NOTE

A Novel Missense Mutation (P366T) of the LHX4 Gene Causes Severe Combined Pituitary Hormone Deficiency with Pituitary Hypoplasia, Ectopic Posterior Lobe and a Poorly Developed Sella Turcica

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Abstract. LIM homeodomain transcription factors regulate many aspects of development in multicellular organisms. LHX4/Lhx4 is a protein that is essential for pituitary development and motor neuron specification in mammals. In human, a heterozygous splicing mutation of the LHX4 gene was reported in a family with combined pituitary hormone deficiencies (CPHD). In addition to CPHD, these patients were characterized by small sella turcica and chiari malformation. Here we report a Japanese patient with CPHD (GH, PRL, TSH, LH, FSH, and ACTH deficiency) due to a novel missense mutation (P366T) of the LHX 4 gene. She showed severe respiratory disease and hypoglycemia soon after birth. Brain MRI demonstrated hypoplastic anterior pituitary, ectopic posterior lobe, a poorly developed sella turcica, and chiari malformation. Sequence analysis of the LHX 4 gene identified a heterozygous missense mutation (P366T) in exon 6, which was present in LIM4 specific domain. Neither of the patient's parents harbored this mutation, indicating *de novo* mutation.

Key words: LHX4, Combined pituitary hormone deficiency (CPHD), Pituitary hypoplasia, Sella turcica

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GENETIC defects affecting the hypothalamic-pituitary-target organ axes can cause a variety of diseases involving restricted or broad disruptions of human development and physiology. Within the hypothalamic-pituitary target organs, there are three main levels at which genetic defects can occur that cause diseases: the level of hypothalamus, the level of the pituitary gland, and the level of the pituitary target organs [1, 2]. The anterior pituitary gland develops from a midline structure contiguous with the primordium of the ventral diencephalons [3, 4]. After proliferation from a

well-defined growth plate, different cell types arise in a distinct spatial and temporal fashion and undergo a highly selective determination and differentiation [3, 4]. Thereafter, numerous cells in the pituitary gland are specialized to produce and secrete specific hormones, such as GH, PRL, TSH, LH, FSH, and ACTH. These processes are controlled by the actions of pituitary-specific and pituitary-enriched transcription factors [1, 2]. These factors include POU1/Pit1, PROP1/Prop1, SF-1/Sf-1, PITX2/Pitx2, NeuroD1, GATA-2, LHX3/Lhx3, TPIT/Tpit, LHX4/Lhx4, and HESX1/Hesx1 [5–15]. Recent investigations in mice and human have clarified that mutations in genes encoding several of these factors can lead to both structural defects of pituitary gland and to deficiencies of anterior pituitary hormones [combined pituitary hormone deficiency (CPHD)] [5–15].

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LHX3/Lhx3 and LHX4/Lhx4 are the LIM homeo-domain protein family and are characterized by two LIM domains and a homeodomain [12, 14, 16]. In general LIM homeodomain genes are widely expressed throughout development in complex and often overlapping patterns, implicating these genes in a variety of regulatory events [12, 14, 16]. Lhx4 of mouse is expressed in the developing neural tube, hindbrain, Rathke's pouch, and pituitary gland [16–18]. Disruption of the mouse Lhx4 gene has demonstrated the defect of proliferation of all anterior pituitary cell lineages and abnormal differentiation of corticotroph, resulting in anterior pituitary hypoplasia [17, 18]. In human, so far only one mutation of the LHX4 gene in a family has been reported to cause CPHD [14].

Here we report a Japanese patient of severe CPHD with a poorly developed sella turcica. In this patient a novel heterozygous missense mutation of the LHX4 gene was identified.

Case Report

The patient is now a 16-month old Japanese girl. She was born after 38 weeks of gestation by cesarean section due to breech position. She was the second child of nonconsanguineous parents. Her birth weight was 2216 g (–2.0 SD for a Japanese girl) and length was 40.5 cm (–2.2 SD for a Japanese girl). Soon after birth she showed tachypnea and cyanosis. At this time she was found to be hypoglycemic (8 mg/dl). Despite oxygen therapy, her respiratory distress deteriorated within 16 hours after birth and the presumed diagnosis of respiratory distress syndrome (RDS) was made. Therefore, mechanical ventilation was started and surfactant was given. Mechanical ventilation was required for 7 days. Neonatal mass screening for congenital hypothyroidism at 10 days of age demonstrated markedly low free T4 and non-elevated TSH levels (free T4 0.2 ng/dl, TSH, 0.17 μ U/mL). Despite improvement of her respiratory condition, she showed failure to thrive. Re-examination of thyroid function in serum at 17 days of age showed very low levels of free T4 and T3 (free T4, 0.21 ng/dl, free T3 0.45 pg/ml, and TSH <0.05 μ U/mL) (Table 1). Central hypothyroidism was evident and thyroid hormone replacement was commenced. At this time, plasma ACTH and serum cortisol levels were also found to be very low (5.6 pg/ml and 1.0 μ g/dl, respectively) (Table 1).

Table 1. Endocrinological findings

free T4 (ng/dl)	0.21
free T3 (pg/ml)	0.45
TSH (μ U/mL)	<0.05
PRL (ng/ml) [#]	<2
IGF-1 (ng/ml)	5.53
Arginine test [#]	
GH (ng/ml)	0.16 → 0.18
GHRH test [#]	
GH (ng/ml)	0.20 → 0.21
Gn-RH stimulation	
LH (mIU/mL) [#]	0.013 → 0.015
FSH (mIU/mL) [#]	0.023 → 0.033
ACTH (pg/ml)	5.6
Cortisol (μ g/dl)	1.0

These values were determined at 14 days of age.

[#] These tests were done at 3 months of age.

Second measurement of serum cortisol and plasma ACTH concentrations remained markedly low. Hydrocortisone (5 mg/day) was initiated to avoid the consequences of adrenal insufficiency. Episodes of severe hypoglycemia, central hypothyroidism, and adrenal insufficiency strongly suggested the existence of congenital hypopituitarism. Brain magnetic resonance imaging (MRI) showed a very small pituitary gland. At 3 months of age further endocrine evaluation was done. Arginine and GHRH stimulation tests did not increase serum GH levels at all (Table 1). PRL level was also lower than detectable limit. There was no response of LH and FSH levels after GnRH stimulation (Table 1). GH replacement therapy was started at this stage. Re-examination of brain MRI at age 15 months demonstrated hypoplastic pituitary, ectopic posterior lobe, a poorly developed sella turcica and chiari malformation (Fig. 1). Now, her body weight is 6500 g (–3.6 SD for a normal Japanese girl) and height is 63 cm (–5.6 SD for a normal Japanese girl).

Informed consent to participate in the study was obtained from her parents. This study was approved by the ethical committee of the Hokkaido University School of Medicine. Each exon of the LHX4 gene was amplified by polymerase-chain-reaction (PCR) using primers according to a previous report [14]. Sequence analysis of the LHX4 gene demonstrated a heterozygous one base substitution in exon 6 (C to A) (Fig. 2A). This substitution changed proline to threonine at residue 366 in the C-terminus of LHX4 (Fig. 2B). Eighty normal Japanese subjects did not have this base

Fig. 1. (A) Sagittal image showed a hypoplastic anterior pituitary and ectopic posterior lobe, and a small sella turcica. (B) Chiari malformation is denoted by an arrow.

Fig. 2. (A) Sequence analysis demonstrated a C to A transition. This change substitutes threonine for proline at residue 366. (B) Schematic representation of the LHX4 and the location of P366T. Two LIM domains N-terminus to a homeodomain and the LHX3 and LHX4 specific C-terminal 35 amino acids characterize LHX4.

change. Family analysis demonstrated that neither of her parents harbored this substitution, indicating that the mutation had occurred *de novo*.

Discussion

We have reported a Japanese patient of severe CPHD with hypoplastic pituitary, ectopic posterior lobe, small

sella turcica and chiari malformation. In the literature, homozygous *Lhx4* knockout mice can form Rathke's pouch with expression of all five cell lineages, although the numbers of these cells gradually decreased, resulting in hypoplastic anterior pituitary [17]. Raetzman *et al.* [18] have reported that this phenomenon is caused by increased apoptosis of cells. This indicates that *Lhx4* is essential for the survival of all five cell lineages in the pituitary. These observations of mice are

concordant with defects of GH, PRL, TSH, LH, FSH and ACTH in our patient. By contrast, a previous study of one family having a mutation of the LHX4 gene has demonstrated less severe phenotype than our case. Affected members have CPHD with deficiencies in GH, TSH and ACTH. Gonadotropin and PRL levels have not been reported; however, one patient was fertile [14]. One possible explanation of this difference is the penetrance. It is well known that genetic defects of transcription factors do not always show the same pattern of deficient hormones and clinical severity [1, 2, 15]. The other explanation for the difference may be associated with the genotype of the LHX4 mutation. A previous mutation was a heterozygous splice-acceptor site in intron 4 [14]. *In vitro* expression study revealed that this splicing mutation produced two fragments by using two cryptic splice-acceptor-sites [19]. One protein lacked the helix II and IV of the homeodomain and the other was truncated by a premature stop codon in exon 5. It is possible that these truncated proteins may compensate for normal LHX4 function to some extent.

Our mutation (P366T) was located in the C-terminus of LHX4. The exact functional of the C-terminus of LHX3 and LHX4 has been unknown; however, the 35-amino acid region at the C-terminus of LHX3 and LHX4 is well conserved and shows no significant similarity to other proteins [12, 16, 20]. This fact suggests that this region may have some specific role in LHX3 and LHX4. Further study of the functional consequence of our mutation (P366T) must be done.

MRI analyses of affected members in a previous literature have shown hypoplasia of the pituitary, small sella turcica, and chiari malformation [14]. Our pa-

tient also showed severe hypoplasia of the pituitary and small sella turcica, and chiari malformation. Since a poorly developed sella turcica has been also identified in our patient, this abnormality may constitute the phenotypic feature that distinguishes CPHD patients with defects of the LHX4 gene from those reported with mutations in other genes.

Finally, her lung disease is of note. Despite term delivery, she showed respiratory failure resembling neonatal respiratory distress syndrome and required surfactant replacement and mechanical ventilation. Li *et al.* [21] reported that early postnatal death in Lhx 4 gene knockout mice was caused by immature lung, which was similar to RDS in human. In that report the exact reason for immature lung was not mentioned. Scommegna *et al.* [22] described 5 patients with pituitary aplasia identified by MRI scan. In addition to symptoms of hypopituitarism, they showed respiratory distress on the first day of life, in spite of uneventful pregnancy, labor, and delivery. Furthermore, Sobrier *et al.* [23] described two patients with an anterior pituitary aplasia caused by mutations of the HESX1 gene. They also presented with respiratory distress complicated by shock at birth. As fetal glucocorticoid is well known to be required for lung maturation during fetal life, cortisol deficiency due to ACTH defect may be partly related to lung disease in neonates of severe pituitary hypoplasia. However, severe congenital isolated ACTH deficiency did not usually show respiratory distress [24]. Considering these facts, other unknown factors might contribute to respiratory distress in patients with severe congenital hypopituitarism. This must be studied further.

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