A novel heterozygous intronic mutation in POU1F1 is associated with combined pituitary hormone deficiency

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Abstract. POU class 1 homeobox 1 (POU1F1) regulates pituitary cell-specific gene expression of somatotropes, lactotropes, and thyrotropes. In humans, two POU1F1 isoforms (long and short isoform), which are generated by the alternative use of the splice acceptor site for exon 2, have been identified. To date, more than 30 POU1F1 mutations in patients with combined pituitary hormone deficiency (CPHD) have been described. All POU1F1 variants reported to date affect both the short and long isoforms of the POU1F1 protein; therefore, it is unclear at present whether a decrease in the function of only one of these two isoforms is sufficient for disease onset in humans. Here, we reported a sibling case of CPHD carrying a heterozygous mutation in intron 1 of POU1F1. In vitro experiments showed that this mutation resulted in exon 2-skipping of only in the short isoform of POU1F1, while the long isoform remained intact. This result strongly suggests the possibility, for the first time, that isolated mutations in the short isoform of POU1F1 could be sufficient for induction of POU1F1-related CPHD. This finding improves our understanding of the molecular mechanisms, and developmental course associated with mutations in POU1F1.

Key words: POU1F1, Short isoform, Exon trapping, Combined pituitary hormone deficiency

POU CLASS 1 HOMEBOX 1 (POU1F1) regulates pituitary cell-specific gene expression of somatotropes, lactotropes, and thyrotropes; therefore, POU1F1 mutations are responsible for deficiencies in corresponding pituitary hormones, i.e., growth hormone (GH), prolactin (PRL), and thyroid-stimulating hormone (TSH) [1, 2]. In humans, two POU1F1 isoforms, the long isoform (317 amino acids, NM_001122757) and the short isoform (291 amino acids, NM_000306), have been identified. Both of these isoforms are generated by the alternative use of the splice acceptor site for exon 2 [3-5].

To date, more than 30 POU1F1 mutations in patients with combined pituitary hormone deficiency (CPHD) have been described (HGMD; http://www.hgmd.cf.ac.uk). All POU1F1 variants reported to date affect both the short and long isoforms of the POU1F1 protein; therefore, it is unclear at present whether a decrease in the function of only one of these two isoforms is sufficient for disease onset in humans. Here, we reported a sibling case of CPHD with a novel heterozygous intronic mutation, c.143-83A>G, in POU1F1. Exon trapping studies showed that the mutation led to exon 2 skipping only in the short isoform of POU1F1, while the long isoform remained intact, indicating the possibility that isolated mutations in the short isoform of POU1F1 could be sufficient for induction of CPHD.

Patients and Methods

Patient

The patient 1 (II-1; Fig. 1a) was a 7-year-old Japanese female patient born at 39 weeks of gestation after an uncomplicated pregnancy and delivery. She had no family history of pituitary dysfunction.
Her parents were nonconsanguineous and phenotypically normal. The father was 172 cm (0.2 SD) tall and mother was 152 cm (-1.2 SD) tall. The patient’s birth length was 44.0 cm (-2.1 SD), and weight was 2,330 g (-1.8 SD). She was referred to our hospital because of poor weight gain at age of 10 days. She was found to have central hypothyroidism (CH) on the basis of a low free T4 (below 0.4 ng/dL; Ref. 0.99–1.91) with an inappropriately normal TSH concentration of 1.32 mU/L (Ref. 0.77–7.3). The diagnosis of CH was confirmed by thyrotropin releasing hormone (TRH) provocation test, performed at age of 11 days (peak TSH 2.09 mU/L, Ref 10–35). Replacement therapy with L-thyroxine was started at age of 12 days. Her basal cortisol concentration, after L-thyroxine replacement was normal. She presented with low serum concentrations of IGF-I (below 10 ng/mL), and impaired GH response on arginine hydrochloride testing, performed at age of 3 months (peak GH 0.06 ng/mL, Ref. >6.0). She was diagnosed as having GH deficiency (GHD) and replacement therapy with recombinant human GH (rhGH) was started at age of 5 months. Her growth responded well to GH replacement. During her last examination at the age of 7 years and 4 months, her height was 123.8 cm (+0.6 SD), her weight was 25.1 kg (+0.4 SD). Her basal prolactin concentration at age of 5 months was very low (below 0.5 ng/mL). The brain MRI exhibited anterior pituitary hypoplasia, while pituitary stalk and posterior pituitary were normal.

Patient 2 (II-2; Fig. 1a) was a younger sister of the patient 1. She was born at 38 weeks of gestation after an uncomplicated pregnancy and delivery. The patient’s birth length was 44.5 cm (-1.9 SD), and weight was 2,515 g (-1.3 SD). She also showed poor weight gain, and was found to have CH on the basis of a low free T4 (below 0.7 ng/dL) with an inappropriately normal TSH concentration of 1.4 mU/L. The diagnosis of CH was confirmed by TRH provocation test (TSH peak 0.4 mU/L, and PRL peak 1.4 ng/mL). Her basal cortisol concentration, after L-thyroxine replacement was normal. She presented with low serum concentrations of IGF-I (below 10 ng/mL), and impaired GH response on arginine hydrochloride testing, performed at age of 4 months (peak GH 0.02 ng/mL). She was diagnosed as having GHD and replacement therapy with rhGH was started at age of 5 months. Her growth responded well to GH replacement. During her last examination at the age of 5 years and 11 months, her height was 112.0 cm (+0 SD), her weight was 19.3 kg (+0 SD). The brain MRI exhibited normal size of anterior pituitary gland with eutopic posterior gland.

**Mutation screening**

After obtaining informed consent, and with the approval of the Institutional Review Board of Tokyo Metropolitan Children’s Medical Center (H25-73), genomic DNA was extracted from peripheral blood leucocytes of the patients and their parents. Due

![Fig. 1](image-url)  
**Fig. 1** Identification of sequence variations of *POU1F1* and RNA analyses  
(a) Pedigree of the patient  
Familial genetic analyses showed that the healthy mother carried the same heterozygous mutation.  
(b) Chromatographs and genomic position of the mutation  
We found a novel heterozygous intronic mutation in *POU1F1* (c.143-83A>G in the short isoform or c.143-5A>G in the long isoform) in both patients. Yellow box indicates exon 2 of the short isoform of *POU1F1* (72 bp, identical in short and long isoforms). Red box indicates additional 78 bp, specific for exon 2 of the long isoform.
to the specific combination of the GH, PRL, and TSH deficiencies, we first analyzed the POU1F1 gene by polymerase chain reaction (PCR) and direct sequencing methods.

Additionally, we sequenced eight genes implicated in CPHD, including PROP1, HESX1, LHX3, LHX4, OTX2, SOX3, SOX2, and GLI2, using the MiSeq instrument (Illumina Inc, San Diego, CA, USA), according to the SureSelect protocol (Agilent Technologies, Santa Clara, CA, USA) as described before [6]. We screened for deletion/duplication involving PROP1, HESX1, LHX3, LHX4, and POU1F1 by multiplex ligation-dependent probe amplification (MLPA) analyses (SALSA MLPA KIT P216; MRC-Holland, Amsterdam, The Netherlands).

Subcloning

In order to demonstrate maternal somatic mosaicism in the unaffected mother of the patient, the PCR product of DNA from her leukocytes was initially cloned into the pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA, USA). And then, we took 100 colonies, screened the mutation, and calculated the ratio of mutant to wild type alleles.

Reverse transcription (RT) PCR

For mRNA analysis, total RNAs were extracted from Epstein-Barr virus-transformed lymphocytes derived from both patients and two independent control subjects. The cDNA produced by reverse transcription (RT) was subjected to PCR amplification with primers encompassing exons 1 to 4 (Forward: 5′-CTGCCGAGTGTCTACCAGTC-3′, and Reverse: 5′-CTCAGCTTCCTCCAGCCATT-3′) and were subsequently processed for direct sequencing.

Exon-trapping experiments

Construction of expression vectors

A 926 bp fragment of the human POU1F1 spanning intron 1, exon 2 (72 bp in short, 150 bp in long isoform) and the first 425 bp of intron 2 was amplified from genomic DNA of an affected individual and a control subject. We used following primers (Forward: 5′-CTCGAGACTATTCTTCTAGTCGGGGAGTTGCAATGT-3′, Reverse: 5′-GCGGCCGCTAGCAGTATTACAACTAACAGTTTGACG-3′) with XhoI and NotI restriction sites (underline). The PCR products were subcloned into the exon trapping vector pET01 (MoBiTec, Goettingen, Germany), and designated as pET01-WT or pET01-Mut. Plasmid integrity was confirmed by DNA sequencing.

Cell culture and transfection

We cultured HeLa cells and transfected cells with pET01-WT or pET01-Mut plasmids by using Lipofectamine™ 2000 reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer’s protocol.

Reverse transcription (RT) PCR

Total RNA was isolated from HeLa cells, which were transfected with pET01-WT or pET01-Mut. The cDNA produced from reverse transcription (RT) of RNA was subjected to PCR amplification, and was subsequently processed for direct sequencing. We used following primers (Forward: 5′-GAGGGATCCGCTTCCTGCCCC-3′, Reverse: 5′-CTGCCGAGTGTCTACCAGTC-3′) for PCR and sequencing.

Results

Mutation screening

We found a novel heterozygous intronic mutation in POU1F1 (c.143-83A>G in the short isoform or c.143-5A>G in the long isoform) in both patients (Fig. 1b). Genetic analyses showed that the healthy mother of the patients carried the same POU1F1 mutation (Fig. 2). The mutation was not detected in 150 healthy Japanese controls and was absent from databases, including dbSNP and the 1000 Genomes Project. Evaluation of the hormonal data for the mother was refused.

Fig. 2 Familial analysis and subcloning of the PCR product from the mother

Genetic analyses showed that the healthy mother of the patients carried the same POU1F1 mutation. The ratio of mutant to wild type alleles was 8/92, indicating somatic mosaicism of the mutation in the mother.
Additionally, we sequenced eight genes implicated in CPHD, including \textit{PROP1}, \textit{HESX1}, \textit{LHX3}, \textit{LHX4}, \textit{OTX2}, \textit{SOX3}, \textit{SOX2}, and \textit{GLI2}, with negative results, and no exon-level deletions or duplications were detected in \textit{PROP1}, \textit{HESX1}, \textit{LHX3}, \textit{LHX4}, and \textit{POU1F1}.

\textbf{Subcloning}

The ratio of mutant to wild type alleles was 8/92, indicating somatic mosaicism of the mutation in the mother (Fig. 2).

\textbf{Reverse transcription (RT) PCR}

RT-PCR generated a product of smaller size than that obtained from a control sample. Sequencing revealed that this product corresponded to a \textit{POU1F1} transcript skipping exon 2 (Fig. 3a), which would generate Δ48-72-\textit{POU1F1}, a previously reported pathogenic protein [7]. The product corresponding to the long isoform was not amplified both in controls and patients.

\textbf{Exon-trapping experiments}

To determine whether this exon 2 skipping transcript was derived from the short or long isoform, we performed exon trapping experiments. RT-PCR of the WT gave the expected correctly spliced 398-bp and 320-bp products corresponding to the long and short isoforms of \textit{POU1F1}, respectively. In contrast, RT-PCR with the mutant yielded only the 398-bp product (Fig. 3b). These results indicated that this intronic mutation led to exon 2 skipping of only the short isoform of \textit{POU1F1}, while the long isoform remained intact.

\textbf{Discussion}

In this study, we described a sibling case of CPHD carrying a heterozygous intronic mutation in \textit{POU1F1}. We detected an exon 2-skipping transcript of \textit{POU1F1} in lymphocytes derived from both patients. It is unclear why RT-PCR of lymphocytes failed to amplify the long isoform of \textit{POU1F1}, which could not be expressed in lymphocytes. Previously, a heterozygous splice site mutation in \textit{POU1F1}, namely c.214+1G>T, which results in exon 2 skipping, was described by Inoue \textit{et al.} [7]. Mutant \textit{POU1F1} (Δ48-72 \textit{POU1F1}) has been shown to result in reduced transcriptional activity, having a dominant-negative effect. As opposed to short isoform, long isoform \textit{POU1F1} has been reported to suppress GH, PRL and TSHb promoters in a pituitary-specific manner, blocking Ras-induced PRL promoter activity [7]. Hence, we believe that the heterozygous intronic variant of \textit{POU1F1} (c.143-83A>G in the short isoform or c.143-5A>G in the long isoform) is pathogenic and could be responsible for the specific combination of GH, PRL, and TSH deficiencies in our cases.

In humans, \textit{POU1F1} has short and long isoforms, both of which are generated by the alternative use of the splice acceptor site for exon 2; exons 1 and 3-6 are identical between the two isoforms [2-3]. Therefore, mutations in exons 1 and 3-6 in \textit{POU1F1} affect both the short and long isoforms of \textit{POU1F1} protein. Only one mutation that affects exon 2 of \textit{POU1F1}, namely c.214+1G>T, has been reported [7]. However, this splice donor site mutation has been shown to affect both the short and long isoforms; therefore, it is unclear whether a decrease in the function of only one of these two isoforms is sufficient for disease onset in humans. Exon trapping studies showed that our c.143-83A>G mutation led to exon 2 skipping only in the short isoform of \textit{POU1F1}, while the long isoform remained intact. This result strongly suggests the possibility, for the first time, that isolated mutations in the short isoform of \textit{POU1F1} could be sufficient for induction of \textit{POU1F1}-related CPHD.

Notably, however, \textit{in vitro} exon trapping studies are artificial and do not necessarily reflect the expression pattern \textit{in vivo}, particularly in the developmental stage of the pituitary. Therefore, further studies are necessary to clarify the contribution of short isoform specific mutations in \textit{POU1F1} to the development of CPHD.

In summary, we report a sibling case of CPHD with a novel heterozygous intronic mutation in \textit{POU1F1}. Exon trapping studies showed that the c.143-83A>G mutation led to exon 2 skipping only in the short isoform of \textit{POU1F1}, while the long isoform remained intact, indicating the possibility that isolated mutations in the short isoform of \textit{POU1F1} could be sufficient for induction of CPHD. These findings improve our understanding of the molecular mechanisms, and developmental course associated with mutations in \textit{POU1F1}.

\textbf{Acknowledgments}

We thank Kazue Kinoshita for technical assistance. We also thank Yukihiro Hasegawa for fruitful discussion. This work was supported by Grants
Mutation in short isoform of POU1F1

Fig. 3  Identification of exon 2 skipping of POU1F1 and exon trapping studies

(a) Identification of exon 2 skipping in the POU1F1 cDNA derived from both patients
The cDNA produced by reverse transcription (RT) was subjected to PCR amplification with primers encompassing exons 1 to 4 and were subsequently processed for direct sequencing. RT-PCR generated a product of smaller size than that obtained from a control sample. Sequencing revealed that this product corresponded to a POU1F1 transcript skipping exon 2. The product corresponding to the long isoform was not amplified both in controls and patients.

(b) Exon trapping experiments
Top, a schematic representation of exon trapping vectors. A 926 bp fragment of the human POU1F1 spanning the intron 1, exon 2, and the first 425 bp of intron 2 was amplified from genomic DNA of a control subject or an affected individual. We subcloned the PCR products into the exon trapping vector using the XhoI and NotI restriction sites.
Lower, RT-PCR of the wild-type (WT) gave the expected correctly spliced 398-bp and 320-bp products corresponding to the long and short isoforms of POU1F1, respectively. In contrast, RT-PCR with the mutant yielded only the 398-bp product. Exon trapping studies showed that the mutation led to exon 2 skipping only in the short isoform of POU1F1, while the long isoform remained intact.
from the Japan Society for the Promotion of Science (16K10007), grants from Takeda Science Foundation, Foundation for Growth Science, the Japanese Society for Pediatric Endocrinology Future Development Grant supported by Novo Nordisk Pharma Ltd, and grants from the Ministry of Health, Labour, and Welfare, Japan (Jitsuyoka Nanbyo-Ippan-014).

**Source of Funding**

This work was supported by grants from the Japan Society for the Promotion of Science (16K10007), grants from Takeda Science Foundation, Foundation for Growth Science, the Japanese Society for Pediatric Endocrinology Future Development Grant supported by Novo Nordisk Pharma Ltd, and grants from the Ministry of Health, Labour, and Welfare, Japan (Jitsuyoka Nanbyo-Ippan-014).

**Disclosure Statement**

The authors have nothing to disclose.

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