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The XLID Protein PQBP1 and the GTPase Dynamin 2 Define a Signaling Link that Orchestrates Ciliary Morphogenesis in Postmitotic Neurons

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SUMMARY

Intellectual disability (ID) is a prevalent developmental disorder of cognition that remains incurable. Here, we report that knockdown of the X-linked ID (XLID) protein polyglutamine-binding protein 1 (PQBP1) in neurons profoundly impairs the morphogenesis of the primary cilium, including in the mouse cerebral cortex in vivo. PQBP1 is localized at the base of the neuronal cilium, and targeting its WW effector domain to the cilium stimulates ciliary morphogenesis. We also find that PQBP1 interacts with Dynamin 2 and thereby inhibits its GTPase activity. Accordingly, Dynamin 2 knockdown in neurons stimulates ciliogenesis and suppresses the PQBP1 knockdown-induced ciliary phenotype. Strikingly, a mutation of the PQBP1 WW domain that causes XLID disrupts its ability to interact with and inhibit Dynamin 2 and to induce neuronal ciliogenesis. These findings define PQBP1 and Dynamin 2 as components of a signaling pathway that orchestrates neuronal ciliary morphogenesis in the brain.

INTRODUCTION

Intellectual disability (ID) is a common developmental disorder with a prevalence of 1%–3% (Bhasin et al., 2006; Larson et al., 2001). No effective treatments are available and thus there is an urgent need to better understand the molecular pathogenesis of ID. Human genetic studies have led to the identification of many genes whose mutations cause ID (Chelly et al., 2006; Ropers, 2010). However, the functions of ID proteins largely remain to be elucidated.

Approximately 30%–50% more males than females are affected with ID, presumably reflecting mutations on the X chromosome. Mutations in more than 90 genes on the X chromosome have been associated with ID (Chiurazzi et al., 2008; Gécz et al., 2009). In many cases, XLID mutations cause syndromes that include constellations of symptoms and signs outside the nervous system. The heterogeneity of genes and associated ID syndromes raises the question as to whether common or disparate pathogenic signaling mechanisms underlie ID. To begin to address this fundamental question, we need to gain a better understanding of the functions of ID-associated proteins. Notably, a substantial portion of XLID genes encode proteins that are predicted to localize in the nucleus (Chiurazzi et al., 2008), providing a starting point for investigating the cell-intrinsic regulation of neuronal development and function by these XLID proteins.

Deregulation of neuronal morphogenesis represents a prominent pathological feature in ID. Impaired development of dendrites and dendritic spines may represent an important aspect of neuronal pathology in the brains of ID patients (Kaufmann and Moser, 2000; Purpura, 1974), supporting the concept that deregulation of neuronal connectivity contributes to the pathogenesis of ID. Consistent with these studies, XLID proteins regulate the development of dendrite arbors and the formation of dendritic spines in neurons (Irwin et al., 2000; Iwase et al., 2007). These observations raise the question as to whether XLID proteins regulate other aspects of neuronal morphogenesis.

The primary cilium represents an intriguing organelle that has received attention in recent years (Gerdes et al., 2009; Singla and Reiter, 2006). Primary cilia play critical roles in early embryonic development and organogenesis in vertebrates by providing a unique cellular domain that facilitates signal transduction in response to morphogens and growth factors (Goetz and Anderson, 2010; Lancaster and Gleeson, 2009). Disruption of the formation or function of primary cilia causes a heterogeneous group of ciliopathies, including Bardet-Biedl syndrome (BBS), Meckel-Gruber syndrome, and Joubert syndrome, which are characterized by overlapping clinical presentations of polycystic kidney, retinal degeneration, cerebellar atrophy, and, notably, ID

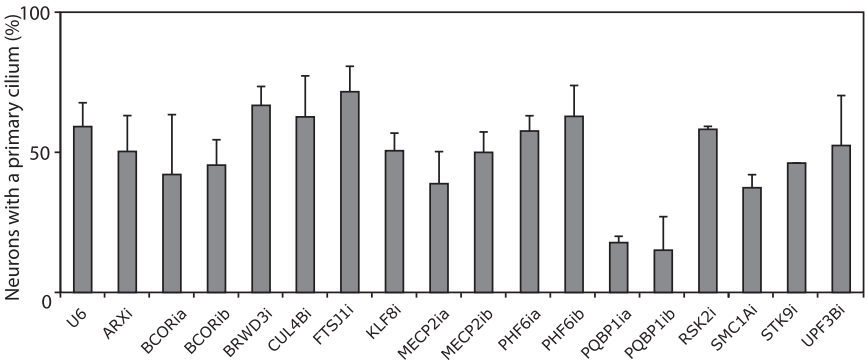

Figure 1. A Targeted RNAi Screen of XLID Genes in Ciliary Morphogenesis in Neurons

Hippocampal neurons were transfected with an RNAi plasmid encoding shRNAs targeting the indicated XLID gene or the control U6 plasmid together with the GFP expression plasmid, and subjected to immunocytochemistry at DIV5 using the AC3 and GFP antibodies. Knockdown of each protein by cognate shRNAs was validated (Figures 2A and S1). Knockdown of PQBP1 consistently reduced the percentage of neurons harboring a primary cilium ($p < 0.05$; ANOVA). A total of 2,646 neurons were quantified.

See also Figure S1.

(Badano et al., 2006; Hildebrandt et al., 2011). These observations support the hypothesis that impairment of neuronal ciliogenesis might represent an important feature in the pathogenesis of developmental cognitive disorders. Growing evidence suggests that the primary cilium functions as a signaling center in neurons (Lee and Gleeson, 2011; Louvi and Grove, 2011). Several G protein coupled receptors (GPCRs) are localized within the neuronal cilium, including the somatostatin receptor 3 (SSTR3), melanin concentrating hormone receptor 1 (MCHR1), serotonin receptor 6 (5HTR6), and dopamine receptors (Domire et al., 2011; Hamon et al., 1999; Händel et al., 1999; Marley and von Zastrow, 2010). In addition, the 12-transmembrane enzyme adenylyl cyclase 3 (AC3), which generates cyclic AMP in response to activation by GPCRs, is localized in the membrane of the neuronal cilium (Bishop et al., 2007). Mice in which the AC3 or SSTR3 gene is disrupted have defects in object recognition memory, suggesting an important role for cilia in regulating signaling events that are critical for cognitive function (Einstein et al., 2010; Wang et al., 2011). Thus, the primary cilium may play a key role in neuronal signaling and brain function.

The fundamental architecture of the primary cilium includes microtubule bundles extending from the basal body and an encasing ciliary membrane on which the ciliary receptors localize. Components of the primary cilium are sorted and trafficked by the intraflagellar transport (IFT) system and ciliary membrane trafficking, which control the formation and function of the cilium (Nachury et al., 2010; Pedersen et al., 2008). Although the mechanisms that control ciliogenesis in nonneuronal cells have been intensely investigated, the mechanisms that specifically orchestrate the development and morphogenesis of the neuronal cilium remain poorly understood.

In this study, we identify a function for the major XLID protein polyglutamine-binding protein 1 (PQBP1) in the morphogenesis of the primary cilium in postmitotic neurons. Knockdown of PQBP1 profoundly impairs the formation of the primary cilium in hippocampal neurons and the mouse cerebral cortex in vivo. PQBP1 is localized at the base of the neuronal cilium as well as the nucleus, and targeting the PQBP1 WW effector domain to the cilium induces ciliary development. We also identify the protein Dynamin 2 as a cytoplasmic target of PQBP1 in neurons. PQBP1 interacts with Dynamin 2 and thereby inhibits the GTPase activity of Dynamin 2. Accordingly, Dynamin 2 knockdown stimulates ciliary morphogenesis and suppresses the PQBP1 knockdown-induced loss of cilia in neurons. Importantly, we also find that a mutation of the PQBP1 WW domain that causes XLID deregulates PQBP1-Dynamin 2 signaling and consequent ciliary morphogenesis in neurons. The identification of the PQBP1-Dynamin 2 signaling link as a critical regulator of neuronal ciliary development in the brain bears potential implications for our understanding of the mechanisms underlying ID.

RESULTS

To investigate the functions of XLID proteins in ciliary morphogenesis, we performed a targeted RNAi screen of XLID genes in primary rat hippocampal neurons. We focused our attention on genes encoding proteins predicted to localize in the nucleus and regulate transcription or RNA processing. Knockdown of each XLID protein by cognate short hairpin RNAs (shRNAs) was validated (see Figures 2A and S1F).

To visualize the primary cilium in hippocampal neurons, we performed immunocytochemical analyses using an antibody that recognizes adenylyl cyclase 3 (AC3). A single bar-shaped ciliary structure extending from the soma of hippocampal neurons was observed (Figure S1A). The base of the cilium was contiguous with the centrosome, with the latter identified by expression of Centrin-GFP (Figure S1A). Although the nonneuronal ciliary marker acetylated tubulin was expressed within the AC3-positive cilia in neurons, it was also detected throughout the cytoplasm in neurons (Figure S1B). The neuronal cilium also displayed expression of somatostatin receptor 3 (SSTR3) and melanin concentration hormone receptor 1 (MCHR1; Figures S1C and S1D). These data establish that primary hippocampal neurons harbor primary cilia. The percentage of hippocampal neurons bearing a cilium increased as neurons matured between 2 days in vitro (DIV2) and DIV7 (Figure S1E).

To assess the effect of knockdown of nuclear XLID proteins on ciliary morphogenesis, we transfected hippocampal neurons with RNAi plasmids targeting each XLID protein together with a GFP expression plasmid at DIV1, and subjected neurons at DIV5 to immunocytochemical analyses using the AC3 and GFP antibodies. Strikingly, we found that knockdown of the XLID protein PQBP1, using two shRNAs targeting distinct regions of PQBP1 messenger RNA (mRNA), consistently and substantially reduced the percentage of neurons harboring a primary cilium (Figures 1, 2A–2C, and S2A). More than 70 affected individuals from more than 20 families with PQBP1 mutations have been