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Review Article

Molecular and Clinical Findings in Patients with *LHX4* and *OTX2* Mutations

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Abstract. The pituitary gland produces hormones that play important roles in both the development and homeostasis of the body. Ontogeny of the anterior and posterior pituitary is orchestrated by inputs from neighboring tissues, cellular signaling molecules and transcription factors. Disruption of expression or function of these factors has been implicated in the etiology of combined pituitary hormone deficiency (CPHD). These include the transcription factors HESX1, PROP1, POU1F1, LHX3, LHX4, OTX2, SOX2, SOX3 and GLI2. This review focuses on summarizing most recent mutations in *LHX4* and *OTX2* responsible for pituitary hormone deficiency. In both genetic defects of *LHX4* and *OTX2*, there is high variability in clinical manifestations even in the same family. In addition, there is no clear phenotype-genotype correlation. These findings indicate that the other genetic and/or environmental factors influence the phenotype. In addition, the variability might reflect a plasticity during pituitary development and maintenance. Over the past two decades, a genetic basis for pituitary hormone deficiency and the mechanism of pituitary development have been clarified. It should be kept in mind that this review is not comprehensive, and defects of other transcriptional factors have been described in patients with CPHD. Furthermore, the causes in many patients with CPHD have not yet been determined. Therefore, continuing efforts for the clarification of the etiology are necessary.

Key words: combined pituitary hormone deficiency, OTX2, LHX4, mutation

Introduction

The anterior pituitary gland is the primary site of endocrine regulation in the control of growth, reproduction and the stress response. The anterior pituitary gland develops from a midline structure contiguous with the primordium of the ventral diencephalon, and the cells undergo a highly selective determination and differentiation in a distinct spatial and temporal fashion (1, 2). Numerous cells in the pituitary gland are specialized to produce and secrete specific hormones, such as growth hormone (GH), prolactin (PRL), thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and adrenocorticotropic hormone (ACTH). These processes are controlled by the actions of pituitary-specific and pituitary-enriched transcription factors and signaling molecules (1–4). The identification and characterization of pituitary developmental factors *in vivo* and *in vitro* has enabled us to clarify a genetic basis

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for combined pituitary hormone deficiency (CP HD) in humans (1–4). These factors include HESX1, LHX3, LHX4, POU1F1, PROP1, SIX6, OTX2, PTX2, GLI2, SOX2, and SOX3 (3–5). Numerous studies demonstrate that mutations and deletions of these transcription factor genes cause a wide range of pituitary phenotypes including severe life-threatening CPHD and isolated GH deficiency (GHD) (3–5). However, as the frequency of reported mutations of these transcription factors remains low, it appears that other multiple genes remain to be identified in human CPHD patients.

This review will focus on the clinical findings and molecular basis of patients who had mutations of the genes encoding for the transcription factors of LHX4 and OTX2.

LHX4

The human *LHX4*, located on chromosomal position 1q25 with six exons encoding 390 amino acids, is a member of the LIM homeodomain of transcription factors. LHX4 contains two LIM domains in its N-terminus and a DNA-binding homeodomain (6, 7). In murine studies, *Lhx4* is expressed in the developing neural tube, hindbrain, Rathke's pouch, and pituitary gland (6, 8, 9). Pituitary development in homozygous *Lhx4* knockout mice proceeds normally until Rathke's pouch rudiment is formed, but demonstrated diminished cell numbers, resulting in a hypoplastic anterior lobe compared with the wild type due to the increased cell apoptosis (8, 9).

In humans, the first patient had c.607-1G>C in intron 4 of *LHX4* (10). Several affected members in this family have CPHD with deficiencies in GH, TSH and ACTH. Gonadotropins and PRL levels have not been reported; however, one patient was fertile. MRI analyses of affected members have shown hypoplasia of the pituitary, a small sella turcica, and chiari malformation. *In vitro* analysis demonstrated that wild-type LXH4 could bind a proximal promoter of another transcription factor, POU1F1, and activate its promoter activity, but the mutant identified by Machinis *et al.* (11) could not. Pfaeffle *et al.* (12) reported three heterozygous missense mutations (p.R84C, p.L190R, and p.A210P) of LHX4. p.R84C was located in the LIM domain, and the other two mutations were in the homeodomain. Two mutations of p.L190R and p.A210P had impaired DNA binding, and could not activate αGSU, POU1F1, and TSHß promoters in a luciferase assay at all. In contrast, p.R84C had normal DNA biding capacity, but had a small amount of activity in a promoter assay. A p.A210P was found in a familial case. Whereas a sister was diagnosed with CPHD, a father and one sister harboring the identical mutation had isolated GHD. Another patient with a p.L190R mutation had GH, ACTH, and TSH, and the remaining patient with p.R84C had GH, TSH and gonadotropin deficiency. MRI findings in this study showed that the four cases exhibited hypoplastic anterior pituitaries but that one case with isolated GHD had a normal sized anterior pituitary. Regarding the posterior pituitary, three showed a normal position, and two showed an ectopic position. No one showed other brain malformations or a small sella turcica. It is of note that two siblings with p.A210P had a pituitary cyst.

Another familial mutation was a one-base insertion (c293_294insC), resulting in frame shift and producing a premature stop codon (p.Thr99fsX53) (13). Functional studies of the mutant LHX4 demonstrated a complete loss of transcriptional activity on the POU1F1 promoter. Two brothers showed GH and TSH deficiency with pituitary hypoplasia and a poorly developed sella turcica. The youngest brother also had corpus callosum hypoplasia and an ectopic posterior lobe. Their father, who also harbored the identical mutation, had only GHD with pituitary hyperplasia. Dateki *et al.* (14) identified the first patient with a *de novo* 0.5-megabase heterozygous deletion including *LHX4*. This patient had a small anterior pituitary, ectopic posterior lobe and underdeveloped sella turcica.

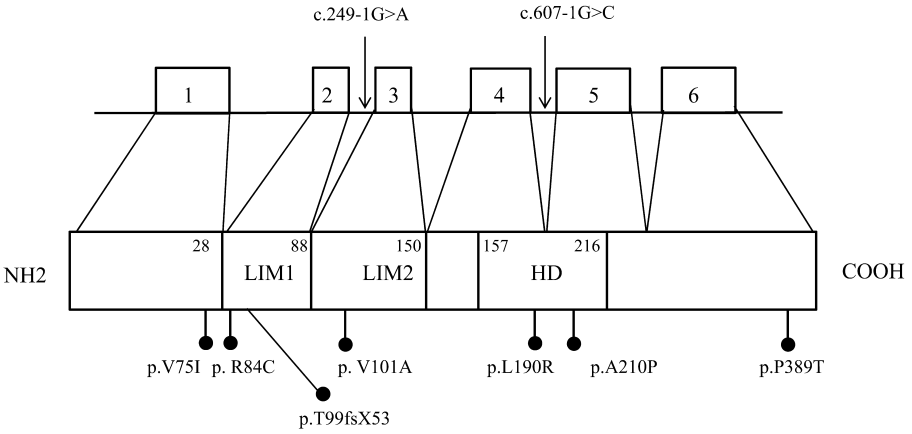

Fig. 1 Schema of *LHX4* genomic organization and protein structure. The location of reported mutations is shown. Mutations of introns are indicated by the arrows. Missense mutations and a frameshift mutation are indicated by black dots. Numbers next to the protein indicate amino acid positions of domain boundaries. White boxes, exon; LIM, LIM domains; HD homeodomain.

Whereas GH, TSH, LH, and FSH were deficient, ACTH secretion was retained when he was evaluated at the age of 17 yr. Filges *et al.* (15) reported a 1.5-megabase heterozygous deletion in 1q25.2-q25.3 including *LHX4*. In this patient, severe respiratory distress, cardiac insufficiency, hypoglycemia, and heart failure were found soon after birth. She also had minor anomalies such as a short nose, short and broad forehead, and nail hypoplasia. Endocrinological evaluation showed complete defect of GH, TSH, LH, FSH and ACTH. Hypoplastic anterior pituitary gland, ectopic posterior lobe and a poorly formed sella were observed by MRI. Her cardiac insufficiency was completely reversed by hormonal replacement, and psychomotor development was within the normal range at 9 mo of age. It is of note that this 1.5-megabase deletion was inherited from an apparently healthy mother. Most recently, Takagi *et al.* (16) reported two patients with *LHX4* mutations. One patient with p. V75I showed GH, TSH, and gonadotropin deficiency. The other patient harboring c.249-1G>A was diagnosed as having isolated GHD at 5 yr of age. Although this patient did not show episodes of adrenal insufficiency, longitudinal follow-up showed that her peak blood cortisol and plasma ACTH levels after insulin tolerance test decreased gradually with age. This mutation was also identified in his apparently normal father and siblings.

We reported two mutations (p.P389T and p.V101A) of *LHX4* in two Japanese patients with CPHD (17, 18). One patient with P389T showed severe respiratory distress and hypoglycemia soon after birth and had defects of all anterior pituitary hormones. These symptoms were improved by hormone replacement. MRI demonstrated a hypoplastic anterior pituitary, ectopic posterior lobe and a poorly developed sella turcica. The second patient with p.V101A in the LIM domain in exon 3 also had a small anterior pituitary and ectopic posterior lobe but had a normal sized sella turcica. *In vitro* functional analysis demonstrated that this mutation could not activate the POU1F1 and FSHβ subunit gene promoter (18).

Mutations of *LHX4* and clinical characteristics are summarized in Fig. 1 and Table 1. As mentioned above, hormone deficiency is variable even in familial cases. Some patients show partial GHD, but some had deficits of all pituitary hormones and developed life-

Table 1 Clinical characteristics and mutations of *LHX4*

	Mutation	Defective hormone	Ant pituitary	Post pituitary	Sella turcica	Others
1-1	c.C607-1G>C	GH, TSH, ACTH	Hypo[a]	Ectopic	Hypo	Chiari[b]
1-2	c.C607-1G>C	GH, TSH, ACTH	Hypo	Ectopic	Hypo	Chiari
1-3	c.C607-1G>C	GH, TSH, ACTH	NA[c]	NA	NA	–
2-1	p.A210P	GH, TSH,LH, FSH, ACTH	Hypo	Ectopic	Normal	Pit Cyst[d]
2-2	p.A210P	GH	Hypo	Ectopic	Normal	Pit Cyst
2-3	p.A210P	GH	Normal	Normal	Normal	–
3	p.L190R	GH, TSH, ACTH	Hypo	Normal	Normal	–
4	p.R84C	GH, TSH, LH, FSH	Hypo	Normal	Normal	–
5-1	c293_294insC p.T99fsX53	GH, TSH	Hypo	Ectopic	Hypo	Corpus callosum hypoplasia
5-2	c293_294insC p.T99fsX53	GH, TSH	Hypo	Ectopic	Hypo	–
5-3	c293_294insC p.T99fsX53	GH, LH, FSH	Swelling	Normal	Normal	–
6	p. P389T	GH, PRL, TSH, LH, FSH, ACTH	Hypo	Ectopic	Hypo	Chiari
7	p. V101A	GH, TSH, LH, FSH, ACTH	Hypo	Ectopic	Normal	Chiari
8	LHX4 gene deletion	GH, PRL, TSH, LH, FSH	Hypo	Ectopic	Hypo	Pit Cyst
9	1q25.2-25.3 micro-deletion	GH, PRL, TSH, LH, FSH, ACTH	Hypo	Ectopic	Hypo	Heart failure ASD
10	c.249-1G>A	GH, ACTH	Hypo	Ectopic	Normal	–
11	p.V75I	GH, TSH, LH, PRL	Hypo	Ectopic	Hypo	–

a, Hypo, hypoplasia. b, Chiari, chiari malformation. c, NA, not available. d, Pit Cyst, pituitary cyst.

threatening symptoms soon after birth. However, there existed apparently normal individuals with identical mutations in familial cases. The range of pituitary malformations in most cases comprises hypoplasia of the anterior pituitary. However, an enlarged or normal sized anterior pituitary has also been described. An early report suggested that a poorly developed sella turcica was a characteristic feature caused by the *LHX4* mutations (10, 17). In about half of the reported cases, the sella turcica is poorly developed. Therefore, although the finding of a poorly developed sella turcica may distinguish CPHD patients with LHX4 defects from those reported with mutations in other genes, this finding is not a universal feature of patients with *LHX4* mutations.

All mutations and deletions of *LHX4* reported to date are heterozygous. In familial cases, the inheritance is autosomal dominant. Moreover, several *in vitro* studies have shown that mutants did not have a dominant negative effect. Taken together, haploinsufficiency of *LHX4* is a probable mechanism for the development of the disease.

OTX2

OTX2 (MIM 600037), a bicoid-type homeodomain gene, is a vertebrate ortholog of the Drosophila gene orthodenticle (Otd), and is located in chromosome 14q and has five exons of which three are coding. There are two known isoforms: a and b; isoform b is the major product of the gene and has eight fewer amino acids than isoform a. Both proteins consist of a homeodomain, and transactivation domains at both the C and N terminal region (19, 20). Mouse *Otx2* is expressed in developing neural and sensory structures, including the brain, ear, nose and eye and has a pivotal role in the development of the brain, face, and skull (20–23). Homozygous *Otx2* knockout mice die at

midgestation with severe brain anomalies (21–23). On the other hand, heterozygous knockout mice reveal variable phenotypes ranging from anencephaly, micrognathia, anophthalmia, and microphthalmia to normal, depending on the genetic background (24).

Consistent with these findings, heterozygous mutations of *OTX2* were first reported in patients with severe ocular malformations and/or brain anomalies, seizures and developmental delay without craniofacial anomalies (25). In this report, none of the patients were subjected to evaluation of pituitary function. However, in humans, a deletion in the 14q22-23 region, including *OTX2*, was reported to cause anophthalmia and hypothalamic-pituitary anomalies (26, 27). Thus, a mutation in the *OTX2* may be the cause of the ophthalmologic anomaly observed in patients with CPHD.

Diaczok *et al.* (28) were the first to report a novel missense mutation (p.N233S) of OTX2 in two unrelated patients without eye malformation. These patients showed deficiency of GH, TSH, LH, FSH, and ACTH. MRI demonstrated a hypoplastic anterior pituitary gland, and ectopic posterior lobe. Because OTX2 binding has been shown on the HESX1 promoter, which is also involved in pituitary development (29), functional analysis of p.N233S was performed using this element. As a result, this mutant was proved to have a dominant negative effect on wild-type OTX2. Dateki *et al.* (30) showed that OTX2 is expressed in the human pituitary and that a de novo frameshift *OTX2* mutation (c. 402dupC, S135fsX) causes bilateral anophthalmia and partial isolated GHD with normal MRI findings. This patient showed developmental delay. The same group of researchers also studied 94 Japanese patients with various ocular or pituitary abnormalities. In this study, heterozygous p.K74fsX103 and p.G188X and a 2.8MB microdeletion involving *OTX2* were identified (31). They showed that p.K74fsX103 and p.G188X lost the activation function in the GNRH1, HESX1, POU1F1, and IRBP (interstitial retinoid-binding protein) gene promoters compared with wild-type OTX2. Two patients with p.K74fsX103 and a 2.8 MB microdeletion showed isolated GHD, and one patient with p.G188X showed CPHD. These three patients had ocular abnormalities and developmental delay. They also reported one patient with p.T178S who had CPHD without ocular abnormality (31). The functional consequence of this amino acid substitution was not determined.

Our group reported p.S136fsX178 mutation in the third translated exon in a patient with severe CPHD, developmental delay, and bilateral anopthalmia (32). MRI demonstrated a small anterior pituitary gland and ectopic posterior lobe. Ashkenazi-Hoffnung *et al.* (33) reported a novel missense mutation (p.R90S) in a patient with unilateral anopthalmia and isolated GHD and a normal MRI. The father of this patient carried the same mutation but had a normal eye structure and a normal height. Henderson *et al.* (34) screened *OTX2* mutations in 142 patients with eye anomalies, and identified one de novo heterozygous mutation (p.S138X) in a patient with isolated GHD and normal MRI findings. The patient had bilateral retinal dystrophy. Schilter *et al.* (35) screened 52 patients with anophthalmia and/or microphthalmia and identified mutations in four families. Among them, two had pituitary abnormalities. One patient with a p.W152X mutation had GH, TSH and ACTH deficiency. The other patient had a c.556–557insTATA mutation (p.S186IfsX187). Whereas the defective hormone in this patient was not described in the literature, MRI showed a small anterior pituitary and absent posterior pituitary glands. Two mutations occurred *de novo*. Two patients also had severe developmental delay.

Most recently, Del Blanco *et al.* (36) screened 92 patients with CPHD and identified a novel missense mutation, p.P134R, of OTX2. This patient had deficiency of GH, TSH, LH, FSH, and ACTH. MRI showed a normal anterior pituitary, invisible pituitary stalk, ectopic posterior lobe,

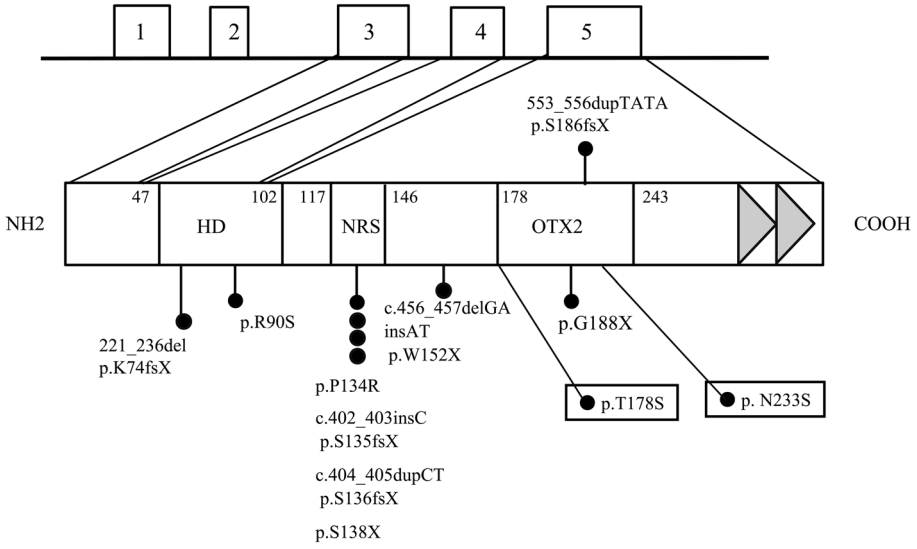

Fig. 2 Schema of OTX2 genomic organization and protein structure. Reported *OTX2* gene mutations associated with human pituitary abnormalities or pituitary hormone deficiency are shown. Missense and frameshift mutations are indicated by black dots. The two missense mutations enclosed in boxes show the dominant negative effect on wild-type OTX2. Numbers next to the protein indicate amino acid positions of domain boundaries. NRS, nuclear retention signal; OTX, OTX family domain; white boxes, exon; HD, homeodomain. The closed triangles represent two tandem repeat conserved transactivation motifs.

and underdeveloped left optic nerve. Moreover, the patient had behavioral problems. *In vitro* analysis demonstrated that this was a second dominant negative mutant on wild-type OTX2. In this family, whereas the patient's father also had an identical mutation, he had no clinical symptoms including hormone deficiency.

Mutations of *OTX2* of patients associated with abnormal pituitary function and structure are shown in Fig. 2. Defective hormones, MRI findings and clinical features in these patients are summarized in Table 2. Mutations of *OTX2* are present throughout the entire gene, and all mutations and a single gene deletion were heterozygous. Most of the reported mutations and the single gene deletion occurred de novo. In familial cases, mutations were transmitted from asymptomatic parents. It is of note that two amino acid substitutions of p.N233S and p.T178S were identified in patients with CPHD without eye malformation. These two mutations are located in the OTX2 family-specific domain. It is possible that any missense mutation in this domain may cause only the pituitary phenotype. To prove this hypothesis, more patients with CPHD are necessary to screen for *OTX2* mutations.

As summarized in Table 2, ocular phenotypes range from bilateral anophthalmia to nearly normal eye development. In regard to hormone deficiency, although several patients showed CPHD, five patients had isolated GHD. This suggests that GH is the most vulnerable pituitary hormone in *OTX2* mutations. Moreover, eight patients showed developmental delay or behavioral problem. These neurological findings suggest that OTX2 is important for the development of the central nervous system.

Table 2 Clinical characteristics and mutations of *OTX2*

	Mutation	Defective hormone	Ant pituitary	Post pituitary	Ocular phenotypes	Others
1	p.N233S[a]	GH, TSH,LH, FSH, ACTH	Hypo[b]	Ectopic	–	–
2	p. N233S	GH, TSH,LH, FSH, ACTH	Hypo	Ectopic	–	–
3	c.402_403insC p.S135fsX	GH	Normal	Normal	Bilaterla ano[c]	Cleft palate, DD[d]
4	c.221_236del p.K74fsX	GH	Hypo	Ectopic	Right ano, Left micro[e]	DD, Retactile testis (right)
5	p.G188X	GH, PRL, TSH, LH, FSH, ACTH	Hypo	Ectopic	Bilateral micr	DD
6	2.8 mega-base deletion	GH	Hypo	Normal	Right micr, left ano	DD
7	p.T178S	GH. TSH, LH, FSH, ACTH	Normal	Normal	–	–
8	c.404_405dupCT p.S136fsX	GH, TSH, LH, FSH, ACTH	Hypo	Ectopic	Bilateral ano	DD
9	p.S138X	GH	NA[f]	NA	Bilateral retinal dystrophy	Tube feeding
10	c.456_457delGAinsAT p.W152X	CPHD	Hypo	NA	Right ano, left micr	Microcephaly, DD
11	553_556insTATA S186fsX	NA	Hypo	Not detected	Bilateral micr	Microcephaly, DD
12	p.R90S	GH	Hypo	Ectopic	Right ano	–
13	p.P134R[a]	GH, TSH, LH, FSH, ACTH	Normal	Ectopic	Left optic nerve hypo	Invisible pituitary stlak, Behavioral problems

a, Dominant negative effect . b, Hypo, hypoplasia. c, ano, anophthalmia. d, DD developmental delay. e, micro, microphthalmia. f, NA, not available.

Conclusion

In recent yr, identification of mutations in pituitary transcription factor genes has markedly advanced our understanding of the mechanisms of pituitary development and congenital pituitary hormone deficiency. The focus of this review is hypopituitarism caused by genetic defects of LHX4 and OTX2. As mentioned, hypopituitarism of these patients ranges from normal to life-threatening severe hypopituitarism. The phenotypic variability may be explained by other environmental factors and modifier genes. Such variability might also reflect a plasticity during pituitary development and maintenance.

Because defects of some transcription factors show a gene-specific phenotype such as a small sella turcica in *LHX4* mutations and ocular abnormalities in *OTX2* mutations, specific gene mutations may be speculated as causes of CPHD, and gene analysis is useful for diagnosis and genetic counseling. Moreover, as many studies indicate that hormone deficiencies caused by transcriptional factor defects may appear with age, regular evaluation of other pituitary hormones is necessary for early recognition of hormone deficiency.

In conclusion, understanding the molecular mechanism of defects of transcriptional factors results in improvement of morbidity in patients with CPHD. Because the causes in many patients with CPHD have not yet been determined, continuing efforts for clarification of the etiology are necessary.

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