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NOTE

Uniparental disomy of chromosome 8 leading to homozygosity of a *CYP11B1* mutation in a patient with congenital adrenal hyperplasia: Implication for a rare etiology of an autosomal recessive disorder

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Abstract. Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder that usually results from paternally and maternally transmitted mutations in genes for steroidogenic enzymes. Recent studies on steroid 21-hydroxylase deficiency, the most common form of CAH, have revealed that a small percentage of patients have a non-carrier parent; uniparental disomy (UPD) and *de novo* mutations were reported as disease-causing mechanisms in these patients. However, it remains unknown whether UPD and *de novo* mutations underlie other forms of CAH. Here, we report a male patient with steroid 11β-hydroxylase deficiency (11OHD) born to a non-carrier mother. The patient was identified by an elevated 17-hydroxyprogesterone level at a neonatal mass-screening test. His clinical features were comparable to those of previously reported patients with 11OHD. Direct sequencing of *CYP11B1* identified a homozygous IVS7+1G>A mutation in the patient, which was not shared by his mother. Comparative genomic hybridization of the patient detected UPD of chromosome 8 [UPD(8)]. Microsatellite analysis indicated non-maternal origin of the UPD(8) and confirmed parentage of other chromosomes. This study shows for the first time that 11OHD can be caused by UPD in the presence of a non-carrier parent. Awareness of such rare cases should improve the accuracy of genetic counseling for families with CAH. Our data support the importance of UPD as an underlying mechanism of autosomal recessive disorders.

Key words: Autosomal recessive disorder, Congenital adrenal hyperplasia, *CYP11B1*, Mutation, Uniparental disomy

CONGENITAL ADRENAL HYPERPLASIA (CAH) is an autosomal recessive disorder that usually results from paternally and maternally transmitted mutations in genes for steroidogenic enzymes [1]. Recent studies on steroid 21-hydroxylase deficiency (21OHD, OMIM #201910), the most common form of CAH, have revealed that a small percentage of patients have a non-carrier mother or father [2, 3]. Uniparental disomy (UPD) leading to unmasking of recessive mutations in *CYP21A2* and *de novo* mutations in the germline were reported as disease-causing mechanisms of such patients. However, it remains unknown whether UPD and *de novo* mutations underlie other forms of CAH.

Steroid 11β-hydroxylase deficiency (11OHD, OMIM #202010) is one form of CAH that accounts for ~1.7% of Japanese patients (Data from the Study Group for Intractable Diseases; http://www.pediatric-world.com/asahikawa/fukujin/) and ~5 – ~8% of patients of other ethnic origin [1, 4]. To date, several mutations in *CYP11B1* on 8q24.3 encoding steroid 11β-hydroxylase

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Abbreviations: 11OHD, steroid 11β-hydroxylase deficiency; 21OHD, steroid 21-hydroxylse deficiency; CAH, congenital adrenal hyperplasia; CGH, comparative genomic hybridization; SNP, single nucleotide polymorphism; UPD, uniparental disomy

have been identified in homozygous or compound heterozygous states in patients and in a heterozygous state in the parents, although mutation analysis of the parental samples remained fragmentally [1, 4]. Here, we report a Japanese male patient with 11OHD born to a non-carrier mother.

Subjects and Methods

Case report

The male patient was born to non-consanguineous parents at 37 weeks gestation after uncomplicated pregnancy. The 30-year-old mother and father were clinically normal and did not undergo assisted reproduction. At birth, weight and length of the patient were 2980 g (-0.5 SD) and 47.5 cm (-1.1 SD), respectively. He showed normal male-type external genitalia without skin pigmentation. A neonatal mass-screening test showed an elevated 17-hydroxyprogesterone level (27.7 ng/mL; cut-off value, 8.0 ng/mL). Laboratory examinations at two weeks of age revealed increased levels of steroid metabolites including 17-hydroxyprogesterone and 11-deoxycortisol (Table 1). Blood ACTH and plasma renin activity were also elevated, while serum electrolytes and urine osmolarity were within the reference range. He was normotensive with a systolic blood pressure of 100 mmHg and had no symptoms of adrenal insufficiency. He showed tall stature (+3.0 SD) at 3.5 years of age. Thus, he was clinically diagnosed as having classic CAH and treated with hydrocortisone from 3.6 years of age. On the latest visit at 7.1 years of age, he showed no clinical abnormalities except for advanced bone age (11.2 years of age).

Molecular analyses

This study was approved by the Institutional Review Board Committee at the National Center for Child Health and Development and performed after obtaining informed consent. We analyzed genomic DNA samples obtained from the patient and his mother. The paternal sample was not available for genetic testing. Direct sequencing was performed for coding exons and their flanking regions of *CYP11B1*. Comparative genomic hybridization (CGH) was carried out using a catalog microarray (human CGH + single nucleotide polymorphism [SNP] array, Sureprint G3, catalog number G4890, 4×180K format, Agilent Technologies, Palo Alto, CA) containing ~120,000 CGH probes and ~60,000 SNP probes. PCR-based genotyping of 18 microsatellite loci was performed using fluorescently labeled forward primers and unlabeled reverse primers. The sequences of primers used in the present study are available upon request.

Results

Molecular analyses

Direct sequencing identified a homozygous nucleotide substitution in *CYP11B1* intron 7 (IVS7+1G>A) in the patient. This substitution was absent from the mother (Fig. 1A). CGH of the patient revealed loss of heterozygosity of SNPs on chromosome 8, indicating complete uniparental isodisomy of this chromosome (Fig. 1B). Microsatellite analysis of the patient and his mother suggested non-maternal origin of chromosome 8 and confirmed parentage of other chromosomes (Table 2).

Table 1 Laboratory findings of the patient

Age at examination	2 weeks	1.5 years	3.5 years
Blood examination			
Na (mEq/L)	136 (134-146)	139 (139-146)	141 (138-145)
K (mEq/L)	4.3 (4.3-7.6)	4.3 (3.0-6.0)	3.4 (3.5-5.6)
Cl (mEq/L)	105 (97-110)	107 (98-106)	**110** (98-106)
ACTH (pg/mL)	**205** (5-46)	**1230** (5-46)	**1530** (5-46)
Plasma renin activity (ng/mL/hr)	**18.8** (0.1-14.8)	< 0.1 (< 0.1-4.3)	< 0.1 (< 0.1-3.74)
17-hydroxyprogesterone (ng/dL)	**1495** (11-173)	**1297** (4-114)	40 (4-114)
11-deoxycortisol (ng/dL)	**26,401** (10-200)	**55,986** (7-210)	**19,377** (7-210)
Cortisol (μg/dL)	**27.0** (3.0-21.0)	**27.6** (5.7-25.0)	12.9 (5.7-25.0)
Aldosterone (ng/dL)	1.7 (2.2-129.6)	0.007 (2.2-25.3)	0.04 (2.2-25.3)
Testosterone (ng/dL)	98.0 (0.58-500.6)	**135.4** (2.0-25.9)	**161.4** (2.0-25.9)

Hormone values above the reference range (shown in parenthesis) are boldfaced, and those below the reference range are underlined.

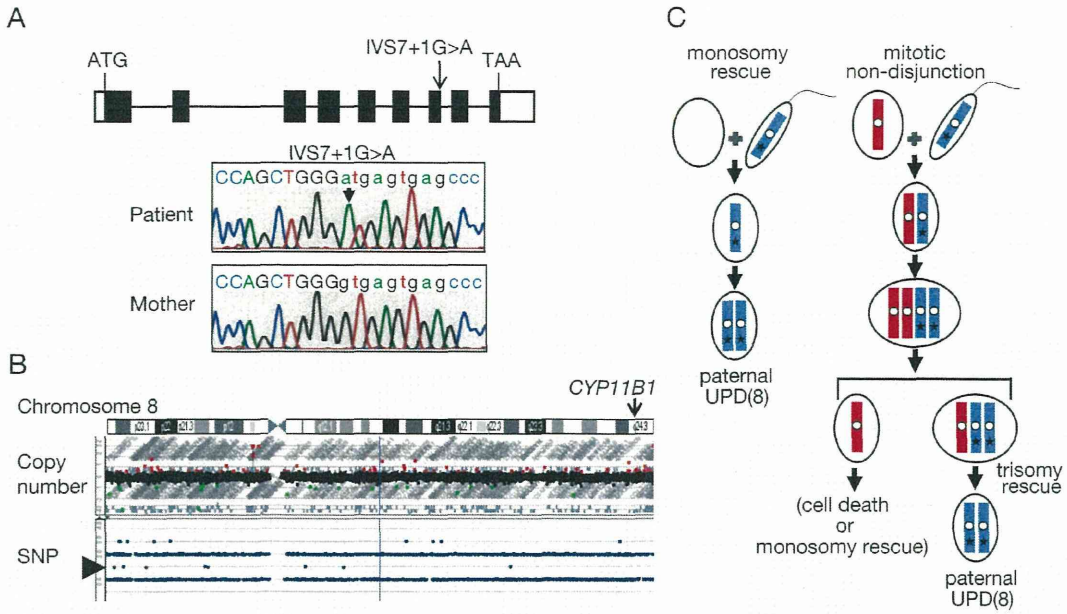

Fig. 1 A. Genomic structure of *CYP11B1* and the position of the mutation identified in the present study. The black and white boxes indicate the coding and non-cording regions, respectively. The patient is homozygous for a nucleotide substitution at the splice donor site in intron 7 (IVS7+1G>A, indicated by an arrow). The mutation is absent from the mother.
B. Comparative genomic hybridization analysis of the patient. No copy-number alteration was identified. Loss of heterozygosity of almost all SNPs on chromosome 8 (an arrowhead) indicates complete uniparental isodisomy.
C. Predicted underlying mechanisms of 11OHD in the patient. The blue and red lines depict the paternally- and maternally-transmitted chromosomes, respectively. The star symbols indicate paternally inherited or *de novo* mutations in *CYP11B1*. UPD(8): uniparental disomy of chromosome 8.

Table 2 Representative results of microsatellite analysis

Locus	Chromosomal position[a]	Mother[b]	Patient[b]	Assessment
D8S264	8p23.3	129	133	isodisomy[c]
D8S260	8q12.2	199/201	195	isodisomy[c]
D8S550	8p23.1	252/272	268	isodisomy[c]
D8S532	8p11.21	238/240	242	isodisomy[c]
D8S1705	8q21.13	192	192	N.I.
D8S1769	8p12	243/255	253	isodisomy[c]
D8S1836	8q24.3	135/151	135	N.I.
D8S256	8q24.22	222/226	222	N.I.
D8S1778	8q22.2	206/208	210	isodisomy[c]
D8S1799	8q24.13	258	254	isodisomy[c]
D15S541	15q11.2	138/148	138	N.I.
D15S542	15q11.2	141/149	135/141	biparental inheritance
D15S1035	15q11.2	236/248	230/236	biparental inheritance
D15S128	15q12	199	199/205	biparental inheritance
D15S117	15q14	131/145	131	N.I.
D15S131	15q23	239/241	239/265	biparental inheritance
D15S205	15q25.3	157	157/159	biparental inheritance
D15S642	15q26.3	206/208	206/210	biparental inheritance

[a], The chromosomal positions are based on Ensembl Genome Browser (http://www.ensembl.org);
[b], The numbers indicate the PCR sizes in bp; [c], Uniparental isodisomy of non-maternal origin.
N.I.: Not informative

Discussion

The patient carried a homozygous IVS7+1G>A mutation in *CYP11B1* and UPD of chromosome 8 [UPD(8)]. The IVS7+1G>A mutation affects the consensus splice donor site of exon 7 and therefore is likely to be a pathogenic mutation. Although IVS7+1G>A has not been described previously, multiple splice-site mutations in *CYP11B1* have been identified in patients with CAH [1, 4]. The results of molecular analyses indicate that CAH in this patient was caused by paternal UPD(8) that unmasked a paternally inherited or *de novo* mutation in *CYP11B1*. Paternal UPD(8) of the patient can be ascribed to monosomy rescue, a condition in which paternal chromosome 8 is replicated in a zygote formed by fertilization of a nullisomic oocyte (Fig. 1C, left panel) [5]. Alternatively, UPD(8) can occur by mitotic non-disjunction; when a segregation error of paternal chromosome 8 takes place during mitosis after normal fertilization, loss of maternal chromosome (trisomy rescue) and subsequent paternal UPD(8) could theoretically occur in one-third of cases (Fig. 1C, right panel) [5]. In this context, although advanced maternal age at childbirth has been suggested as a predisposing factor for oocyte aneuploidy and subsequent UPD [6, 7], this was not applicable to the present case. Thus, hitherto unknown factors may have played a role in the development of paternal UPD(8) in our patient.

Despite carrying UPD(8), the patient manifested no clinical features other than steroidogenic defects. Indeed, hormone values of the patient were comparable to those of previously reported patients with *CYP11B1* mutations [4], and his tall stature is consistent with androgen excess due to 11OHD [4]. These findings, together with a previous report of apparently normal phenotype except for unmasked autosomal recessive lipoprotein lipase deficiency in a patient with paternal UPD(8) [8], indicate that paternal UPD(8) would not result in imprinting defects in disease-causing genes. However, the phenotype of maternal UPD(8) remains to be clarified [9, 10].

Recent studies have revealed that UPD is a fairly common phenomenon which occurs in ~1/3500 live births [5]. Although UPD can be a clinically neutral event, it can also lead to genetic diseases by unmasking recessive mutations or dysregulating imprinted genes [2, 5]. UPD of various chromosomes, together with germline *de novo* mutations, have been implicated in several autosomal recessive disorders [2, 5]. Finkielstain *et al.* identified UPD and *de novo* mutations in 0.9% and 1.9% of patients with 21OHD, respectively [3]. These findings argue against the classical concept that the parents of patients with autosomal recessive disorders are carriers of the disease-causing mutations [2]. Our data provide a novel example of autosomal recessive disorders resulting from UPD. Given the rise in the number of births associated with advanced maternal age that represents a possible predisposing factor of oocyte aneuploidy and UPD [6, 7, 11], UPD should be regarded as an important cause of congenital disorders. Indeed, carrier status of the parents should be examined before genetic counseling of families with autosomal recessive disorders including CAH, because the recurrence risk of such disorders is 25% in the majority of cases, and low or negligible when the disease is associated with UPD or *de novo* mutations.

In summary, the present study shows for the first time that 11OHD can be caused by UPD in the presence of a non-carrier parent. Awareness of such rare cases should improve the accuracy of genetic counseling for families with CAH. Furthermore, our data suggest the importance of UPD as an underlying mechanism of autosomal recessive disorders.

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Disclosure

None of the authors have any potential conflict of interest associated with this research.

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Novel Insights from Clinical Practice

HORMONE RESEARCH IN PÆDIATRICS

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De novo Frameshift Mutation in *Fibroblast Growth Factor 8* in a Male Patient with Gonadotropin Deficiency

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Established Facts

- Missense, nonsense, and splice mutations in *Fibroblast Growth Factor 8 (FGF8)* have been identified in patients with hypothalamo-pituitary dysfunction and craniofacial anomalies.

Novel Insights

- *FGF8* frameshift mutations account for a part of the etiology of hypothalamo-pituitary dysfunction and craniofacial anomalies.
- Micropenis in patients with *FGF8* mutations can be ascribed to gonadotropin deficiency and impaired outgrowth of the anlage of the penis.

Key Words
Fibroblast Growth Factor 8 · Frameshift mutation · Gonadotropin deficiency · Hypothalamo-pituitary dysfunction

Abstract

Background/Aims: Missense, nonsense, and splice mutations in the *Fibroblast Growth Factor 8 (FGF8)* have recently been identified in patients with hypothalamo-pituitary dysfunction and craniofacial anomalies. Here, we report a male patient with a frameshift mutation in *FGF8*. ***Case Report:*** The patient exhibited micropenis, craniofacial anomalies, and ventricular septal defect at birth. Clinical evaluation at 16 years and 8 months of age revealed delayed puberty, hyposmia, borderline mental retardation, and mild hearing difficulty. Endocrine findings included gonadotropin deficiency and primary hypothyroidism. ***Results:*** Molecular analysis identified a de novo heterozygous p.S192fsX204 mutation in the last exon of *FGF8*. RT-PCR analysis of normal human tissues detected *FGF8* expression in the genital skin, and whole-mount in situ hybridization analysis of mouse embryos revealed *Fgf8* expression in the anlage of the penis. ***Conclusion:*** The results indicate that frameshift mutations in *FGF8* account for a part of the etiology of hypothalamo-pituitary dysfunction. Micropenis in patients with *FGF8* abnormalities appears to be caused by gonadotropin deficiency and defective outgrowth of the anlage of the penis.

E. Suzuki, S. Yatsuga and M. Igarashi contributed equally to this work.

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Introduction

Fibroblast growth factor (FGF) 8 (FGF8, NP_149353.1) is the major ligand of FGF receptor 1 (FGFR1) and plays a critical role in formation of the anterior midline in the forebrain [1–5]. Animal studies have indicated that FGF8 regulates the development of GnRH neurons in a dose-dependent manner [4, 5]. Recently, multiple missense mutations as well as two nonsense and one splice mutation in *FGF8* (NM_033163.3) have been identified in patients with various types of hypothalamo-pituitary dysfunction and craniofacial anomalies [1, 3, 4–9]. The mutation-positive patients invariably manifest gonadotropin deficiency and/or delayed puberty, indicating that GnRH neurons are highly vulnerable to impaired function of FGF8 [1, 3, 4–9]. Furthermore, mutations in several genes involved in the FGF8–FGFR1 network have been shown to underlie gonadotropin deficiency [1].

However, given the small number of reported patients, further studies are necessary to clarify the mutation spectrum and phenotypes of *FGF8* abnormalities. For example, frameshift mutations in *FGF8* have not been identified, and the underlying mechanisms of genital anomalies in patients with *FGF8* mutations have poorly been investigated. Here, we report a male patient with a de novo frameshift mutation in *FGF8*.

Subjects and Methods

Case Report

The male patient was born to non-consanguineous Japanese parents at 38 weeks' gestation. At birth, the patient manifested micropenis, phimosis, and hypoplastic scrotum. He also exhibited cleft lip and palate, strabismus, and ventricular septal defect. Hypospadias and cryptorchidism were absent. From 6 months of age, he underwent surgical interventions for facial and genital abnormalities. He had multiple episodes of convulsions from 12 years of age, and was treated with anticonvulsants. From infancy to early teens, his stature followed the –2.0 SD growth curve for Japanese males (fig. 1a).

At 16 years and 8 months of age, the patient was referred to our clinic for delayed puberty. Clinical assessment revealed a high-pitched voice and pubic and axillary hair of Tanner stage 1–2. His penile length was 1.5 cm (fig. 1b). Bilateral testes of ~1 ml were palpable in the scrotum. His stature and weight were 154.0 cm (–2.8 SD) and 52.9 kg (–0.8 SD), respectively. He had mild hearing difficulty and borderline mental retardation with a WISC-III IQ score of 71. Although he showed a response to a smell test using intravenous injection of combined vitamins (Alinamin; Takeda Pharmaceutical Co. Ltd, Osaka, Japan), he was unable to identify various odorants. Thus, he was suspected as having hyposmia. Brain magnetic resonance imaging delineated no structural abnormalities in the hypothalamus, pituitary, or olfactory bulbs (fig. 1b). The patient manifested no symptoms associated with the heart anomaly, although cardiac evaluation showed a ventricular septal defect of approximately 2 mm. His father, mother and elder brother were clinically normal, and had heights of 164 cm (–1.2 SD), 143 cm (–2.7 SD), and 167 cm (–0.7 SD), respectively.

Endocrine examinations indicated multiple hormone deficiencies in the patient (table 1). Blood LH values were low at baseline and poorly responded to GnRH stimulation. FSH values were low-normal. Slightly elevated TSH levels and mildly decreased free T_4 levels indicated primary hypothyroidism. The IGF-1 level was low-normal. Blood levels of prolactin, ACTH, and cortisol were within the normal range. Anti-thyroperoxidase and anti-thyroglobulin antibodies were negative. Thyroid technetium-99m scintigram revealed no abnormalities. After initiation of levothyroxine supplementation therapy (50 μg/day), TSH and free T_4 values remained within the normal range.

Mutation Analysis

This study was approved by the Institutional Review Board Committee at the National Center for Child Health and Development and performed after obtaining written informed consent. A genomic DNA sample from the patient was analyzed for mutations in 13 genes that have been implicated in gonadotropin deficiency: *FGFR1*, *KAL1*, *FGF8*, *PROK2*, *PROKR2*, *TAC3*, *TACR3*, *KISS1*, *KISS1R*, *GNRHR*, *GNRH1*, *CHD7*, and *NELF* [1, 7, 10, 11]. Mutations were screened by the Haloplex method (Agilent Technologies, Palo Alto, Calif., USA) on a MiSeq next-generation sequencer (Illumina, San Diego, Calif., USA). An *FGF8* mutation indicated by the screening analysis was confirmed by Sanger sequencing with primers, 5′-GCGAGTTGTGAGGGATTAGAGA-3′ and 5′-GGGTGCCCTACAGGATGAG-3′. To verify the heterozygous mutation, the PCR product was subcloned into a TOPO TA cloning vector (Life Technologies, Carlsbad, Calif., USA) and the mutant and wild-type alleles were sequenced separately. Genomic DNA samples from the parents and brother were examined for the presence or absence of the *FGF8* mutation.

Expression Analysis for FGF8/Fgf8 in Normal Human Tissues and Mouse Embryos

We investigated mRNA expression of *FGF8* in normal human tissues by PCR. Human cDNA samples were purchased from Clontech (Palo Alto, Calif., USA) or prepared by RT-PCR. PCR analysis of *FGF8* was performed with primers, 5′-AGTCCGAGGAGCCGAGA-3′ and 5′-AAGTGGACCTCACGCTGGT-3′. As an internal control, we amplified *GAPDH* with primers, 5′-CCACCCATGGCAAATTCCATGGCA-3′ and 5′-TCTAGACGGCAGGTCAGGTCCACC-3′.

We also examined the expression of *Fgf8* in the developing external genitalia of mouse embryos by whole-mount in situ hybridization. An antisense cRNA fragment corresponding to nucleotide 1–978 of mouse *Fgf8* (BC048734) was utilized as a probe. The procedures were performed as described previously [12].

Results

Mutation Analysis

The patient carried a heterozygous frameshift mutation in the last exon of *FGF8* (p.S192fsX204, c.574delT)

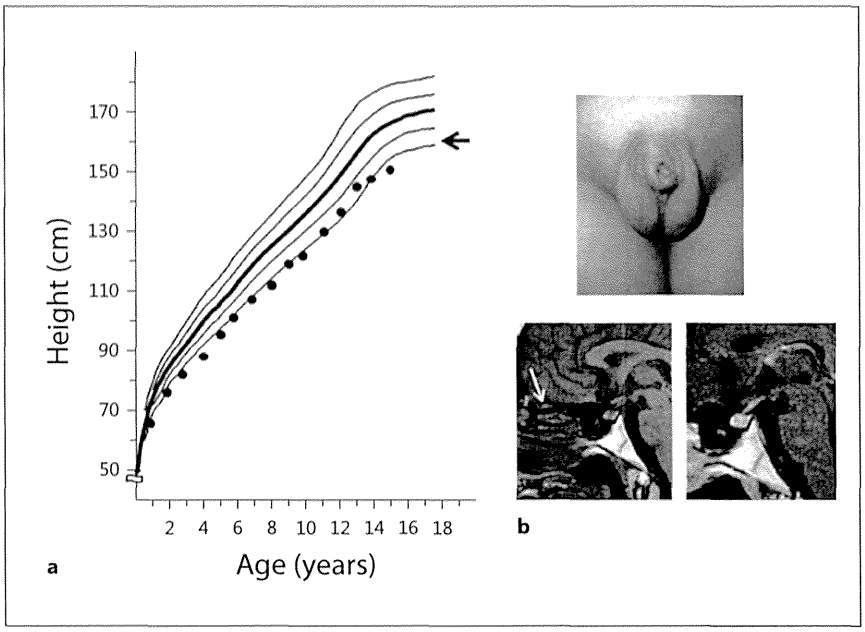

Fig. 1. Clinical findings of the patient. **a** Growth chart. Actual height of the patient is plotted against the growth curve for Japanese boys (the mean, ±1.0 SD and ±2.0 SD). The arrow indicates the midparental height. **b** Upper panel: genital appearance at 16 years and 8 months of age. Lower panels: brain magnetic resonance imaging. No abnormalities are detected in the hypothalamus, pituitary, or olfactory bulbs (arrow).

Table 1. Endocrine data of the patient

	Stimulus (dosage)	Patient		Reference values[1]	
		baseline	peak	baseline	peak
At diagnosis					
LH, mIU/ml	GnRH (100 μg)[2]	**0.5**	**4.6**	0.8–4.2	18.2–38.0
FSH, mIU/ml	GnRH (100 μg)[2]	3.0	9.5	2.9–10.8	5.8–22.3
GH, ng/ml	Insulin (3 U)[2]	0.87	**3.18**[3]	0.6–8.7	>6.0
Prolactin, ng/ml	TRH (350 μg)[2]	4.3	14.4	1.1–9.5	–
TSH, μU/ml	TRH (350 μg)[2]	*8.3*	23.0	0.6–5.9	5.8–30.6
IGF-1, ng/ml		301[4]	–	250–680	–
ACTH, pg/ml	CRH (100 μg)[2]	9.5	50.3	5.3–51.1	17.2–153.3
Cortisol, μg/dl	CRH (100 μg)[2]	8.4	25.8	3.9–21.3	13.1–35.6
Free T_4, ng/dl		**0.79**	–	1.0–1.7	–
Free T_3, pg/ml		2.8	–	2.1–4.1	–
Testosterone, ng/ml	HCG (4,000 U)[5]	**0.12**	**0.38**	2.8–7.0	11.0–13.1
On levothyroxine treatment[6]					
TSH, μU/ml		0.68	–	0.6–5.9	–
Free T_4, ng/dl		1.14	–	1.0–1.7	–

The conversion factors to the SI unit: LH, 1.0 (IU/l); FSH, 1.0 (IU/l); GH, 1.0 (μg/l); prolactin, 43.48 (pmol/l); TSH, 1.0 (mIU/l); IGF-I, 0.131 (nmol/l); ACTH, 0.22 (pmol/l); cortisol, 27.59 (nmol/l); free T_4, 12.87 (pmol/l); free T_3, 1.54 (pmol/l), and testosterone, 3.47 (nmol/l). Hormone values above the reference range are italicized and those below the reference range are boldfaced.

[1] Reference values in age-matched males. [2] GnRH, insulin and TRH i.v.; blood sampling at 0, 30, 60, 90, and 120 min. [3] Low GH values of the patient may be due to insufficient hypoglycemic stimulation; blood glucose was 89 mg/dl at 0 min, and 58 mg/dl at 30 min. [4] –1.7 SD. [5] HCG i.m. for 3 consecutive days; blood sampling on days 1 and 4. [6] Levothyroxine 50 μg/day.

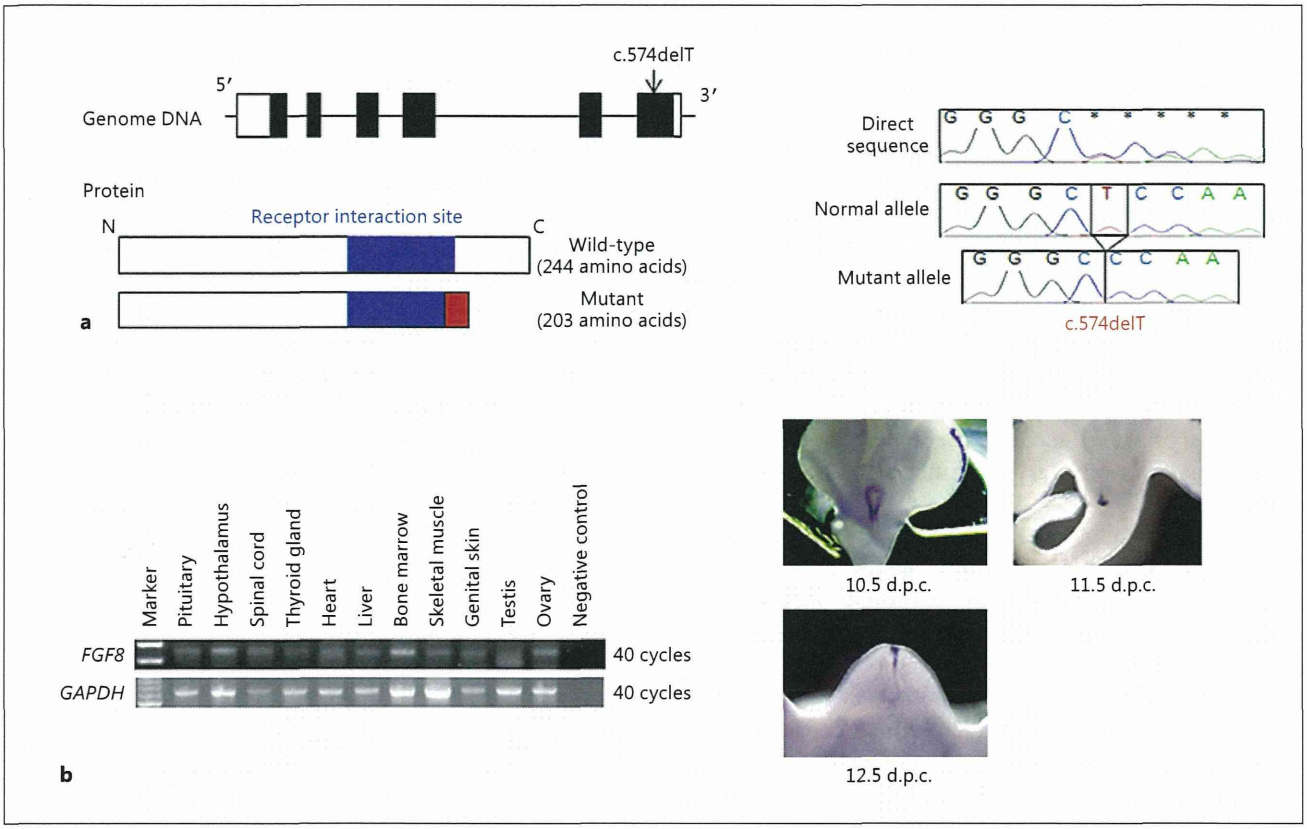

Fig. 2. Mutation analysis of *FGF8* and expression studies of *FGF8/Fgf8*. **a** *FGF8* mutation identified in the patient. The positions of nucleotides and amino acids correspond to NM_033163.3 and NP_149353.1, respectively. Left panel: the genomic and protein structures of FGF8. The white and black boxes in genome DNA indicate the non-coding and coding regions, respectively. The blue boxes in the protein depict the receptor interaction site at codons 83–207 and the red box indicates truncated amino acids. Right panel: chromatograms of the c.574delT mutation. **b** Expression analyses of *FGF8/Fgf8*. Left panel: PCR-based cDNA screening for human *FGF8*. After 40 cycles, PCR products for *FGF8* were detected in all tissues examined. *GAPDH* is utilized as an internal control. Right panel: whole-mount in situ hybridization analysis in mouse embryos. Purple signals indicate expression of mouse *Fgf8*. d.p.c. = Days post-coitum.

(fig. 2a). No pathogenic mutations were identified in other tested genes. The p.S192fsX204 mutation was predicted to truncate the C-terminus of FGF8 by replacing 53 amino acids with 12 aberrant amino acids (fig. 2a). This mutation affected a part of the receptor interacting site of FGF8 (codons 83–207). The *FGF8* mutation was not identified in the parents or brother.

Expression Analysis for FGF8/Fgf8 in Normal Human Tissues and Mouse Embryos

PCR-based cDNA screening indicated that human *FGF8* is expressed in a range of tissues including the hypothalamus, pituitary, thyroid gland, heart, and genital skin (fig. 2b). Whole-mount in situ hybridization indicated that mouse *Fgf8* is expressed in the epithelium of the outmost part of the urogenital sinus before outgrowth of the anlage of the penis (genital tubercle) (10.5 days post-coitum) and in the epithelium of distal urethral plate during development of the genital tubercle (11.5 and 12.5 days post-coitum) (fig. 2b).

Discussion

We identified a de novo *FGF8* frameshift mutation in a Japanese patient with gonadotropin deficiency and multiple complications. The p.S192fsX204 mutation resides in the last exon of *FGF8* and is likely to escape non-

sense-mediated mRNA decay [13]. However, the mutation is predicted to alter the C-terminal structure of the protein and affect the receptor interacting site. In this context, Falardeau et al. [4] indicated that a missense mutation at the 229th codon is sufficient to reduce in vitro activity. Thus, although in vitro functional assays have not been conducted for p.S192fsX204, this mutation appears to markedly impair the function of FGF8. Consistent with this, the patient manifested gonadotropin deficiency, hyposmia, and craniofacial anomalies comparable to the phenotypes of previously reported patients with nonsense, missense, and splice mutations of *FGF8* [1, 3–9]. These data indicate for the first time that frameshift mutations of *FGF8* account for a part of the etiology of hypothalamo-pituitary dysfunction and craniofacial anomalies.

We cannot exclude the possibility that the patient carries additional mutations in other genes involved in hypothalamo-pituitary function. Although *FGF8* abnormalities are known to cause gonadotropin deficiency mostly as monoallelic mutations, they can also appear in an oligogenic condition [1]. Therefore, although our patient has no mutations in the 13 known causative genes for gonadotropin deficiency, he may have mutations in other unexamined genes. Indeed, several genes including *FGF17*, *IL17RD*, *DUSP6*, *SPRY4*, and *FLRT3* have recently been implicated in gonadotropin deficiency [1].

The Japanese patient manifested severe micropenis. This phenotype is consistent with severe gonadotropin deficiency [14]. In addition, defective formation the penis during fetal period may have played a role in the development of micropenis, because outgrowth of the genital tubercle in mouse embryos primarily depends on *Fgf8* signaling [12]. Indeed, we found expression of *FGF8/Fgf8* in human genital skin and in the epithelium of the mouse genital tubercle.

The patient manifested primary hypothyroidism and a congenital heart anomaly, neither of which has been reported in patients with *FGF8* mutations. Although we detected expression of *FGF8* in the human thyroid gland and heart, and several studies have revealed that FGF8 plays an essential role in formation of the cardiovascular system and thyroid gland in mice [15–20], it remains unknown whether thyroid and heart abnormalities of the patient are associated with the *FGF8* mutation.

In summary, we identified the first frameshift *FGF8* mutations in a patient with gonadotropin deficiency. The results indicate molecular diversity of *FGF8* abnormalities.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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