The *PHF6* Mutation c.1A>G; pM1V Causes Börjeson-Forsman-Lehmann Syndrome in a Family with Four Affected Young Boys

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Key Words

Börjeson-Forsman-Lehmann syndrome · Intellectual disability · Mental retardation · *PHF6* · Whole-exome sequencing · X-linked inheritance

Abstract

The family presented with 4 boys, 2 sets of brothers, with unexplained intellectual disability. Numerous analyses had been conducted over more than a decade, without reaching a final clinical or molecular diagnosis. According to the pedigree, an X-linked inheritance pattern was strongly suspected. Whole-exome sequencing (WES) with targeted analysis of the coding regions of the X chromosome was carried out in the 4 boys, their mothers, and their shared grandmother. A filtering process searching for nonsynonymous variants and variants in the exon-intron boundaries revealed one variant, c.1A>G; pM1V, in the first codon of the *PHF6* gene. The variant was hemizygous in the 4 boys and heterozygous in the 2 mothers and the grandmother. Mutations in the *PHF6* gene are known to cause Börjeson-Forsman-Lehmann syndrome (BFLS). The boys were reexamined after the finding of the mutation, and the phenotype fitted perfectly with

BFLS. The mutation found in the *PHF6* gene is causative for the intellectual disability in this family. We also conclude that WES of the X chromosome is a powerful tool in families where an X-linked inheritance pattern is suspected.

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The mental retardation syndrome Börjeson-Forsman-Lehmann syndrome (BFLS) was described for the first time in 1962 in 3 related young men [Börjeson et al., 1962]. The inheritance pattern was expected to be X-linked recessive, which was supported in the following decades [Ardinger et al., 1984; Carter et al., 2009]. The clinical phenotype of the males with this syndrome is variable, and is characterized by severe intellectual disability, hypogonadism, hypotonia, poor feeding, truncal obesity, gynecomastia, big ears with fleshy lobes, broad feet with short toes, tapered fingers, and coarsening of facial features [Turner et al., 2004; Géczy et al., 2006; Carter et al., 2009]. Female carriers are usually not affected due to X-chromosome inactivation, but might be mildly affected due to skewed X-chromosome inactivation [Crawford et al., 2006; Zweier et al., 2013]. In 1989, the

Table 1. Clinical features of the 4 boys

	Patients			
	III:1	III:2	III:4	III:5
Year of birth	2002	2010	2010	2010
Pregnancy	normal	normal	maternal gestational diabetes	maternal gestational diabetes
Delivery	normal	caesarean	caesarean	caesarean
Birth weight	2,960 g	2,955 g	2,655 g	2,155 g
Feeding problems in infancy	NR	yes	NR	NR
External genitalia	normal	normal	normal	normal
Neonatal tonus	hypertonic LE and UE	hypertonic LE, hypotonic UE	hypertonic	hypertonic
Current tonus	hypotonic	hypotonic	hypotonic	hypotonic
Motor milestones	delayed	delayed	delayed	delayed
Spoken language	no	no	no	no
Sign language	yes	yes	NR	NR
Intellectual disability	yes	yes	yes	yes
Ears	low-set, big	low-set, flat	NR	NR
Sandal gap	NR	NR	NR	NR
Short toes	NR	NR	NR	NR
Tapered fingers	NR	NR	NR	NR
Gynecomastia	NR	NR	NR	NR
Truncal obesity	NR	NR	NR	NR
Coarsening of facial features	reported	reported	reported	reported
Micro- or macrocephaly	no	no	NR	NR
Seizures	yes	no	no	no
Short stature	low normal	low normal	NR	NR
Other features	umbilical hernia, hoarse voice, retinopathy	heart defect, kidney anomaly, 1p21.1 deletion	hypermetropia	hypermetropia, unilateral hearing loss

LE = Lower extremity; NR = not reported; UE = upper extremity.

Fig. 1. The family pedigree with the 4 affected boys, III.1, III.2, III.4 and III.5, depicted as solid black squares. The healthy carrier mothers, II.2 and II.3, and their shared grandmother, I.2, are circles with a dot.

syndrome was linked to Xq26q27, and in 2002 the associated gene was found to be the PHD-finger gene 6 (*PHF6*) [Mathews et al., 1989; Turner et al., 1989; Lower et al., 2002]. *PHF6* is highly conserved in vertebrates, but the cellular function of *PHF6* is not known [Lower et al., 2002; Voss et al., 2007]. The protein has 4 nucleolization signals [Voss et al., 2007] and is believed to be involved in transcription and/or in nucleosome assembly [Todd and Picketts, 2012]. To date, at least 17 families with 41 affected males have been described with mutations in *PHF6* [Zweier et al., 2013].

Materials and Methods

The family counseled was a Danish family with 4 boys, 3–12 years of age, with developmental delay (the pedigree is shown in fig. 1). All 4 had intellectual disability, coarsening of facial features, hypotonia, delayed motor milestones, and no spoken language (table 1). Prior to the diagnosis, none of the boys were described as having hypogonadism, broad feet with short toes or tapered fin-

Table 2. Variants after filtering

Variant	Gene	Classification	Exon	HGVS coding	HGVS protein
X:123034511-SNV	<i>XIAP</i>	nonsynonymous SNV	6	c.1268A>C	p.Gln423Pro
X:123554646-SNV	<i>ODZ1</i>	synonymous	25	c.4497A>G	p. =
X:130678596-SNV	<i>OR13H1</i>	synonymous	1	c.549C>G	p. =
X:133511648-SNV	<i>PHF6</i>	initiating codon	2	c.1A>G	p.Met1Val

Variants according to the Genome Reference Consortium (GRCh37). HGVS = Human Genome Variation Society.

Fig. 2. Filtering of variants. After filtering, 7 variants were found to be present in all family members. Four of them were splice-site or exonic variants. Two of these 4 were synonymous, leaving only 2 potentially disease causing variants in the *XIAP* and *PHF6* genes, respectively.

gers in the pediatric and genetic files. All 4 were tested by array CGH. In III:2, a 2.5-Mb deletion of 1p21.1 was detected and thought to be the cause of his symptoms. However, the 4 boys had a similar phenotype, and in the other 3 boys this deletion was not detected. III:1 was also tested for metabolic diseases and fragile X syndrome. III:1 and III:2 were karyotyped. III:4 and III:5 were tested for mitochondrial diseases by muscle biopsy. Based on the pedigree and the similar phenotype, X-linked inheritance was strongly suspected.

Whole-Exome Sequencing

Sequencing of the coding regions and the exon/intron boundaries of the X chromosome was carried out using whole-exome

sequencing (WES). WES was performed in the 4 affected boys III:1, III:2, III:4, and III:5; their respective mothers II:2 and II:3, and their maternal grandmother I:2. In brief, 3 µg of DNA was fragmented through acoustic sonication on a Covaris® S2 system and enriched using Agilent SureSelect Whole Exome Capture kit prior to 75 bp forward sequencing on SOLiD Wildfire 5500xl (Life Technologies).

Library Preparation and Sequencing

3 µg of DNA was fragmented through acoustic sonication on a Covaris® S2 system according to the Fragment Library Preparation Using the AB Library Builder™ system 5500 Series Solid™ Systems User Guide.

The fragmented DNA was then used for Fragment Library Preparation Using the AB Library Builder™ System adding 1.7 µl of adaptor P1 and barcoded adaptors followed by nick translation and 6 cycles of amplifications according to the protocol.

The libraries were quantified using SOLiD™ Library TaqMan Quantitation Kit. The 7 barcoded libraries were pooled in equal amounts in 2 tubes, one with 4 barcoded libraries (125 ng of each) and one with 3 barcoded libraries (167 ng of each). To each tube of pooled libraries, 5 µl human Cot-1 DNA (0.1 mg/ml), 5 µl P1 blocker (ABI) and 5 µl barcode 1–16 blocker (ABI) were added. The mix was dried down in a Savant Speed Vac (ThermoScientific) at medium drying rate, and hereafter resuspended in 9 µl H₂O, ready for use with the SureSelect^{XT} Target Enrichment System for Solid 5500 Multiplexing Sequencing v1. The 9 µl library pool was denatured at 95°C for 5 min followed by the addition of SureSelect hybridization buffer (13 µl), RNase block (2 µl 25% dil.) and 5 µl SureSelect capture library (Agilent SureSelect Whole Exome Capture kit V5). The solution was incubated for 24 h at 65°C.

The captured library was collected by binding to Dynabeads MyOne Streptavidine T1 and washing of beads was carried out according to the SureSelect protocol.

The 30 µl post-capture beads with DNA library was then converted to a 5500 wildfire library using 10 cycles of amplification according to the Conversion of 5500 Library to 5500 W Library protocol.

4 nM of the pool of 7 barcoded libraries were sequenced with 50 bp forward sequencing on the SOLiD W 5500xl (Life Technologies) resulting in between 77 and 167 million raw reads for each library.

Fig. 3. Photos showing the clinical features of the 2 brothers III.1 and III.2. The facial photos show the enlarged ears with fleshy lobes. The hands show tapering of the finger, and the feet are broad with short toes.

Data Analyses and Results

The raw reads in the XSQ files were first joined by an in-house program into one XSQ file. The joining step allows LifeScope (Life Technologies) to produce one BAM file for each sample. The joined XSQ file was then run in LifeScope Software 2.5 (Life Technologies) by using the Whole Exome Fragment Resequencing workflow. In brief, the workflow contains color space error correction, filtering of short- and low-quality reads, sequence alignment, calculate statistics of the aligned reads, and variant calling.

LifeScope could identify a total of 86,926 variants for all 7 subjects. Tertiary data analysis on variants was done with SNP & Variation Suite (Golden Helix Inc.). Before we performed further data analysis, all variants outside of the X chromosome were filtered out, leaving a total of 1,649 variants (fig. 2). After filtering variants by genotypes, 7 variants were kept. By coding and noncoding classification, 3 variants were excluded.

Of the 4 remaining variants, the 2 nonsynonymous variants were the most likely candidates to be the cause of the phenotype in the 4 boys (table 2). A missense variant was found in the *XIAP* gene. Pathogenic mutations in the *XIAP* gene cause X-linked lymphoproliferative syndrome, which is not consistent with the observed phenotype in the 4 boys.

The second nonsynonymous variant was in the *PHF6* gene where pathogenic mutations are known to cause BFLS. The c.1A>G is a nonsynonymous missense variant in the first codon of the *PHF6* gene. There is no downstream alternative start codon, and we therefore expect complete lack of protein production. A different mutation affecting the first amino acid in the *PHF6* gene has been reported twice previously as the causal variant in a BFLS family [Lower et al., 2002; Crawford et al., 2006]. The variant was confirmed by Sanger Sequencing.

Discussion

After the diagnosis of BFLS, the 4 boys were reexamined. All 4 had a weak suck at infancy, hypotonia, developmental delay, no language, hypogonadism, tapered fingers, big ears with fleshy lobes, broad feet with short toes, and a wide gap between the first and second toe (fig. 3). Due to the young age of the 4 affected boys, none of them have developed gynecomastia or truncal obesity yet. The oldest affected boy and his younger brother have no spoken language, but have reasonable perceptive abilities and a continuously developing sign language. Reports from other patients with BFLS also describe a very small vocabulary of only a few words but an ability to use sign language [Zweier et al., 2013].

Fig. 4. Reported pathogenic variants in the *PHF6* gene. Schematic view of the different mutations described in the *PHF6* gene giving rise to BFLS. The mutation found in this family is printed in red [Lower et al., 2002, 2004; Baumstark et al., 2003; Turner et al., 2004; Vallée et al., 2004; Visootsak et al., 2004; Crawford et al., 2006; Carter et al., 2009; Chao et al., 2010; Berland et al., 2011; Zweier et al., 2013].

It has taken 12 years with several counseling sessions and numerous analyses to finally reach a diagnosis for this family. A syndrome being responsible for the phenotype in the oldest boy was suspected before his first birthday. Diagnosing intellectual disability often proves to be difficult due to the heterogeneity of the disease. Intellectual disability shows a variable clinical presentation, and often mutations in different genes give rise to the same or a similar phenotype. This complicates both the clinical diagnosis as well as the genetic diagnosis.

WES has shown it is worth reaching a diagnosis in families where an extensive diagnostic effort such as SNP arrays and targeted sequencing has failed. The benefit of WES is that it covers the entire coding region which is most likely to contain the disease causing variant. WES gives the opportunity to consider large gene panels as well as looking beyond known susceptibility genes and pathways [de Ligt et al., 2012]. However, in our experience WES also has challenges, such as not covering all areas of the exome because of uneven coverage due to the nature of the sequence or capture/amplification of the sequence, which may result in areas not covered at all. Another challenge is heterozygous variants being missed during data analysis if the frequency of the variant is well below 50%.

A Finnish study examined the X chromosome by WES approach in 14 families suspected of X-linked intellectual disability, and in 6 of the 14 families, genetic and clinical diagnoses was found [Philips et al., 2014]. In the 8 unresolved cases, 5 could also be explained by autosomal recessive inheritance, since only brothers from one set of parents were affected. Our study shows that analysis of the X chromosome by WES is suitable for families suspected of having X-linked intellectual disability. Especial-

ly, if there are boys affected in several branches of the family, reducing the possibility of a rare autosomal recessive variant being the cause of disease.

Conclusion

A novel mutation c.1A>G; p.M1V in the *PHF6* gene was found hemizygous in all 4 boys (III:1, III:2, III:4, and III:5) and heterozygous in the 2 mothers (II:2 and II:3) and the grandmother (I:2). The mutation alters the start codon, where a p.M1T mutation has been previously reported. The mutation caused BFLS in the 4 affected boys with 3 unaffected female carriers. The genotype status of the unaffected sister III.3 is unknown. Several other variants, including nonsense, frameshifts, missense and splice site mutations in the *PHF6* gene have been shown to cause BFLS (listed in fig. 4) [Lower et al., 2002, 2004; Baumstark et al., 2003; Turner et al., 2004; Vallée et al., 2004; Visootsak et al., 2004; Crawford et al., 2006; Carter et al., 2009; Chao et al., 2010; Berland et al., 2011; Zweier et al., 2013].

We conclude that sequencing of the X chromosome by WES in families with boys having suspected X-linked intellectual disability is a good choice with a reasonable probability of finding the disease-causing mutation.

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Statement of Ethics

The parents of the underaged participants have given written consent for WES analysis and for the publication of the results.

Disclosure Statement

The authors have no conflict of interest to declare.

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