A novel *LHX4* mutation is associated with hypogonadotropic hypogonadism, not combined pituitary hormone deficiency

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Take Home Message

We showed for the first time that *LHX4* mutation is associated with hypogonadotropic hypogonadism (HH), not combined pituitary hormone deficiency (CPHD).

Backgrounds

Several transcription factor genes have been linked to the pathogenesis of CPHD, including *POU1F1*, *PROP1*, *HESX1*, *LHX3*, *OTX2*, *SOX2*, *SOX3*, *GLI2*, and *LHX4*.

In addition to these genes, some causative genes for Kallmann syndrome (KS), which is defined by HH with anosmia, have been identified in a small number of CPHD and septo optic dysplasia (SOD)[1-2].

1)Reynaud et al. 2012 J Clin Endocrinol Metab 2)McCabe et al. 2013 J Clin Endocrinol Metab

On the other hand, mutations in *HESX1*[3], *SOX3*[4], responsible for CPHD have been identified in a small number of HH.

3) Newbern *et al*. 2013 Fertil Steril. 4) Izumi *et al*. 2014 Fertil Steril

These findings strongly suggest that the genetic overlap between CPHD, SOD, and KS/HH is significant.

Objectives

We report an infant clinically diagnosed as HH with a novel missense mutation in *LHX4* (R221W).

Materials & Methods

Case Report

A 2-month-old boy was referred because of micropenis (stretched penile length 1.0 cm) with intrascrotal testes (right 1 mL, left 1 mL).

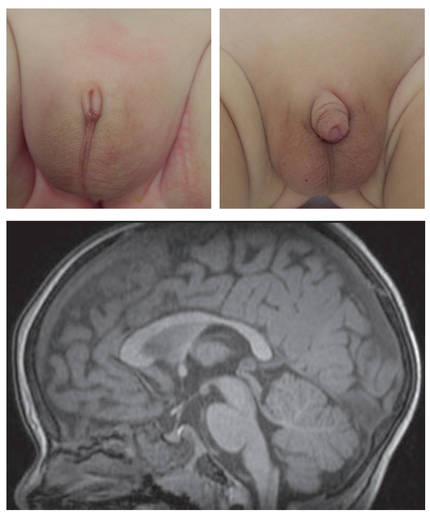
Hormone assays revealed very-low plasma testosterone concentrations (0.06 ng/mL). LHRH stimulating test performed at the age of 3 months (minipuberty) revealed LH peak 7.3 mIU/mL, and FSH peak 20.7 mIU/mL, suggesting pre-pubertal response.

Plasma concentrations of thyroxine and insulin-like growth factor-1 were within normal ranges. No episode of hypoglycemia was noted. Brain MRI showed a normal size anterior pituitary with a visible stalk.

⇒ He was diagnosed as HH clinically. He responded well to testosterone enanthate therapy (im 25 mg every 4 wk for three doses).

At the last examination at age 20 months, his height and weight were -0.2 SD, and +1.3 SD, respectively.

	2 month	Reference
IGF-1 (ng/ml)	67	Male:11-149
TSH (μU/ml)	3.776	
Free T4 (ng/dl)	1.22	0.91-1.99
Free T3 (pg/ml)	3.81	
		Adult
LH (mIU/ml)	1.9	Male: 2.2-8.4
FSH (mIU/ml)	8.8	Male: 1.8-12
Testosterone (ng/ml)	0.104	Male: 2.01-7.50



LHRH and hCG stimulating test

	Basal	Peak
LH (mIU/ml)	1.0	7.3
FSH (mIU/ml)	6.9	20.7
Testosterone (ng/ml)	0.06	4.179

Mutation Screening

Using a next-generation sequencing strategy, we sequenced 9 genes implicated in CPHD, including *POU1F1*, *PROP1*, *HESX1*, *LHX3*, *OTX2*, *SOX2*, *SOX3*, *GLI2*, *LHX4*, and 12 genes implicated in HH, including *CHD7*, *FGFR1*, *FGF8*, *GNRH1*, *GNRHR*, *KAL1*, *KISS1*, *KISS1R*, *PROK2*, *PROKR2*, *TAC3*, and *TACR3*.

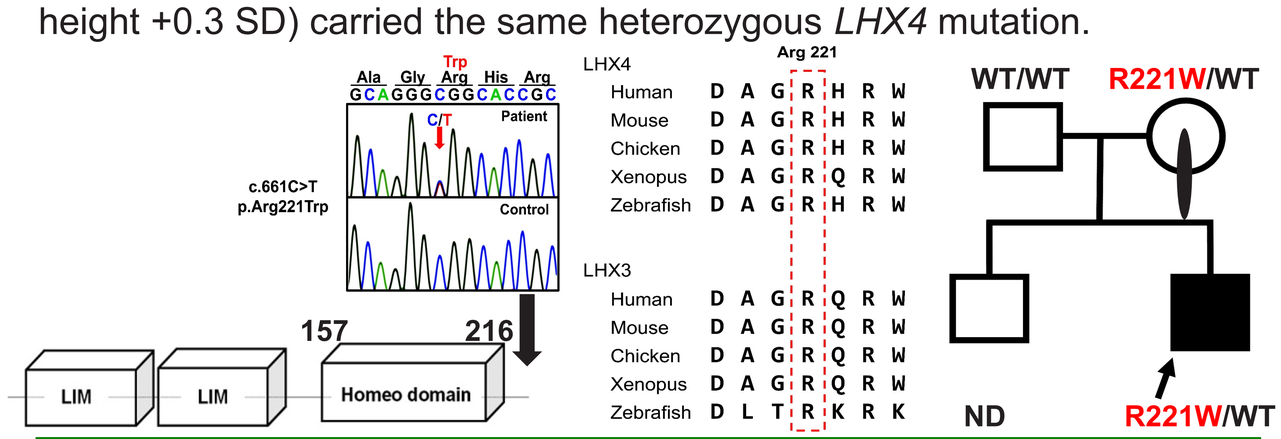
Functional Studies

Transcriptional activity of identified *LHX4* variant was evaluated by luciferase reporter assays constructed by inserting the *POU1F1* or *αGSU* promoters. We also performed subcellular localization analyses and EMSA assays.

Results①

We identified a novel heterozygous *LHX4* sequence variation, c.661C>T (p.R221W). The R221 in LHX4 is evolutionarily highly conserved amino acid located immediately 3 ′of the Homeo domain.

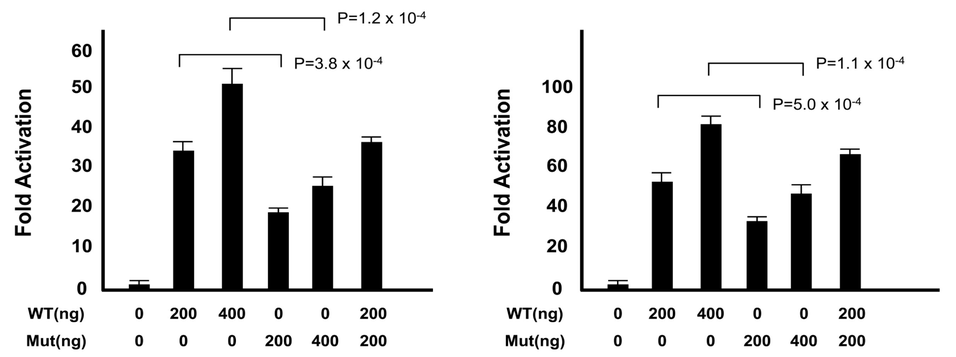
Genetic analyses showed that the clinically normal mother (adult height +0.3 SD) carried the same heterozygous *LHX4* mutation.



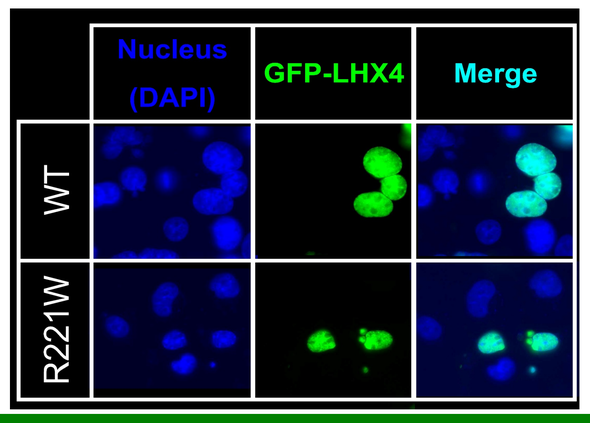
Results②

R221W had reduced transactivation, and had no dominant negative effect (FIG.A). Subcellular localization revealed no significant difference between WT and R221W (FIG.B). WT LHX4 showed specific binding to the elements, which was competed by excess amount of cold competitors. EMSA experiments showed that the R221W LHX4 had reduced DNA-binding ability (FIG.C).

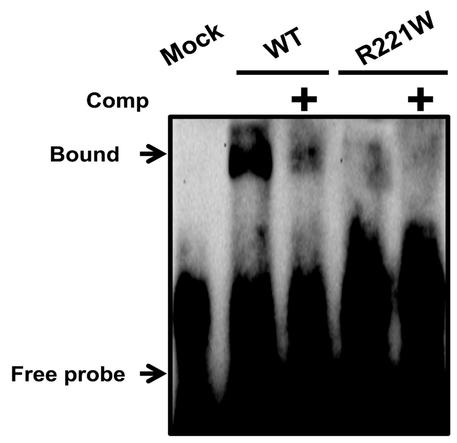
A. Transactivation assays



B. Subcellular localization



C. EMSA experiments



Discussion

1. We characterized a novel mutant (R221W) of the LHX4 transcription factor that is associated with HH. Arginine 221 is a highly conserved amino acid located immediately 3 ′of the homeodomain, suggesting that substitution of arginine 221 to tryptophan, which is predicted to lose a residue-DNA contact, results in defective interactions with DNA. Indeed, EMSA studies showed that the mutant LHX4 protein had reduced DNA-binding affinity. The partial transcription activity suggests that the R221W mutation is a hypomorphic mutation that retains residual activity.

2. We showed for the first time that *LHX4* mutation is associated with HH, not CPHD. This unique and/or mild phenotype could be due to residual LHX4 activity. The findings in this patient emphasize the importance of testing for *LHX4* mutations in HH individuals. This study extends our understanding of the phenotypic features, molecular mechanism, and developmental course associated with mutations in *LHX4*.

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