match the 9/10 exon junction found here for the GH receptor. Thus, it would appear that the prolactin and GH receptor will have at least some similarity in their exon structures. This coupled with an overall amino acid identity of the two receptors of about 25% shows that the two receptors have evolved by duplication and divergence of a common ancestral gene.

We thank Ellen Heath-Mannig for providing fibroblasts for the three U.S. LTD patients, Jeroen Knops for the initial analysis of patient 1 DNA, and the Genentech organic synthesis group for oligonucleotide probes. Z.L. is incumbent of the Irene and Nicholas Marsh Chair for Pediatric Endocrinology and Diabetes at Tel Aviv University. L.R.M. was supported by a J. N. Goddard Research Fellowship and by a National Institutes of Health Fellowship (5 T32 DK0 7298-09); J.P.G. was supported by a National Institutes of Health Training Grant (DK 07120). This work was supported by grants from the March of Dimes (Clinical Research Grant 6-454, J.S.P. and Z.L.), from the National Institutes of Health (R01 HD24960, J.S.P.; and P01 HD20805, P.S.R.), and from the Emory Egleston Children's Research Center (J.S.P.) and by Genentech, Inc.

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