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Case report

A Novel Nonsense Mutation of *PHF6* in a Female with Extended Phenotypes of Borjeson-Forssman-Lehmann Syndrome

Running head: Female patient of BFLS with extended phenotypes.

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What is already known on this topic?

Borjeson-Forssman-Lehmann syndrome (BFLS) is a rare X-linked disease caused by *PHF6* mutations. Classic BFLS is featured by intellectual disability, developmental delay, obesity, epilepsy, characteristic face and anomalies of fingers and toes. Endocrine deregulation in BFLS was reported but not well delineated.

What this study adds?

We report a female with a novel nonsense mutation c.673C>T(p.R225X) of *PHF6* gene. she exhibits certain features beyond the classic BFLS, including complete deficiency of growth hormone and horseshoe kidney, and adverse effects were elicited after growth hormone treatment in this patient, which was not reported before and cautions the use in this condition. We also reviewed all the BFLS case reports and summarized endocrine presentations and treatment in literature for the first time.

Abstract

Borjeson-Forssman-Lehmann syndrome (BFLS) is a rare X-linked disease caused by *PHF6* mutations. Classic BFLS is featured by intellectual disability (ID), developmental delay (DD), obesity, epilepsy, characteristic face and anomalies of fingers and toes. Endocrinological phenotypes and relevant outcome of treatment in this condition remains to be delineated. Here we report a patient with presentations beyond the classic BFLS - the patient exhibited complete growth hormone deficiency, and adverse effects were elicited after hormonal treatment. Horseshoe kidney was found in this patient, which is also atypical in BFLS. A heterozygous nonsense mutation c.673C>T(p.R225X) of *PHF6* gene was identified in the patient, inherited from her unaffected mother. Both the patient and her mother showed highly skewed X-inactivation. We reviewed the phenotypes of all reported BFLS cases, and summarized the endocrine presentations in literature. The report of this first Asian patient with extended phenotypes further delineated the genetic and phenotypic spectrum of BFLS, and the adverse effect presented in this case cautions the use of hormonal treatment in this condition.

Keywords: Borjeson-Forssman-Lehmann syndrome, *PHF6*, X-inactivation, Growth hormone deficiency, rhGH treatment, hypogonadism

Introduction

Borjeson-Forssman-Lehmann syndrome (BFLS), first described in 1962, is a rare X-linked disease [1]. So far, about 33 families or sporadic cases have been reported, with 64 patients total [2-4]. It is characterized by moderate to severe ID, DD, obesity, epilepsy, hypogonadism, characteristic face, anomalies of fingers and toes [5]. This X-linked condition usually affects males, but mild to severe symptoms are presented in female carriers and most of them have highly skewed X-inactivation [6]. In 2002, Lower et al identified *plant homeodomain finger 6(PHF6)* as the causal gene of BFLS [7]. Since then, 29 different mutations have been reported in *PHF6,* and among these, 14 mutations were identified in affected females [2-4,8-12]. All the patients and variants identified were from European ethnicity. In addition, 27 of the BFLS patients were reported to have endocrine abnormalities [10,13-25]. These hormonal abnormalities have not been well summarized so far.

Here we report a Chinese female with a nonsense mutation in *PHF6* gene inherited from her mother. With a thorough review of all reported BFLS cases, we identified a few features in this patient beyond classic BFLS presentations. We also reviewed and summarized the endocrine aspect of BFLS patients in literature for the first time. A discussion was included to further delineate the genetic and phenotypic spectrum of BFLS.

Case report

A 9 year 1 month girl came to the genetic endocrinology clinic for intellectual disability and short stature. Her height was 123cm(-2SD), and height age was 7 years old. Her weight was 23kg(-1.3SD), and BMI was 15.2(P25-P50). The height of her father and mother were 168cm and 157cm respectively, and the familial target

height of the patient was 156cm (-0.85SD). She was born by caesarean at post-term, with no history of asphyxia. Her birth weight and body length were 4.25kg and 50cm respectively. Severe developmental delay was noticed at toddler stage - she walked alone at the age of 3 and could speak a few simple words at 5. She presented the typical facial features of BFLS, including coarse face, hair sparse, narrow forehead, ptosis, deep-set eyes, broad nasal tip, short nose, malformed teeth, and large ears with moderate earlobes (Figure 1A-C). She had tapering fingers and the bilateral fifth fingers were curved (Figure 1D). She also had flatfoot and the forth toes were shorter than the fifth (Figure 1E). Extensive hyperpigmentation was observed all over the body especially the feet and legs. The patient did not present secondary sexual characteristics at the time of examination. The breast and pubic hair were in the period of B1 and P1 respectively (stage I according to the Tanner scale).

The assay on thyroid and liver function revealed normal results, but she suffered from a complete deficiency of growth hormone (Table 1). Her stature was below three percentile, and her bone age was 7 year 10 month. Because our patient had a complete lack of growth hormone, the clinicians tried rhGH (recombinant human growth hormone) injections for 0.036mg/kg/day to improve her stature, but after 3 weeks she developed edema of both lower extremities and the hormonal treatment was halted.

Ultrasound showed that she had fused kidney at the lower end (horseshoe kidney). The brain MRI revealed periventricular leukomalacia and hyaline compartment formation. The pituitary appeared thinner than girls of the same age, though definitive measurement of the pituitary size was not performed. Her karyotype is 46,XX and chromosomal microarray did not reveal pathogenic variants. Her mother was unaffected based on oral reports. Clinical information of the patient was collected in Shanghai Children's Medical Center at 2012 (see Table 2). Written consent was obtained from the patient's parents.

For whole exome sequencing, genomic DNA was extracted from ethylendiaminetetra acetic acid (EDTA)-treated peripheral blood. Library preparation was performed on the proband with xGen Exome research panel v1.0 (IDT). The captured DNA fragments were subsequently sequenced by Illumina HiSeq 4000. Data was analyzed as previously described [11]. The pattern of X-chromosome inactivation in our patient and her mother was evaluated by assays of differential methylation in the genes between the active and the inactive chromosome X based on methylation-specific PCR [27].

Results

The clinical features of the proband were presented in Figure 1A-E and Table 2. For comparison with previously reported phenotypes, we reviewed the description of a total of 20 female and 43 male BFLS patients in literature (Table 2), and summarized the endocrinological presentations (Table 3). Whole exome sequencing revealed a heterozygous nonsense mutation c.673C>T; p.R225X (the transcript accession number is NM_001015877) of *PHF6* gene in the proband. Sanger sequencing of the proband and her parents demonstrated that the heterozygous mutation was inherited from her mother. No other rare variant with clinical significance was identified. Methylation-specific PCR of peripheral blood DNA indicated a highly skewed X-inactivation in the patient (98:2) and in her mother (95:5) (Figure 1F).

Discussion

BFLS is an X-linked syndromic disorder caused by *PHF6* variations [7,8]. The most prevalent features observed in BFLS (in >80% subjects described) were ID, delay of walking, delay of speech, coarse face, dental abnormalities, large ears and fingers deformities in females. Additionally, genital anomalies and gynecomastia were frequently reported in male BFLS patients.

The phenotypes presented in our patient largely conforms to the description of BFLS based on patients of European ancestry. However, complete deficiency of growth hormone was rarely reported before. Her stature was below three percentile, also a relatively infrequent feature in female BFLS patients (14% of reported cases) [3]. She developed edema in lower extremities after injection of rhGH (before the *PHF6* mutation was identified). Peripheral edema was reported in 1:100-1:10000 of the patients using rhGH therapy [28], possibly due to the impact on fluid homeostasis and retention of water and sodium [29]. So far, a total of 5 BFLS patients were reported to have growth hormone deficiency in literature (Table 3), with two of them presenting multiple pituitary hormone deficiency. The authors reported no improvement of stature after GH treatment [15]. Together with the adverse effect elicited in our patient, GH use in this condition may not be helpful and should be of particular caution, especially considering the recent research showed *PHF6* mutation was associated with pediatric leukemia [30].

Genital anomalies were observed in 59% (27/46) of patients. Early literature reported that hypogonadism was caused by hypophyseal dysfunction [1], but recent report in a male patient with low testosterone, elevated LH and FSH, and another patient with abnormal testicular tissue, suggested that both central and gonadal deregulation might be involved [23]. Based on our summary, the level of estradiol was reduced in 2/4 of the female patients, testosterone reduced in 12/15 of the male patients, gonadotrophin reduced in 8/23 patients, and hypothyroidism in 6/15 patients, also suggesting that both central and end-organ dysfunction may play a role in BFLS.

The phenotype of hyperpigmentation was commonly seen in female BFLS patients - 10 out of 13 female patients presented hyperpigmentation based on previous reports [3,13]. Most of them presented linear pigmentation in the extremities or individual spots in the armpit [3,13]. The hyperpigmentation was extensively distributed over the feet and legs in our patient. Mosaicism may be accountable for the differential presentation, but detailed mechanism of hyperpigmentation was unknown.

One additional feature of our patient that does not fit the description of classic BFLS is horseshoe kidney. In an earlier report, clinical phenotypes of BFLS were noticed to partially overlap with Coffin-Siris syndrome (CSS) [12], particularly in infancy among female patients [4]. CSS is characterized by ID, typical facial features, hypoplasia/aplasia of the fifth digit or finger/toenail, organ malformations including horseshoe kidney [4,12]. Our patient exhibited many phenotypes overlapping with CSS, of particular the presence of horseshoe kidney. It is well established that *PHF6* interacts with the nucleosome remodeling and deacetylation(NuRD) complex implicated in chromatin remodeling, thus functional interaction may exist between *PHF6* and SWI/SNF complex proteins, which are main factors responsible for CSS [3]. This may explain the overlapping features of these two syndromes.

Based on our summary (Table 2), the penetrance in female carriers is about 43.8% (21/48). 38/43 of females with *PHF6* mutations had highly skewed X-inactivation, but only 18 of them were affected. Our patient and her mother had same genotype and similar skewing in X-inactivation but their clinical manifestations were quite different, suggesting mosaicism as a contributing factor to the variable expression of the phenotype [12]. At the same time, this phenomenon suggests that in obligate carriers of *PHF6* mutations, the level of X-inactivation skewing measured in peripheral blood cells may not be a reliable predictor of the expression of BFLS phenotypes [5].

The limitation of this report is that the manifestation of complete GH deficiency and horseshoe kidney was based on only one patient, and more cases are warranted to fully evaluate the outcome of hormonal treatment in this condition.

In conclusion, we report a female with a novel nonsense mutation c.673C>T(p.R225X) of *PHF6* gene, the patient exhibits certain features beyond the classic BFLS, including horseshoe kidney and complete deficiency of growth hormone. Adverse effect was elicited after GH treatment, cautioning the use of GH in this condition. Both she and her unaffected mother has skewing of X-inactivation indicating X-inactivation assay may not reliably predict the expression of BFLS phenotypes. These clinical and genetic information collected may contribute to improve our understanding of BFLS and also future diagnosis and genetic counseling of the condition.

Ethics

Informed Consent: Written consent was obtained from the patient's parents.

Peer-review: Externally peer-reviewed.

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Authorship Contributions Clinical data collection: Yongguo Yu, Xuefan Gu. Genetic testing and analysis: Xia Zhang, Yanjie Fan, Xiaomin Liu, Yu Sun, Yunjuan He, Xiantao Ye. X-chromosome inactivation assay: Hui Yan. Design: Yanjie Fan, Yongguo Yu. Writing: Xia Zhang, Yanjie Fan.

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Figure 1A-E: Pictures of our patient at 9 years old. A-B: Facial characteristics of our patient. C: Dental abnormalities of the patient. D-E: Hand and foot of the patient. The fifth fingers are short and curved, and the forth toes are short. 1F: Results of the methylation-specific PCR assay. The inactivated X chromosome sequence was amplified by the M-primer, the activated X chromosome sequence was amplified by the U-primer. The result indicated a highly skewed X-inactivation in the patient (98:2) and in her mother (95:5).

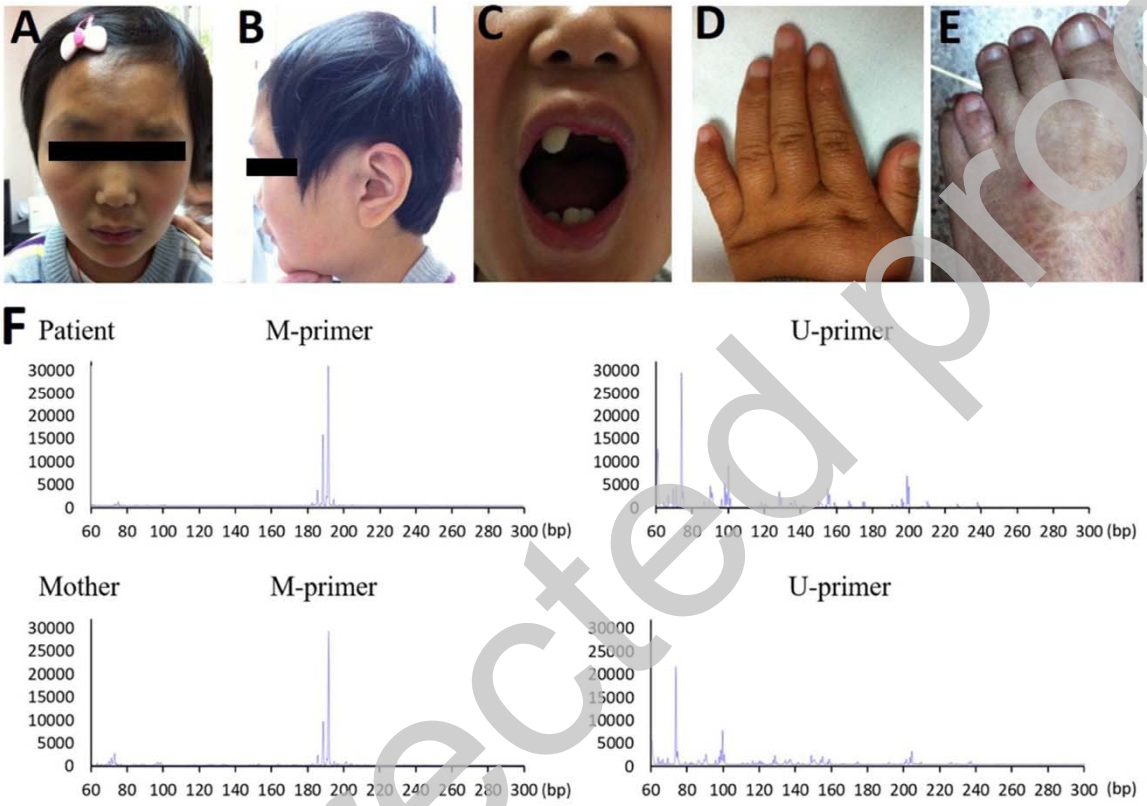

Table 1. Summary of the laboratory results of endocrine tests

Thyroid function			Growth hormone stimulation test (ng/ml)		
FT3	6.99pmol/L (3.8-9.4)			Arginine	Clonidine
FT4	16.72pmol/L (7.9-16.0)		0min	1.902	0.450
TSH	3.28uIU/ml (0.3-5.6)		30min	0.362	0.083
IGF-1	IGF-BP3		60min	0.122	1.005
48.5ng/ml (84-495)	3.4ug/ml (3.4-11.8)		90min	0.260	0.347

Table 2. Clinical information of our patient and the BFLS patients in literature

Patients	Our patient	Frequency in females	Frequency in males
Gender	Female	21 females affected (among 48 carriers)	43 males
Inheritance	Maternally inherited	9 maternally inherited 12 de novo	41 maternally inherited
Age	9y1m	2y-32y	10m-59y
Growth			
Birth weight	Normal, 4.25kg	/	/
Birth length	Normal, 50cm	/	/
Abnormal weight	<P10	0/19 low weight	3%(1/32) low weight
		37% (7/19) obesity	72% (23/32) obesity
Short stature	<P3	14%(3/21)	35%(13/37)
Abnormal Bone age	+, 7y10m	/	/
Neurological abnormalities			
Intellectual disability	+	86%(18/21)	100%(43/43)
Delay of walking	+	92% (12/13)	91%(21/23)
Delay of speech,	+	91% (10/11)	92% (23/25)
Epilepsy	-	19%(4/21)	8%(3/39)
Behavioral anomalies	-	29%(6/21)	36%(4/11)
Vision anomalies	-	67%(8/12)	/
Hearing loss	-	23%(3/13)	/
Craniofacial features			
Coarse face	+	92%(12/13)	84%(27/32)
Hyperpigmentation	+	77%(10/13)	/
Sparse hair	+	62%(8/13)	50%(5/10)
Narrow forehead	-	54%(7/13)	10%(1/10)
Ptosis	+	8%(1/13)	/
Synophrys	-	29%(6/21)	/
Deep-set eyes	+	44%(4/9)	100%(31/31)
Thick eyebrows	-	38%(8/21)	/
Arched eyebrows	-	62%(8/13)	/
Eyelid narrow	+	14%(3/21)	71%(5/7)
Broad nasal tip	+	85%(11/13)	64%(7/11)
Short nose	+	85%(11/13)	50%(4/8)
Large mouth	-	15%(2/13)	13%(1/8)
Dental abnormalities	+	92%(11/12)	100%(2/2)
Cleft palate	-	10%(2/21)	0
Large ears	+	86%(18/21)	100%(25/25)
Hirsutism	-	19%(4/21)	0
Skeletal features			
Tapering finger	+	67%(4/6)	75%(6/8)
Deformity of fingers	+	90%(9/10)	89%(8/9)
Deformity of toes	+	57%(12/21)	78%(7/9)
Viscera development			
Genital anomalies	-	19%(4/21)	92%(23/25)
Gynecomastia	/	/	97%(31/32)
Abnormal Brain MRI	+	55%(6/11)	/
Cardiac defect	-	18%(2/11)	/
Renal anomalies	+	83%(5/6)	/
Skewed X-inactivation in blood	+	94%(17/18) (among patients)	/
		88%(38/43) (among carriers)	

y-years, m-month, +-positive, --negative, /-not known.

Table 3. Summary of hormone levels in BFLS patients

Reference	Thyroid function	LH	FSH	E2	T	GH	PRL	Other
Female								
Our patient	-	/	/	/	/	↓	/	
Carter MT et al. 2009 [13]	-	↓	↓	-	/	/	/	
Crawford J et al.2006 [10]	↓	/	/	/	/	/	/	
Birrell G et al. 2003 [15]	↓	/	/	/	/	/	/	
Petridou M et al. 1997 [17]	-	-	-	/	/	-	-	
Matsuo K et al. 1984 [22]	-	-	-	-	/	/	/	
Robinson LK et al. 1983 [23] #1	↓	↓	↓	↓	-	↓	↓	
Robinson LK et al. 1983 [23] #2	↓	↓	↓	↓	-	↓	↓	
Male								
de Winter CF et al. 2009 [14]	/	/	/	/	-	/	/	
Carter MT et al. 2009 [16] #1	/	-	-	/	/	/	/	
Carter MT et al. 2009 [16] #2	/	-	-	/	↓	/	/	
Birrell G et al. 2003 [15] #1	↓	↓	↓	/	/	↓	/	ACTH↓
Birrell G et al. 2003 [15] #2	↓	↓	↓	/	/	↓	/	ACTH↓
Baumstark A et al. 2003 [18] #1	/	-	↑	/	↓	/	/	
Baumstark A et al. 2003 [18] #2	/	-	-	/	↓	/	/	
Baumstark A et al. 2003 [18] #3	/	-	-	/	↓	/	/	
Baumstark A et al. 2003 [18] #4	/	-	-	/	-	/	/	
Turner G et al. 1989 [19] #1	-	↓	-	/	↓	/	/	
Turner G et al. 1989 [19] #2	/	-	/	/	↓	/	/	
Dereymaeker AM et al. 1986 [20]	-	-	-	/	/	-	/	Cortisol-
Ardinger HH et al.1984 [21] #1	/	-	↓	/	↓	/	/	
Ardinger HH et al.1984 [21] #2	/	-	↓	/	↓	/	/	
Ardinger HH et al.1984 [21] #3	/	-	↑	/	↓	/	/	
Ardinger HH et al.1984 [21] #4	/	-	↑	/	↓	/	/	
Ardinger HH et al.1984 [21] #5	/	-	-	/	/	/	/	
Robinson LK et al. 1983 [23]	-	↑	↑	-	↓	↓	↑	
Veall RM et al. 1979 [24]	-	/	/	/	-	-	-	
Weber FT et al.1978 [25]	-	-	-	/	↓	/	/	Cortisol-

--normal, ↑-increase, ↓-decrease, /-not known.

LH: luteinizing hormone; FSH: follicle-stimulating hormone; E2: estradiol; T: testosterone; GH: growth hormone; PRL: prolactin; ACTH: adrenocorticotropic Hormone.