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Further characterization of Borjeson-Forssman-Lehmann syndrome in females due to *de novo* variants in *PHF6*

Running title: female Borjeson-Forssman-Lehmann syndrome

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Not relevant

STATEMENT OF CONTRIBUTIONS

C.B.G., A.F., O.B., S.B., M.D., M.H., I.H., P.M.-R., P.R.M., F.M.-C., I.M., D.K., K.S., A.W., M.Z. and C.Z. collected mutational and clinical data. A.F. performed targeted testing of *PHF6*. H.S. performed structural modeling. C.B.G. and C.Z. wrote the manuscript, which was read and revised by all co-authors.

ETHICAL STATEMENT

The study was approved by the ethics committee of the medical faculty of the Friedrich-Alexander-University Erlangen-Nuremberg (approval 142_15B). Testing in all but one individual was performed in a diagnostic setting. Individual 10 was investigated in the frame of a research study approved by the ethical review board of the canton Zurich and respective consent was retrieved from the family. Informed consent for publication of mutational and clinical data and particularly for publication of patient photographs was obtained from the parents or legal guardians.

URLs

https://gnomad.broadinstitute.org

http://www.ncbi.nlm.nih.gov/clinvar/

https://cadd.gs.washington.edu/score

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https://www.mutationtaster.org

ABSTRACT

While inherited hemizygous variants in *PHF6* cause X-linked recessive Borjeson-Forssman-Lehmann syndrome (BFLS) in males, *de no*vo heterozygous variants in females are associated with an overlapping but distinct phenotype, including moderate to severe intellectual disability, characteristic facial dysmorphism, dental, finger and toe anomalies and linear skin pigmentation.

By personal communication with colleagues, we assembled eleven additional females with BFLS due to variants in *PHF6*. We confirm the distinct phenotype to include variable intellectual disability, recognizable facial dysmorphism and other anomalies. We observed skewed X-inactivation in blood and streaky skin pigmentation compatible with functional mosaicism. Variants occurred *de novo* in ten individuals, of whom one was only mildly affected and transmitted it to her more severely affected daughter. The mutational spectrum comprises a 2-exon deletion, five truncating, one splice-site and three missense variants, the latter all located in the PHD2 domain and predicted to severely destabilize the domain structure. This observation supports the hypothesis of more severe variants in females contributing to gender-specific phenotypes in addition to or in combination with effects of X-inactivation and functional mosaicism.

Therefore, our findings further delineate the clinical and mutational spectrum of female BFLS and provide further insights into possible genotype-phenotype correlations between females and males.

Keywords: PHF6, X-chromosomal, *de novo*, Borjeson-Forssman-Lehmann syndrome

INTRODUCTION

X-linked recessive Borjeson-Forssman-Lehmann syndrome (BFLS, OMIM#301900) was first described in 1962.[1] In 2002, variants in the gene encoding PHD finger protein 6 (*PHF6*) were identified as the underlying cause.[2] PHF6 contains two extended atypical PHD-like zinc finger domains (PHD1 and PHD2), two nuclear and one nucleolar localization sequences[2] and is assumed to play a role in transcription, ribosomal RNA transcription and neuronal migration.[3-5] Affected males present with developmental delay, moderate to severe intellectual disability (ID), truncal obesity, hypogonadism, tapering fingers, toe anomalies, and a typical facial gestalt with long ears or prominent earlobes and prominent cheek bones.[6,7] Some of the female carriers in these families show mild aspects of BFLS such as learning difficulties, mild facial features or toe and finger anomalies.[7-9] Skewing of X-inactivation (XI) in female carriers in these reports was inconsistent and did not correlate with clinical findings.[9-11]

In 2013, a series of seven females with *de novo* variants in *PHF6* was reported.[12] Affected individuals presented with a neurodevelopmental disorder overlapping with BFLS in males, but also displaying additional distinct features.[12] Next to moderate to severe intellectual disability, a characteristic facial gestalt with long shaped ears, bitemporal narrowing, prominent supraorbital ridges, high eyebrows, a short nose and a bulbous nasal tip was delineated. Furthermore, oligomenorrhea, more prominent finger and toe deformities, dental anomalies and linear skin hyperpigmentation occurred. In accordance with streaky skin pigmentation, skewed XI in blood samples and random XI in fibroblasts indicated functional mosaicism of the active and inactivated mutant allele.[12] Up to now, a total of twelve female individuals with such *de novo* germline deletions, duplications or single nucleotide variants in *PHF6* were reported.[10,12-17]

Male individuals with BFLS predominantly harbor missense variants and only a few truncating variants distributed all over the gene/protein,[2] while in females with *de novo* variants, mostly deletions, (likely) truncating aberrations and only one missense variant located within the second PHD zinc finger domain were identified to date.[12,17,18] Observing differential cellular localization between “male” and “female” variants *in vitro* and predicting more severe effects of the single female missense variant compared to male missense variants in the PHD2 zinc finger on domain stability, a possible genotype-phenotype correlation between nature and localization of variants and gender-specific phenotypic manifestation was recently suggested.[18]

We now further delineate the mutational and clinical spectrum of female BFLS by assembling eleven additional cases with aberrations in *PHF6*. Variants occurred *de novo* in ten individuals, of whom one was only mildly affected and transmitted it to her more severely affected daughter. Identification of three further missense variants within the PHD2 domain and subsequent structural modeling support the previously suspected genotype-phenotype correlation.

MATERIAL AND METHODS

Patient material and data

Personal communication with colleagues following the initial reports[12,16] enabled us to collect clinical and mutational details on eleven female individuals with BFLS due to variants in *PHF6*. The study was approved by the ethics committee of the medical faculty of the Friedrich-Alexander-University Erlangen-Nuremberg (approval 142_15B). Testing in the majority of individuals was performed in a diagnostic setting. Individual 10 was analyzed within a study to unravel the diagnosis of patients with developmental disorders. Informed consent for publication of mutational and clinical data and particularly for publication of patient photographs was obtained from the parents or legal guardians.

PHF6 analysis and structural modeling

In four individuals, targeted analysis of *PHF6* (NM_032458) based on clinical suspicion was performed by Sanger sequencing and/or MLPA, as described previously.[12] Further details on primer and probe sequences and conditions are available on request. Trio-exome sequencing was performed in one, panel sequencing in two and single exome sequencing in three individuals (Table 1). Segregation analysis in the non-trio cases was performed by Sanger sequencing or MLPA, respectively. XI analysis in blood samples was performed in seven individuals in the respective centers within routine diagnostics. VIPUR scores[19] of the three novel and one published[20] missense variants were determined as described previously.[18] VIPUR is designed to distinguish between neutral (score < 0.5) and deleterious (score > 0.5) protein variants by modeling their effect on the three-dimensional protein structure. Thus, high scores indicate a large effect of the respective protein variant on the protein structure.

RESULTS

Clinical Spectrum

For a summary of clinical details, see also Table 1. Age at last investigation of the eleven affected individuals ranged from 10 months to 36 years. Developmental delay was variable. The age of unsupported walking was between ten months and not yet at seven years. Age of first words ranged from normal to lack of speech at age 7 or 15 years in two individuals, respectively. Three of the individuals communicated in simple sentences, one with correct grammar.

All but one of the individuals at informative ages presented with intellectual disability, ranging from mild/moderate (7/10) to severe/profound (3/10). Formally tested IQs were not available.

Of note, the mother of the familial case had normal motor and speech development and later only learning difficulties at school.

Behavioral anomalies such as verbally and physically abusive behavior were observed in a single individual. A happy and friendly demeanor was described in two other individuals.

MRI of the brain was performed in six individuals and revealed unspecific abnormalities in five of them, such as white matter lesions. Subcortical nodular heterotopia was observed in one individual. Neurological aspects such as muscular hypotonia or seizures only occurred in two or a single individual, respectively. Retinal depigmentation with maculopathy was reported in two individuals. Other ophthalmological anomalies as well as further organ abnormalities or cleft palate, occurred in single cases.

Ten individuals in this study showed a distinctive facial gestalt with long shaped ears with prominent earlobes, bitemporal narrowing, prominent supraorbital ridges, synophrys, a high nasal root and bulbous nasal tip. The mildly affected mother of family 2 showed rather subtle facial aspects with a bulbous nasal tip. Eight individuals had sparse scalp hair, in combination with fine hair texture during infancy (Figure 1).

Linear skin hyperpigmentation was present in five individuals affecting different body parts. One individual presented with hypertrichosis. Teeth anomalies were described in six individuals and included hypodontia, enamel defects, rather small teeth or large frontal teeth with long roots or misalignment. All but one of the individuals showed finger anomalies, including campto-, brachy-, clinodactyly or tapering. Nine out of eleven individuals presented with toe anomalies such as syndactyly II/III (5x), brachy-, clino- and camptodactyly, broad or hypoplastic toes. Dysplastic or hypoplastic finger or toe nails were reported in three and four individuals, respectively (Figure 1). Obesity occurred in three individuals.

Mutational spectrum

For a summary of identified variants, see Figure 2, Table 1 and Supplementary Table S1.

Three missense and six truncating variants, including a splice-site variant, in *PHF6* were detected in the eleven herewith described individuals. The deletion of exons 6 and 7 in one individual was predicted to be frameshifting and thus truncating. Nine of the variants were shown to have occurred *de novo*. In one case, maternal inheritance was excluded, and the father was not available for testing. One individual inherited the variant from her mildly affected mother, in whom the variant was shown to have occurred *de novo*. Sanger sequencing in blood was not indicative for mosaicism in her (Supplementary Figure S1).

While the truncating variants were distributed all over the gene/protein, the three missense variants clustered within the PHD2 domain (Figure 2). To our knowledge, none of the variants has been reported as pathogenic before in literature or ClinVar. They are not observed in gnomAD. The missense variants affect highly conserved amino acids and are predicted to be deleterious by at least three of the used *in silico* prediction programs (Supplementary Table

S1). According to ACMG guidelines,[21] all identified variants were classified as pathogenic or likely pathogenic (Supplementary Table S1).

XI pattern in blood samples was tested in seven individuals and was skewed (>90%) in all of them. Of note, both the mildly affected mother and the more severely affected daughter of the familial case had a similarly skewed degree of XI of more than 90% (Table 1).

Structural modeling of the missense variants

We used the VIPUR score,[19] integrating sequence analysis and structural modeling, to assess the effect of the identified missense variants on the three-dimensional protein structure. All three missense variants in our cohort showed a high VIPUR score of >0.86, thus predicting a strong destabilizing effect on the protein structure (Figure 2, Supplementary Table S1). Also, the published missense variant c.823G>A, p.(Gly275Arg) within the PHD2 domain[20] was predicted to have a strong effect with a VIPUR score of 0.99.

DISCUSSION

PHF6 belongs to the increasing number of X-chromosomal genes in which both inherited variants in males with an X-chromosomal recessive neurodevelopmental disorder (NDD) and *de novo* variants in females with a comparable severe but distinct NDD were identified. Female variant carriers in the X-linked recessive families are mostly asymptomatic but may display mild and infrequent clinical aspects (Table 1). While the male BFLS phenotype has been known for several decades,[1,2] the distinct female phenotype associated with *de novo* variants in *PHF6* was only delineated in 2013.[12] Thus, the available information on the latter is still limited and based on twelve published cases so far.[10,12-17] By reporting on eleven further individuals with the female form of BFLS, we further characterize the phenotypic and mutational spectrum.

With this study, we confirm the very distinct phenotype of BFLS in females caused by *de novo* variants in *PHF6* to include variable intellectual disability, a characteristic facial gestalt, acral and dental anomalies and linear skin hyperpigmentation. While the variable degree of intellectual disability is comparable to that of affected male individuals, some of the facial aspects, as well as the presence of dental and pigmentation abnormalities are rather specific for the female phenotype. Finger and toe abnormalities are similarly frequent in both novel and published females and males with BFLS (>90%). However, in males, mainly tapering of fingers has been observed, while finger deformities in females are more prominent and diverse with tapering, campto-, clino-, brachydactyly and hypoplastic nails (Table 1). While sandal gaps have been reported more frequently in males, syndactyly of toes and hypoplastic toe nails occurs more frequently in females (Table 1). The recognizability of the female phenotype is also demonstrated by the fact that targeted testing of *PHF6* was performed in four of the individuals based on a specific clinical suspicion.

Furthermore, we confirmed or observed novel or previously under-recognized aspects of female BFLS in this cohort. Whereas non-specific ophthalmological abnormalities such as ametropia, nystagmus or strabismus were frequently reported in about half of the previously published[12,16,17] and the new cases, more specific, retinal findings such as dystrophy or depigmentation were only observed once previously[12] and now additionally in two of the herewith reported individuals.

In general, neither structural brain abnormalities nor neurological features such as epilepsy seem to be a frequent feature of either male or female BFLS. Two adult females with a similar duplication of exons 4-5 were previously reported with a specific brain phenotype resembling band heterotopia and with adult-onset epilepsy.[22] Apart from these, MRI data has been only infrequently available for affected individuals with BFLS. In our cohort, MRIs were performed in six individuals, indicating brain anomalies in five of them. These included mainly non-specific signs such as white matter abnormalities, dysplastic pons and enlarged ventricles. Interestingly, frontal subcortical heterotopia was observed in a single individual. In accordance with the findings by Kasper et al.,[22] and observations in mice,[3] this might support a role of PHF6 in neuronal migration. Epilepsy occurred in only one of the herewith reported individuals. Whether the specific brain and epilepsy phenotype in the two previously reported females[22] reflects a genotype-phenotype correlation regarding the shared exon 4-5 duplication therefore remains elusive and would require further cases with a similar duplication and/or brain phenotype.

While general or truncal obesity was described in more than 90% of males with BFLS,[1,6,9] this has been only observed in 20% of females with BFLS both in the published and the herewith reported individuals (Table 1). Also hypogonadism has been described as one of the prominent features in males with BFLS,[1,9] and variable endocrinological abnormalities were observed in individuals carrying *PHF6* variants.[23] Oligomenorrhea, frequently observed in females with *de novo* variants in *PHF6*, might also reflect hypogonadism.[12,14] While hypogonadotropic hypogonadism was confirmed in a single female individual,[14] detailed endocrinological testing was not performed or is not available for other female individuals with *de novo* variants in *PHF6*, thus currently limiting the characterization of a endocrinological phenotype in female BFLS. Of note, in the current cohort, a mildly affected female with oligomenorrhea gave birth to a daughter.

Several factors are assumed to contribute to the phenotypic differences between genders and between unaffected and affected female *PHF6* variant carriers. Functional mosaicism of the active and inactive mutant *PHF6* allele is discussed as a contributing pathomechanistic factor in females with *de novo* variants in *PHF6*.[12,13,18] Streaky skin pigmentation has been observed in the majority of previously reported affected females[12-17] but not in the unaffected carrier

females in X-recessive families.[7-9] Of note, in this study, only half of the females showed skin pigmentation anomalies, and the presence of these was not correlating with the severity of disease manifestation. Random XI might be another indicator of functional mosaicism, supported by a previous report showing skewed XI in blood samples but random XI in fibroblasts.[12] In blood, there is a high frequency of skewed XI both in asymptomatic carriers and symptomatic females.[6,9,12,16,23] For two affected females with *de novo* variants preferential inactivation of the mutant allele in blood was demonstrated (so far unpublished data, Supplementary Figure S2). Thus, the "direction" of XI in blood cells does not provide an explanation for the presence or severity of phenotypes. Furthermore, in the herewith reported and two other published familial cases with the transmission of a *PHF6* variant from a mildly affected or asymptomatic mother to a severely affected daughter,[20,23] XI pattern in blood was similar in mothers and daughters. However, no data is available if the same allele was preferentially inactivated in both individuals. In summary, previous and new observations demonstrate that determining the degree of XI from blood samples might have only a weak predicting effect and allows no conclusions on the pattern of XI in more relevant tissues such as the brain.

Location and "severity" of the variants in *PHF6* were discussed as another factor contributing to phenotypic differences. Although a severe effect by near complete loss of PHF6 protein expression has been demonstrated for the recurrent c.1024C>T, p.(Arg342Ter) variant in males,[24] the frequency of truncating variants is still significantly higher in females with BFLS than in males.[18] Furthermore, "male" and "female" missense variants behave differently in *in vitro* assays and regarding protein domain stability.[18] While a missense variant in the PHD2 domain identified in a female was predicted to have a strong destabilizing effect, male missense variants in the same domain were predicted to have a milder effect.[18] Missense variants from males in the PHD1 domain were shown to have a strong destabilizing effect[18] and were shown to result in reduced protein expression,[24] but were so far exclusively observed in X-linked recessive families and not as *de novo* variants in females with the full clinical picture of BFLS. Missense variants in the PHD1 domain were therefore postulated to be less deleterious than missense variants in the PHD2 domain, and within the PHD2 domain females missense variants to be more deleterious than male missense variants.[18] By now, we find further evidence for this previously discussed genotype-phenotype correlation. Three additional *de novo* missense variants identified in females were all located in the PHD2 domain and predicted to result in severe destabilization of the domain, similar to the previously published female variant[18] and more severe compared to male missense variants in the same domain.[18]

This is also supported by another published missense variant detected in a female, c.823G>A p.Gly275Arg,[20] which is also located in the PHD2 domain and predicted to have a strong

destabilizing effect. Surprisingly, however, this variant was transmitted from an asymptomatic mother to a more severely affected daughter.[20] There is another case of female-to-female transmission of a truncating variant in the literature[23] and additionally in the herewith reported family 2 (see also above). Similar degrees of XI skewing in blood in mildly affected mothers and more severely affected daughters[20,23] does not provide an explanation for the phenotypic differences. In family 2 and in one of the published cases[20] the variant was shown to be *de novo* in the mothers, however, without indication of mosaicism for the variant in them. Still, this cannot be excluded as an explanation for the mild presentation. Post-zygotic mosaicism associated with a milder phenotypic presentation has been reported in a female individual before.[15]

In total, the phenotypic manifestation of BFLS in females can not be attributed to a single factor but seems to result from a complex and variable interplay of different contributing factors including XI, functional mosaicism, as well as localization, nature and severity of variants.

Our study confirms that BFLS caused by *de novo* variants in *PHF6* is a distinct, recognizable neurodevelopmental disorder in females, and we further delineate the clinical and mutational spectrum. By confirming a genotype-correlation between males and females and between symptomatic and asymptomatic female carriers regarding localization of consequences of missense variants, we support the hypothesis that nature and localization of variants in *PHF6* are contributing factors to the female BFLS phenotype.

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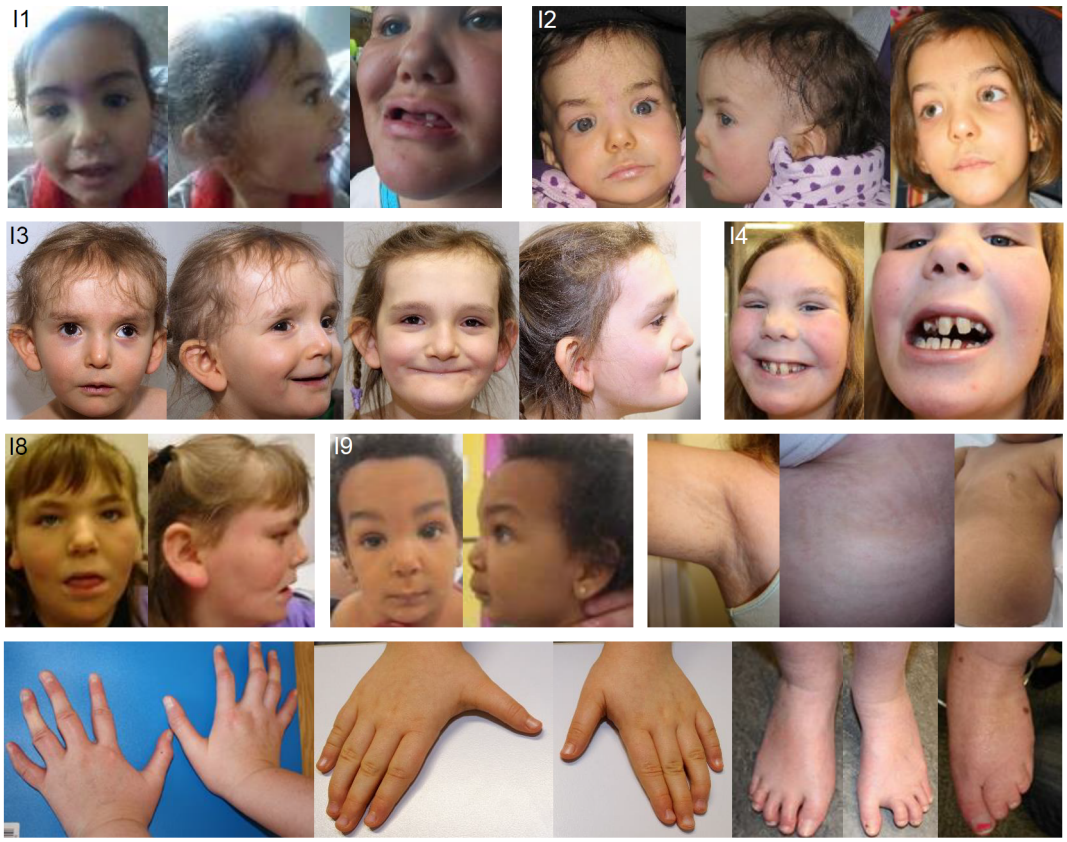
FIGURE LEGENDS

Figure 1 Morphological aspects of females with BFLS due to *de novo* variants in *PHF6*. Note the characteristic facial appearance with sparse hair in infancy, long-shaped ears, bitemporal narrowing, prominent supraorbital ridges, synophrys and a short nose with bulbous nasal tip. Additionally, irregularly shaped or missing teeth, linear skin hyperpigmentation and finger and/or toe anomalies occur.

Figure 2 Schematic drawing of *PHF6* (NM_032458) with location of variants identified in females. Coding exons are colored in dark blue, non-coding exons in light blue. Red squares mark exons encoding the extended plant homeodomain 1 and 2 (PHD1/2). Above the gene, published deletions and variants[10,12-17,20] are indicated, below the scheme novel variants. VIPUR score[19] for all female missense variants in the PHD2 domain is higher than 0.86 indicating a severe deleterious effect of the variants on the protein stability.



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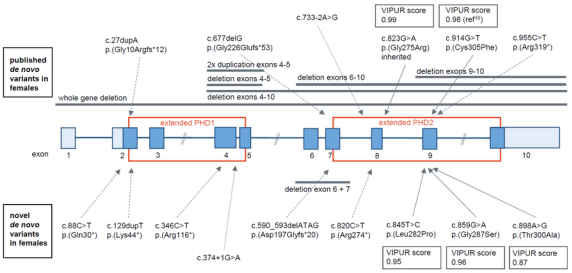

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Further characterization of Borjeson-Forssman-Lehmann syndrome in females due to *de novo* variants in *PHF6*

delineating the mutational and clinical spectrum of female BFLS by assembling eleven additional cases with aberrations in *PHF6*

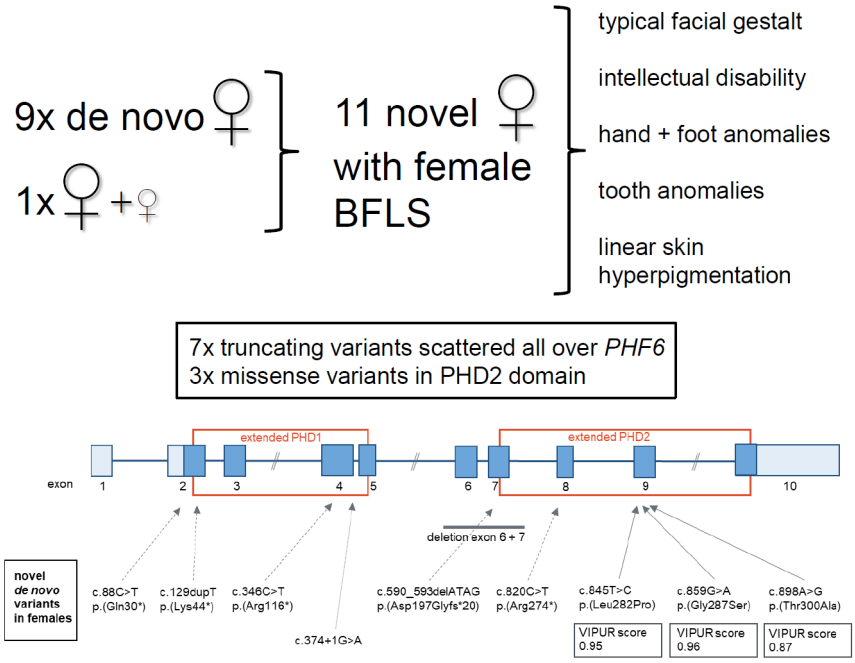

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Table 1. Mutational and clinical details of females with BFLS due to variants in *PHF6* and comparison to published males and female carriers

patient #	**1**	**2.1 (index)**	**2.2 (mother)**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**female BFLS n=27[1-9], current study**	**male BFLS n=62[2,10-22]**	**female carriers in XLR families n=63[2,10-18,20-22]**
***PHF6* variant (NM_032458)**	**exon 6-7 deletion**	**c.88C>T p.(Gln30*)**	**c.88C>T p.(Gln30*)**	**c.129dup p.(Lys44*)**	**c.346C>T p.(Arg116*)**	**c.374+1G>A r.spl**	**c.590_593del p.(Asp197Glyfs*20)**	**c.820C>T p.(Arg274*)**	**c.845T>C p.(Leu282Pro)**	**c.859G>A p.(Gly287Ser)**	**c.898A>G p.(Thr300Ala)**	19x truncating 5x missense	7 families truncating 15 families missense	NA
de novo	yes	maternal	yes	excl. in the mother	yes	yes	yes	yes	yes	yes	yes	23/26	1/56	1/43
method	MLPA	panel	Sanger segr.	Sanger	panel	exome	Sanger	exome	Sanger	exome	trio exome	NA	NA	NA
skewed XI blood	NA	XI 97.2	XI 91.4	XI 100	XI 100	XI 100	XI 100	NA	NA	XI 100	NA	22/22 100%	NA	23/29 79.3%
sex	female	female	female	female	female	female	female	female	female	female	female	female	male	female
age	4y	7y 8m	36y 2m	6y 10m	10y 10m	12m	12m	20m	15y	3 y	12y 7m	several m to 41 y	10 m to 62 y	NA
body measurements														
gestational week	NA	39	term	38	NA	NA	NA	NA	38	39	NA	NA	NA	NA
birth weight (g)	4564	2850	2800	2950	3230	2300	2410	2140	2739	2644	3100	NA	NA	NA
birth length (cm)	NA	49	50	49	49	47.5	49	44	NA	47	47	NA	NA	NA
OFC at birth	NA	36	34	36.5	NA	31	32	31.5	NA	NA	36.5	NA	NA	NA
weight (kg) / SD	NA	22 / -0.58	88 / NA	24.3 / 0.63	57.9 / 2.18	6.5 / -2.69	8.5 / -0.42	10.8 / 0.12	NA	12.1 / -1.82	67.7 / 2.01	2x underweight, 15x normal, 5x obese	2x underweight, 3x normal, 6x overweight	14x normal, 4x obese
heigth (cm) / SD	NA	119 / -0.99	180 / 2.56	117.9 / -0.36	158.6 / 2.22	70.5 / -1.37	76 / 0.77	80 / -0.9	NA	92 / -2	161.2 / 0.98	2x short, 17x normal, 4x tall	9x short, 12x normal	2x short, 16x normal
OFC (cm) / SD	NA	51 / -0.63	NA	51.9 / 0.38	53.3 / 0.26	43.5 / -1.86	46 / 0.3	45 / -2.25	NA	49 / -0.71	58.8 / 3.54	4x microcephaly, 14x normocephaly, 2x macrocephaly	5x microcephaly, 25x normocephaly, 6x macrocephaly	3x normal
obesity	no	no	no	no	yes	no	no	no	yes	no	yes	5/25 20%	44/48 91.7%	4/19 21.1%
characteristic facial gestalt	yes	yes	no (subtle)	yes	yes	yes	yes	yes	yes	yes	yes	23/25 92% typical female BFLS	47/47 100% typical male BFLS	16/23 69.6% subtle male BFLS
development														

age at walking	18m	supported (7y)	10m	1y 10m	15m	unsteady sitting (1y)	NA	not yet (1y 8m)	NA	sitting (2y 2m) not yet (3y)	1y 1m	1y to not yet at 7 y	1y 11m to 4 y	NA
first words	2y	no words	normal	3y	1y 6m	not yet	not yet	not yet	no words	no words	3y 6m	15 m to not speaking	4 y to not speaking	NA
current speech ability	NA	only vocalizations	normal	2-3 word sentences	simple sentences	NA	NA	hoarse voice	growls	NA	simple sentences	NA	NA	NA
intellectual disability	moderate	severe/ profound	no	moderate	mild-moderate	mild	moderate	severe	severe	moderate	moderate	24/27 88.9%	54/54 100%	6/32 18.8% learning disability
neurological														
seizures	no	no	no	no	no	no	no	yes	no	no	no	5/23	3x	2x
MRI anomalies	NA	prominent outer cerebrospinal fluid space	NA	NA	periventricular white matter lesions, frontal subcortical heterotopia	small pituitary gland	NA	delayed myelination, white matter loss, dysplastic pons, 3rd ventriculo-megaly	NA	abnormal position of the cerebellar tonsil	normal MRI	10/15	NR	NR
behavioral anomalies	no	too impaired to assess	no	no	earlier physically, now more verbally abusive	no	NA	too impaired to assess	NA	happy demeanor	shy, anxious, friendly demeanor	6/15	13/34	1x
extremities														
finger anomalies	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	24/25 96%	16/16 100% reported as common in the rest	8/21 38.1%
clinodactyly	V	IV	IV	IV + V	no	IV + V	no	V	no	no	no	16/25 64.0%	5/16 31.2%	0/21 0%
brachydactyly	V	no	no	no	no	no	no	V	V	no	V	13/25 52.0%	3/16 18.8%	0/21 0%
camptodactyly	no	no	no	IV + V	several fingers	no	no	no	no	no	no	3/25 12.0%	0/16 0%	0/21 0%
tapering fingers	no	no	no	no	yes	no	no	no	no	yes	yes	7/25 28.0%	11/16 68.8%	7/21 33.3%
hypoplastic nails	no	no	no	no	no	no	dysplastic, brittle	hypoplastic V	hypoplastic V	no	no	8/25 32.0%	2/16 12.5%	1/21 4.8%
toe anomalies	no	yes, crowded toes	yes, crowded toes	yes, II broad	yes	yes	yes	yes	yes	yes	yes	21/23 91.3%	21/22 95.5%	9/21 42.8%
brachydactyly	no	no	no	no	no	no	no	yes	no	no	IV + V	5/23 21.7%	12/22 54.5%	8/21 36.4%
camptodactyly /hammer toes	no	no	yes	III, IV + V	II + III	no	no	no	no	no	no	6/23 43.5%	common in a sample of 25[22]	0/21 0%

sandal gap	no	no	no	no	no	no	no	no	no	no	yes	2/23 8.7%	13/22 59.1%	1/21 4.8%
syndactyly	no	no	no	no	no	II-IV right, II-III left	minimal II-III	no	II-III	II-III	II-III	10/23 43.5%	4/22 18.2%	0/21 0%
hypoplastic nails	no	dysplastic V	dysplastic V	no	no	no	dysplastic, brittle	hypoplastic	no	no	no	7/23 30.4%	1/22 4.5%	1/21 4.8%
other														
linear skin hyper-pigmentation	no	yes (both thighs)	no	no	yes (groin + armpits)	no	yes (legs)	no	yes (arm + trunk)	yes (trunk, bottom + legs)	no	17/25 68.0%	NR	0/1, NR
dental anomalies	no	small teeth, misalignment	misalign-ment	enamel defect	hypodontia, large roots	NA	small teeth	hypodontia	NA	NA	yes	18/20 90.0%	1x small teeth, widely spaced	0/1, NR
oligo-/ amenorrhea (age at menarche)	NA	NA	yes (13y)	NA	NA	NA	NA	NA	NA	NA	not yet	10/10 100%	44/45 97.8% hypogonadism or small external genitalia	1/15 6.7% oligo-menorrhea
genital anomalies	no	hypoplastic labia minora	no	no	no	no	no	no	hypoplastic clitoris	no	NA			
eye anomalies	no	strabism, hyperopia, excavation of the papilla	myopia	strabism, impaired stereo vision, astigmatism	progressive retinal depigmenta-tion, maculopathy	no	NA	no	no	NA	nystagm, hyperopia, retinal depigmenta-tion, maculopathy	abnormalities in 13/15	abnormalities in 3/5	NR
other	no	muscular hypotonia	no	ectopic kidney, initially muscular hypotonia	unilateral hydro-nephrosis, congenital umbilical hernia, mild hearing impairment	renal pilocalyceal dilatation	hyertrichosis at the back	cleft hard and soft palate, possible hearing impairment	no	feeding difficulties	muscular hypotonia	NA	NA	NA

XLR, x-linked recessive (obligate female carriers with proven variant either in the female herself or in the family); y, years; m, months; NA: not available or not applicable; NR: not reported; XLR: X-linked recessive; II, III, IV, V, 2nd, 3rd, 4th, 5th finger or toe, respectively

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