Identification of novel mutations in *FGFR1* and functional characteristics in patients with isolated gonadotropin-releasing hormone deficiency

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Yena Lee[1], Juyoung Huh[2], Arum Oh[1], Gu-Hwan Kim[3], Han-Wook Yoo[1], Jin-Ho Choi[1]

Department of Pediatrics[1], Asan Institute for Life Sciences[2], Medical Genetics Center[3], Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Korea

Introduction

- Isolated gonadotropin-releasing hormone (GnRH) deficiency (IGD) is caused by a deficiency in GnRH production, secretion or action.
- IGD is a highly heterogeneous disorder with wide phenotypic spectrum including Kallmann syndrome (KS) with anosmia and normosmic idiopathic hypogonadotropic hypogonadism (nIHH).
- Over the last 20 years, significant progress has been made in the understanding of the molecular genetics of IGD. More than 30 different causative genes have been identified in several studies.
- *FGFR1* mutations have been identified in about 3–10% of patients with Kallmann syndrome or nIHH.

Objectives

- This study was performed to investigate the clinical phenotypes and functional characteristics of *FGFR1* mutations in patients with IGD.

Methods

- Molecular analysis was performed in 49 subjects with IGD using targeted gene panel for 69 genes (n = 34) or whole exome sequencing (n = 15).
- The impact of the identified mutations on FGFR1 function was assessed by using *in silico* prediction program, and then confirmed by *in vitro* functional studies.
- Clinical characteristics and hormonal findings of the patients with *FGFR1* mutations were obtained by retrospective chart review.

Results

Clinical characteristics of patients with *FGFR1* mutations

- Six novel heterozygous mutations in *FGFR1* were identified in 6 unrelated patients (12.2%): p.Y210*, p.Y339H, p.S681I, c.1855-1G>A, c.1663+2T>G, and c.551dup (p.N185Kfs*16).
- The clinical phenotypes of these patients were diverse, ranging from KS (N = 1), nIHH (N = 4), and a prepubertal male with anosmia.
- Four male patients had history of undescended testis, micropenis, or delayed puberty, while a female subject presented with primary amenorrhea at the age of 19 years.
- A 7-year-old male presented with anosmia and his brain magnetic resonance imaging (MRI) revealed the absence of olfactory bulbs. He is currently 8 years old without any signs of puberty.
- Subject 1 and 5 had osteoporosis and finger syndactyly, respectively.

Table 1. Clinical characteristics and laboratory findings of patients with *FGFR1* mutations

Proband		1	2	3	4	5	6
Sex		F	M	M	M	M	M
Age at diagnosis (yr)		19	19	14.1	14	18	7
Current age (yr)		25	27	14.6	18	32	8
Initial Presentation		Primary amenorrhea	Micropenis	Delayed puberty	Micropenis History of Cryptorchidism	Micropenis Delayed puberty	Anosmia History of Cryptorchidism
LH (mIU/mL)	Basal	1.9	1.1	0.85	0.8	< 2	0.07
	Peak	ND	1.6	5.6	1.6	2.8	0.74
FSH (mIU/mL)	Basal	1.0	0.72	1.0	0.1	< 2	< 0.3
	Peak	ND	2.2	5.2	2.6	4.0	10.56
Testosterone (ng/mL) or Estradiol (pg/mL)		10.0	0.36	0.27	0.11	0.4	0.11
Anosmia		-	+	-	-	-	+
Brain MRI		ND	Small olfactory bulbs	ND	Normal	Normal	Absence of olfactory bulbs
Diagnosis		nIHH	KS	nIHH	nIHH	nIHH	Prepubertal state
Other findings		Osteoporosis	-	-	-	Finger Syndactyly	-

LH; Luteinizing hormone, FSH; Follicle-stimulating hormone, ND; Not done, nIHH; normosmic idiopathic hypogonadotropic hypogonadism, KS; Kallmann syndrome

Molecular analysis of the *FGFR1* gene

- Six novel *FGFR1* mutations were identified in 6 patients. Three of 6 *FGFR1* mutations were likely pathogenic, and the other three mutations were classified as a variant of uncertain significance.
- Two novel missense variant, p.Y339H and p.S681I, was predicted to be deleterious by PolyPhen-2 and SIFT.

Table 2. Novel *FGFR1* mutations identified in studied patients

No.	Nucleotide change	Amino acid change	Intron/exon	ACMG/AMP guideline	Clinical diagnosis
1	c.1015T>C	p.Y339H	8	Likely pathogenic	nIHH
2	c.551dup	p.N185Kfs*16	5	Likely pathogenic	KS
3	c.630T>A	p.Y210*	6	Likely pathogenic	nIHH
4	c.2042G>T	p.S681I	15	Uncertain significance	nIHH
5	c.1855-1G>A	Splice site	13	Uncertain significance	nIHH
6	c.1663+2T>G	Splice site	12	Uncertain significance	-

In vitro functional analysis of the novel *FGFR1* mutations

- Wild-type (WT) and *FGFR1* mutants (p.Y339H, p.N185Kfs*16 and p.S681I) were transiently transfected into L6 myoblasts with an FGFR1-responsive osteocalcin promoter luciferase construct.
- FGF8 treatment of WT FGFR1 induced an increase in LUC reporter gene expression. The maximum receptor signaling capacity of the *FGFR1* mutants were reduced compared to WT.

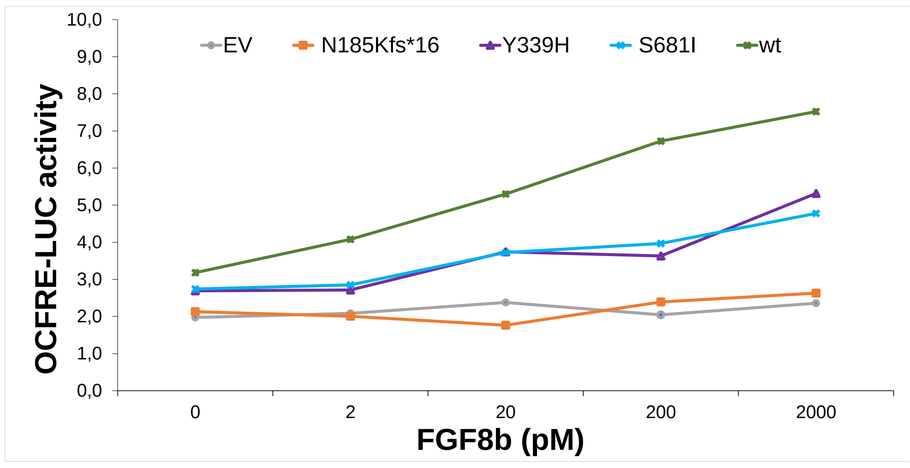

Fig 1. Transcription reporter activity of WT and mutants *FGFR1*. Luciferase reporter assay showing reduced signal capacity of mutant *FGFR1*

- Total RNA was extracted from peripheral blood using a PAXgene blood RNA kit and RT-PCR was performed in the patients with a c.1663+2T>G mutation, resulting a skipping of exon 12.

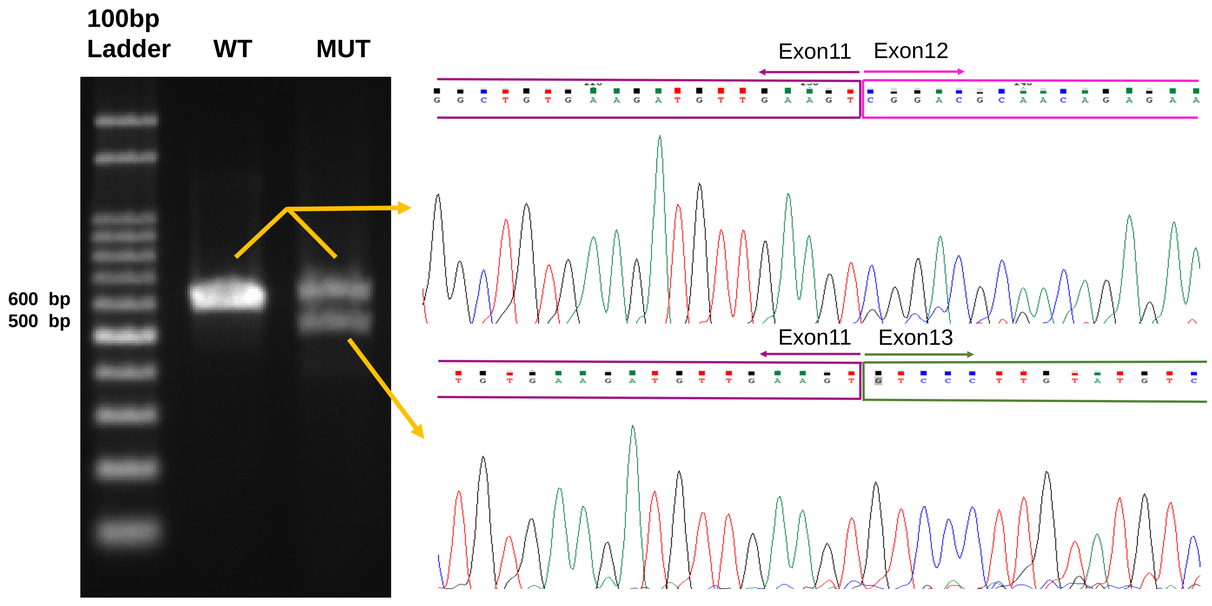

Fig 2. PCR analysis and cDNA sequencing of *FGFR1* revealed the skipping of exon 12 in a prepubertal male patient caused by the intronic c.1663+2T>G mutation

Conclusions

- This study identified six novel mutations in *FGFR1,* which account for 12.2% of causative gene for KS and nIHH.
- Probands carrying an *FGFR1* mutation displayed a wide phenotypic spectrum ranging from KS to anosmia. A prepubertal male with anosmia should be followed up to assess pubertal development.

Disclosure statement

The authors have nothing to disclose.