Novel Insights from Clinical Practice

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A Novel Germline Mutation, IVS4+1G>A, of the *POU1F1* Gene Underlying Combined Pituitary Hormone Deficiency

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Established Facts

- Up to now, 23 mutations of the *POU1F1* gene in combined pituitary hormone deficiency patients have been reported. Most are missense mutations in the DNA-binding domains, with no reports of splice site mutations.
- Both autosomal recessive and autosomal dominant patterns of POU1F1 abnormality have been reported.

Novel Insights

- We described a combined pituitary hormone deficiency patient who was homozygous for *POU1F1* IVS4+1G>A. It is the first splice site mutation in the *POU1F1* gene described to date. Five other family members, who were heterozygous for the mutation, were unaffected suggesting that this mutation, which lies within the DNA-binding domain, is inherited in an autosomal recessive fashion.
- This mutation was associated with the development of severe hypothyroidism in the first year of life, implying a major deficit in POU1F1 function.

Key Words

POU1F1 · Pituitary development · Combined pituitary hormone deficiency · Secondary hypothyroidism · Growth hormone deficiency

Abstract

Background: POU1F1 is a pituitary transcription factor that plays a pivotal role in pituitary development and expression of the *GH*, *PRL* and *TSHβ* genes. Therefore, abnormalities of the *POU1F1* gene are known to be responsible for a phenotype causing combined pituitary hormone deficiency (CPHD) involving growth hormone, prolactin and thyrotropin. ***Methods:*** We described an 18-year-old Thai man, from a consanguineous family, who presented with short stature and cognitive deficit. He underwent endocrinological and molecular investigations. ***Results:*** Hormonal studies showed that the patient had GH deficiency and secondary hypothyroidism, consistent with CPHD. Direct DNA sequencing revealed a novel homozygous mutation at the splice site of exon 4,

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IVS4+1G>A. It is the first splice site mutation in the *POU1F1* gene described to date. Of the 7 other family members studied for this mutation by restriction enzyme digestions, 5 were heterozygous. They were all unaffected, suggesting a recessive pattern of inheritance. ***Conclusions:*** We described a novel *POU1F1* splice site mutation, IVS4+1G>A, the first of its kind, in a Thai patient with CPHD. Recessive inheritance is suggested. We also noted preventable morbidities which resulted from delay in diagnosis of concomitant pituitary hormone defects in newborns suspected of CPHD.

Introduction

The mature human anterior pituitary gland is populated by at least five highly differentiated cell types: somatotropes, lactotropes, thyrotropes, gonadotropes, and corticotropes. They secrete growth hormone (GH), prolactin (PRL), thyrotropin (TSH), follicular-stimulating hormone (FSH) and luteinizing hormone (LH), and corticotrophin (ACTH), respectively. These cells emerge from a common ectodermal primodium and arise in a precise spatial and temporal expression fashion in response to intrinsic and extrinsic signaling molecules [1]. Pituitary transcriptional factors are one of the key regulators of this multistep process for morphogenesis and organogenesis of the pituitary gland. Mutations in these factors in mice and humans result in combined pituitary hormone deficiencies (CPHD), characterized by GH deficiency and may be associated with other pituitary hormone deficiencies or anatomical abnormalities, confirming their roles in pituitary development [2, 3].

POU1F1, also known as *PIT-1* (pituitary-specific transcription factor 1) or *GHF-1* (growth hormone factor-1), locates on chromosome 3p11-12, which is 17 kb long and contains 6 exons [4]. The *POU1F1* gene acts as a transactivator for *GH*, *PRL* and *TSHβ* genes in the anterior pituitary gland [5, 6]. Its corresponding protein, 291 amino acids, is a member of the POU-family transcription factors which play a pivotal role in the development and differentiation of somatotropes, lactotropes and thyrotropes. Therefore, the abnormalities of the *POU1F1* gene known so far have been shown to be responsible for a phenotype of CPHD involving GH, PRL and TSH deficiencies [7, 8].

As a member of the POU family, POU1F1 protein contains an N-terminal transcriptional activation domain and a C-terminal DNA-binding region, POU-specific (POU-S) domain and POU-homeodomain (POU-H) [7]. X-ray crystallography evidence suggests there are four α helices in the POU-S domain (75 amino acids) and three α helices in the POU-H domain (60 amino acids), connected by a 15-amino-acid flexible linker [9]. POU1F1 usually binds to multiple sites on target genes, and dimerization of POU1F1 on DNA elements is important for high-affinity DNA bindings [5].

Up to now, 23 different genetic lesions – 20 missense/nonsense mutations, 2 small deletions and 1 small insertion – have been reported in the *POU1F1* gene [10]. The mutations are spread throughout the gene, usually occurring in POU domains, corresponding to exons 4 and 6. These are important for the ability to dimerize and bind to DNA. However, a splice site mutation of the *POU1F1* gene has never been reported. In this study, we report a novel homozygous mutation, G>A substitution, at the splice donor site of exon 4 of the *POU1F1* gene, in a young Thai male having GH deficiency and secondary hypothyroidism. The clinical phenotypes and findings on hormonal and molecular genetic studies are illustrated.

Case Report

The proband is the second child of consanguineous Thai parents. Both parents and his family members are healthy and of normal height according to the known percentile of the Thai population. He was born at term after an uncomplicated pregnancy. The birth weight and length were 3 kg (–0.18 SDS) and 50 cm (–0.56 SDS), respectively, and his cranial circumference was 35 cm (–0.49 SDS). No abnormalities were found in the postnatal TSH screening. At the age of 1 year, he presented with delayed psychomotor development and constipation. A diagnosis of cretinism was given. Thyroid function tests revealed a low serum thyroxine (T_4) (1.1 μg/dl; normal 6–12) and an inappropriately low TSH (0.22 μU/ml; normal 0.3–4.1). Thyroid hormone replacement was commenced but he was lost to follow-up. During childhood period, he had a learning disability. There was no history of hypoglycemia. At 11 years of age, he started receiving testosterone therapy from another hospital, supposedly to increase his height. Subsequently, he entered puberty at the age of 13 years.

The proband was referred to our department for the first time at age 18 years, for investigation of his short stature and delayed psychomotor development. Physical examination revealed short stature with a height of 116 cm (–9.8 SDS) and weight of 22.5 kg (–5.6 SDS). He had a prominent forehead, a depressed nasal bridge, a small facial part of his skull with childish voice. Secondary sex characteristics were Tanner stage 3, with testes in the scrotum, 15 ml in volume and of normal consistency. His psychomotor development was impaired (WAIS IQ test 58). Blood and urine analyses were normal except for a low fasting plasma glucose of 51 mg/dl. Pituitary hormone assessment found an undetectable basal GH level, remaining low GH level after an intravenous bolus of 0.05 U/kg insulin (<1 ng/ml), low insulin-like growth factor-I

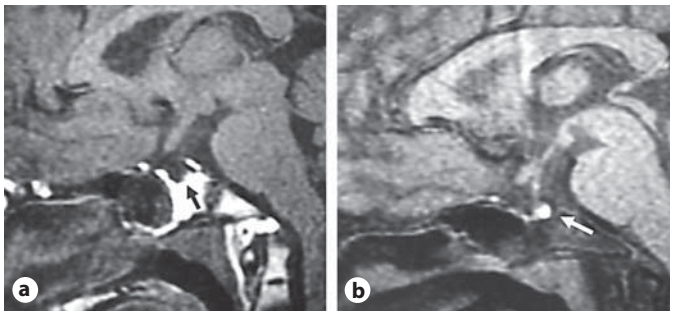

Fig. 1. MRI of the brain, sagittal T_1-weighted (**a**) and with fat suppression (**b**), showing hypoplasia of adenohypophysis (black arrow) of the pituitary gland, and intact stalk with normal posterior lobe (white arrow).

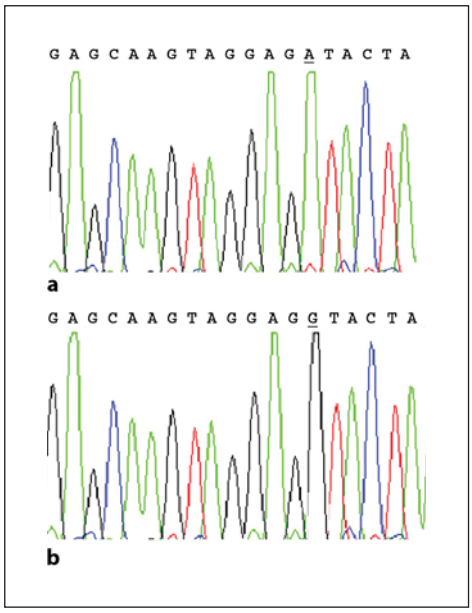

Fig. 2. The sense sequencing of *POU1F1* gene of our patient (**a**) showing the homozygous IVS4+1G>A change and of the wild type (**b**).

level (17.9 ng/ml; normal 60–320), low free thyroxine (FT_4) (0.3 ng/dl; normal 0.8–1.8) with suppressed TSH (0.12 μU/ml; normal 0.3–4.1) and an undetectable prolactin level (normal 2–25 ng/ml). Morning cortisol level was 25 μg/dl, while during a hypoglycemic state it was 36 μg/dl. ACTH and FSH/LH and testosterone were within normal ranges of his age. TRH stimulation test was not performed. Pituitary magnetic resonance imaging (MRI) revealed hypoplasia of the anterior pituitary gland with normal signal and location of the posterior pituitary gland (fig. 1). Bone age was 18 years, according to Greulich and Pyle. His mother, father, brother and sister were of normal heights and psychomotor and sexual development.

Genomic DNA Analysis of the POU1F1 *Gene*

Informed consent was obtained from the proband and his family members. Genomic DNA (gDNA) was extracted from peripheral blood leukocytes by the phenol-chloroform method. All six exons of the *POU1F1* gene with their flanking introns were amplified using a set of primers as previously described [11]. Sequencing reactions were performed in a MJ Research PTC-225 Peltier Thermal Cycler using an ABI Prism Big Dye TM Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (FS enzyme) (Applied Biosystems), following the protocols supplied by the manufacturer.

Confirmation of the Mutation by Restriction Fragment Length Polymorphism

For restriction fragment length polymorphism (RFLP) analysis, a 393-bp PCR fragment of exon 4 was amplified from gDNA of the proband and their family members, using forward primer (5′-AAA GTT GGA GCT GAT GGT C-3′) and reverse primer (5′-CCA CAC TTA CAT TGG CCT T-3′). The PCR products were digested with the restriction enzyme *Rsa*I (Toyobo, Osaka, Japan), according to the manufacturer's instructions and analyzed by 2% agarose gel.

Results

PCR-sequencing analysis of the entire coding region of the *POU1F1* gene in the proband revealed a homozygous G>A substitution in the intron 4, IVS4+1G>A (fig. 2). The mutation was confirmed by RFLP, in which the mutant allele eliminates the *Rsa*I restriction site of the wild type (fig. 3). By RFLP, the proband's unaffected parents, grandmothers, brother and his sister were shown to be heterozygous for the IVS4+1G>A. This mutation was not found in 50 controls.

Discussion

Over the past decade, there has been an advance in knowledge of the genetic cascade in hypothalamic-pituitary development. This has led to the identification of mutations in a number of genes, especially five major transcriptional factors *(HESX1, LHX3, LHX4, PROP1,* and *POU1F1)* in individual with CPHD [10, 12–14]. In this report, we present the proband having the clinical features of the *POU1F1* gene mutation: short stature and delayed psychomotor development due to congenital GH and TSH deficiency, and a hypoplastic pituitary gland by MRI study.

PCR-sequencing and RFLP analyses indicated that our patient was homozygous for a mutation, IVS4+1G>A, in the *POU1F1* gene. This has never been previously re-

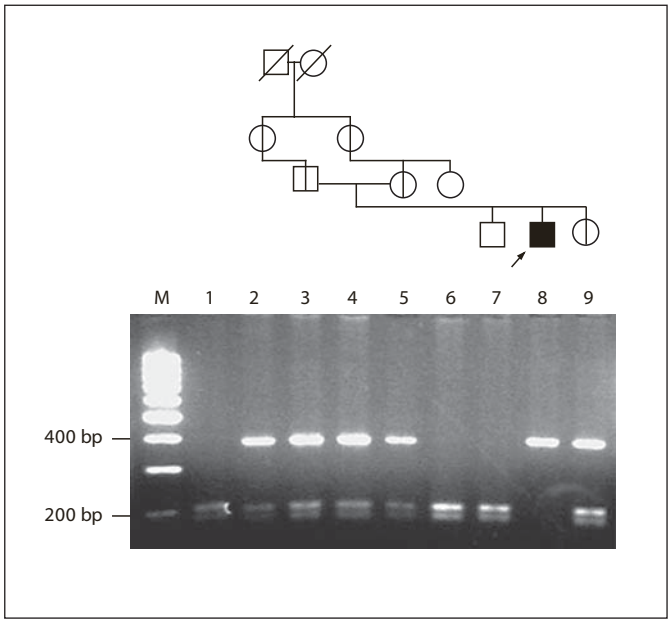

Fig. 3. Restriction enzyme analysis of the IVS4+1G>A mutation of the *POU1F1* gene. The size of PCR product of exon 4 is 393 bp (not shown). After *Rsa*I treatment, two restriction products of 204 and 189 bp of the wild type were found in control (lane 1) while the undigested 393-bp fragments of the mutant allele were found in only the proband (lane 8). A subject who carried a heterozygous allele would demonstrate 3 bands, 393, 204 and 189 bp, respectively. Each lane represents the individuals in the proband's family. M represents the 100-bp marker; lane 2: paternal grandmother (height 0.45 SDS), lane 3: father (height 0.81 SDS), lane 4: maternal grandmother (height −0.23 SDS), lane 5: mother (height −0.42 SDS), lane 6: mother's sister (height 0.14 SDS), lane 7: brother (height 0.69 SDS), lane 9: sister (height 0.45 SDS).

ported and is the first splice site mutation in the *POU1F1* gene described to date. Because the *POU1F1* gene is expressed only in pituitary tissues which are practically and ethically inaccessible, *POU1F1* RNA of the proband was unavailable hindering us from directly studying the effect of the splice site mutation. Nonetheless, based on similar mutation types in other genes, we speculate that the most likely possible effect is that the mutation affects both the structure and amount of POU1F1 protein. This presumably results in a decreased amount of a truncated protein [15]. Of note, our patient's phenotypes are similar to patients having nearby *POU1F1* truncated mutations, R172X and 747delA, leading to severely impairment or loss of function of the POU-H domain, an essential part to direct the protein into the DNA elemental response [16, 17].

Patients with the *POU1F1* gene mutation will develop complete GH, TSH and PRL deficiency eventually but the phenotype seems to be varied in respect to the initial severity of hypothyroidism [18]. Our patient had severely delayed psychomotor development from congenital hypothyroidism, which is not a common presentation in patients with the *POU1F1* gene mutations. The congenital onset of hypothyroidism may have resulted from severely impaired or loss of function of the POU1F1 protein, while the cognitive deficits can be partly due to a delayed diagnosis of cretinism which was missed by the neonatal screening programs relied solely on TSH measurement.

The *POU1F1* gene mutations can be inherited in either an autosomal dominant or an autosomal recessive mode partly depending on their position on the gene. Previous studies suggested that mutations lying within the DNA-binding domains cause autosomal recessive inherited CPHD, whereas CPHD caused by mutations outside these two specific regions (P24L, R143Q, K216E, and R271W) may follow the autosomal dominant pattern of inheritance [10, 19]. A few exceptions are the C-terminal located mutation (V272ter) and the N-terminal located mutation in the *POU1F1* gene (Q4ter). Therefore, the dominant mechanism, probably caused by competitive inhibition of the mutant homodimer and/or the mutant/wild-type heterodimer as opposed to the wild-type homodimer, leads to a block in transcription [20–22]. As only our patient had a phenotype while the heterozygous mutation in his consanguineous family relatives had a normal phenotype as well as a normal hormonal profile, a recessive pattern of this mutation is suggested.

In conclusion, we reported a CPHD patient with a novel homozygous *POU1F1* IVS4+1G>A mutation, inherited in an autosomal recessive fashion. We also emphasized on the importance of a rapid and accurate diagnosis for patients suspected of CPHD as severe morbidities can be prevented.

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