P1-D3-193

A novel mutation of *OTX2* associated with neonatally diagnosed combined pituitary hormone deficiency (CPHD) and bilateral microphthalmia

Aya Shimada[1)], Masaki Takagi[1)], Kentaro Miyai[1)], Takahiro Nakamura[2)], Yukihiro Hasegawa[1)]

Tokyo Metropolitan Children's Medical center 1) Department of Endocrinology and Metabolism 2) Department of Cardiology

Take Home Message

OTX2 mutation can cause ACTH deficiency from neonatal period.

Backgrounds

Orthodenticle homeobox 2 (OTX2) is a transcription factor implicated in pituitary, ocular, and craniofacial development.

OTX2 mutation can cause congenital hypopituitarism (CH) ranging from isolated growth hormone deficiency (IGHD) to CPHD. However, CPHD including ACTH deficiency from neonatal period was rare among the previous reports.

Objectives

To examine pathogenicity of novel mutation of *OTX2*

Case : 0 year-old , boy

【History of Present illness】

Born at 40w, BW 3178g (Mean), Height 49cm (Mean), Apgar Score 9/9

He had congenital cardiac malformations, bilateral microphthalmia, and micropenis. At the age of 5 days, he was diagnosed as having CPHD on the basis of multiple low anterior pituitary hormones (Table 1).

【MRI】(Fig.1)

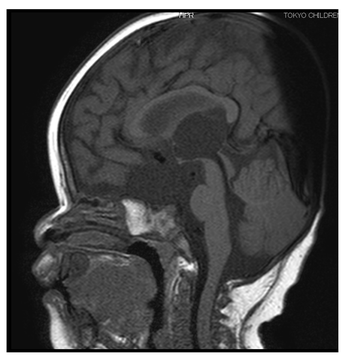
Bilateral microphthalmia, hypoplastic anterior pituitary gland

Ectopic posterior pituitary lobe

Table 1. Endocrinological findings (Day 9)

	Data		Data
TSH (μIU/ml)	1.181	GH (ng/mL)	＜0.03
fT4 (ng/dl)	0.11		
fT3 (pg/ml)	1.04	LH (mIU/ml)	≦0.10
ACTH (pg/ml)	≦2.0	FSH (mIU/ml)	≦0.05
Cortisol (μg/dl)	＜1.0	T (ng/ml)	≦0.03

Fig 1. MRI (9 month)



G-banding : 46,XY　　Array CGH (180kb) : no deletion

Material & Methods

This study was approved by the Institutional Review Board of Tokyo Metropolitan Children's Medical Center.

① Genetic Analysis

We sequenced all coding exons and flanking introns of *OTX2* in patient and his family members. We also examined all the coding exons and franking introns of other known causative genes of CPHD (*POU1F1, PROP1, HESX1, LHX3, LHX4, SOX2, GLI2,* and *SOX3*)

② Functional Analysis

We performed western blotting, nuclear localization analysis, DNA binding analysis, and transactivation analysis. Transcriptional activity of the mutation was evaluated by using *HESX1*, *POU1F1*, and *GnRH* as reporters.

Results ① Genetic Analysis

We identified a novel heterozygous mutation (c.266G>C, p.R89P) of *OTX2* in the patient and in his healthy father (Fig2-A, 2-B). This mutation was not detected in 100 healthy controls. This mutation located in the homeobox domain of *OTX2* (Fig 2-C). There was no mutation in other genes detected.

Fig 2-A

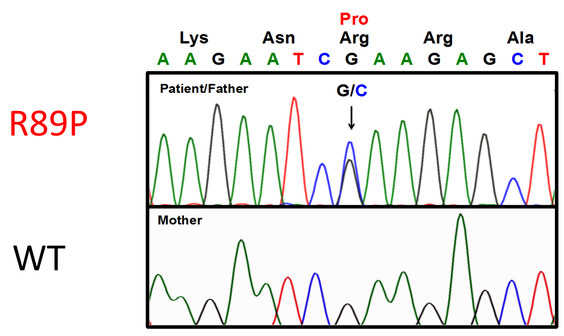


Fig 2-B

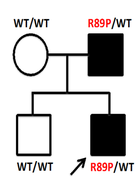
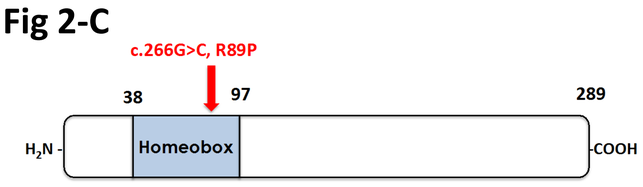


Fig 2-C



Results ② Functional analysis for R89P OTX2

Results of functional analysis for R89P OTX2 were as follows:

1) In transcriptional analysis, R89P showed decreased transactivation. With *POU1F1* reporter, R89P showed decreased transactivation with dominant negative effect (Fig.3-A)
2) EMSA experiment showed that R89P lost DNA binding capacity (Fig 3-B).
3) In nuclear localization analysis, there was no significant difference between WT and R89P (Fig 3-C).
4) In crystal structural modeling, R89P predicted to lose DNA binding capacity (Fig 3-D).

Fig.3-A Luciferase Assay

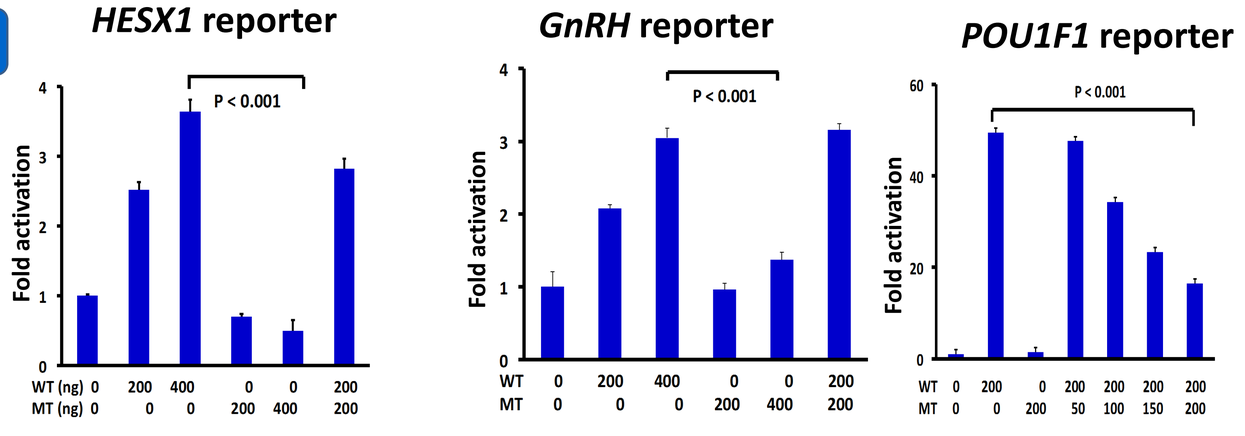


Fig.3-B EMSA assay

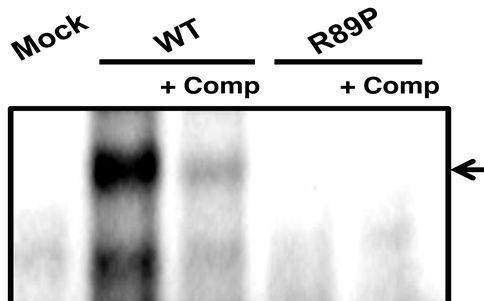


Fig.3-C Nuclear localization Analysis

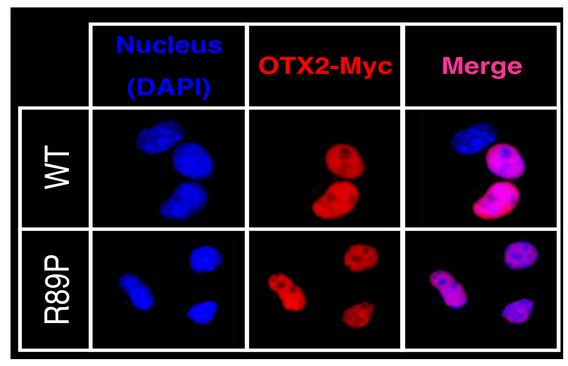
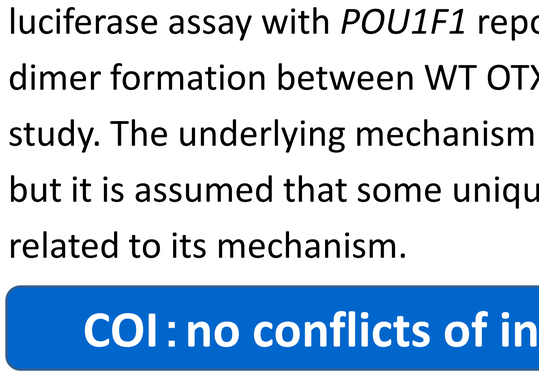


Fig.3-D Crystal structural modeling



Discussion

We showed a case with ACTH deficiency carrying a novel mutation in *OTX2*.

In previous reports, only two cases of ACTH deficiency from neonatal period were reported with mutated OTX2. Our case showed another proof that *OTX2* mutation can cause multiple anterior pituitary hormone deficiency including ACTH.

In this study. it is also confirmed that *OTX2* mutation can present wide phenotypic diversity even if a mutation is a non-functional one. In familial analysis, patient and his healthy father carries the same mutation though the R89P OTX2 was considered to be a non-functional mutation.

In functional analysis, dominant negative effects are noted only in luciferase assay with *POU1F1* reporter. Dominant negative effect due to the dimer formation between WT OTX2 and mutant OTX2 is not confirmed in our study. The underlying mechanism of dominant negative effect is still unclear, but it is assumed that some unique co-factor with *POU1F1* reporter may related to its mechanism.

COI : no conflicts of interest

Contact Adress :
aya_shimada@tmhp.jp