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Pathogenesis of Börjeson-Forssman-Lehmann Syndrome: Insights from PHF6 Function

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Abstract

Intellectual disability encompasses a large set of neurodevelopmental disorders of cognition that are more common in males than females. Although mutations in over 100 X-linked genes linked to intellectual disability have been identified, only a few X-linked intellectual disability proteins have been intensively studied. Hence, the molecular mechanisms underlying the majority of X-linked intellectual disability disorders remain poorly understood. A substantial fraction of X-linked intellectual disability genes encode nuclear proteins, suggesting that elucidating their functions in the regulation of transcription may provide novel insights into the pathogenesis of intellectual disability. Recent studies have uncovered mechanisms by which mutations of the gene encoding Plant Homeodomain (PHD)-like Finger protein 6 (PHF6) contribute to the pathogenesis of the X-linked intellectual disability disorder Börjeson-Forssman-Lehmann syndrome (BFLS). PHF6 plays a critical role in the migration of neurons in the mouse cerebral cortex *in vivo*, and patient-specific mutations disrupt the ability of PHF6 to promote neuronal migration. Interestingly, PHF6 physically associates with the PAF1 transcriptional elongation complex and thereby drives neuronal migration in the cerebral cortex. PHF6 also interacts with the NuRD chromatin remodeling complex and with the nucleolar transcriptional regulator UBF, though the biological role of these interactions remains to be characterized. In other studies, PHF6 mRNA has been identified as the target of the microRNA miR-128 in the cerebral cortex, providing new insights into regulation of PHF6 function in neuronal migration. Importantly, deregulation of PHF6 function in neuronal migration triggers the formation of white matter heterotopias that harbor neuronal hyperexcitability, which may be relevant to the pathogenesis of intellectual disability and seizures in BFLS. Collectively, these studies are beginning to provide key insights into the molecular pathogenesis of BFLS.

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Keywords

X-linked intellectual disability; Börjeson-Forssman-Lehmann syndrome; PHF6; PAF1 complex; Neuronal positioning; Transcription; Heterotopia

X-linked Intellectual disability is a prevalent neurodevelopmental disorder

X-linked intellectual disability (XLID) affects 1 to 3 percent of the population (Bhasin et al., 2006; Larson et al., 2001; van Bokhoven & Kramer, 2010). Clinically, XLID is characterized by a deficit in intellectual function with an intelligence quotient (IQ) of less than 70 before the age of 18 and impairment of adaptive behaviors leading to deficient communication and social interactions (van Bokhoven & Kramer, 2010). Based on IQ score, intellectual disability may be classified as follows: profound (IQ<20), severe (IQ 20–34), moderate (IQ 35–49) and mild (IQ 50–69) (Ropers, 2010). XLID affects more males than females (Ropers, 2010). XLID has been grouped into syndromic and nonsyndromic forms. Nonsyndromic XLID is characterized by intellectual disability in the absence of other symptoms or signs, whereas syndromic XLID patients display intellectual disability together with other developmental abnormalities (Kleine-Kohlbrecher et al., 2010). Gene deletions, duplications, inversions and point mutations on the X chromosome have been associated with XLID. Thus far, mutations in over 100 genes on the X chromosome have been associated with intellectual disability. These mutations affect proteins with functions in transcription, translation, signal transduction and cell cycle regulation (Kleine-Kohlbrecher et al., 2010).

Characterization of XLID-associated proteins has opened up new fields of study in brain development and disease. The X-linked fragile X and Rett syndromes represent two major examples of XLID syndromes that have spawned major lines of research at the intersection of development and cognitive disorders of the brain. Characterized by intellectual disability, autistic features, developmental delay, hyperactivity and attention deficit behavior (Coffee et al., 2008; Huber et al., 2002; Leigh & Hagerman, 2013), fragile X syndrome is caused by expansion of CGG nucleotide repeats in the 5′ untranslated region of the *Fmr1* gene, leading to its transcriptional silencing (Verkerk et al., 1991; Yu et al., 1991). A large number of studies have elucidated functions for the *Fmr1*-encoded protein, FMRP in protein translation and synaptic plasticity (for review, see Leigh & Hagerman, 2013). In mutant mice lacking FMRP, long-term depression triggered by metabotropic glutamate receptors activation is enhanced (Bhakar et al., 2012; Huber et al., 2002). In the cerebral cortex, FMRP knockout mice have increased dendritic spine number with more immature long shaped spines but fewer spines with mature mushroom shape (Irwin et al., 2000). Thus, FMRP regulates both synaptic development and plasticity. At a molecular level, FMRP operates as an RNA-binding protein to control mRNA stability (Zalfa et al., 2007). FMRP may enter the nucleus and assemble with targeted RNAs into mRNP and undergo export to dendrites and regulate local protein synthesis (Eberhart et al., 1996). In fragile X syndrome patients, loss of FMRP function causes deregulation of localized mRNA synthesis, leading to defects in synapse function (Comery et al., 1997; Jin & Warren, 2000).

Rett syndrome patients have normal psychomotor development for the first 6 months after birth but subsequently fail to attain psychomotor milestones and eventually lose language and hand skills (Samaco & Neul, 2011). Mutations in methyl CpG binding protein 2 (MeCP2) cause Rett syndrome (Amir et al., 1999). MeCP2 knockout mice behave normally before weaning age (Guy et al., 2001), but by week eight these mice have unstable gait and reduced spontaneous movement followed by hindlimb clasping (Guy et al., 2001). Induced pluripotent stem cell (iPSC)-derived neurons from patients with Rett syndrome have reduced spine density, soma size and glutamatergic synapses (Marchetto et al., 2010), mimicking neuropathological features of the brain in Rett syndrome patients (Kaufmann & Moser, 2000; Reiss et al., 1993; Samaco & Neul, 2011). MeCP2 binds to methylated cytosine within CpG motifs and has been implicated in the regulation of transcription (Nan et al., 1997). MeCP2 may silence transcription by recruiting co-repressors including histone deacetylases (Hdacs) (Nan et al., 1998; Skene et al., 2010). Remarkably, MeCP2 binds to methylated CA motifs within long genes (>100 kb) to repress transcription, and upon MeCP2 depletion long gene expression is increased (Gabel et al., 2015). Although MeCP2 accumulates in post-mitotic neurons, MeCP2 may also play a role in glial cells and mutations of MeCP2 in glial cells may cause non-cell autonomous defects in neurons (Ballas et al., 2009).

Studies of FMRP and MeCP2 illustrate that significant advances in our understanding of neural development and pathogenesis of intellectual disability can be achieved from systematic studies of individual XLID proteins. Therefore, characterization of Plant Homeodomian (PHD)-like Finger protein 6 (PHF6), which is mutated in the X-linked Börjeson-Forssman-Lehmann syndrome (BFLS), is likely to yield novel insights into our understanding of brain development and intellectual disability.

Börjeson-Forssman-Lehmann syndrome (BFLS)

The Börjeson-Forssman-Lehmann syndrome (BFLS) was first described as an X-linked intellectual disability syndrome by Mats Börjeson and colleagues in 1962 (Börjeson et al., 1962). Since then, additional cases of BFLS patients have been identified. BFLS patients typically have normal birth weight, head circumference and delivery (Lower et al., 2002). However, the main clinical features of BFLS become apparent with age and show considerable variation among different patients (de Winter et al., 2009; Lower et al., 2002). A summary of the clinical features of BFLS is presented in Table 1. Diagnosis of BFLS is typically made clinically based on history and physical examination, and can be confirmed by sequencing of the *PHF6* gene. MRI data in patients with BFLS are lacking. In the few BFLS cases where MRI data are available in the literature, enlarged ventricles have been reported in once case (Table 1). Developmental delay usually appears before the first birthday. All BFLS patients have intellectual disability, ranging from mild to severe, as well as small genitalia and short stature (Gécz et al., 2006; Lower et al., 2002). Approximately 75% of the patients have obesity emerging in late childhood; BFLS patients display microcephaly or macrocephaly at a frequency of 6% and 15% respectively; Epileptic seizures have been observed in approximately 8% of patients (Carter et al., 2009; Di Donato et al., 2014; Mangelsdorf et al., 2009; Zweier et al., 2013). Other features of the syndrome include tapered fingers and broad shortened toes. Adult BFLS patients display coarsening of

facial features with prominence of the supraorbital ridges and deep-set eyes (Gécz et al., 2006; Lower et al., 2002). Other abnormalities observed uncommonly in BFLS patients include mild polyneuropathy, hearing impairment, cleft lip and hypopituitarism (Gécz et al., 2006). Female carriers of BFLS may have learning problems and show physical manifestations including shortened toes, thickened fleshy ear lobes, pronounced supraorbital ridges and deep-set eyes (Gécz et al., 2006; Lower et al., 2002).

Molecular genetics studies have led to the identification of multiple mutations in the *PHF6* gene on the X chromosome in BFLS patients (Berland et al., 2011; Carter et al., 2009; Lower et al., 2002; Turner et al., 2004). Most *PHF6* mutations in BFLS are either missense or lead to truncation of PHF6 protein. Five *PHF6* mutations in BFLS are recurrent including c.2T>C/p.M1T, c134G>A/p.C45Y, c769A>G/p.R257G, c.999-1001delTGA/p.D333del and c1024C>T/p.R342X, found in 21% of patients. The truncation mutation c.1024C>T/p.R342X is found in five different BFLS families (Chao et al., 2010; Crawford et al., 2006; Lower et al., 2002, 2004). The majority of *PHF6* mutations occur in exon 2, which encodes the N-terminus of PHF6 protein. A fundamental question that remains to be addressed is how PHF6 mutations lead to BFLS.

Mechanisms of PHF6 function

The *PHF6* gene, which is located on Xq26-27 between the markers DXS425 and DXS105, is comprised of 11 exons that is transcribed into a 4.5 kb mRNA and translated into a 41 kDa protein with 365 amino acids. Two major isoforms of PHF6 mRNA, depending on whether intron 10 is spliced out, give the same protein product (Gécz et al., 2006). PHF6 protein is highly conserved in vertebrates. Structurally, PHF6 protein contains two plant homeodomain (PHD)-like zinc fingers, two nuclear localization sequences and a nucleolar localization sequence (Liu et al., 2014), suggesting that PHF6 may play a role in the regulation of transcription. PHD zinc fingers are structurally conserved modules that interact with chromatin or mediate protein-protein interactions (Sanchez & Zhou, 2011). The Cys_4-His-Cys_3 motifs within the PHD domain of PHF6 coordinate two Zn^{2+} ions and stabilize PHF6 protein structure (Liu et al., 2014). The evolutionary conserved PHF6 cysteine residues C45, C99 and C305 are targeted by missense mutations in BFLS. PHD fingers also recognize specific histone modifications (Aasland et al., 1995; Sanchez & Zhou, 2011; Wysocka et al., 2006), and regulate the function of epigenome readers in transcription via recruitment of multi-protein complexes. However, unlike other PHD domain proteins, PHF6 contains two imperfect PHD domains (Liu et al., 2014). Therefore, it is unclear whether the PHD domains in PHF6 function similarly as other PHD domains to recognize specific chromatin marks.

Employing an unbiased computation-assisted proteomics approach, Zhang et al. discovered that PHF6 associates with subunits of the PAF1 transcription elongation complex including PAF1, LEO1, CTR9, and CDC73 (Zhang et al., 2013). The interaction of PHF6 with the PAF1 complex plays a critical role in PHF6 function in neuronal migration in the cerebral cortex. Knockdown of PAF1 mimics the PHF6 knockdown-induced phenotype of impaired neuronal migration in the mouse cerebral cortex (Zhang et al., 2013). The PAF1 complex occupies the promoter and gene body of actively transcribed genes and associates with RNA polymerase II (PolII) to promote transcriptional elongation and transcription-coupled histone

modifications (Chen et al., 2009; Krogan et al., 2002; Marton & Desiderio, 2008; Rondon et al., 2004; Squazzo et al., 2002). The C-terminal domain (CTD) phosphorylation of PolII is thought to mediate PAF1 recruitment to PolII. The PAF1 complex may directly stimulate RNA PolII transcription or act synergistically with other factors to promote transcriptional elongation (Jaehning et al., 2010). During transcriptional elongation, the PAF1 complex may recruit the histone methyltransferase Set2 to induce trimethylation of H3K36 and the ATPase Chd1 to remodel chromatin structure (Tomson & Arndt, 2013). In yeast, the PAF1 complex also promotes H2B K123 monoubiquitylation by recruiting the ubiquitin-ligase Rad6-Bre1. The H2B moniubiquitination facilitates dimethylation and trimethylation of H3K4 and H3K79 by the histone methyltransferases Set1 and Dot1 (Tomson & Arndt, 2013; Wood et al., 2003). Recent studies have shown that PAF1 may also regulate promoter-proximal pausing of PolII (Chen et al., 2015). PAF1 regulates PolII pausing approximately 50 nucleotides downstream of the transcription start site (TSS) where significant PolII occupancy is found. Upon PAF1 knockdown, PolII is released to the gene body (Chen et al., 2015). Together, these studies indicate the PAF1 complex regulates multiple aspects of transcription. By interacting with the PAF1 complex, PHF6 is thus poised to influence transcription. Interestingly, both of PHF6 and the PAF1 complex were shown to induce the expression of the gene encoding Neuroglycan C, which in turn mediates the ability of PHF6 and PAF1 complex to promote neuronal migration (Zhang et al., 2013). Knockdown of Neuroglycan C phenocopies the effect of PHF6 knockdown and PAF1 knockdown on neuronal migration, and expression of Neuroglycan C in PHF6-depleted progenitors rescues the defects in neuron migration (Zhang et al., 2013). These observations suggest that PAF1 and PHF6 may share a core network of target genes.

PHF6 also interacts with the nucleosome remodeling and deacetylation (NuRD) chromatin remodeling complex (Todd & Picketts, 2012). The NuRD complex is composed of the histone deacetylase Hdac1 or Hdac2 and ATPase Chd3 or Chd4, with combinatorial assembly of other subunits, including histone-binding protein RbAp46 and RbAp48, scaffold protein Mta1/2, DNA-binding protein Mbd3, and GATA zinc finger domain-containing protein 2A Gatad2a and Gatad2b (Zhang et al., 1998, 1999). The NuRD complex has been widely studied in cancer and embryonic stem (ES) cells (Lai & Wade, 2011). In acute promyelocytic leukemia (APL), the NuRD complex alters DNA methylation levels leading to gene repression. Upon removing the NuRD subunit Mbd3 in leukemia cells, DNA methylation levels at the RARb2 gene are significantly reduced (Morey et al., 2008). In addition, depletion of Mbd3 allows reprogramming of somatic cells to induced pluripotent stem (iPS) cells (Rais et al., 2013). The Mbd3-NuRD complex is thought to be recruited to the OSKM (Oct4, Sox2, Klf4, and Myc) target genes and inhibit robust reprogramming. Upon Mbd3 depletion, OSKM proteins interact with other pluoripotency-inducing chromatin regulators to drive efficient reprogramming (Rais et al., 2013). Recently, two *de novo* mutations in the NuRD subunit Gatad2a have been described in patients with severe intellectual disability (Willemsen et al., 2013), suggesting that the NuRD complex plays a critical role in brain development.

The NuRD complex is highly expressed in the developing brain. Recent studies by Yamada, Yang et al. have identified a novel function for the NuRD complex in neuronal connectivity in the brain (Yamada et al., 2014). The NuRD complex decommissions a subset of gene

promoters by removing H3K9/14 acetylation marks and thereby represses the expression of developmentally regulated genes to trigger granule neuron parallel fiber/Purkinje cell synaptic connectivity in the mammalian brain. Upon depletion of Chd4, the core ATPase subunit of the NuRD complex, these developmental genes are derepressed, leading to defects in granule neuron presynaptic bouton formation (Yamada et al., 2014). Whether the interaction of PHF6 with the NuRD complex influences gene expression and establishment of neuronal connectivity remains unexplored.

PHF6 also interacts with the nucleolar transcription factor upstream binding factor (UBF) in cells (Wang et al., 2013). UBF recruits RNA polymerase I to rDNA promoter regions. Upon PHF6 depletion, HeLa cells arrest at the G2/M phase of the cell cycle and have increased levels of UBF and ribosomal RNA synthesis. PHF6 interacts with UBF and is recruited to rDNA promoters to repress rDNA expression. Knockdown of PHF6 derepresses the rDNA promoter, leading to increased ribosomal RNA levels (Wang et al., 2013). However, the mechanism of rDNA repression by the PHF6-UBF interaction localized to the rDNA promoter remains unclear.

In situ RNA hybridization and expression analyses have shown that PHF6 is highly expressed in the developing central nervous system (CNS) and declines along the course of development (Voss et al., 2007). These observations suggest that PHF6 expression is tightly regulated during brain development. Recent studies by Franzoni et al provide insights into the mechanisms underlying the regulation of PHF6 expression (Franzoni et al., 2015). Downregulation of *PHF6* expression is correlated with an increase in the expression levels of miR-128, suggesting that miR-128 may regulate PHF6 expression during brain development. Consistent with this interpretation, expression of miR-128 in cortical neurons triggers the downregulation of PHF6, an effect that is mediated by miR-128 binding sites within the 3′UTR of PHF6 mRNA (Franzoni et al., 2015). A prediction of these results is that miR-128 might influence neuronal migration in the cerebral cortex. Consistent with this prediction, expression of miR-128 by *in utero* electroporation in the mouse brain arrests the migration of cortical neurons in layers III and IV–VI (Franzoni et al., 2015), which phenocopies the migration defects observed upon PHF6 knockdown (Zhang et al., 2013). Importantly, expression of PHF6 reverses the miR-128-induced migration defects (Franzoni et al., 2015), suggesting that PHF6 operates downstream of miR-128 in the regulation of cortical migration. Whether miR-128 regulation of PHF6 expression influences other aspects of brain development remains to be explored.

PHF6 in BFLS Pathogenesis

Identification of a critical role for PHF6 in radial neuronal migration in the cerebral cortex raises the important question of whether deregulation of PHF6 function in migration contributes to BFLS pathogenesis. Proper migration and positioning of neurons is a prerequisite for correct neuronal connectivity (Ayala et al., 2007; Morgan-Smith et al., 2014; Nadarajah & Parnavelas, 2002). Radially migrating neurons in the cerebral cortex are born in the ventricular zone (VZ) and sub-ventricular zone (SVZ), migrate along the radial glia scaffold to reach the cortical plate (Ayala et al., 2007; Kriegstein & Noctor, 2004; Vaillend et al., 2008). The oldest neurons reside in the deep layer of the cortical plate, and newly

generated neurons pass the deep layers to reside in more superficial layers (Greig et al., 2013). Upon PHF6 knockdown, neurons arrest in the intermediate zone, and the ectopic cells have neuronal hyperexcitability (Zhang et al., 2013). Introduction of an RNAi-resistant form of wild type-PHF6 into PHF6 knockdown mice rescues the migration defects, suggesting that the RNAi-induced phenotype is the result of specific knockdown of PHF6 rather than off target effects (Zhang et al., 2013). *In vivo* structure-function analyses of PHF6 demonstrate that BFLS patient-specific mutations impair the ability of PHF6 to promote neuronal migration (Zhang et al., 2013). Strikingly, neuropathological studies performed in the first identified BFLS patient have revealed defects of neuronal migration in the cortical plate including coarse gyri, cortical dysplasia with indistinct lamination and heterotopic neurons observed in the subcortical white matter (Brun et al., 1974).

PHF6 knockdown in the mouse cerebral cortex also triggers the formation of white matter heterotopias with aberrant neuronal activity (Zhang et al., 2013). Importantly, electrophysiological recordings reveal hyperexcitability of heterotopic neurons. Whole-cell patch clamp recordings reveal an aberrant pattern of activity in heterotopic neurons in PHF6 knockdown animals (Zhang et al., 2013). The membrane potential of heterotopic neurons oscillates, leading to frequent firing of action potentials. Further, knockdown of Neuroglycan C, a PHF6 target gene, also leads to formation of heterotopias that harbor neuronal hyperexcitability (Zhang et al., 2013). Heterotopias have been postulated to contribute to seizure susceptibility (Ackman et al., 2009). Heterotopias may serve as loci that initiate seizure activity. Studies in both genetically induced animal models and depth electrode recordings from patients have suggested that heterotopic loci may function to generate epilepsy (Aghakhani et al., 2005; Kothare et al., 1998, Manent et al., 2009). Together, these data suggest that inhibition of the PHF6 pathway triggers the formation of heterotopias in which neurons are hyperexcitable. These findings may be relevant to reduced seizure threshold in BFLS patients.

Although recent studies of PHF6 function have focused on its role in the regulation of neuronal migration and positioning in the cerebral cortex, it is likely that PHF6 has additional functions in the developing brain owing to its expression pattern (Voss et al., 2007; Zhang et al., 2013). PHF6 is expressed throughout the cerebral cortex and although levels of PHF6 drop with maturation, PHF6 is poised to regulate other aspects of neuronal development beyond neuronal positioning. In addition, PHF6 may have developmental roles outside of the cerebral cortex that may be relevant to BFLS pathogenesis.

Perspectives

Recent studies have advanced our understanding of the underlying molecular mechanisms of PHF6 function and its relevance to BFLS pathogenesis. These studies suggest that PHF6 is required for proper neuronal migration and that deregulation of PHF6 function in neuronal positioning contributes to the pathogenesis of BFLS. Analyses of PHF6 signaling in cells, primary neurons, and in the developing mouse brain suggest a role for PHF6 in transcription. Although progress has been made in identifying PHF6 binding partners through proteomic analyses of cells in which PHF6 is expressed, comprehensive proteomic analyses of PHF6 partners during neuronal development or in BFLS-derived iPSC cells should advance our

understanding of the mechanisms by which PHF6 regulates brain development as well as provide novel insights into BFLS pathogenesis. In addition, mapping genome wide targets of PHF6 in the developing brain should provide insights on the transcriptional roles of PHF6 specifically in the brain. Therefore, further research is required to gain a better understanding of PHF6 functions in brain development and pathogenic mechanisms underlying BFLS.

The finding that migration arrest in the PHF6 knockdown animals leads to the formation of heterotopias has important implications in the pathogenesis of BFLS. Clinically, heterotopia formation is associated with epileptic activity in the brain, which has been documented in BFLS patients (Robinson et al., 1983). Additionally, heterotopic neurons are hyperexcitable (Ackman et al., 2009). In human neuropathological studies, in addition to evidence of whiter matter heterotopias, the cerebral cortex displays dysplasia with indistinct or absent lamination (Brun et al., 1974). Whether cortical dysplasia due to impairment of PHF6 function is relevant to BFLS requires further study.

The identification of a novel transcriptional PHF6/PAF1 pathway in the developing brain has opened new avenues for the study of the molecular mechanisms that underlie XLID, and has also brought new insights into the regulation of transcription. The association of PHF6 with the PAF1 complex suggests an important role for PHF6 in the regulation of transcriptional elongation during neuronal development. Intriguingly, nascent transcriptional initiation is widespread across the genome, and the transition from transcriptional initiation to elongation represents a critical step of gene regulation (Guenther et al., 2007; Krumm et al., 1995; Muse et al., 2007; Nechaev & Adelman, 2012). Previous studies have revealed that the PAF1 complex cooperates with DSIF and the elongation factor SII/TFIIS, and thereby promotes elongation of PolII along the gene body (Chen et al., 2009; Zhu et al., 2005). In view of the findings that PHF6 may function to negatively or positively regulate gene expression (Todd & Picketts, 2012; Wang et al., 2013; Zhang et al., 2013) and PAF1's critical role in gene pausing (Chen et al., 2015), it would be interesting to investigate whether PHF6 regulates gene pausing, pause release, or both. The PAF1 complex appears to be critical for histone modifications including H3K4 tri-methylation (H3K4me3) or histone H2B (ubH2B) ubiquitination (Krogan et al., 2003; Wood et al., 2003). Whether PHF6 promotes modifications of histones via interactions with the PAF1 complex remains an open question.

The identification of Neuroglycan C as a target gene of PHF6 and the PAF1 complex provides new insights in neuronal migration and intellectual disability (Zhang et al., 2013). The Neuroglycan C gene, a member of the neuregulin family, encodes a transmembrane chondroitin sulfate glycoprotein, which has been implicated in the regulation of cortical radial migration (Anton et al., 1997; Brandt et al., 2007; Kinugasa et al., 2004; Rio et al., 1997). The Neuroglycan C gene might represent a locus associated with schizophrenia, a neuropsychiatric disorder in which impaired neuronal migration may play a pathogenic role (Impagnatiello et al., 1998; Lee et al., 2011; So et al., 2010; Tomita et al., 2011). These observations raise the possibility that mutations of PHF6 may contribute to the pathogenesis of neuropsychiatric disorders beyond intellectual disability. It will be also interesting to

determine whether deregulation of Neuroglycan C might play a role in intellectual disability and epilepsy.

Beyond the role of the PHF6-PAF1 interaction in neuronal positioning, it will be important to characterize the significance of the PHF6 interaction with the NuRD complex in brain development. Recently, two *de novo* mutations in the NuRD subunit Gatad2a have been reported in patients with severe intellectual disability (Willemsen et al., 2013). The NuRD complex represses transcription of developmentally regulated genes and thereby drives synaptogenesis in the mammalian brain (Yamada et al., 2014). Whether the NuRD complex regulates neuronal migration in the cerebral cortex remains unclear. In granule neurons of the cerebellum, the NuRD complex removes H3K9/14 acetylation marks at a subset of genes during development to drive synaptogenesis (Yamada et al., 2014). However, how the NuRD complex and PHF6 might regulate the epigenetic landscape of the genome in the cerebral cortex remains unknown.

Identification of the PHF6/UBF interaction in HeLa cells suggests that PHF6 may function in the nucleolus to regulate rDNA transcription (Wang et al., 2013). The nucleolus is a prominent subnuclear domain implicated in ribosomal RNA (rRNA) transcription, which accounts for up to 80% of RNA synthesis in eukaryotic cells (Nemeth & Langst, 2011). Transcription of rDNA is essential during the cell cycle (Roussel et al., 1996); however, its importance in post-mitotic neurons remains to be elucidated. Recently, intellectual disability-linked proteins including FMRP and the RNA methyltransferase NSUN2 have been shown to be transported to nucleoli (Khan et al., 2012; Nemeth & Langst, 2011). Whether PHF6 may harbor regulatory roles in the nucleolus has been unexplored.

Future studies of PHF6 should focus on elucidation of the biochemical functions of PHF6 including the role of its PHD domains and pathological relevance. Mouse models of the disease will assist in understanding the pathological consequences of PHF6 inactivation and their impact on clinical features including intellectual disability and seizures. In addition, it will be important to explore whether PHF6 harbors additional biological functions in brain development including neural progenitor proliferation, differentiation, synaptic development and circuit integration, and determine whether deregulation of such functions contributes to the pathogenesis of BFLS.

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Highlights

- Mutations of the *PHF6* gene are linked with Börjeson-Forssman-Lehmann syndrome (BFLS).
- PHF6 regulates transcription via interactions with the PAF1 complex, NuRD complex, and UBF.
- miR-128 controls neuronal positioning by inhibiting PHF6 in the cerebral cortex.
- Deregulation of the PHF6/PAF1 complex triggers formation of white matter heterotopia.

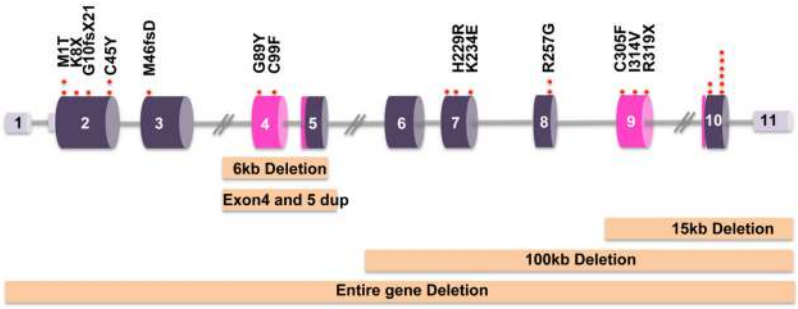

Figure 1.

PHF6 gene with patient mutations. The small blocks in light purple represent the 3′ and 5′ UTR of *PHF6* gene. The blocks in dark purple represent the coding exons with PHD domains shown in pink. Point mutations in BFLS are shown as red dots. Five large domain deletions in BFLS are also shown.

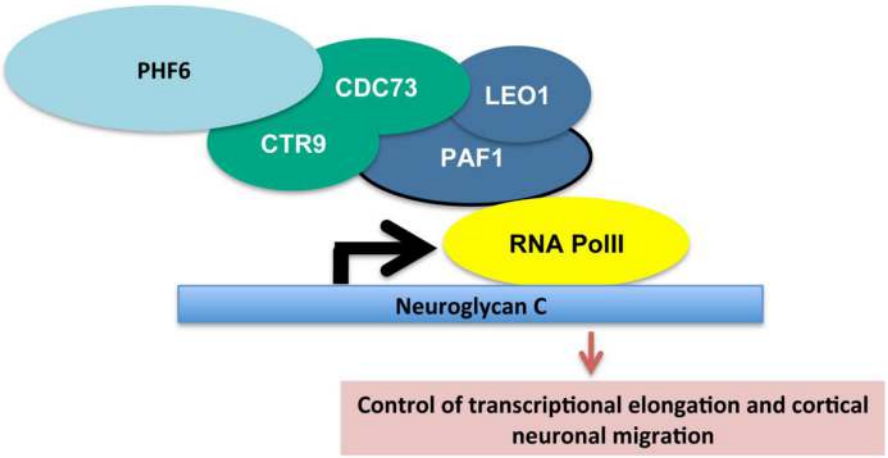

Figure 2.

A proposed model for PHF6-PAF1 complex in regulation of neuronal migration during cortical development. During critical period of neuronal migration, PHF6 interacts with PAF1 transcriptional elongation complex to regulate downstream targets including Neuroglycan C to regulate neuronal migration and positioning.

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Table 1

Summary of PHF6 mutations in BFLS patients in different individuals from different families. Classical features of BFLS includes mild to severe mental retardation, hypogonadism, hypometabolism, obesity with marked gynecomastia, facial dysmorphism, narrow palpebral fissure, large and fleshy ears, tapered fingers.

F/M	Nucleotide	Amino Acid	Mutation	Reported clinical features	Behavioral disturbances	Ref
M	c. 2T>C	p.M1T	Missense	Classical features of BFLS with no detailed clinical records.	N/A	(Lower et al., 2002)
M	c. 2T>C	p.M1T	Missense	Classical features of BFLS. Born at term after uneventful pregnancy, delayed early milestones, walked at age 4, began saying a few words at age 5, height of 140cm (<3rd centile), weight of 45kg (5th centile), and occipital-frontal head circumference of 53.3cm (10th-25th centile) at age 15, short stature, triangular face, hyperplastic supraorbital digits, deep-set eyes, large ears, gynecomastia, lower abdominal obesity, and small penis and testes with no evidence of pubertal change.	N/A	(Crawford et al., 2006)
M	c.134G>A	p.C45Y (familial)	Missense	Classical features of BFLS with no detailed clinical records.	N/A	(Lower et al., 2002)
M	c.134G>A	p.C45Y (de novo)	Missense	Classical features of BFLS with no detailed clinical records.	N/A	(Lower et al., 2002)
M	c.266G>T	p.G89Y	Missense	Classical features of BFLS. At age 22, height 173cm, weight 108kg, head circumference 61cm. Moderate intellectual disability; delayed development, no epileptic seizures. Brother has similar symptoms with severe intellectual disability, Female carrier in the family has shown mild intellectual disability.	Good tempered with occasional aggressive attacks	(Mangelsdorf et al., 2009)
M	c.296G>T	p.C99F	Missense	Classical features of BFLS. Female carrier has mild clinical features of BFLS including obesity, large ears, amenorrhea, hypothyroidism, epilepsy, and learning difficulties.	N/A	(Lower et al., 2002)
M,F	c.686A>G	p.H229R	Missense	Classical features of BFLS with no detailed clinical records.	N/A	(Lower et al., 2002)
M	c.700A>G	p.K234E	Missense	Classical features of BFLS with no detailed clinical records.	N/A	(Lower et al., 2002)
M	c.769A>G	p.R257G	Missense	Classical features of BFLS with no detailed clinical records.	N/A	(Lower et al., 2002)
M	c.769A>G	p.R257G	Missense	Classical features of BFLS. Severe intellectual disability, self-injurious behavior, stooped hyposcoliotic posture, central obesity, marked gynecomastia, hypogonadism, and hand and feet anomalies. Hodgkin's lymphoma	N/A	(Vallée et al., 2004) (Carter et al., 2009)
M	c.940A>G	p.I314V	Missense	Classical features of BFLS. Psychomotor developmental delay, language delay, mild intellectual disability (IQ of 61). At age 18, height 171.5 cm, head circumference 55.7cm, moderate obesity, marked gynecomastia.	Lack of inhibition in sexual contact; limbs showing hypermobility.	(Crawford et al., 2006)

F/M	Nucleotide	Amino Acid	Mutation	Reported clinical features	Behavioral disturbances	Ref
M	c.22A>T	p.K8X	Nonsense	Classical features of BFLS.		(Lower et al., 2002)
F	c.955C>T	p.R319X	Nonsense	Classical features of BFLS. Head circumference 34cm (−0.5SD), delayed walking at age 2.5. During first years, frontotemporal alopecia, nystagmus, strabismus, small teeth, hypoplastic labia minora and clitoris. At age 6, head circumference of 49cm (−1.63SD), moderate to severe intellectual disability, facial dimorphism, and tapering fingers. Normal MRI.	Lack of swallowing coordination; Sociable and happy	(Zweier et al., 2013)
M	c.1024C>T (original family)	p.R342X	Nonsense	Classical features of BFLS. Developmental delay, upslanting palpebral fissures, large ears, a high palate, hypotonia, cryptorchidism, and an inguinal hernia. At age 15, talk sentances consisting of three or four words, short stature and obesity, large ears, protruding cheeks,. Female carrier has normal cognitive function. Big hands, ears and feet, with some sensory impairment in the legs. Normal MRI.	Female carrier is clumsy when running or jumping.	(Gécz, Turner, Nelson, & Partington, 2006; Lower et al., 2004)
M	c.1024C>T	p.R342X	Nonsense	Delayed developmental milestones, height on 25th centile, weight over 97th centile, head circumference over 98th centile, narrow bitemporal diameter, upslanting eyes, prominent supraorbiral ridges, hypotelorism, long ears/earlobes, a prominent chin, obese, marked gynecomastia, a hypoplastic scrotum with retractile testes, cubitus valgus, tapered fingers. Female carrier is obese, has deep-set eyes, prominent supraorbital ridges, large ear lobes, tapered fingers, broad feet and short toes.	N/A	(Lower et al., 2002)
M	c.1024C>T	p.R342X	Nonsense	Classical features of BFLS.	N/A	(Lower et al., 2002)
M	c.1024C>T	p.R342X	Nonsense	Classical features of BFLS.	N/A	(Crawford et al., 2006)
M	c.1024C>T	p.R342X	Nonsense	Classical features of BFLS.	N/A	(Crawford et al., 2006)
M	c.1024C>T	p.R342X	Nonsense	Classical features of BFLS and T cell acute lymphoblastic leukemia at age 9	N/A	(Chao et al., 2010)
F	c.27dupA	p.G10fsX21	Frameshift	Classical features of BFLS. Delayed speech, normal IQ of 87, high level of thyroid stimulating hormone, low level of thyroxin at age 7–8; at age 14, height 180cm, weight 115 kg, shallow forehead, fleshy earlobes, deep set eyes, broad feet, hammertoes, no menstruation at age 14.	Poor coordination; Tactile defensive and emotionally labile, poor spatial awareness, and startles to noise	(Crawford et al., 2006)
M	IVS2-8A>G	M46fsD exon3	Frameshift	Classical features of BFLS with mild to moderate intellectual disability, and global developmental delay.	N/A	(Vallée et al., 2004)
F	c.677delG	p. G226fsE53	Frameshift	Classical features of BFLS Diagnosed with Coffin Siris Syndrome in early infancy. At 9 years old, height 1.7SD, weight 30kg, head circumference	Shy and intolerance for being alone; anxiety; sleeping difficulties	(Wieczorek et al., 2013; Zweier et al., 2013)

F/M	Nucleotide	Amino Acid	Mutation	Reported clinical features	Behavioral disturbances	Ref
				54cm (1.4SD). Mild intellectual disability, mild clinodactyly, short toes, and facial dysmorphism.		
F	c.914G>T	p. C305F	Missense	Classical features of BFLS, previously diagnosed with Coffin Siris Syndrome, unstable gait on stairs, head circumference of −1.0SD at age 11, susceptibility to urinary tract infections, frequent constipation, facial dysmorphism, and tapered fingers.	Aggressive behavior	(Wieczorek et al., 2013; Zweier et al., 2013)
F		Dosage gain of exon 4, 5	Duplication	Classical features of BFLS, delayed milestones, delayed speech, conductive hearing loss, potential retinal dystrophy, and seizures at age 20. At age 22, head circumference 56.5(1.4 SD), facial dysmorphism, tapering fingers.	Unstable gait; behavioral problems including short attention span, hyperactivity, compulsive behavior, sleeping difficulties, and lack of emotional detachment.	(Zweier et al., 2013)
F		Dosage gain of exon 4, 5	Duplication	Classical features of BFLS. Head circumference of 34cm (1.4 SD) at birth; At age 3–4 month, several single seizures, delayed walking and language, constipation, and neurogenic bladder. At age 32, head circumference 54cm (−0.3 SD).	Sociable behavior	(Zweier et al., 2013)
F	6kb deletion	Affecting exon 4, 5	Deletion	Classical features of BFLS with moderate intellectual disability. Head circumference 53cm (2.2SD), linear skin hyperpigmentation, and facial dimorphism.	Sociable behavior,	(Zweier et al., 2013)
F	100kb deletion	Deletion of last 5 coding exons	Deletion	Classical features of BFLS. Delayed developmental milestone, feeding difficulties, muscular hypotonia, single horseshoe kidney, obstructive uropathy, recurrent pyelonephritis, and multiple febrile seizures.	N/A	(Di Donato et al., 2014)
F	15kb deletion	Deletion of last 3 exons	Deletion	Classical features of BFLS with mild intellectual disability. At age 7, mild hearing loss, and recurrent middle ear infections; At age 16, head circumference 56.6cm (75th centile), slightly widened ventricles and increased white matter signal abnormality on MRI.	N/A	(Berland et al., 2011)
M	c.999-1001 delTGA	p.D333del	Deletion	Classical features of BFLS with delayed milestones.	N/A	(Baumstark et al., 2003)
M	c.999-1001 delTGA	p.D333del	Deletion	First patient description. Delayed milestones, severe mental deficiency with IQ of 20, seizures, large ears, short stature, small genitalia, and small pituitary gland. Coarse gyri, widened ventricular system, cortical dysplasia with indistinct lamination, and heterotopias.	Hyperactive and aggressive behaviors	(Börjeson, Forssman, & Lehmann, 1962; Gécz et al., 2006)
F	Entire gene deleted		Deletion	Classical features of BFLS. Head circumference 35.5 (0.5SD) at birth, delayed motor milestones and facial dysmorphism. muscular hypotonia, ectopic left kidney, strabismus, hyperopia, bilateral sensorineural hearing loss, enlarged ventricles on MRI.	Compulsive eating behavior; Severe sleeping difficulties,	(Zweier et al., 2013)
F	270kb deletion		Deletion	Classical features of BFLS. Mild muscular hypotonia, mild speech delay, narrow external auditory canal, mild learning disability; IQ within borderline to normal range.	N/A	(Di Donato et al., 2014)