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Genomic Structure, Chromosomal Localization, and Expression Pattern of the Human LIM-Homeobox3 (*LHX 3*) Gene

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Lhx3 is a LIM-homeobox protein essential for pituitary development in mice. The human homologue gene spans 7.2 kb and contains 7 exons, including two alternatively spliced first exons. This structure encodes two distinct protein isoforms, LHX3a and LHX3b, that differ exclusively in their amino-terminus. The *LHX3* gene was localized at 9q34.2–34.3. The predicted protein sequence is highly homologous to other known Lhx3 proteins, the highest degree of homology being in the conserved domains. The highest expression of *LHX3* was found in pituitary gland, spinal cord, and lung. Among different pituitary cell types, corticotrophs appear to express preferentially LHX3b isoform, suggesting a distinct role of the b-form in the development of this cell lineage. Although the human *LHX3* gene structure would provide a ground for clarification of the molecular basis of complete anterior pituitary deficiency, we were unable to identify any mutation in the *LHX3* gene of 46 such patients.

The pituitary gland is a central endocrine organ. Hormones released from this gland are crucial for physiological processes such as body growth, metabolism, CNS development, stress response, reproduction, parturition, lactation, and water homeostasis. During development, the anterior lobe in human is formed from a fold of oral ectoderm called the Rathke's pouch. The posterior lobe, associated with the anterior pituitary in the mature gland, is an extension of the neuroectoderm from the brain.

Pituitary morphogenesis occurs in three ontogenic steps that are controlled by distinct gene functions. The first step in pituitary organogenesis is the formation of the rudimentary pouch, the second event is the formation of the definitive pouch, and the third is the transformation of the Rathke's pouch into a nascent pituitary gland. At this stage individual hormone secreting cells emerge from the pouch in a sequential order and commit themselves to a particular lineage, corticotrophs secreting ACTH; melanotrophs, MSH; thyreotrophs, TSH; gonadotrophs secreting, FSH and LH; somatotrophs, GH; and, finally, lactotrophs producing prolactin. Many lines of evidence suggest that contact and signaling between the floor of the dien cephalon and Rathke's pouch is crucial for proper determination and commitment of these structures in the early stages of development. For example, FGF8 (Fibroblast Growth Factor-8) from the ventral diencephalon initiates expression of a pair of LIM-Homeobox proteins, called Lhx3 and Lhx4 (1, 2). Lhx3 and Lhx4 in turn, regulate the last two developmental steps, formation of a definitive pouch and organ fate commitment. These transcription factors are characterized by two N-terminal LIM domains and a homeobox DNA-binding domain. The LIM-domain is a protein-protein interaction motif whose structure resembles the double Zinc-finger configuration of the DNA-binding region of nuclear receptors and GATA-class transcription factors.

Data derived from the *Lhx3* −/− and *Lhx3/Lhx4* double knock out mice demonstrate that commitment to the pituitary gland fate and subsequent growth of the pouch are developmental decisions solely dependent on Lhx3 (3). Four of the six anterior pituitary cell lineages—thyrotrophs, somatotrophs, gonadotrophs, and lactotrophs—are specifically depleted in *Lhx3* −/− mice, while corticotrophs fail to proliferate, demonstrating that pituitary organ fate commitment is a function of Lhx3, which is not replaceable by Lhx4. Based on the phenotype of the *Lhx3*−/− mice, we might predict that the phenotype of patients lacking LHX3 will include morphological abnormalities of the anterior pituitary (hypoplasia or aplasia) with several degrees of hypopituitarism at presentation. Since some patients with PROP-1 deficiency develop ACTH deficiency later in life, the distinguishing feature of a

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TABLE 1

Oligonucleotides Used for Genomic DNA Amplification of Human *LHX3* Gene

Primer number	Primer symbol	Sequence (5′ → 3′)	Length (nucleotides)	Location (Accession No./nt)
1a	hlhx3a	CGGGATCCATGCTGCTGGAAACGGGGCT	28	AF156888
1b	Begex1b	GCGGATGGAGATGGCCACCCAAGGCGG	27	AF211944/1–27
2	Ex1-D	ATGGAGGCGCGCGGGGAGCTGGGCCCG	27	AF211944/37–63
3	Int1/2-R	GGGAGGCTGGCTCGCGTTGGGGTGGG	27	AF211945/25–51
4	Int 1/2-D	GGCCTGAGGATCTCCTGGTCTCCCCGG	27	AF211946/255–281
5	Int 2/3-R	CCCTGGGGCAGGCGTGCCCTCCGC	26	AF211946/544–567
6	Int 2/3-D	GGGCGAAATGAGCCTCGCGCTTCCGC	24	AF211947/170–195
7	Int3/4-R	GCCCCATTTTTTTCAGACCAGGAAAGG	27	AF211947/452–478
8	Int 3/4-D	GGGGGTCCGGCCGGGGCCGGAGGGGC	26	AF211948/175–200
9	Int 4/5-R	GGCTCTGCGGGGGCCCCCAAGGCCGCG	27	AF211948/413–439
10	Int 4/5-D	CGCGGCCTTGGGGGCCCCCGCAGAGCC	27	AF211948/413–439
11	Int 5/6-R	CCGAGCTCCGCGATCCCTCCGCC	23	AF211948/641–664
12	Int 5/6-D	GGCGGAGGGATCGCGGAGCTCGG	25	AF211948/639–664
13	Int 5/6-D2	GGGGCCGCAGGTGGAGGGCAGGCGC	25	AF211949/242–266
14	Ex 6-R	CATGGGTGGGGGCCCGCCGGGG	22	AF211950/295–316
15	Endcds	GCCGCCCACCCAGGGGCAGCTCCCTCG	27	AF211950/466–489
16	Hombo	CGAGTGGTGCAGGTGTGGTT	20	L38248/713–732
17	R1	TCGCTGTCTTGTCCCTCCTG	20	L38248/854–873
18	Limend-XL	CGGACTACGAAACAGCCAAGCAGCG	25	AF211947/400–424 L38248/864–890
19	R1-XL	CTGTCCTGCCCCTCCTGAACGCTG	24	AF211948/592–615
20	Hombeg601D	CAGCCAAGCGGCCGCGCACGACC	23	AF211948/412–428
21	GAG911R	GAGCCTTCCTTGGCGGAAATGGGCC	25	AF211950/35–56

LHX3 deficient patient should be lack of conjunction of the anterior and posterior pituitary lobes, with consequent ectopy of the neuropituitary.

Lhx3 proteins are highly conserved among species. Overall identity in the primary structure of Lhx3 proteins ranges between 75% (mouse-chicken) and 84% (chicken-xenopous), indicating that Lhx3 is an evolutionarily conserved protein. To examine conservation of the Lhx3 structure and analyze its role in human pituitary gland formation, we characterized the primary structure, chromosomal location and gene expression pattern of the human *LHX3* gene.

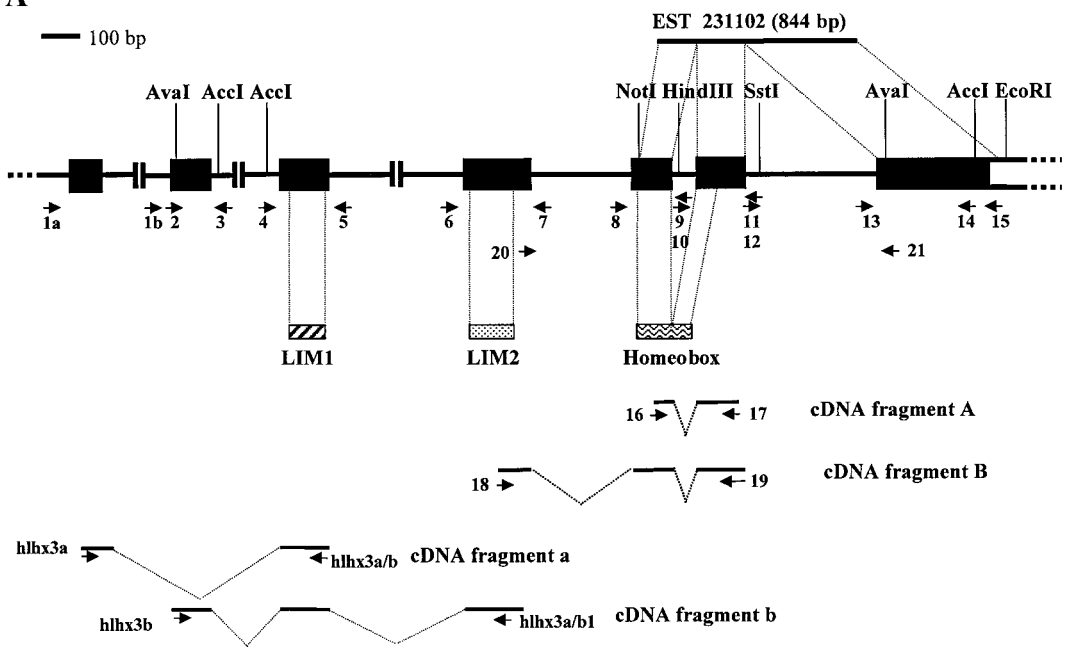
MATERIALS AND METHODS

Reverse transcriptase-polymerase chain reaction (RT-PCR). To identify human cDNA fragments, 100 ng of human pituitary polyA+ RNA (Clontech, Basel, Switzerland) was reverse-transcribed using SuperScript reverse transcriptase (Gibco-BRL). PCR amplification was performed using the following oligonucleotides designed to be complementary to the mouse sequence: the direct primer $^{5'}$ CGAGTGGTGCAGGTGTGGTT $^{3'}$ (no. 16 in Fig. 1A) and the reverse primer $^{5'}$ TCGCTGTCTTGTCCCTCCTG $^{3'}$ (no. 17 in Fig. 1A) were annealed to the single stranded cDNA under the cycling conditions: 94°C 1′, 56°C 2′, 72°C 1′ × 40 cycles. A 168 bp long product (Fig. 1A, product A) was obtained and sequenced. Based on its sequence, human-specific primers were synthetized and the amplification of the reverse-transcribed pituitary mRNA was extended. The human specific direct primer $^{5'}$ CGGACTACGAAACAGCCAAGCAGCG $^{3'}$ (no. 18, Fig. 1A) and the reverse primer $^{5'}$ CTGTCCTGCCCCTCCTGAACGCTG $^{3'}$ (no. 19 in Fig. 1A) were used to perform this second amplification applying the cycling conditions: 94°C 30″, touch down 72°C to 57°C 1′; 94°C, 30″, 57°C, 1′, 72°C, 1′ × 20 cycles. The amplification was carried out using the Advantage cDNA Polymerase mix (Clontech, Basel, Switzerland). The obtained 230 bp product (Fig. 1A, product B) was sequenced for confirmation.

Expressed sequence tags (ESTs) and BAC-genomic library screening. Using the sequence of product B as a template, we found two expressed sequence tags (EST) derived from a human pineal gland cDNA library (231102-3′ and 231103-5′, accession no. H52572 and H52168). The EST clone was purchased from the IMAGE consortium. Resequencing of the plasmid clone was performed for confirmation. The 844 bp insert representing the 3′ region of the *LHX3* cDNA was then used as reference and probe for hybridization. Using the EST clone sequence, we were able to design oligonucleotides we used to amplify human genomic DNA, (direct $^{5'}$ CAGCCAAGCGGCCGCGCACGACC $^{3'}$, no. 20 in Fig. 1A; reverse $^{5'}$ GGCCCATTTCCGCCAAGGAAGGCTC $^{3'}$, (no. 21 in Fig. 1A) under the following PCR conditions: 95°C 1′; 94°C 10″, 68°C 3′ × 25 cycles; 68°C 3′. This procedure generated a product of about 1400 bp. The same PCR method was utilized to screen a BAC genomic library and isolate one BAC-clone (clone 23180, Genome System Inc., St. Louis). To confirm the presence of our putative *LHX3* sequence in the 23180 clone, after plasmid DNA extraction (QIAGEN Large-Construct Kit, QIAGEN, Germany), we amplified and partly sequenced the 1400 bp product C out of the 23180 clone. The 5′-region of the putative human *LHX3* cDNA was obtained by pituitary gland cDNA screening. For confirmation of these sequences we amplified and sequenced human genomic DNA from 20 normal individuals using primers designed to be complementary to the coding sequence (No. 1–15, Fig. 1A). Sequences of the primers are available from the authors upon request. Human genomic DNA was extracted from peripheral blood leukocytes of 20 subjects using the QIAamp Blood kit (QIAGEN, Germany).

Protein sequence alignment. The analysis of similarities between protein sequences was performed according to the Needleman-Wunsch algorithm. For multiple sequence alignments, the Clustal W method was employed (4).

A



B

Exon	3' splice site	Coding (bp)	5' splice site	Intron	Intron size (kb)
1a		79	GGC ACC CGA **gt**ggttcaga G T R 26	1a	1.8
1b		94	CTG CGC CGA G **gt**cccgtaga L R R 31	1b	2.2
2	gtgcccac**ag** AG ATC CCG E I P 32	173	TTT TTC AAG tgagccccga F F K 89	2	0.8
3	cgtcccgc**ag** CGC TTC GGG R F G 90	202	AAG CAG CGA G **gt**cagccgag K Q R 156	3	0.4
4	cgccgcgc**ag** AG GCC GAG E A E 157	152	GTG GTG CAG **gt**aagctttc V V Q 207	4	0.086
5	ccgcccccc**ag** GTT TGG TTC V W F 208	169	TCC TTC CCC G **gt**aggcggag S F P 263	5	0.55
6	ttgtagcc**ag** AT GGC CTT D G L 264	404			

FIG. 1. (A) Physical map of *LHX3* gene. Black boxes represent the exonic coding region, solid lines the intronic sequence. The EST 231102 is also depicted. The conserved protein domains-encoding sequences are indicated together with their respective motifs (LIM1, LIM2, and homeobox). Some restriction sites useful for subcloning are also outlined. The oligonucleotides used as primers for RT-PCR and genomic DNA amplification in their 5′-3′ orientation are represented by arrows. The numbers correspond to those shown in Table 1. (B) Exon/intron boundary sequences of the human *LHX3* gene. Exon sequences are in uppercase and intron sequences in lowercase letters. Consensus splice sites are depicted in bold.

Fluorescence in situ hybridization (FISH). Human metaphase spreads were analyzed using the BAC clone containing the putative LHX3 gene as a probe. Human metaphase chromosomes were prepared from peripheral blood according to standard cytogenetic procedures. As hybridization probe, 1 μg of plasmid DNA (BAC 23180) was nick-translated with biotin-14-dATP (Life Technologies). Ten μl

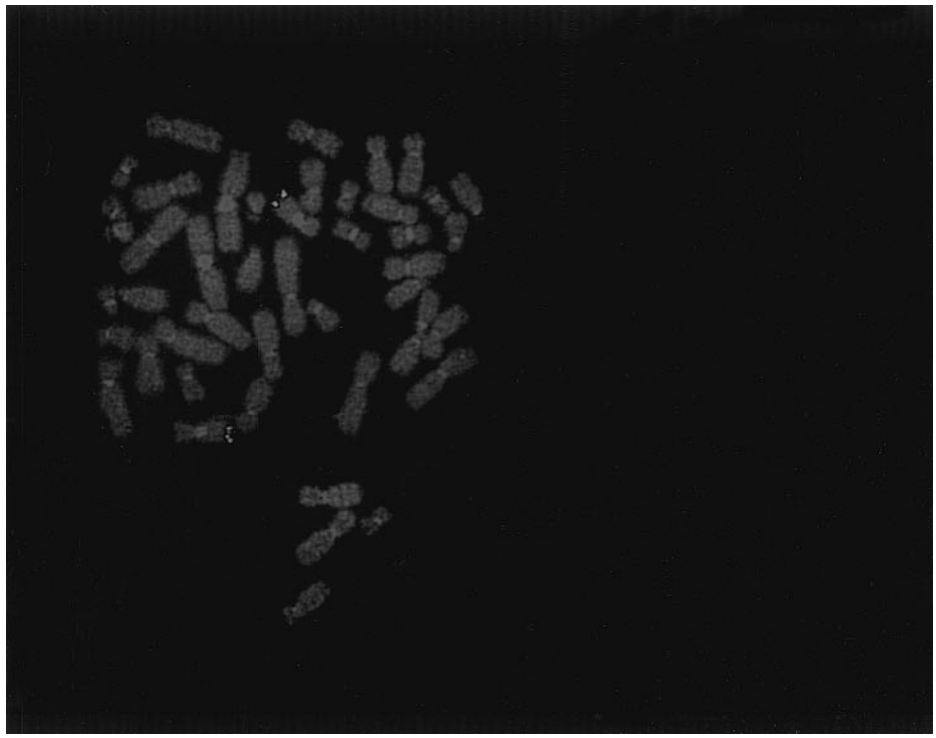


FIG. 2. Chromosomal localization of the human *LHX3* gene by FISH. A human metaphase spread is shown after in situ hybridization using the BAC clone containing the human *LHX3* gene as probe. Chromosomes were visualized with DAPI. The stain is clearly located on chromosome 9q34.2–34.3.

of probe mixture was applied to the washed and denatured metaphase spreads, and hybridized overnight at 37°C. Detection of the hybridized plasmid probe was performed by indirect immunofluorescence using FITC-conjugated avidin in combination with biotinylated goat anti-avidin (Vector Laboratories) as described (5).

LHX3 expression pattern. Dot blot: A commercially available human RNA dot blot (Human RNA master Blot, Clontech, Switzerland) was hybridized with the labeled EST clone (844 bp). The labeling was performed using the Prime-a-gene kit (Promega). RT-PCR: to investigate the differential expression of the isoforms LHX3a and LHX3b, total RNA was extracted from confluent AtT20, α-TSH, 2C-4B/C, GH3 cells (American Type Culture Collection, Manassas, VA) using the RNeasy minikit (QIAGEN, Germany) and reverse transcribed as described above. The same procedure was used to extract and reverse transcribe total RNA from human tissue samples (normal human liver and pituitary; pituitary tumors). 100 ng of total RNA were used for the RT. The PCR amplification was performed using LHX3a and b specific direct primers, and common a/b reverse primers (Fig. 1A; for hlhx3a, hlhx3b and hlhx3a/b sequence see ref. 6; hlhx3a/b1 $^{5'}$TCGCTGCTTGGCTGTTTCGTAGTC$^{3'}$) under the PCR conditions: 94°C 30″, 61°C 1′, 72°C 1′ × 30 cycles. The a- and b-specific products have a predicted length of 257 and 424 bp respectively.

Patients. 46 patients suffering from hypopituitarism were selected based on the presence of anterior pituitary hypo-aplasia and obligate ectopy of the posterior pituitary, detected by Magnetic Resonance Imaging (MRI). Among these individuals, two were first degree related (brother and sister). The degree of hormonal deficiencies was variable at the time of diagnosis, with all patients displaying GH deficiency.

Genomic DNAs from these children were extracted as described above. PCR amplification was performed using primers designated 1–15 and shown in Table 1 and Fig. 1A. Direct cycle sequencing of the PCR products was performed using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit and analyzed by electrophoresis on the ABI Prism 310 Genetic Analyzer (Perkin Elmer Applied Biosystems, Rotkreuz, Switzerland).

RESULTS AND DISCUSSION

Isolation and characterization of the human LHX3 gene. To isolate the human LHX3 gene, we first attempted to perform Southern hybridization with human genomic DNA and to screen a human pituitary cDNA library with the mouse Lhx3 cDNA homologue as a probe (Accession No. L38248). Since both approaches failed to give any specific hybridization signals, we performed PCR with human pituitary reverse-transcribed mRNA as template and two oligonucleotides complementary to the mouse sequence (Fig. 1A, no. 16 and 17). These oligonucleotides were localized in the highly conserved region encoding part of the homeobox (aa 204–220). The RT-PCR generated a 168 bp fragment (Fig. 1A, product A) whose sequence was similar to that of the corresponding mouse cDNA and coded for aminoacid 204–256 of the human LHX3 protein. The sequence of fragment A was then used to synthesize human-specific primers, allowing us to obtain a longer fragment (230 bp, Fig. 1A, product B) encompassing the entire homeobox-encoding sequence (aa 149–256). This sequence was then used to screen the EST-databases and identify 2 homologous expressed sequence tags (231102-3′ and 5′). The 3′ EST

clone comprises part of the presumptive exon 4, exon 5, the entire coding region of exon 6, and 117 bp of the 3′ untranslated sequence. The 5′ sequence, obtained *via* screening of a human pituitary cDNA library, includes exons 1–3. Using oligonucleotides designed on the basis of the EST sequence, we were able to obtain a 1400 bp genomic product. The same amplification protocol was then used to screen a human genomic library.

BAC human PCR screening. To identify genomic sequences containing the human *LHX3* gene, a BAC clone (23180) was obtained using a PCR procedure. An insert of about 85 Kb was isolated from the pBeloBAC11 vector and confirmed to contain the putative *LHX3* sequence by amplification and sequencing of products B and C (not shown). The BAC clone 23180 was also used as a probe for chromosomal localization (see below).

Gene and predicted protein sequence. The human LHX3 gene is constituted by 7 exons separated by 6 intervening sequences in a pattern almost identical to that found in the mouse gene. The length of the coding region is 1194 bp. The complete sequence of the coding region, obtained by direct sequencing of PCR-amplified human genomic DNA, was shown to be almost identical to that of the ESTs and the 5′-region combined. The sequence obtained from DNA of 30 normal individuals was proven to contain the proper Open Reading Frame (ORF). There are two alternatively spliced first exons, 1a and 1b, separated by a 1.8 kb intron. A physical map of the LHX3 gene, the size of the exonic sequence, of the IVSs and the structure of the intron-exon boundaries are shown in Fig. 1 (A and B). The complete coding sequence is available under the GenBank Accession Nos. AF211944, AF211945, AF211946, AF211947, AF211948, AF211949, and AF211950. In the human *LHX3* gene, the two LIM domains are encoded by separate exons, whereas the homeodomain-encoding sequence is interrupted by a short 86 bp intron. This structure is again identical to that of the mouse gene. Furthermore, the position of all intron-exon boundaries are exactly the same as in the mouse and zebrafish *Lhx3* genes (7).

Based on alignment with other known Lhx3 proteins we were able to identify the three expected conserved domains: LIM1 ranges from amino acid (aa) 36 to 89; LIM2 from aa 94 to 149 and the homeobox from aa 161 to 220. The LIM domain is a specialized double zinc-finger motif present in several proteins, thought to be involved in protein-protein interactions and, in the current view, the LIM domains appear to inhibit DNA binding by the homeobox. The homeobox is a DNA binding motif first identified in a number of *Drosphila* homeotic and segmentation proteins, but now known to be well-conserved in many other animals, including vertebrates (8–10). A putative proline-rich region (PRR), spanning aa residues 332 to 398, was also identified. PRR are often found on the surface of globular proteins being a flexible hydrophilic structural element that play an important role in protein-protein interaction, e.g., with the SH3 (Src homology 3). This suggests

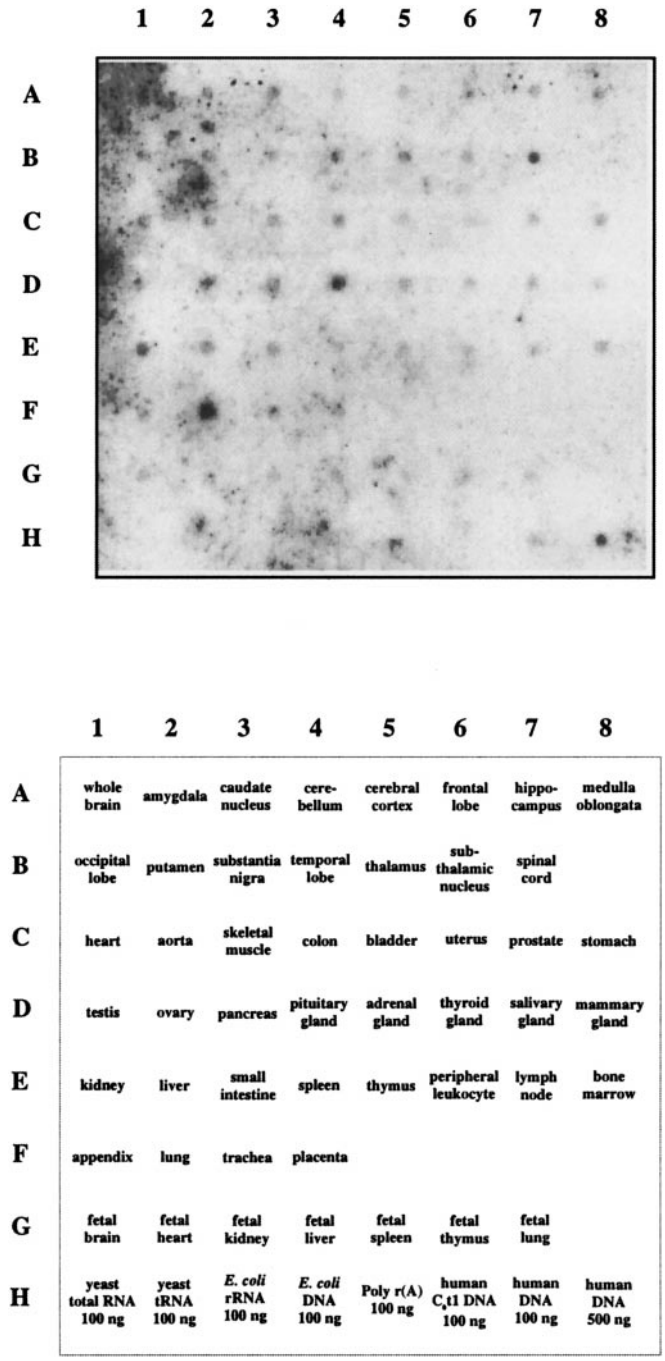
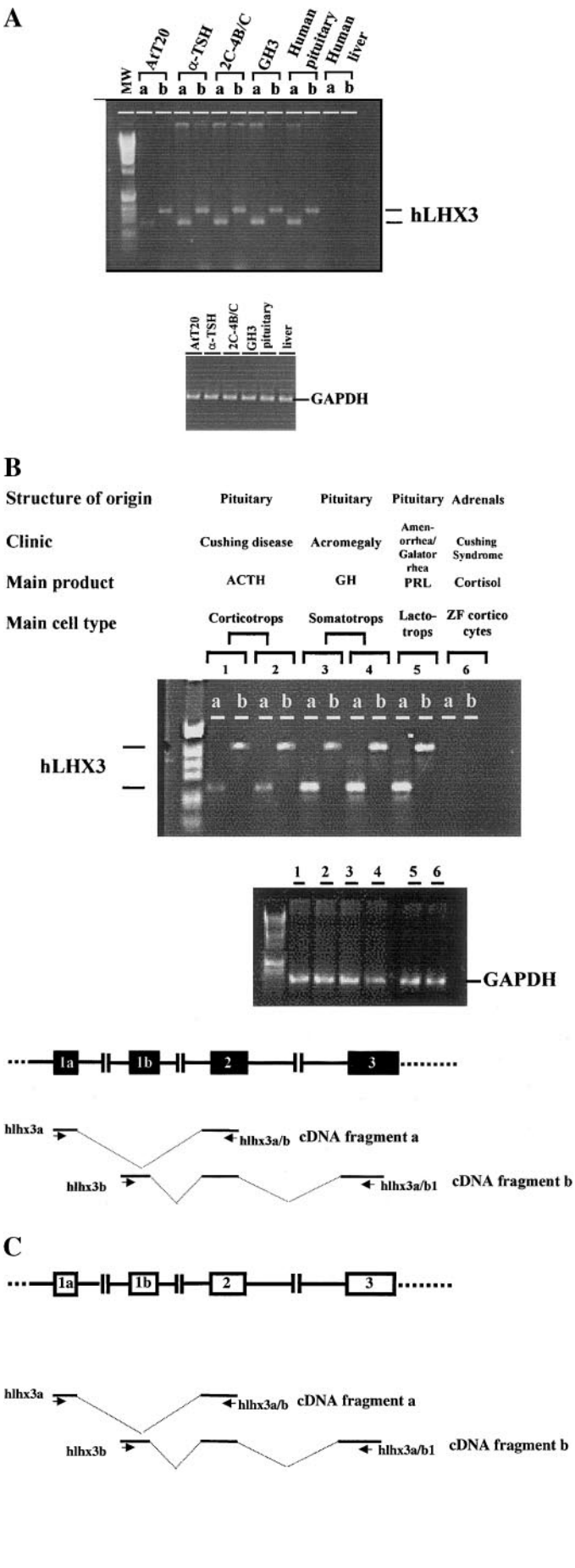


FIG. 3. Expression pattern of *LHX3* in several human tissues. A commercially available human RNA dot blot was hybridized using the labeled EST as a probe. This EST contains part of exon 4, exon 5, the coding sequence of exon 6, and additional 117 bp of the 3′ untranslated region. The lower panel depicts the loading diagram. The highest *LHX3* expression was seen in adult pituitary gland (D4), spinal cord (B7) and lung (F2).



a role of the C-terminus of LHX3 in signal transduction and indicates that this region may function as an element crucial for transactivation.

The predicted protein sequence revealed a high degree of identity among several Lhx3 proteins derived from human, mouse, pig, chicken, *Xenopous laevis*, and zebrafish species. The overall identity ranges from 65% (human-xenopous) and 73% (human-mouse). The degree of homology is striking higher when the single conserved domains are compared: the LIM2 domains (aa 94–149 in human) are between 92% (human-xenopous) and 96% (human-mouse) identical, whereas the homeobox domain (aa 161–220 in human) identity ranges from 90% (human-xenopous) to 96% (human-mouse). The putative C-terminal PRR (aa 332–398 in human) showed significant identity, ranging from 68% (human-xenopous) to 89% (human-pig) (not shown). Although the conserved domains show a high degree of identity among different species, there are distinctions between the human LHX3 protein and those of other organisms. The LIM1 domains, for example, diverge in primary structure much more than the LIM2 and Homeobox regions do. The divergences between the human LHX3 protein and those of other species might reflect differences in LHX3 functions in the tissues, including the pituitary gland. Thus, defining the phenotype of putative LHX3 deficient patients should be done with care.

Chromosomal localization. Using the BAC clone 23180 as a probe, the LHX3 gene was localized to chromosome 9q34.2–34.3 (Fig. 2), a region corresponding to mouse chromosome 2 (cM pos. 15.0), where *Lhx3* is localized (7). The LHX3 specific signal, shown as a light spot in the figure, was pseudo-colored in green with FITC in the original. No evidence of any cross reaction signal was observed, suggesting that the BAC clone does not contain any similar sequence such as LHX4.

Expression pattern. As expected, the expression pattern of LHX3 revealed high expression in the pituitary gland. High expression was also found in the adult lung and in several regions of the adult brain, the

FIG. 4. (A) Expression of the LHX3a and LHX3b isoforms in different cell lines: AtT20, mouse corticotrophs; α-TSH, mouse thyreotrophs; GH3, rat GH/PRL secreting cells; RC-4B/C, rat LH, FSH, and β-TSH secreting cells. Human pituitary, normal adult pituitary (aged 36–74) total RNA pool from 5 gland; human liver, normal adult liver (ppol from 3 biopsies). GAPDH was used as internal control for RNA amount. (B) Expression of the LHX3a and LHX3b isoforms in different human pituitary tumors (adult). Samples 1 and 2 derive from two distinct ACTH-producing tumors; samples 3 and 4 from GH-secreting tumors; sample 5 was a prolactinoma. Sample 6, derived from an adrenal cortex tumor, was used as negative control. (C) RT-PCR strategy used to specifically amplify the two distinct isoforms (see text for details).

strongest signal being in the spinal cord (Fig. 3). The expression of *LHX3* in the CNS is not surprising, since there is evidence that Lhx3, Lhx4 may have both early and late functions in motor neuron development. No data are available on the functional implication of the pulmonary expression of *LHX3*. The *Lhx3*−/− mice do not display any lung defect, suggesting that the function if any of Lhx3 in this organ is not crucial. On the other hand, the cognate protein Lhx4 (also known as Gsh4) was shown to play a critical role in the development of respiratory control mechanisms and in the normal growth and maturation of the lung in mice (11). Nevertheless, the expression of *LHX3* in the lung is unlikely to be due to unspecific cross-reaction with *LHX4*, as the same sequence used to perform the chromosomal FISH analysis presented no cross hybridization.

We confirmed at the genomic level the presence of two first exons, alternatively spliced in the mRNA. The two differently spliced exons, 1a and 1b, encode two N-terminal regions that allow the distinction of two different isoforms called LHX3a and LHX3b. Given the fact that gene activation studies demonstrate characteristic functional differences (6), we decided to determine whether there is a differential expression of the two isoforms in distinct pituitary cell types. The RT-PCR experiments demonstrate that normal adult pituitary expresses both LHX3 isoforms. Mouse thyrotrophs (α-THS), gonadotrophs (2C-4B/C) and rat somatotrophs (GH3) seem to express similar amounts of the two isoforms, whereas mouse corticotrophs (AtT20) appear to express the b isoform. The negative control human liver does not express any LHX3 (Fig. 4A). To confirm these findings, we performed the same RT-PCR analysis on human pituitary mRNA samples, selected based on the clinical picture and main histological cell type. Similarly to the cell lines, tumors characterized mainly by corticotrophs (samples 1 and 2) again appear to express more LHX3b than LHX3a. Somatotrophs (samples 3 and 4) and lactotrophs (sample 5) seem to express equal amounts of both isoforms. Human adrenal tumor cells, used as negative control, do not express LHX3 (Fig. 4B). All the tested cell types (cell lines and cells derived from primary pituitary tumors) express both isoforms apparently at the same level, with the exception of the corticotrophs, that express predominantly the b-isoform, but at low levels. The relative low level of expression of LHX3 in corticotrophs is consistent with the observation that Lhx3−/− mice have some corticotrophs but lack all other pituitary cell types. That demonstrates that LHX3a is not essential for corticotrophs differentiation. It is appealing to speculate that the more abundant LHX3b isoform in corticotrophs plays a unique, although unknown, role in the development of this cell type.

Molecular analysis of patients with hypopituitarism. Genomic DNA of 46 patients with hypopituitarism due to pituitary hypoplasia or aplasia and ectopy of the posterior lobe were PCR-amplified and directly sequenced. No structural mutation was found in the coding region or the intron-exon boundaries of the 46 patients analyzed. There are several possible explanations for this phenomenon. First, the rarity of the disease limits the access to the patients. More individuals are currently being analyzed to increase the chances of identifying a mutant. Second, we are looking at the wrong phenotype. In contrast to the Lhx3−/− mice, the patients might be heterozygote and display different or milder clinical features and not be included in our selection of individuals who therefore suffer from the defect of another factor. Third, given the similarity in expression pattern and function between Lhx3 and Lhx4, it would be conceivable that LHX4 replaces a nonfunctional LHX3. As a consequence, no clinical phenotype would be identifiable, and again the patients we selected are affected by the defect of another factor. Although it is possible that some degree of redundancy exists between LHX3 and LHX4 in some tissues and in specific stages of development, the data from the *Lhx3* −/− animals demonstrate that growth of the Rathkes's pouch depends exclusively on Lhx3. It is therefore unlikely that LHX4 can completely substitute LHX3 function.

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