A Novel E250X Mutation of the PIT1 Gene in a Patient with Combined Pituitary Hormone Deficiency

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Abstract. PIT1 abnormality is defined as a genetic abnormality in the PIT1 gene that encodes a pituitary specific transcription factor, Pit-1/GHF-1. PIT1 abnormality indicates combined deficiency of thyrotropin (TSH), growth hormone (GH) and prolactin (PRL), and has been reported in several cases. We studied the PIT1 gene in a patient with combined deficiency of TSH, GH and PRL. A novel mutation substituting a termination codon for Glutamate at 250th codon (E250X) was identified in the homozygous state in the patient. Both of the healthy parents harbored this mutation in the heterozygous state. This nonsense mutation results in complete loss of helix 3 of the POU homeodomain of Pit-1/GHF-1. As helix 3 of the homeodomain is involved directly in DNA binding, the mutant Pit-1/GHF-1 may lose the DNA binding activity of the POU homeodomain and lose its transcriptional activation. The E250X mutation is therefore considered to be the cause of the combined deficiency of TSH, GH and PRL in this patient.

Key words: PIT1, Pit-1/GHF-1, Pituitary hormone deficiency, POU homeodomain, Mutation

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PIT-1/GHF-1, a 291 amino acid protein encoded by the PIT1 gene, is a pituitary specific transcription factor. It is indispensable for pituitary development and pituitary hormone expression in vertebrates [1-4]. Pit-1/GHF-1 transactivates the thyrotropin β-subunit (TSHβ), growth hormone (GH) and prolactin (PRL) genes [5]. The human PIT1 gene is 17 kb in size, and separated into six protein coding exons [6]. Its chromosomal localization is 3pl1-12 [7]. The PIT1 gene belongs to a member of the POU domain gene family, PIT1.
of the patient resulting in a defect in helix 3 of the POU homeodomain. Pedigree analysis showed that this mutation is homozygous in the patient and heterozygous in the parents.

Materials and Methods

Patient

We studied a Thai girl with severe growth retardation and her parents after informed consents. She was an offspring of a short mother (140 cm, −2.5 SD) and a father of normal stature. She was born at full term without any problems. Her mother showed no abnormality during pregnancy and delivery. Her birth weight was 2750 g and birth length was 40 cm. She had poor feeding, and was brought to the hospital at four months of age because she had poor weight gain with chronic constipation. She weighed 4200 g and showed hypothyroidism. The serum levels of thyroxine and TSH were 0.87 µg/dl (normal values: 5-12 µg/dl) and 0.66 µU/ml (normal values: 0.3-3.5 µU/ml), respectively. In spite of thyroxine replacement, she showed severe growth retardation. Then she was referred to Ramathibodi Hospital for further investigation. On admission, she was 16 months old, weighed 4300 g and was 56 cm long. The precise clinical features were reported in a previous article [14]. As shown in Table 1, she lacked TSH, GH and PRL, but LH and FSH were intact. ACTH was not measured, but her basal plasma cortisol level was not decreased. Magnetic resonance imaging (MRI) of the brain demonstrated no abnormality in the pituitary or the rest of the brain. The patient grew well after thyroid hormone replacement and human growth hormone therapy. The mother showed no abnormality in endocrinological tests. For the father, endocrinological tests were not done.

PCR-direct sequencing

Analyses of the PIT1 gene in the patient and the parents were performed by the PCR-direct sequencing method as previously described [15]. In brief, genomic DNA was extracted from the peripheral blood. Each of the six protein coding exons of the PIT1 gene was amplified by PCR by using specific primers. The amplified products were gel-purified and submitted for direct sequencing. For the parents, only exon 6 was analyzed.

Results

In this patient, only one point mutation was identified in the entire protein coding sequences of the PIT1 gene (Fig. 1). This was a novel mutation, a G to T transition in exon 6 causing a termination codon for Glutamate at the 250th codon (E250X) in the POU homeodomain. To determine how this nonsense mutation was transmitted in the family, pedigree analysis was performed. As shown in Fig. 1, the patient harbored this mutation in the homozygous state, while the parents harbored it in the heterozygous state.

Discussion

In this study, we presented a novel E250X mutation of the PIT1 gene in a patient with combined deficiency of TSH, GH and PRL. The E250X mutation results in complete loss of helix 3 of the POU gene.

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Table 1. Anterior pituitary functions of the patient

<table>
<thead>
<tr>
<th></th>
<th>TSH (µU/ml)</th>
<th>GH (ng/ml)</th>
<th>PRL (ng/ml)</th>
<th>LH (mIU/ml)</th>
<th>FSH (mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>provocation tests</td>
<td>TRH</td>
<td>GRF</td>
<td>TRH</td>
<td>LH-RH</td>
<td>LH-RH</td>
</tr>
<tr>
<td>base</td>
<td>&lt;0.04 L</td>
<td>&lt;0.6 L</td>
<td>&lt;0.6 L</td>
<td>4.8</td>
<td>32</td>
</tr>
<tr>
<td>peak</td>
<td>0.04 L</td>
<td>1.2 L</td>
<td>0.6 L</td>
<td>175</td>
<td>146</td>
</tr>
</tbody>
</table>

All the tests except TSH secretion test were performed during euthyroid state in the patient. L, low basal value or hyporesponse.
The E250X mutation is a recessive mutation because the patient harbored this mutation in the homozygous state and both of the parents with no endocrinological abnormality harbored it in the heterozygous state. As the parents came from the same region of Thailand, the mutation might have...
derived from a common ancestor.

Thus the E250X mutation is considered to be the cause of the combined deficiency of TSH, GH and PRL in this patient, although we are unable to rule out other additional abnormalities, such as a decreased level of mRNA, abnormal splicing, or alterations of some other genes.

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References


