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CLINICAL CASE SEMINAR

A Novel *LHX3* Mutation Presenting as Combined Pituitary Hormonal Deficiency

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Context: *LHX3* encodes LIM homeodomain class transcription factors with important roles in pituitary and nervous system development. The only previous report of *LHX3* mutations described patients with two types of recessive mutations displaying combined pituitary hormone deficiency coupled with neck rigidity.

Objective: We report a patient presenting a unique phenotype associated with a novel mutation in the *LHX3* gene.

Patient: We report a 6-yr, 9-month-old boy born from a consanguineous relationship who presented shortly after birth with cyanosis, feeding difficulty, persistent jaundice, micropenis, and poor weight gain and growth rate. Laboratory data, including an undetectable TSH, low free T_4, low IGF-I and IGF binding protein-3, prolactin deficiency, and LH and FSH deficiency were consistent with hypopituitarism. A rigid cervical spine leading to limited head rotation was noticed on follow-up examination. Magnetic resonance imaging revealed an apparently structurally normal cervical spine and a postcontrast hypointense lesion in the anterior pituitary.

Results: Analysis of the *LHX3* gene revealed homozygosity for a novel single-base-pair deletion in exon 2. This mutation leads to a frame shift predicted to result in the production of short, inactive LHX3 proteins. The results of *in vitro* translation experiments are consistent with this prediction. The parents of the patients are heterozygotes, indicating a recessive mode of action for the deletion allele.

Conclusions: The presence of a hypointense pituitary lesion and other clinical findings broadens the phenotype associated with *LHX3* gene mutation. (***J Clin Endocrinol Metab* 91: 747–753, 2006**)

IN HUMANS, THE pituitary gland is composed of two major parts, the anterior and posterior lobes. The posterior pituitary arises during development from the ventral diencephalon and secretes the hormones oxytocin and vasopressin. The anterior pituitary gland is derived from Rathke's pouch, an outgrowth of the oral ectoderm. It contains five hormone-secreting cell types: somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, and corticotrophs, which secrete GH, prolactin (PRL), TSH, the gonadotropin hormones (LH and FSH), and ACTH, respectively (1, 2). After early inductive events, the actions of specific transcription factors are important for the determination and differentiation of these specialized cell types (2–5). Mutations in the genes encoding these transcription factors cause complex hormone deficiency diseases in humans and animal models (5, 6).

Several LIM-homeodomain class transcription factors, including ISL1, LHX3, and LHX4, play critical roles in pituitary and nervous system development (7). These regulatory proteins have two LIM domains in the amino terminus that are involved in protein/protein interactions and a centrally located homeodomain that mediates interactions with DNA recognition sequences in target genes (7). The human *LHX3* gene contains seven coding exons and six introns on chromosome 9q34 and encodes three protein isoforms of the LHX3 transcription factor (8–10). The three protein isoforms are LHX3a and LHX3b, which both contain the LIM domains and homeodomain but have different amino termini, and a shorter isoform (M2-LHX3) that lacks the LIM domains but retains some gene regulatory activities (8–10). In mice, the orthologous gene, *Lhx3,* is expressed in the embryonic nervous system and in the developing and mature pituitary (11–13). Transgenic mice with a homozygous deletion of *Lhx3* display incomplete pituitary development, aberrant motor neuron specification, and perinatal death (14–16). In these animals, some ACTH is detectable, indicating at least partial corticotroph cell function, but the characteristic hormones of the other anterior pituitary cells are not found, indicating that *Lhx3* is required for development of four of the five specialized cell types (16). Molecular studies have demonstrated that LHX3 can activate pituitary expressed genes, such as

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Abbreviations: CPHD, Combined pituitary hormone deficiency; EMG, electromyography; IGFBP-3, IGF binding protein-3; MRI, magnetic resonance imaging; PRL, prolactin.

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those encoding the α-glycoprotein subunit (αGSU), PRL, FSHβ, TSHβ, the GnRH receptor, and the Pit-1 transcription factor (9, 10, 13, 17–19).

To date, only one report has described molecular defects in *LHX3* associated with a complex combined pituitary hormone deficiency (CPHD) syndrome (20). Two recessive mutations were reported: a missense mutation altering a single, conserved amino acid in the LIM domains (Y111C in the LHX3a protein) and a 23-bp deletion predicted to result in the translation of a truncated protein (20). The patients were from two unrelated, consanguineous families that presented with GH, TSH, LH, FSH, and PRL deficiencies and a short, rigid cervical spine with limited head rotation. Magnetic resonance imaging (MRI) demonstrated small anterior pituitaries in two patients, whereas another had an enlarged pituitary gland (20). These mutations are predicted to lead to the production of proteins that are ineffective in *trans*-activation of pituitary hormone gene promoters such as *αGSU* and *PRL* (21, 22).

In this study, we describe a patient with a novel *LHX3* gene mutation, CPHD with neck rigidity, a hypointense pituitary lesion radiologically consistent with a microadenoma, and other features including possible focal amyotrophy and speech delay. A homozygous single-base-pair deletion was found in exon II of *LHX3*. This mutation is predicted to result in the production of nonfunctional protein(s). The patient's parents are cousins and are heterozygous for the mutation, consistent with a recessive mode of inheritance.

Patient and Methods

Hormonal testing

A CRH stimulation test was performed with 1 μg/kg of ovine CRH as previously described (23). Serum cortisol was analyzed by HPLC tandem mass spectrometry method. ACTH was measured by the immunochemiluminometric assay (ICMA) method; LH and FSH were measured by an immunochemiluminometric assay method. IGF-I and IGF binding protein-3 (IGFBP-3) were measured with blocking RIA after acid/alcohol extraction and RIA in dilute serum methods (Esoterix, Calabasas Hills, CA).

Diagnostic procedures to detect mutations in LHX3

After consent, peripheral blood was collected in a green-top heparin vacuum tube and frozen. The study protocol was approved by the Institutional Review Board of Indiana University. Informed consent was obtained from the parents of all of the subjects and from the subjects themselves if they were 8 yr of age or older. Genomic DNA was extracted from blood using a QIAamp Blood Midi Kit (QIAGEN, Valencia, CA). *LHX3* exons were amplified by the PCR using primers recognizing intronic sequences as we have described (Fig. 1B) (24). Each PCR contained 2.5 U *PfuUltra* DNA polymerase (Invitrogen, La Jolla, CA), 10 mM

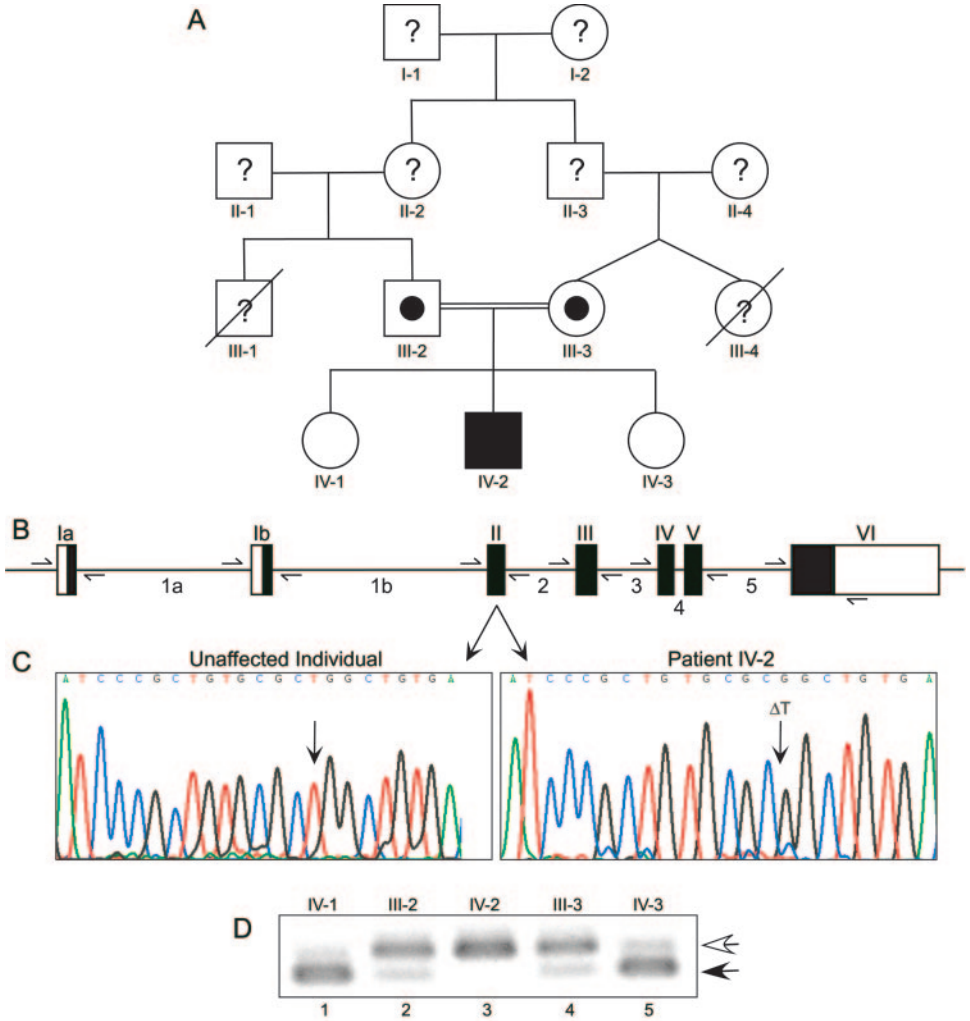

FIG. 1. A novel *LHX3* mutation is found in a patient with CPHD and other symptoms. A, Family pedigree of the patient (IV-2) who is homozygous for the affected allele (*solid black square*). The father (III-2) and mother (III-3) are heterozygous for the affected allele (*symbols with black dots*). The sisters of the proband (IV-1, a 7-yr-old, and IV-3, a 4-yr-old) are homozygous for the unaffected allele. The father's brother (III-1) died in childhood for unknown reasons. The mother's twin sister (III-4) died in infancy of unidentified causes. B, Diagram of the human *LHX3* gene with the sequencing strategy. *Arrows* indicate PCR primers. Exons are in *Roman numerals*. Introns are in *Arabic font*. *Black boxes* indicate translated exons; *white boxes* are untranslated exons. C, Example DNA sequence chromatograms representing exon II from an unaffected individual (*left*) and from the patient (IV-2, *right*). The nucleotide missing in patient IV-2 is indicated (*arrow*). The mutation is g.159delT (based on GenBank reference sequence AF367086). D, Results of *Cac*8I restriction enzyme digestion of an exon II PCR product for each immediate family member. The *open arrow* depicts an undigested fragment in which the mutation has abolished the restriction site. The *closed arrow* shows the products resulting from *Cac*8I cleavage. The slight upper bands in lanes 1 and 5 and the higher intensity of the upper bands in lanes 2 and 4 reflect a small fraction of the PCR amplicons that are resistant to *Cac*8I restriction enzyme digestion (incomplete digestion).

dATP, dCTP, dGTP, and dTTP, approximately 200 ng genomic DNA, and 10 pmol of each forward and reverse primer. Primers to amplify each exon were as follows: exon Ia, 5′-tgacctcggaggagcgcgtct-3′ and 5′-caaccgctgtcccgcactctt-3′; exon Ib, 5′-gaaagttcgggactggagagt-3′ and 5′-cagtgccacaacctcactca-3′; exon II, 5′-tacgaggtgacccagaactt-3′ and 5′-cctggccttggtgattgtga-3′; exon III, 5′-tttcagaccaggaaaggtgg-3′ and 5′-cgaaatgagcctcgcgcttc-3′; exons IV and V, 5′-gctgccgcgcctcaccgct-3′ and 5′-aggagtccactaactccatg-3′; and exon VI, 5′-cgctgactgagcctctgctt-3′ and 5′-cctcgtgtgaggtgcagggt-3′. Touchdown PCR cycling parameters were as follows: 94 C for 2 min; 94 C for 10 sec, 66 C for 10 sec, and 72 C for 2 min for two cycles; 94 C for 10 sec, 64.5 C for 10 sec, and 72 C for 2 min for two cycles; 94 C for 10 sec, 63 C for 10 sec, and 72 C for 2 min for two cycles; 94 C for 10 sec, 61.5 C for 10 sec, and 72 C for 2 min for two cycles; and 94 C for 10 sec, 60 C for 10 sec, and 72 C for 2 min for 20 cycles. If required, reactions amplifying GC-rich regions were supplemented with MasterAmp PCR optimization buffers (Epicenter, Madison, WI). Reaction products were analyzed on 1.5% agarose, Tris-borate gels and subsequently ligated into the pCR4-TOPO-Blunt vector (Invitrogen, Carlsbad, CA). Plasmid minipreps were performed, and insert-containing plasmids were sequenced on both strands by automated DNA sequencing using a Perkin-Elmer DNA sequencer (Biochemistry Biotechnology Facility, Indiana University School of Medicine) and M13 forward and M13 reverse primers. At least six PCR products of each amplified exon were sequenced from each patient to ensure more than 98% confidence of characterization of both alleles. The candidate mutation in exon II of patient AM was then confirmed by sequencing of amplicons representing another two independent PCRs.

The genotype of family members was assayed by three approaches. First, exon II of *LHX3* was amplified from genomic DNA samples and cloned amplicons were sequenced as described above. Second, exon II amplicons were directly sequenced to check the genotype. In this procedure, after PCR amplification, 20% of the PCR was treated with 0.5 U exonuclease I (USB Corp., Cleveland, OH) for 60 min at 37 C, and then the enzyme was heat-killed at 80 C for 15 min. The PCR product was then purified by ethanol precipitation and resuspended in water for automated DNA sequencing. Sequence chromatographs were analyzed using Chromas 2.23 (Technelysium Pty Ltd., Tewantin, Australia). Third, because the observed mutation disrupts a *Cac*8I restriction site in exon II, 10% of an exon II PCR was digested using 2 U *Cac*8I restriction endonuclease (New England Biolabs, Beverly, MA) and appropriate buffer at 37 C for 1 h [as similarly used by Turton *et al.* (25) to monitor *POU1F1* mutant alleles]. Reaction products were separated using 10% acrylamide or 2.5% agarose gel electrophoresis in Tris-borate buffers. DNA fragments were visualized using SYBR Gold nucleic acid stain (Molecular Probes/Invitrogen, Carlsbad, CA).

Site-directed mutagenesis

The deletion mutation was introduced into expression vectors containing human *LHX3a* and *LHX3b* cDNAs (9) using the QuikChange site-directed mutagenesis protocol (Stratagene, La Jolla, CA) as we have described (26). Mutagenic oligonucleotide sequences were 5′-gagagatc ccgctgtgcgcggctgtgaccagcacatcc-3′ and 5′-ggatgtgctggtcacagccgcgcacagcgggatctctc-3′.

In vitro transcription/translation

Radiolabeled wild-type and mutant LHX3 proteins were synthesized *in vitro* from pcDNA3.1-based expression vectors by a modification of methods that we have described (22). Translation reactions included plasmid substrates, T7 RNA polymerase, TnT rabbit reticulocyte lysates (Promega, Madison, WI), and [^{35}S]cysteine (GE/Amersham, Piscataway, NJ). Labeled proteins were separated using SDS-PAGE followed by treatment of gels with Amplify fluorography reagent (Amersham) and visualized using a Storm phosphorimager (GE/Amersham/Molecular Dynamics).

Patient description

The patient is a 6-yr, 9-month-old male. He was born full-term with a birth weight of 2.8 kg (5th percentile) and a length of 46.6 cm (<5th percentile) to healthy consanguineous parents of Sephardic-Jewish descent originally from Azerbaijan (Fig. 1). He presented on the first day of life with cyanosis, poor feeding, persistent jaundice, and a micropenis (stretched penile length of 1.5 cm). His examination showed a large anterior and posterior fontenelle, mild pulmonic stenosis, and hypertelorism. Initial evaluation was consistent with central hypothyroidism (Table 1), and thyroid replacement therapy was started at d 4 of life. The patient remained hospitalized for poor weight gain. Serum IGF-I and IGFBP-3 levels were low, consistent with GH deficiency. A random serum cortisol was normal at 2 wk of age (Table 1). During the first year of life, the patient lacked the physiological LH, FSH surge, suspicious for gonadotropin deficiency (Table 1). Cryptorchidism was noticed, and orchidopexy was performed at 1 yr of age. The peak of ACTH rise was normal after CRH stimulation, consistent with normal corticotroph function (Table 2).

An MRI of the pituitary done soon after birth revealed a postcontrast hypointense lesion. A repeat MRI performed at 6 yr, 8 months of age confirmed the hypointensity, which is radiologically consistent with a microadenoma leading to the appearance of an enlarged anterior pituitary size (Fig. 2).

At 6 yr, 8 months he had neck rigidity with an inability to turn his neck. Cervical spine x-rays and MRI showed normal alignment and configuration of cervical spine without any apparent structural abnormalities. Rotation of the patient's neck was limited to approximately 90° (compared with a normal rotation of ~180°).

He was diagnosed with mental retardation by school psychoeducational assessment and is currently functioning 30–40% behind an age-appropriate level, particularly in reading and math skills. He is unable to speak words clearly or to construct sentences. The speech delay is a result of the global mental retardation. He is receiving speech, physical, and occupational therapy.

On neurological exam, there was mild hypotonia and immature prehensile skills. There was a suggestion of bilateral deltoid weakness and weakness in neck muscles including sternocleidomastoids. Mild weakness was also noted at the pelvic girdles, but the gait was normal and no fasciculations were seen. The cranial nerves were normal and the tendon reflexes were preserved.

Electromyography (EMG) of median, ulnar, and common peroneal nerves and deltoid, biceps, and vastus lateralis muscles was performed.

TABLE 1. Hormonal profile of the patient shows deficiencies of TSH, PRL, IGF-I, and IGFBP-3

	Cortisol (μg/dl)	ACTH (pg/ml)	FSH (IU/liter)	LH (IU/liter)	Prolactin (ng/ml)	IGFBP-3 (mg/liter)	IGF-I (ng/ml)	TSH (μU/ml)	Free T_4 (ng/ml)
NI data	3–21	10–60	0.26–3.0	0.02–0.3	3–18	1.5–3.4	51–288	0.5–4.8	0.8–2.2
Patient's age									
2 d								<0.06	0.3
2 wk	22					0.5	12		
2 months	8.0		0.11	0.02	0.4	1.0	42	0.02	2.8
8 months			0.25	0.03				0.10	1.4
5 yr, 1 month			0.18	0.02	0.5			0.07	1.3
6 yr, 2 months	2.5	25							

The absence of a LH and FSH surge between 2 and 8 months of age is suspicious for gonadotropin deficiency. NI, Normal individual. Convert to SI units as follows: for cortisol, to convert to nmol/liter multiply by 27.6; for ACTH, to convert to pmol/liter multiply by 0.22; and for IGF-I, to convert to nmol/liter multiply by 0.13.

TABLE 2. CRH stimulation test done at 6 yr, 8 months of age shows an adequate response of ACTH

CRH administration	Cortisol (μg/dl)	ACTH (pg/ml)
Baseline	3.6	16
60 min	11	95
120 min	12	124
180 min	13	96

Convert to SI units as follows: for cortisol, to convert to nmol/liter multiply by 27.6; and for ACTH, to convert to pmol/liter multiply by 0.22.

EMG of the deltoid muscle showed moderate fasciculation potentials and occasional fibrillation potentials suggestive of a dysfunction at the anterior horn cell. Motor units and recruitment could not be adequately assessed because of poor cooperation. The paraspinal EMG could not be assessed because of poor cooperation. Nerve conduction studies were normal.

The parents' anterior pituitary hormone panel (PRL, IGF-I, free T_4, TSH, LH and FSH) was normal. The father's brother died in childhood, and the mother's twin sister died during infancy of unknown causes.

Results

Molecular analysis of the LHX3 gene

The hormone deficiency profile and neck abnormality of the patient suggested that it may be caused by an inactivating mutation of the *LHX3* gene. To test this hypothesis, genomic DNA was extracted from a peripheral blood sample and all *LHX3* gene exons were amplified by the PCR using primers recognizing intronic sequences (Fig. 1B). Sequence analysis of cloned PCR products revealed a deletion of a single thymidine residue in exon II of both alleles (Fig. 1C). The mutation is g.159delT based on GenBank reference sequence AF367086 (the mutation is referred to as exon II delT hereafter). All other exons were normal in patient IV-2, and the entire gene was normal in an unrelated patient with similar hormone deficiencies but without the limited neck rotation (Fig. 1C and data not shown). The mutation was then confirmed by sequencing of amplicons representing another two independent PCRs using genomic DNA from patient IV-2.

The genotypes of the parents of patient IV-2 and his two sisters were then determined. Three approaches were taken. First, exon II of *LHX3* was amplified from genomic DNA samples and cloned amplicons were sequenced as described above. Second, exon II amplicons were directly sequenced in bulk to determine the genotype. Lastly, because the observed mutation disrupts a *Cac*8I restriction site in exon II, these amplicons were digested with this restriction endonuclease, and reactions products were separated by electrophoresis. These experiments demonstrated that the parents of patient IV-2 were heterozygous for the *LHX3* exon II delT mutation, and his sisters (IV-1 and IV-3) were both homozygous for the wild-type allele (Fig. 1, A and D, and data not shown).

The *LHX3* exon II delT mutation found in patient IV-2 is predicted to cause a frame shift in translation of proteins from *LHX3* mRNAs causing the production of short, approximately 18-kDa protein products lacking the LIM domains and the homeodomain of LHX3 (Fig. 3A). To confirm that the deletion would have this effect, the mutation was engineered into expression vectors containing full-length human *LHX3a* and *LHX3b* cDNAs by site-directed mutagenesis. These plasmids were used as substrates for *in vitro* transcription/translation reactions using rabbit reticulocyte lysates and [^{35}S]cysteine (as a source of radiolabel). Labeled proteins were separated by electrophoresis and visualized using a phosphorimager. As predicted, wild-type LHX3a migrated with an apparent mass of approximately 44 kDa, and the protein from the mutated cDNA migrated with an apparent mass of approximately 18 kDa (Fig. 3B). Similar data were observed for LHX3b (data not shown). In these experiments, the signal from the protein produced from the mutated cDNA is notably weaker than the wild-type protein (Fig. 3B). This result is likely a result of reduced labeling of the mutant protein because it has less than half the number of cysteines present in the wild-type proteins. It is also possible, however, that the aberrant protein has decreased stability.

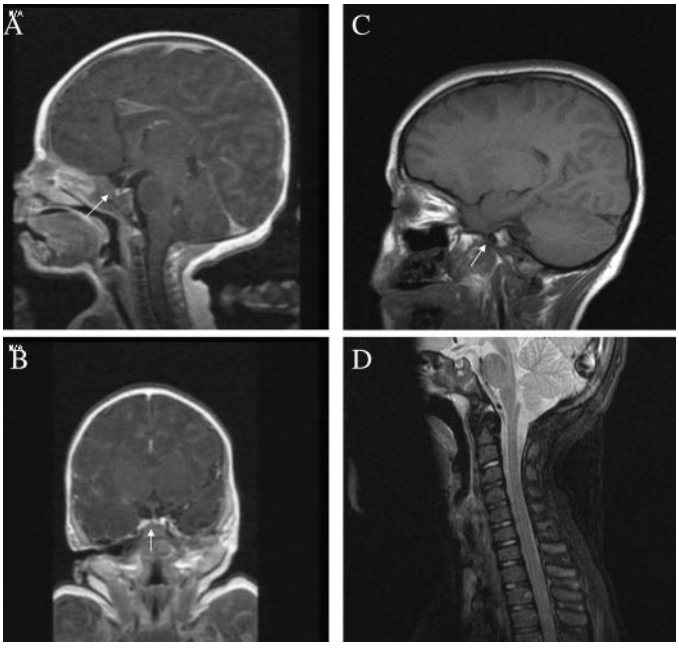

FIG. 2. Imaging of the pituitary gland and the cervical spine. A, A T1 sagittal section post gadolinium MRI performed shortly after birth revealed a hypointensity in the anterior pituitary consistent with a microadenoma (*arrow*). B, A T1 coronal section post gadolinium image taken at the same time as A also shows a hypointensity in the anterior pituitary consistent with microadenoma (*arrow*). C, T1 sagittal section post gadolinium MRI performed at 6.5 yr shows a hypointensity consistent with 4 × 8 × 5 mm adenoma (*arrow*). Pituitary data: anterior-posterior diameter = 6.3 mm, mediolateral dimension = 18.1 mm, height = 5 mm, pituitary volume = 570.15 mm^3 (mean normal pituitary volume for 6 yr = 226 mm^3; 95% reference interval, 142–359 mm^3) (28). D, STIR (Short TI Inversion Recovery) sagittal sections show a normal alignment of the vertebral bodies, a normal disc space, and a normal intensity of the spinal cord.

Discussion

The patient described has several features that have been associated with mutations of the *LHX3* gene. The hormonal profile is consistent with one previous report to date; *LHX3* mutation is associated with deficiencies of GH, PRL, LH, FSH, and TSH, with intact corticotroph function (20). A rigid cervical spine with limited head rotation is also a notable feature of this syndrome (20). This feature is thought to be

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LHX3a   MLLETGLERDRARPGAAAVCTLGGTR 26
Pat     MLLETGLERDRARPGAAAVCTLGGTR 26

LHX3b   MEARGELGPARESAGGDLLLALLARRADLRR 31
Pat     MEARGELGPARESAGGDLLLALLARRADLRR 31

                              LIM Domain 1
LHX3a/b EIPLCAGCDQHILDRFILKALDRHWHSKCLKCSDCHTPLAERCFSRGESVYCKDDFFKRFGTK
Pat     EIPLCAAVTSTSWTASSSRLWTATGTASVSSAATATRHWPSAASAEGRAFTARTTFSSASGPS

                              LIM Domain 2
LHX3a/b CAACQLGIPPTQVVRRAQDFVYHLHCFACVVCKRQLATGDEFYLMEDSRLVCKADYETAKQREAEA
Pat     APRASWASRPRRWCAAPRTSCTTCTALPASCASGSWPRATSSTSWRTAGSCARRTTKPPSSERPRP

                              Homeodomain
LHX3a/b TAKRPRTTITAKQLETLKSAYNTSPKPARHVREQLSSETGLDMRVVQVWFQNRRAKEKRLKKDAG
Pat     RPSGRARPSPPSSWRR* 171

LHX3a/b RQRWGQYFRNMKRSRGGSKSDKDSVQEGQDSDAEVSFPDEPSLAEMGPANGLYGSLGEPTQALGR
LHX3a/b PSGALGNFSLEHGGLAGPEQYRELRPGSPYGVPPSPAAPQSLPGPQPLLSSLVYPDTSLGLVPSGA
LHX3a/b PGGPPPMRVLAGNGPSSDLSTGSSGGYPDFPASPASWLDEVDHAQF* 397/402
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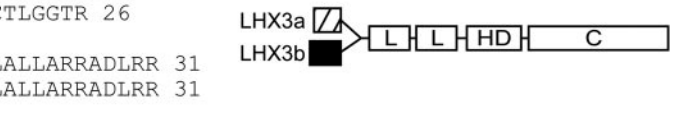

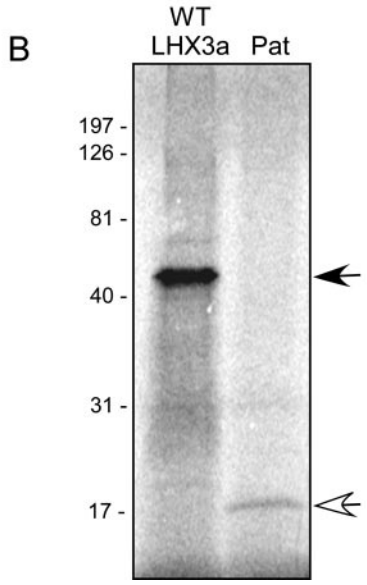

FIG. 3. Result of the deletion mutation on LHX3 protein translation. A, Comparison of the wild-type LHX3a and LHX3b proteins with the altered amino sequence encoded by the mutated *LHX3* gene of patient IV-2 (Pat). *Numbers* indicate amino acids. The LIM domains and the homeodomain are *underlined*. The missense sequence in the affected patient is shown in *italic*. The schematic on the *right* shows the protein domain organization of the LHX3a and LHX3b protein isoforms. L, LIM domain; HD, homeodomain; C, carboxy terminus. B, *In vitro* transcription/translation of an *LHX3a* cDNA in which the g.159delT mutation was introduced by site-directed mutagenesis (Pat) produces a small protein of the predicted size (~18 kDa, *open arrow*). The *closed arrow* shows the migration position of wild-type (WT) LHX3a (~44 kDa, *closed arrow*). The migration positions of protein markers (in kDa) are shown.

linked to the fact that *LHX3* is expressed in specific neurons in the central nervous system, and a mutation could thus lead to this presentation (27). The morphology of the pituitary was reported to be abnormal in three patients; two patients had hypoplastic anterior pituitaries, and one patient presented with an enlarged pituitary (20). By contrast, our patient has a hypointensity in the anterior pituitary radiologically consistent with the presence of a nonsecreting microadenoma leading to the appearance of enlarged pituitary size (compared with normal volumes) (28). These findings were observed on an MRI performed shortly after birth and a later image taken at 6.5 yr of age (Fig. 2). This patient despite neck rigidity also has a normal cervical spine configuration and normal alignment in contrast to at least one of the previously reported patients who displayed an abnormal steepness of the cervical spine (20). In the previous report, the EMG was normal in two patients (20); however, the EMG findings in our patient suggest an anterior horn cell disease process that may be localized to the lower cranial and cervical anterior horn cells, but there is otherwise no overt cranial nerve involvement. This assumption is based on the presence of moderate fasciculations; unfortunately, motor unit morphology could not be obtained because of poor patient cooperation.

In summary, the patient has mental retardation and a webbed neck with the suggestion of weakness about the rigid neck muscles. The mutation may result in focal amyotrophy, and other patients with *LHX3* mutations will need to be assessed with detailed EMG studies to determine whether this represents an associated neurological phenotype. In mice, *Lhx3* is expressed in several regions of the nervous system including the brain stem, pons, medulla oblongata, pineal, and hindbrain (11–13). Thus, the association between *LHX3* mutations and nervous system pathologies such as amyotrophies, speech difficulties, and mental retardation is plausible, but additional investigations in other subjects with *LHX3* mutations are needed.

Two different mutations were identified in the first report; three patients from one family had an A to G transition that resulted in a Y111C nonconservative substitution in the second LIM domain of LHX3a (Y116C in LHX3b) (20). Another patient from an unrelated family had a homozygous deletion of 23 bp involving the last three bases of exon 3 and the adjacent splice-donor site (20). The inheritance pattern for both mutations was found to be autosomal recessive (20). The patient reported here is homozygous for g.159delT (based on reference sequence AF367086) in exon 2 of *LHX3*, having inherited a copy of the recessive mutated gene from his parents who are both heterozygous.

The *LHX3* gene exon II delT mutation described here causes a frame shift that is predicted to result in an altered reading frame and the addition of 139 aberrant amino acid residues after the 32nd residue of LHX3a (Fig. 3A). The aberrant protein is ended by the occurrence of a premature termination codon that lies in exon 4 of the gene, resulting in a short protein product of approximately 18 kDa that is indeed observed after transcription/translation of *LHX3* cDNAs into which the deletion has been engineered (Fig. 3B). This protein would lack the LIM domains and the DNA-binding homeodomain and would therefore be predicted to be functionally inactive, as we have previously described for a truncated form of LHX3 (22). *In vivo*, it is possible that no significant protein product is made because of nonsense-mediated decay of *LHX3* mRNAs. The location of the premature termination codon in the fourth exon, *i.e.* in a location 5′ to the last 50 nucleotides of the penultimate exon is consistent with the likely targeting of this condition for nonsense-mediated decay (29, 30). However, there are exceptions to the defined rules for nonsense-mediated decay, and some transcripts appear to be especially prone or resistant to this process (29, 30), so the outcome in this case is speculative. The normal phenotype of the heterozygous parents of the described patient is consistent with the idea that, if any protein is made, the produced peptides are inactive, rather than having any significant negative activity. In either case, the evidence favors the hypothesis that this patient lacks *LHX3* gene function.

The effects of pituitary transcription factor gene mutations on pituitary cell growth, both prenatally and after birth, appear to be complex. In the original report of *LHX3* gene mutations, although some patients were described with hypoplastic pituitaries, one patient developed an enlarged anterior pituitary gland later in life (20). By contrast, some patients with mutations in the *PROP1* gene (a gene encoding Prophet of Pit-1, a key pituitary transcription factor) display hyperplastic pituitary glands that later become hypoplastic (31). Longitudinal studies of pre- and postnatal pituitary development in normal and *Prop1*-deficient dwarf mice have examined the biology underlying this observation (32). These experiments reveal that fetal pituitaries lacking *Prop1* function have an abnormal morphology because mutant anterior pituitary progenitor cells fail to leave the perilumenal region of Rathke's pouch and do not differentiate (32). Later, the lack of progenitors in the anterior lobes of the mutant pituitaries is associated with enhanced apoptosis and reduced proliferation (32). The *LHX3*-deficient patient described here appears to perhaps have abnormal proliferation of pituitary cells, and this finding was observed both shortly after birth and more than 6 yr later. LHX3 has been implicated in the proliferation of the hormone-secreting cell types during pituitary development in mice (15). It is possible, however, that under some circumstances, an absence of *LHX3* function could also result in inappropriate proliferation of pituitary cells. Further study of the nature of the lesion present in this patient would be necessary to provide additional data supporting this idea.

This study demonstrates that there is phenotypic variation in CPHD patients with mutations in *LHX3*. The actions of other genes within the genomes of these patients are likely to affect the phenotypic outcome of inactivating *LHX3* mutations. Studies in animal models have demonstrated the influence of genetic background on the phenotypes associated with an individual (mutant) pituitary transcription factor allele. For example, it has been reported that the pituitary disease phenotypes associated with a mouse $Lhx4^{-/-}$ genotype are variable (33). In addition, the phenotype of homozygous mice carrying a null allele of *Prop1* is markedly influenced by the genetic background (34). Genetic modifiers therefore likely play a pivotal role in the disease symptoms of patients with inactivating *LHX3* mutations.

This study confirms the importance of *LHX3* gene function in pituitary development and is consistent with a role in the development of the human nervous system. More detailed clinical case reports with special attention to cognition and detailed EMG studies will elucidate the role of this mutation in neuronal development and function. The presence of a hypointense pituitary lesion, possible amyotrophy, and mental retardation in this patient extends the phenotypic profile associated with *LHX3* gene mutations.

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