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LETTER

1024C>T (R342X) is a recurrent *PHF6* mutation also found in the original Börjeson–Forssman–Lehmann syndrome family

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The Börjeson–Forsmann–Lehmann syndrome (BFLS, (MIM 301900)) was first described by one of us in 1962 in a family with severe X-linked mental retardation, short stature, obesity, hypogonadism, narrow palpebral fissures and extremely large ears with prominent ear lobes.[1] Since then an autopsy on one of the cases in the family has been reported,[2] highlighting CNS malformation and endocrine organ hypoplasia as histological features of BFLS. Two new affected males have been born into the family (V-1 and V-2; Figure 1).

In 2002, Lower *et al*[3] identified mutations in the *PHF6* gene, as the cause of BFLS in seven familial and two sporadic cases. In one of these families[4] (referred to as Family 2), the mutation was found to be 1024C>T (R342X).[3] We have now shown that this mutation is also present in the original BFLS family (referred to as Family 1) and in a newly identified family from Western Australia (referred to as Family 3).

One of the new cases in the original BFLS family (V-I, Figure 2a) was born at term with a birth weight of 3020 g. Developmental delay was recognised early and assessment at 10 months showed upslanting palpebral fissures, large ears, a high palate, hypotonia, cryptorchidism and an inguinal hernia. Now at age 15 years, he can talk with three or four word sentences, write his name and dress himself. He has short stature and obesity, with a height 2 standard deviations (SD) below the mean and a weight 3 SD above the mean for his age. He has large ears and earlobes,

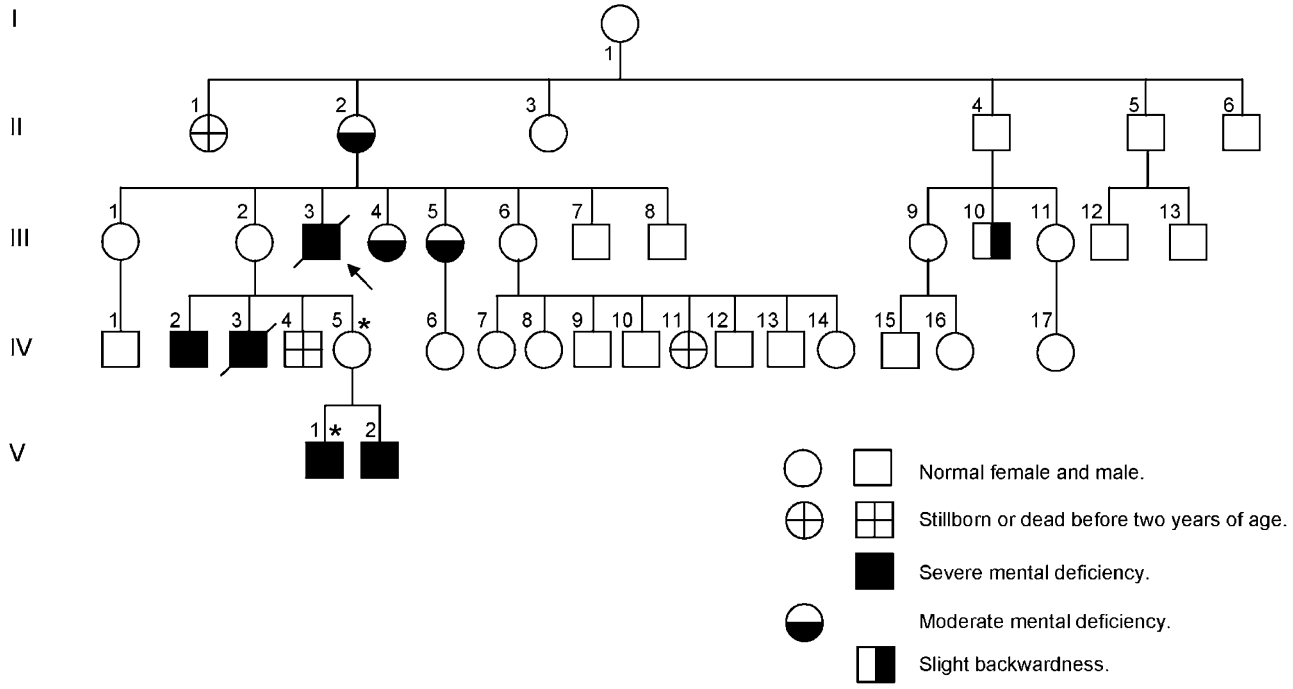

Figure 1 Redrawn and updated pedigree of the original family reported by Börjeson *et al.*[1] Only one new generation was added, generation V, with two affected boys (V-1 and V-2), sons of IV-5. Individual IV-4 died neonatally and his affected status is not known. Asterisks denote individuals shown in Figure 2 and the original proband in the family is indicated by an arrow.

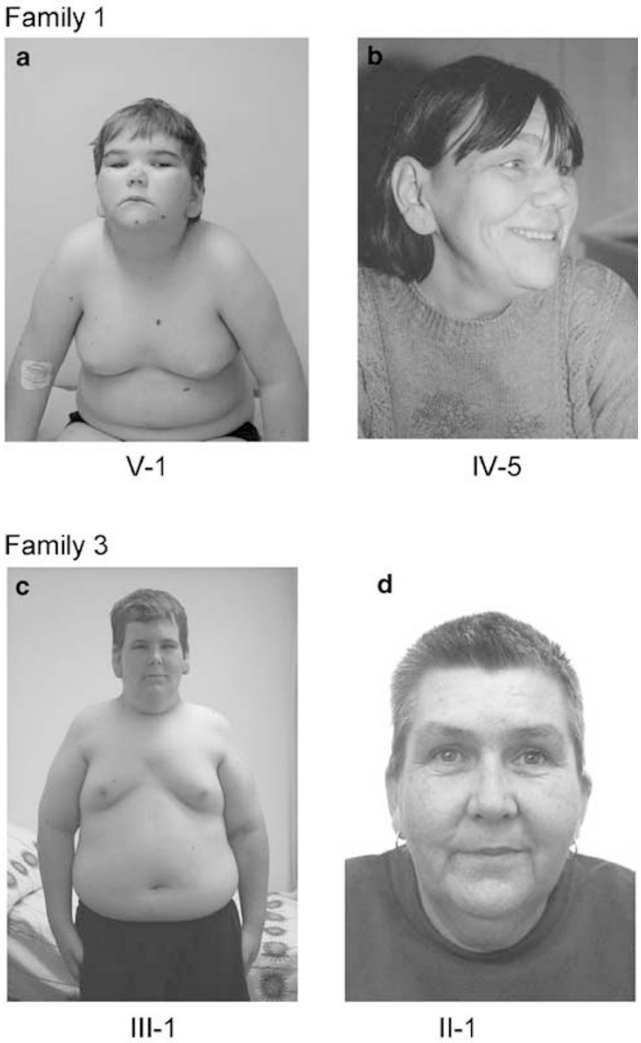

Figure 2 Photographs of affected BFLS males and carrier females from Families 1 (the original Swedish BFLS family) and three (the new Western Australian family). Family 1: (**a**) left panel, affected boy (V-1 in Figure 1) at 15 years of age; (**b**) right panel, mother (IV-5 in Figure 1); Family 3: (**c**) left panel, affected boy at 16 years of age (III-1 in Figure 3); (**d**) right panel, mother (II-1 in Figure 3).

protruding cheeks, broad feet with bilateral syndactyly between toes 2 and 3 and underdevelopment of toes 4 and 5. His mother (Figure 2b) has normal cognitive function and reflexes but has always been clumsy in running and jumping. She has big hands, big ears and feet with some sensory impairment in the legs. MRI scan and EEG were normal but nerve conduction studies showed a moderate axonal polyneuropathy. Similar evidence of a polyneuropathy was found in her brother with BFLS and in both her affected sons. This has not been reported in other BFLS families,[5] but it may well have been overlooked. It may prove to be a previously unrecognised manifestation of the syndrome.

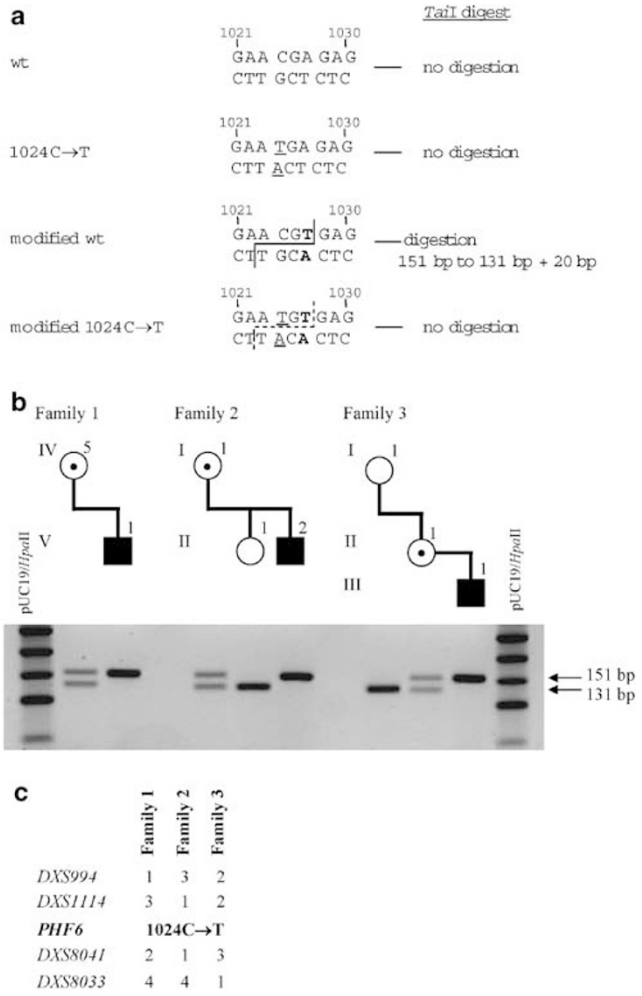

Figure 3 Restriction digest and haplotype analysis of the 1024C>T mutation in all three families. (**a**) A modified reverse PCR primer was designed such that a *Tai*I restriction site (5′-ACGT-3′) was created at bp position 1023–1026 of the wild-type (wt) *PHF6* gene. For this purpose, the A nucleotide at position 1026 was changed via the reverse PCR primer to T, shown in bold. Presence of the 1024C>T mutation (underlined) results in the abolition of this restriction site. (**b**) Following *Tai*I digestion of the 151 bp PCR product, the wild-type allele gives a 131 bp and a 20 bp band, the 1024C>T allele gives a 151 bp undigested band, and carriers show all bands after separation on a 1.5% agarose gel. Partial pedigrees of the three BFLS families carrying this mutation are shown above the gel picture. Nucleotides are numbered in respect to cDNA sequence, with 1 being the A in the start methionine ATG. The 20 bp band is not shown. (**c**) Haplotype analysis of *PHF6* alleles carrying the 1024C>T mutation from three families (Family 1, Family 2 and Family 3). Four flanking microsatellite markers from the proximity of the *PHF6* gene (two each side) were used. The approximate distances between individual markers and *PHF6* are as follows: *DXS994* ~ 3000 kb-*DXS1114*-200 kb-*PHF6*-140 kb-*DXS8041*-390 kb-*DXS8033*.

The proband in Family 3 (Figure 2c) was born normally at 36 weeks gestation with a birth weight of 2985 g. He was hypotonic and jaundiced in the newborn period and was slow to achieve his developmental milestones. His mother described him as a 'very good baby' who never cried and only woke for feeds. Now at 16 years, he attends a special school. His height is on the 25th centile, weight is 14 kg above the 97th centile and head circumference is 0.5 cm above the 98th centile. He has a narrow bitemporal diameter with deep-set upslanting eyes, ptosis, prominent supraorbital ridges, hypotelorism, very long ears (8 cm) and ear lobes (3 cm) and a prominent chin. He is obese with marked gynaecomastia, a hypoplastic scrotum with retractile testes and a small penis. He has cubitus valgus, tapered fingers with short fourth and fifth metacarpals and clinodactyly of his fifth fingers. He has an increased gap between his first and second toes with a short right fourth metatarsal such that his fourth toe is set back in his foot. The rest of his toes are short and his feet are flat. His mother (Figure 2d) is obese with deep-set eyes, prominent supraorbital ridges, large ear lobes, tapered fingers, broad feet and short toes. Illustrations and clinical descriptions of a mother and son in Family 2 are documented in Turner *et al*.[4]

Mutation analysis of *PHF6* identified the same 1024C>T mutation within exon 10 in all three families. We designed a simple test to screen for this mutation. A modified exon 10 reverse primer E10Rm, 5′-TCCTCGGCTTTTACTCTCAC-3′, where T at the penultimate 3′ end position is changed to A-underlined, used in conjunction with the E10Fw primer (Lower *et al*,[3] 5′- CATCCACTAATGTTGGCAGG-3′) generates a *Tai*I restriction site on a normal allele and not on the 1024C>T allele (Figure 3a).

Further investigation showed that the 1024C>T mutation is likely to have appeared *de novo* in Family 3 since the grandmother does not carry it (Figure 3b). Genealogical and microsatellite data (Figure 3c) confirm that the 1024C>T mutation is not identical by descent, but rather arose independently in all three families. The vast majority (~68%) of human genetic mutations are single-base pair substitutions and about 30% of these occur at CpG dinucleotides.[6,7] Point mutations at arginine residues (R) account for nearly 15% of missense mutations.[8] Four out of six arginine codons (CGA, CGU, CGC, and CGG) contain 5′-CpG dinucleotides potentially subject to methylation. Based on our observation of three unrelated 1024C>T (R342X) mutations, it is likely that the 5′-CpG dinucleotide of *PHF6* at position 1024–1025, which is part of the arginine 342 codon (R342, CGA), is methylated; however, this was not investigated further.

The phenotype within and between families with BFLS shows more variation than previously appreciated, and genotype/phenotype correlations have not yet clearly emerged.[5]

In conclusion, we have found a *PHF6* mutation in the family, which defined BFLS 42 years ago. As 25% (three out 12) of all the families with BFLS so far known have this same mutation, and the mutation occurs in a hypermutable 5′-CpG dinucleotide codon for amino-acid arginine (CGA), we speculate that this may be a mutational hot spot in the gene.

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