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

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Take Home Message

OTX2 mutation can cause ACTH deficiency from neonatal period.

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).

Objectives

To examine pathogenicity of novel mutation of OTX2

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.

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 microprosoph. 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)

<table>
<thead>
<tr>
<th>Data</th>
<th>TSH (μIU/ml)</th>
<th>GH (ng/ml)</th>
<th>FT4 (ng/dl)</th>
<th>FT3 (pg/ml)</th>
<th>ACTH (pg/ml)</th>
<th>Cortisol (μg/dl)</th>
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</thead>
<tbody>
<tr>
<td>Data</td>
<td>1.181</td>
<td>1.181</td>
<td>0.11</td>
<td>1.04</td>
<td>≦0.03</td>
<td>≦1.0</td>
</tr>
</tbody>
</table>

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

Table 1. Endocrinological findings (Day 9)

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

Material & Methods

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 flanking introns of other known causative genes of CPHD (POU1F1, PROP1, HESX1, LHX3, LHX4, SOX2, GLI2, and SOX3). 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.

② Functional Analysis

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.

Discussion

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

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

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