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## SHORT REPORT

Mutation screening in Börjeson-Forssman-Lehmann syndrome: identification of a novel de novo *PHF6* mutation in a female patient

J Crawford, K M Lower, R C M Hennekam, H Van Esch, A Mégarbané, S A Lynch, G Turner, J Géczy



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**Background:** Börjeson-Forssman-Lehmann syndrome (BFLS; MIM 301900) is an infrequently described X linked disorder caused by mutations in *PHF6*, a novel zinc finger gene of unknown function.

**Objective:** To present the results of mutation screening in individuals referred for *PHF6* testing and discuss the value of prior X-inactivation testing in the mothers of these individuals.

**Results:** 25 unrelated individuals were screened (24 male, one female). Five *PHF6* mutations were detected, two of which (c.940A→G and c.27\_28insA) were novel. One of these new mutations, c.27\_28insA, was identified in a female BFLS patient. This was shown to be a de novo mutation arising on the paternal chromosome. This is the first report of a clinically diagnosed BFLS female with a confirmed *PHF6* mutation. In addition, the X-inactivation status of the mothers of 19 males with suggested clinical diagnosis of BFLS was determined. Skewed (≥70%) X-inactivation was present in five mothers, three of whom had sons in whom a *PHF6* mutation was detected. The mutation positive female also showed skewing.

**Conclusions:** The results indicate that the success of *PHF6* screening in males suspected of having BFLS is markedly increased if there is a positive family history and/or skewed X-inactivation is found in the mother.

that approximately 50% of XLMR carriers showed markedly (≥80%) skewed patterns of X-inactivation, compared with approximately 10% of the control population. In several instances where obligate carriers were found to have pronounced skewing, the pattern of skewing was consistent throughout the family. Screening of *PHF6* in individuals with a suggested clinical diagnosis of BFLS immediately following characterisation of the gene identified mutations in all seven of a group of familial BFLS cases, but in only two of 13 sporadic cases.<sup>2</sup> Based on our initial observation of frequently and consistently skewed X-inactivation in *PHF6* mutation carriers,<sup>2</sup> we investigated the use of X-inactivation screening in the mothers of males with a suggested clinical diagnosis of BFLS as an indicator of their *PHF6* mutation carrier status. Here we outline our experience with *PHF6* mutation screening, supply a brief family history of the previously unreported BFLS patients identified in this study, and compile all currently reported *PHF6* mutations.

## METHODS

## Patients

Since publication of the BFLS gene *PHF6*,<sup>2</sup> 25 patients with clinical features suggestive of BFLS have been referred for mutation testing to our laboratory. Photographs and detailed clinical history of the five patients found to have a *PHF6* mutation were obtained from the referring clinicians before mutation detection. A brief family history of the three unpublished patients is outlined in the results section of this paper. Patient consent and ethics approval were obtained locally by the referring clinician for all cases.

## X-inactivation studies

X chromosome inactivation status was studied in DNA extracted from white blood cells from the mothers of boys with a suggested clinical diagnosis of BFLS, and in the single girl clinically diagnosed as having BFLS (QIAamp DNA blood maxi kit, Qiagen Inc, Valencia, California, USA). The X-inactivation analysis was undertaken using three polymerase chain reaction (PCR) based assays: *HpaII* digestion of DNA followed either by amplification of the androgen receptor (*AR*) gene polymorphic CAG repeat,<sup>6</sup> or of the *FMRI* gene variable CGG repeat,<sup>7</sup> or amplification of the phosphoglycerate kinase-1 (*PGK-1*) gene followed by digestion of the polymorphic *BstXI* site.<sup>8</sup> *PGK-1* analysis was carried out only when the patient was uninformative at both *AR* and *FRAXA* loci. Initial analysis of *AR* and *FMRI* repeat regions involved incorporation of radioactive <sup>32</sup>P into each amplicon and electrophoresis of resultant labelled products on denaturing

Börjeson-Forssman-Lehmann syndrome was first described in 1962 in a family with severe intellectual disability, characteristic craniofacial features, microcephaly, hypogonadism, obesity, short stature, and epilepsy.<sup>1</sup> Identification of the BFLS gene, *PHF6*,<sup>2</sup> has enabled the causative mutation in this original family to be determined.<sup>3</sup> A recent clinical review of 25 affected males from nine unrelated families with *PHF6* mutations showed that the phenotype is seldom as severe as that seen in the original patients. Clinical manifestations are quite variable, with the most consistent features being initial hypotonia, mild to moderate intellectual disability, large fleshy ears, underdeveloped genitalia, gynaecomastia, truncal obesity, tapering fingers, and shortening of the fourth and fifth toes.<sup>4</sup> Heterozygote females may have a milder similar clinical phenotype, which can include hypothyroidism; however, many carrier females appear unaffected. Our initial research indicated that the majority of females carrying known *PHF6* mutations show highly skewed X-inactivation in their blood leucocytes.<sup>2</sup> Plenge *et al*<sup>5</sup> analysed X-inactivation skewing in female carriers from 24 families segregating 20 distinct X linked mental retardation (XLMR) disorders. They found

**Abbreviations:** AR, androgen receptor; BFLS, Börjeson-Forssman-Lehmann syndrome; ORF, open reading frame; XLMR, X linked mental retardation

**Table 1** *PHF6* specific primers used for polymerase chain reaction amplification and direct sequencing

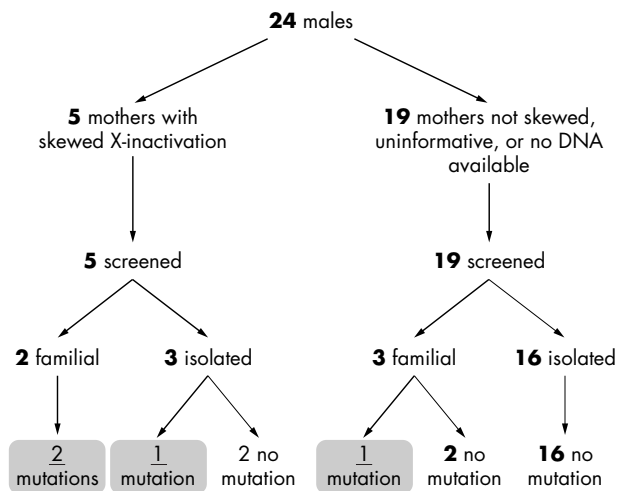
Primer name	Sequence (5' to 3')	Size (bp)	Product (bp)
PHF6ex1-F	GTA TCA ACG CTC TGT GGG TC	20	273
PHF6ex1-R	GGC CAC CGC CAT CCT CC	17	
PHF6ex2-F	AAA ATT AAC ATT GTC GCC CTT C	22	287
PHF6ex2-R	GAA CAT TCA TGT GTT ATT AAG AG	23	
PHF6ex3-F	GCC ATT TTT ACT AGA AAA TTA CC	23	235
PHF6ex3-R	TTC TCA AGA GGC AGA AAT ACC	21	
PHF6ex4-F	TTT TCA ATA ACC AAT TTG TTT TCC	24	310
PHF6ex4-R	TTT TTA CAA AAG CCC AAA GAA AG	23	
PHF6ex5-F	TTG GGT GAA GTG TAC TGC TC	20	245
PHF6ex5-R	GAA TGT TGA GAT ATG TCT TAT GG	23	
PHF6ex6-F	AAC TAA TAC TTA TTT TGA GAT TGG	24	314
PHF6ex6-R	CAT TTC AAA TGA TGA ACT TTA CC	23	
PHF6ex7-F	ATG TTA AGT AAG CTT GAA ATA CC	23	250
PHF6ex7-R	CAA AAT TGG GCT TAA AAG AAC C	22	
PHF6ex8-F	CAT TTA ATG TTT CTC TCA TAA GG	23	265
PHF6ex8-R	ACT TTA AAT TTT CTG ATG ACT GG	23	
PHF6ex9-F	TCT TTT TCA ATA GAA AAT AGC TG	23	276
PHF6ex9-R	TAA ACT AAT GTC ATC TAT TTA AGG	24	
PHF6ex10-F	CAT CCA CTA ATG TTG GCA GG	20	261
PHF6ex10-R	ATA TAT CAG TGT GTA TTG TAT CC	23	
PHF6polyA-F	TTT TGT TCT AAC AGA CAT TTA GG	23	285
PHF6polyA-R	AAA GCA AGA CTA AAA GGA ACA C	22	
PHF6cDNA $\alpha$ -F	CAT TTC TTG AGA CTT AAA GTG G	22	605
PHF6cDNA $\alpha$ -R	GTT CCA TGG GAG GAT GTG G	19	
PHF6cDNA $\beta$ -F	AAC TGG AGC CCT CAT CAC C	19	756/1086*
PHF6cDNA $\beta$ -R	GGC AGT AAA AAG TTA CAA ACC C	22	

\*Product sizes differ depending on the alternative splicing of intron between exons 10 and 11 (330 bp). bp, base pair.

5% acrylamide gels. We have also used *AR* and *FMRI* amplification with fluorescently labelled primers (FAM and HEX, respectively) with subsequent analysis of labelled amplicons on a 3100-Avant genetic analyser (Applied Biosystems, Foster City, California, USA). The percentage of skewed X-inactivation in the original cell population from which the genomic DNA was derived was then determined by comparison of peak areas in digested versus undigested samples.<sup>9</sup> Digestion conditions, primer sequences, and PCR conditions are available on request.

**PHF6 mutation screening**

Mutation screening was undertaken on either the genomic DNA or EBV transformed lymphoblast cell line RNA of the individuals with a suggested clinical diagnosis of BFLS. Genomic DNA was isolated from patient’s white blood cells using the QIAamp DNA blood maxi kit (Qiagen). Screening was undertaken on exons 2 to 10, a region that encompasses the entire *PHF6* coding region. Primers were designed in intronic sequences such that coding sequences and a minimum of 20 base pairs (bp) of flanking intronic sequence could be analysed by direct sequencing (table 1). Cycling conditions for exons 2, 3, 5, 7, and 8 were as follows: 10 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, followed by 25 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. Exons 4, 6, 9, and 10 were amplified with 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds for 35 cycles. Final extension for all *PHF6* genomic PCRs was 72°C for seven



**Figure 1** Schematic representation of our experience with screening of the *PHF6* gene in patients clinically diagnosed with Börjeson-Forssman-Lehmann syndrome.

minutes. Total RNA was isolated, when available, from patient lymphoblast cell lines with the RNeasy mini kit (Qiagen); 1 µg of total RNA was oligo-dT reverse transcribed using SuperScript II RNase H- reverse transcriptase (Gibco BRL, Gaithersburg, Maryland, USA) following the manufacturer’s instructions. The *PHF6* open reading frame (ORF) was

amplified in two PCR products with *PHF6*-cDNA specific primers (table 1), under the following cycling conditions: initial denaturation at 94°C for two minutes, followed by 35 cycles of 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for one +minute. All amplifications were carried out on 100 ng of genomic DNA or 40 ng of cDNA, in a total PCR volume of 50 µl. PCR products were column purified (QIAquick PCR purification kit, Qiagen) and one or both strands were directly sequenced using Big Dye Terminator v3.0 or v3.1 sequencing chemistry (PE Applied Biosystems).

The following GenBank reference sequences were used: for *PHF6* cDNA, NM\_032335; for *PHF6* protein, NM\_032335; and for *PHF6* genomic DNA, AL591668.

## RESULTS

### X-inactivation analysis of *PHF6* mutation carrier females

We had access to DNA from 19 mothers of the 25 clinically referred BFLS patients. We have identified five mothers with skewed (>70%) X-inactivation, three of whom have sons with *PHF6* mutations. In addition, we tested for, and found, highly (>90%) skewed X-inactivation in the only one BFLS diagnosed female. This pick up rate differs significantly from that seen for isolated males with a suggested clinical diagnosis of BFLS for whom the mother's X-inactivation status was unknown, uninformative, or not skewed. In the 16 such cases screened, we failed to detect any *PHF6* mutation (fig 1).

### Mutation analysis in *PHF6*

We have conducted mutation screening in a cohort of 25 unrelated individuals clinically suspected of having Börjeson-Forssman-Lehmann syndrome. Screening of the *PHF6* ORF has identified mutations in five of these individuals, four male and one female. Two of the males had the recurrent p.R342X mutation and are published elsewhere.<sup>3</sup> A brief clinical history of the other two males and the female BFLS patient is outlined below. The c.2T→C/p.M1T mutation seen in case 2 has been identified in a previously reported family.<sup>2</sup> Both the c.940A→G/p.I314V and c.27\_28insA/p.G10fsX21 mutations, identified in cases 1 and 3 respectively, are unique to this study. The c.940A→G mutation (case 1) results in a replacement of isoleucine for valine at position p.314. This isoleucine is 100% conserved among man, orang-utan, cow, chicken, rat, mouse, xenopus, pufferfish, and zebrafish (data not shown). Photographs of the BFLS female patient (case 3) are presented in fig 2A, reproduced with permission. Mutation identification in this individual is outlined in fig 2B and described in further detail below. Family history appears

to be a strong indicator of subsequent *PHF6* mutation detection, with mutations identified in three of the five familial cases studied (fig 1). There were no *PHF6* nucleotide changes detected in the other 20 patients screened and none of the mutations identified in this study was seen on at least 50 control chromosomes.

### Identification of a novel *PHF6* mutation in a female BFLS patient

Case 3 was unique in that the BFLS presenting proband was female (fig 2A). We first assessed the X-inactivation of this patient. Upon finding highly (93%) skewed X-inactivation, we screened her *PHF6* locus on genomic DNA. Exonic sequencing revealed a single nucleotide insertion in exon 2 (c.27\_28insA, fig 2b), presumably leading to a subsequent frameshift and addition of 21 novel amino acids to the *PHF6* protein (p.G10fsX21). This mutation, which apparently results in a premature stop codon in exon 2, was the only sequence variant detected in the *PHF6* open reading frame. Sequencing of DNA from the proband's parents showed that the c.27\_28insA mutation arose de novo, as it was not present in either parent. X-inactivation analysis indicated that the proband had the maternal chromosome active in 93% of peripheral blood cells (data not shown). The mother of the proband also had skewed X-inactivation (28:72). To determine the parental chromosome on which the c.27\_28insA arose, we have taken advantage of an intronic SNP (intron 2, IVS2+203G/A) in close distance from the exon 2, where the c.27\_28insA mutation lies. While the paternal chromosome had allele G at position IVS2+203 and the maternal chromosomes were both IVS2+203A, the proband is a IVS2+203G/A heterozygote. Subcloning and subsequent sequencing of genomic PCR products across exon 2, intron 2, and exon 3 (primers *PHF6*x2-F and *PHF6*x3-R, 597 bp product) showed that the c.27\_28insA mutation was on the chromosome with the IVS2+203G allele—that is, on the paternal chromosome (results not shown).

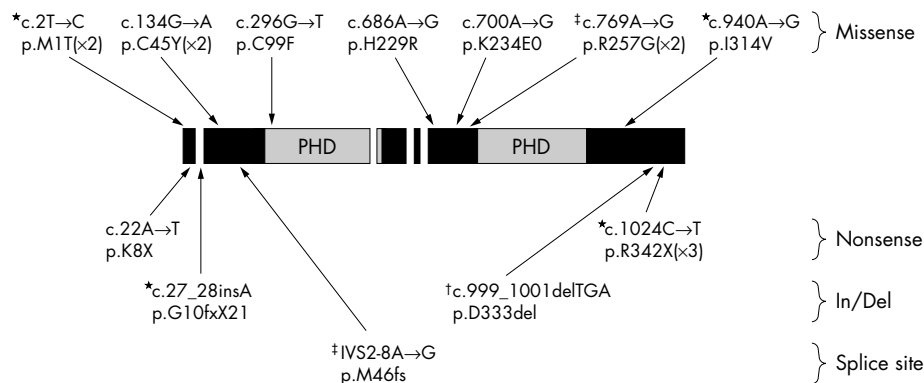
### Brief family history of newly identified BFLS patients

#### Case 1 (c.940A→G/p.I314V)

The proband was born after 42 weeks with a birth weight of 3750 g. Psychomotor developmental delay was recognised early; he did not walk until two years three months and did not begin to talk until four years. He has an IQ of 61 and has major behavioural problems, one of the most pronounced being lack of any inhibition in sexual contacts. At 18 years he was 171.5 cm tall with a head circumference of 55.7 cm, moderate obesity, and marked gynecomastia. External



**Figure 2** Female patient with Börjeson-Forssman-Lehmann syndrome and an identified *PHF6* mutation (case 3). (A) Two photographs of the patient, a side profile showing a shallow forehead, large ears, and fleshy ear lobes, and a frontal photograph showing a shallow forehead, deep set eyes, and prominent supraorbital ridges. (B) Sequencing traces illustrating reverse sequencing reactions of *PHF6* exon 2 product of case 3 together with a control normal male trace; n, normal allele; m, mutant allele with the single nucleotide insertion, c.27\_28insA; the arrow indicates the position of insertion of "A" where the sequences of the n and m alleles start to differ; arrowheads indicate the orientation of the sequencing reaction; the filled circle indicates the proband, the empty square a control male. The written permission of the patient's parents was obtained for reproducing the photographs in panel A.



**Figure 3** Schematic representation of the *PHF6* gene with all mutations identified to date that cause the Börjeson-Forsman-Lehmann syndrome (BFLS). Mutations identified in this study (c.2T→C, case 2; c.940A→G, case 1; and c.27\_28insA, case 3). †Mutation reported by Baumstark *et al.*<sup>10</sup> ‡Mutations recently published by Vallee *et al.*<sup>11</sup> Unmarked mutations are those previously reported by our group.<sup>2</sup> Respective parts of the *PHF6* gene open reading frame coding for nuclear localisation sequences are shown in white and those coding for PHD domains indicated. Four mutations are recurrent, with p.M1T, p.C45Y and p.R257G each found in two unrelated BFLS families, and p.R342X found in three unrelated BFLS families.

genitalia showed normal post-pubertal development for age. His limbs showed hypermobility with Y-shaped cutaneous syndactyly on both feet. The X-inactivation testing on the mother of the proband showed a 100% skewed X-inactivation pattern.

#### Case 2 (c.2T→C/p.M1T)

The proband from this family was born at term after an uneventful pregnancy. His early milestones were delayed; he walked at four years and began saying a few words at five years. At approximately 15 years his height was 140 cm (<3rd centile), weight was 45 kg (5th centile), and occipito-frontal head circumference (OFC), 53.3 cm (10th–25th centile). On physical examination there was a proportionate short stature, triangular face, hyperplastic supraorbital ridges, deep set eyes, large ears, gynaecomastia, and lower abdominal obesity. External genital examination revealed a small penis and small testes with no evidence of pubertal change. The X-inactivation testing on the mother of the proband showed random pattern of X-inactivation.

#### Case 3 (c.27\_28insA/p.G10fsX21)

This proband is a girl who presented with BFLS-like symptoms. The pregnancy was uneventful. Her birth weight was 3972 g. She never crawled but she walked at one year. However, her coordination was poor and she fell several times. Her speech was delayed and she attended a speech therapist at three years. She had words at 12–15 months, but these were indistinct. She had a full psychometric test (WPPSI-R) aged four years eight months and her overall IQ was scored at 84 (verbal scale IQ, 87; performance IQ, 84). Her thyroid function was detected as abnormal at seven to eight years; she has had a high level of thyroid stimulating hormone with a low thyroxine (T4). Her S-thyroglobulin antibodies were weakly positive (385; normal range 0 to 325). At 14 years the patient is 180 cm tall (>98th centile) and weighs 115 kg (>98.6th centile). She has an OFC of 62 cm (>97th centile) with a remarkably shallow forehead. She has fleshy earlobes, deep set eyes with a wide neck and face (see fig 2a). She has broad feet and hammer toes. At the time of examination (14 years) it was noted she had auxiliary and pubic hair but had not yet begun menstruation. She is tactile defensive and emotionally labile, has poor spatial awareness and startles easily to noise. Testing of her blood DNA showed a 93% skewed X-inactivation pattern.

#### *PHF6* mutation summary

Amalgamating the results of our study with those published by others gives a total of 12 familial and five sporadic cases of BFLS recognised so far with the identified *PHF6* gene mutation.<sup>2 3 10 11</sup> There are currently 12 different *PHF6* mutations identified from 17 unrelated BFLS families, with four of these mutations (c.2T→C; c.134G→A, c.769A→G, and c.1024C→T) being recurrent (fig 3). Missense mutations are the most frequent ones, found in 10 of the 17 cases. Interestingly, *PHF6* mutations are not clustered and they do not seem to predominantly affect the only two recognised functional domains of the protein, the PHD-like zinc fingers. In fact just the opposite is seen, with the majority of mutations found outside the PHD finger regions. These observations, along with the lack of notable genotype-phenotype correlations, lead us to propose that the majority, if not all, *PHF6* mutations might cause loss of function of the *PHF6* protein.

#### DISCUSSION

Since the identification of the causative gene for BFLS in 2002,<sup>2</sup> we have analysed 25 clinically diagnosed cases received from colleagues worldwide (fig 1). Given the size of the gene (10 exons) we preferentially screened affected individuals on cDNA generated from lymphoblast cell line (LCL) RNA; however, as LCLs were not available for every patient, we also undertook screening on genomic DNA (supplementary table 1, which can be viewed on the journal website: <http://www.jmedgenet.com/supplemental>). Analysis of mutation detection outcomes indicated that, as expected, family history was a strong indicator of subsequent *PHF6* mutation detection, with mutations identified in three of the five referred familial cases. Skewed X-inactivation in carrier females was another attribute that indicated increased likelihood of *PHF6* mutation detection, with *PHF6* mutations being carried by three of the five mothers with skewed X-inactivation. The single clinically diagnosed BFLS female in whom we identified a *PHF6* mutation also showed a skewed pattern of X-inactivation. A detailed flow chart of the results of *PHF6* screening is shown in fig 1.

Based on the analysis of the initial BFLS families<sup>2</sup> we expected to see concordance between BFLS carrier status and skewing of X-inactivation. However, as the number of characterised BFLS cases has increased, the percentage of obligate carrier females with highly skewed (>90%) X-inactivation has decreased. Combining the data from this and our previous study,<sup>2</sup> we have now data on carrier females

from 13 unrelated BFLS families. Of these, in three families the carrier females show random X-inactivation and in a further three the carrier females show approximately 70% skewing.<sup>2,3</sup> However, carriers in the seven remaining families show >90% skewed X-inactivation as tested on blood leucocyte DNA. Taken together, in about 50% of BFLS families obligate carriers show highly (>90%) skewed X-inactivation. There does not seem to be a correlation between the X-inactivation and the type or location of the *PHF6* mutation. While in three families with the c.1204C→T (p.R342X) mutation we see occasionally skewed (>70%), but more frequently highly skewed (>90%), X-inactivation in blood of obligate carriers,<sup>2,3</sup> for the c.2T→C (p.M1T) mutation one obligate carrier is 100% skewed and one shows random X-inactivation (2 and case 2 in this study).

It must be recognised that although knowledge of X-inactivation skewing in the mother of the proband, together with familial occurrence of the BFLS phenotype, is the best predictor of the detection of a *PHF6* mutation, some *PHF6* mutations (including de novo mutations) may be missed if screening is based solely on these criteria. There also remains the question of the genetic cause in patients with clinically diagnosed BFLS for whom we have not detected a *PHF6* mutation. Underlying genetic heterogeneity (well known for XLMR disorders) provide one explanation. However, no BFLS families suggesting this have been reported. Another, more likely explanation is that the clinical features that raise the possibility of a BFLS diagnosis are so protean that over-diagnosis and overlap with Prader-Willi, Wilson-Turner, Cohen, and Klinefelter syndromes may be common.<sup>4</sup> Alternatively, individuals diagnosed with BFLS and tested negative for *PHF6* mutations in the coding region could harbour mutations not detected by our screening strategy, such as changes in short or long range regulatory elements, copy number imbalances, or other rearrangements. Our current and previous<sup>2</sup> gene resequencing analyses of controls and patients have failed to detect any single nucleotide variation within the coding region of the *PHF6* gene, allowing us to rule out potential "splicing spoilers" as causative of BFLS.<sup>12</sup>

The *PHF6* mutations identified thus far are not clustered within a particular domain, for instance within the PHD-like zinc finger domains (in fact only one mutation occurs in this region), nor are they concentrated in any other region of the protein. The mutations occur along the length of *PHF6*, and include missense, nonsense, splice site, deletion, and insertion mutations (fig 3). There is no clear link between mutation type or location, and the severity of the disease.<sup>4</sup> There is one potential mutational hot spot in exon 10, c.1024C→T, which has been found in three cases (of the 17 known) carrying a *PHF6* mutation. The nucleotide C at position c.1024 is part of a CpG dinucleotide, known to be often mutated in humans.<sup>13</sup>

The identification of a female BFLS patient with a *PHF6* mutation (case 3) is novel. Unlike many female *PHF6* mutation carriers studied so far, she is affected. Varying expression of clinical features in female carriers had been reported<sup>4,14</sup> and as such this case may represent an extreme. A likely explanation of such a varying heterozygous expression may lie in differences in X-inactivation skewing between tissues (for example, brain versus blood), or a probable non-cell-autonomous effect of the *PHF6* mutation harbouring cells on those with normal *PHF6*, similar to the observations made in Rett syndrome.<sup>15,16</sup> The identification of additional female BFLS cases or the generation of a suitable animal model will help address this important issue.

In conclusion, we have screened a total of 25 unrelated individuals with suggested clinical diagnosis of BFLS (24 males and one female) and detected five *PHF6* mutations,

two of which have not been identified previously (case 1 and case 3, fig 2). We assessed the X-inactivation status of the mothers of 19 of the clinically diagnosed BFLS patients. We saw skewed ( $\geq 70\%$ ) X-inactivation in five of these cases, with three *PHF6* mutations subsequently detected (two p.R342X and one p.I314V (case 1)). We identified a further *PHF6* mutation in a family where the obligate carrier mother did not show a skewed pattern of X-inactivation (p.M1T (case 2)). The fifth mutation was identified in a female BFLS patient (p.G10fsX21; case 3, fig 2) and was identified as a de novo mutation arising on the paternal chromosome. Before the ascertainment of case 3, we had identified carrier females (ranging in phenotype from asymptomatic to mildly affected) only through an affected male relative. Case 3 was the only referred female BFLS patient. Clinically diagnosed female BFLS patients have previously been reported,<sup>17-19</sup> but never with subsequent genetic confirmation of the diagnosis. Based on this finding and the published reports, mutation screening of the *PHF6* gene in clinically diagnosed BFLS female patients should be considered, particularly if they show a skewed pattern of X-inactivation.

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The supplementary table can be seen on the journal website (<http://www.jmedgenet.com/supplemental>)

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Conflicts of interest: none declared

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## CORRECTION

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In the Original article titled, Oculocerebrocutaneous syndrome: the brain malformation defines a core phenotype (*J Med Genet* 2005;**42**:913-921) the supplementary figures were missing from the paper. The supplementary figures are available on the JMG website at <http://www.jmedgenet.com/supplemental>.