GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

A female patient with normosmic idiopathic hypogonadotropic hypogonadism carrying a novel mutation in *FGFR1*

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Genet. Mol. Res. 13 (4): 9472-9476 (2014)
Received October 16, 2013
Accepted March 18, 2014
Published November 11, 2014
DOI http://dx.doi.org/10.4238/2014.November.11.12

ABSTRACT. Mutations in the fibroblast growth factor receptor 1 gene (*FGFR1*) have been reported in patients with Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism (nIHH). Here, we report an nIHH patient with a novel mutation in *FGFR1*. The patient was a 19-year-old female who presented the nIHH phenotype with primary amenorrhea, cleft lip and palate, mixed hearing disorders, and skeletal malformations. Coding regions of 12 genes that have been implicated in nIHH were analyzed by direct sequencing. Mutation analysis revealed a novel mutation at exon 10 of the *FGFR1* gene, 1422 C>G, and a C→G transition in codon 476, which resulted in the replacement of aspartic acid with glutamic acid. The patient's family members did not possess this mutation. We briefly reviewed *FGFR1* variants found in Chinese subjects. These results indicate that the mutation in *FGFR1* is a cause of nIHH, which is associated with specific non-reproductive phenotypes.

Key words: Normosmic idiopathic hypogonadotropic hypogonadism; Amenorrhea; *FGFR1*; Mutation

INTRODUCTION

Idiopathic hypogonadotropic hypogonadism (IHH) is characterized by the failure of pubertal development because of the impaired secretion of luteinizing hormone and follicle-stimulating hormone; however, the mechanism remains unknown (Seminara et al., 1998). IHH may present as anosmia/hyposmia, which is known as Kallmann syndrome (KS) (Seminara et al., 1998), or with a normal sense of smell known as normosmic IHH (nIHH) (Boyar et al., 1976). Genetic heterogeneity exists in IHH (Bianco and Kaiser, 2009). Among the causative gene mutations of IHH, mutations in the fibroblast growth factor receptor 1 (*FGFR1*) and fibroblast growth factor 8 (*FGF8*) genes are causes of KS associated with some non-reproductive phenotypes, particularly cleft lip and palate (Dode et al., 2003; Dode and Hardelin, 2009; McCabe et al., 2011). *FGFR1* mutations have also been reported in male patients with nIHH (Pitteloud et al., 2006; Raivio et al., 2009). Here, we report a female patient who presented the nIHH phenotype with primary amenorrhea, cleft lip and palate, mixed hearing disorders, and skeletal malformations, carrying a novel mutation in *FGFR1*. We examined the effects and the consequences of pulsatile gonadorelin using an infusion pump.

MATERIAL AND METHODS

Patient and family

The patient was a 19-year-old Chinese woman. She had a history of cleft lip and palate, nasal collapse, and nasal septum deviation, which had been corrected by surgery. When the patient was 18 years old, she was treated at the First Affiliated Hospital of China Medical University because of primary amenorrhea. Her height was 165 cm, weight was 40 kg, body mass index was 14.7 kg/m^2, and arm span was 170 cm. The magnetic resonance imaging examination was negative for tumors and abnormalities of the hypothalamic-pituitary region and olfactory bulbs, but a platybasia was observed. She had cubitus valgus and chest deformity. The karyotype was 46,XX. The patient had normal adrenocorticotropic hormone-cortisol rhythm and thyroid function. Other serum hormone levels are listed in Table 1. She underwent simultaneous intravenous infusions of 100 µg gonadorelin for 1 and 5 days. Gonadotropin responses were blunted after infusion for 1 day, but luteinizing hormone peaked at 7.35 mIU/mL after infusion for 5 days (Table 2). She received hormone replacement therapy for 3 months and then received pulsatile gonadorelin by infusion pump for 4 months at a dose of 10 µg/90 min. Pulsatile gonadorelin therapy was discontinued because of a poor response (Table 1) and replaced with hormone-replacement therapy.

This study was approved by the hospital Ethics Committee, and the patient signed the informed consent form.

Table 1. Patient serum hormone assay data during follow-up.

Time	FSH (mIU/mL)	LH (mIU/mL)	E2 (pg/mL)	E3 (pg/mL)	GH (mIU/L)	PRL (ng/mL)	P (nM)
2012.07.24	<0.10	0.13	<73.4	<0.24	18.80	214	<0.64
2012.09.27	1.46	0.13	110	<0.24	-	-	<0.64
2013.01.05	1.05	0.11	<73.4	<0.24	-	-	0.75

Table 2. Results of gonadorelin stimulation tests.

Time after *im* (min)	100 μg GD *im* for 1 day		100 μg GD *im*. for 5 days	
	FSH (mIU/mL)	LH (mIU/mL)	FSH (mIU/mL)	LH (mIU/mL)
0	<0.10	<0.10	0.61	0.61
15	<0.10	1.16	1.85	5.03
30	0.33	1.81	2.74	7.35
60	0.91	2.47	4.1	7.15
90	1.46	1.80	3.89	6.37
120	1.6	1.55	3.99	4.98

GD = gonadorelin hydrochloride.

Mutation analysis

Genomic DNA was extracted from peripheral blood leukocytes using the TIANamp blood DNA kit (TIANGEN BIOTECH, Beijing, China). The coding regions of 12 genes that have been implicated in nIHH, including *FGFR1*, *FGF8*, *PROK2*, *PROKR2*, *GNRHR*, *GNRH1*, *KISS1R*, *KISS1*, *TAC3*, *TACR3*, *CHD7*, and *WDR11*, were amplified from the DNA from the patient's blood as described previously (Laitinen et al., 2011, 2012). Amplified products were sequenced in both directions using a 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Gene sequences were identified using the ENSEMBL genome database.

RESULTS

The patient had a novel missense mutation in exon 10 of the *FGFR1* gene, 1422 C>G, a C→G transition in codon 476, which resulted in the replacement of aspartic acid with glutamic acid (Figure 1). This missense mutation was not observed in the patient's family members or 100 normal controls. No pathogenic mutations were identified in other tested genes.

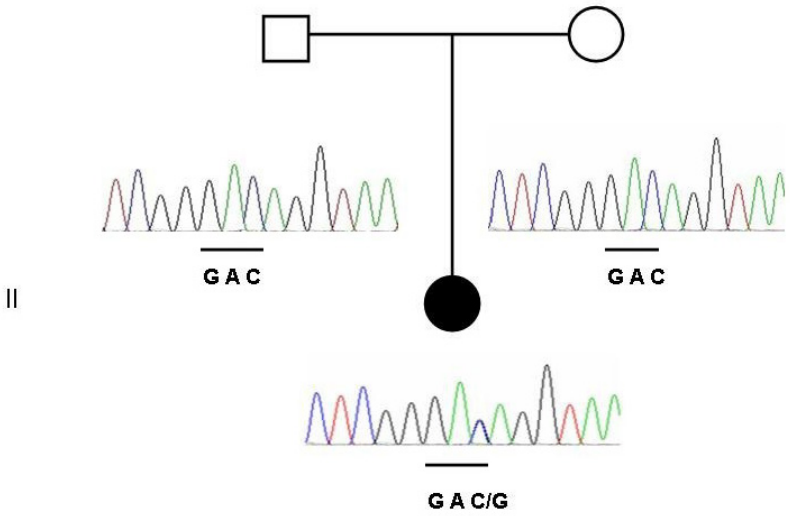

Figure 1. Pedigree of patient's family and the results of *FGFR1* sequence analysis. The patient had a novel mutation in exon 10 of *FGFR1* (c.1422 C>G), which resulted in the replacement of aspartic acid by glutamic acid (p.D476E). Her parents did not have this mutation.

DISCUSSION

We identified a novel homozygous D476E mutation in *FGFR1* in a Chinese female patient with nIHH and other non-reproductive phenotypes such as primary amenorrhea, cleft lip and palate, mixed hearing disorders, and skeletal malformations. The novel nonsense mutation in exon 10 of *FGFR1* is located in the protein kinase domain and is conserved among various species (Figure 2). Other kinase domain mutations such as R470L and Q680X leading to loss of function of *FGFR1* have been previously described (Pitteloud et al., 2006; Raivio et al., 2009). Thus, although functional studies have not been conducted for D476E, this mutation appears to impair the function of *FGFR1*, which is consistent with the previously reported phenotype-genotype correlation (Dode et al., 2003). These data indicate that this novel mutation in *FGFR1* accounts for the etiology of nIHH and the specific non-reproductive phenotypes.

By searching relevant articles available in a Chinese database, studies of *FGFR1* mutations in Chinese patients with IHH appear to be very limited. The main features of these cases are summarized in Table 3. Most of the mutations were observed in male patients, and some were novel. However, fortunately no specific non-reproductive phenotypes have been reported.

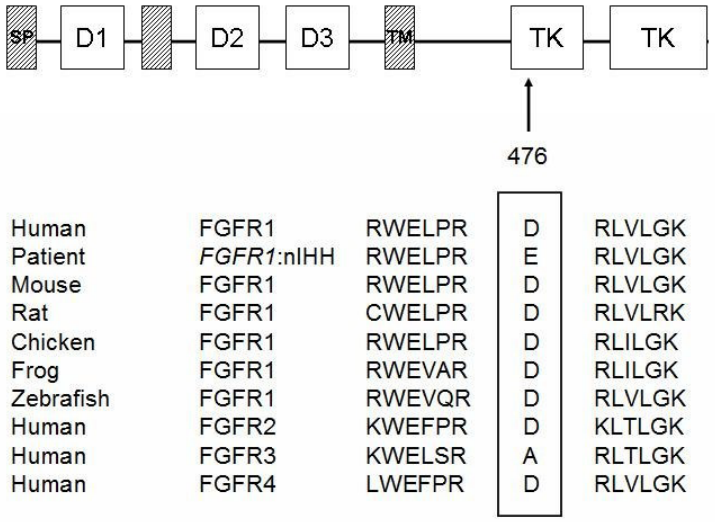

Figure 2. Location and conservation of *FGFR1* mutation. Alignment of vertebrate *FGFR1s* and human *FGFRs*.

Table 3. *FGFR1* mutation in Chinese patients with IHH.

Patients			Molecular analysis				Clinical features
Age (years)	Gender	Inheritance	Position	Nucleotide change	Amino acid change	Remark	
22	Female	Sporadic	Exon 3	c.251A>T	p.E84V	Novel	Normosmia
18	Male	Sporadic	Exon 3	c.320C>T	p.S107L	Recurrent	Normosmia
18	Male	Sporadic	Exon 3	c.346G>A	p.V116I	Novel	Hyposmia
20	Male	Sporadic	Exon 5	c.569G>C	p.W190S	Novel	Hyposmia
22	Male	Sporadic	Exon 6	c.709G>A	p.G237S	Recurrent	Anosmia
23	Male	Familial	Exon 7	c.748C>T	p.R250W	Recurrent	Hyposmia
19	Male	Sporadic	Exon 16	c.2172C>G	p.N724K	Recurrent	Hyposmia

Loss-of-function mutations in *FGFR1* may cause variable non-reproductive phenotypes, particularly cleft lip and palate (Dode et al., 2003; Dode and Hardelin, 2009). Skeletal malformations have also been studied in KS patients by Jarzabek et al. (2012). Interestingly, our patient had platybasia, cubitus valgus, and chest deformity, which are not frequently presented by IHH patients according to previous studies. This indicates that the presence of cleft lip and palate as well as bone malformation in IHH patients should direct clinicians to search for mutations in *FGFR1*.

We cannot exclude the possibility of additional mutations in other novel genes involved in nIHH, such as *FGF17*, *IL17RD*, *DUSP6*, *SPRY4*, and *FLRT3*, which were recently reported (Miraoui et al., 2013).

Although the gonadorelin stimulation test showed relatively normal pituitary function, the response to pulsatile gonadorelin therapy for 4 months was poor. This likely resulted from the relatively higher dose of gonadorelin used for the very low body weight (250 ng/kg) (Martin et al., 1990), and may decrease the luteinizing hormone receptor number on the pituitary. We will consider using estrogen-progestin treatment for this patient again to maintain secondary sex characteristics, followed by a lower dose of pulsatile gonadorelin therapy if the patient and her family desire fertility.

In summary, we reported a female patient with nIHH, including clinical evaluation, hormone assays, and gene mutation research. A novel D476E mutation was observed in *FGFR1*. We also briefly reviewed the *FGFR1* variants found in the Chinese population. These results indicate that this novel mutation in *FGFR1* is a cause of nIHH. When nIHH is associated with these specific non-reproductive phenotypes, clinicians should search for mutations in *FGFR1*.

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