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Primary cilia in neurodevelopmental disorders

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Abstract

Primary cilia are generally solitary organelles that emanate from the surface of almost all vertebrate cell types. Until recently, details regarding the function of these structures were lacking; however, extensive evidence now suggests that primary cilia have critical roles in sensing the extracellular environment, and in coordinating developmental and homeostatic signalling pathways. Furthermore, disruption of these functions seems to underlie a diverse spectrum of disorders, known as primary ciliopathies. These disorders are characterized by wide-ranging clinical and genetic heterogeneity, but with substantial overlap among distinct conditions. Indeed, ciliopathies are associated with a large variety of manifestations that often include distinctive neurological findings. Herein, we review neurological features associated with primary ciliopathies, highlight genotype–phenotype correlations, and discuss potential mechanisms underlying these findings.

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Competing interests

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Introduction

In the past decade, a class of disorders known as ciliopathies has become recognized, comprising a unique spectrum of genetic syndromes. These disorders are caused by dysfunction of primary cilia—small non-motile, hair-like organelles that are found to protrude from the surface of nearly all vertebrate cell types, at a frequency of one per cell, and are highly conserved throughout evolution. The cilium comprises a microtubule-based core, the axoneme, which extends from a specialized centriole at the base of the cilium, termed the basal body, and a region between the axoneme and the basal body known as the transition zone (Figure 1).

The specialized structure of the primary cilia offers a unique opportunity for partitioning of sensory and signalling proteins away from the main body of the cell in a different cytoplasmic environment to enable fine tuning of biological responses to various stimuli, such as mechanical stimuli and light. For instance, flow-induced passive bending of cilia present on kidney tubular epithelial cells mediates the mechanosensation of extracellular urine flow, while in retinal photoreceptors, a specialized primary cilium connects the inner segment, which contains the cellular nucleus, with the outer segment, which contains the photopigment. Furthermore, many receptors expressed on the primary cilium surface are necessary to bind specific hormones (for example, somatostatin), growth factors (for example, platelet derived growth factor) or morphogens (for example, sonic hedgehog [SHH] and Wnt), which have essential roles especially during embryonic development. Indeed, primary cilia sense and transduce many extracellular signals to influence a wide variety of processes, such as cell proliferation and polarity, developmental processes and neuronal growth.¹

Defects in the primary cilia can lead to a wide array of clinical phenotypes, and in fact ciliopathies can affect nearly every major body system, including the brain, eyes, liver, kidneys, skeleton and limbs.² Advances in our understanding of the biology of primary cilia have provided important insights into the unifying features of these distinct disorders. In turn, elucidation of the genetic basis of many ciliopathies has informed our understanding of ciliary biology, and helped identify many of the key molecular components that underlie cilium formation and function.

The syndromes described herein have long been recognized as distinct clinical entities, although many of these disorders share common clinical features as well as common causative genes (Table 1). In some instances, the wide clinical heterogeneity associated with different mutations within the same ciliary gene can be explained by a correlation between the type of mutation and the severity of the phenotype; for example, loss-of-function mutations of *TMEM67* are markedly enriched in patients with lethal ciliopathies, whereas the presence of at least one hypomorphic mutation in this gene, which causes only partial loss of function, is usually associated with milder, non-lethal phenotypes.³ However, such a relation ship does not always hold true, and often the same genetic mutations can result in clinically distinct ciliopathies, even among siblings, highlighting the complexity of genotype–phenotype correlations.⁴

Only in the past decade has the basis of this complexity begun to be understood, with the finding that most ciliopathies are not transmitted in a purely Mendelian fashion, but might follow a multiallelic mode of inheritance. According to this multiallelic model, the inherited recessive mutations in one major gene are not sufficient *per se* to fully explain the phenotype; the concurrent presence of additional mutations, rare allelic variants or even common polymorphisms in other ciliary genes contributes to determine the ‘mutational load’, which eventually modulates the phenotypic expression of the ciliopathy in each patient.⁵ For instance, the heterozygous p.R830W polymorphic variant of *AHII* has been shown to influence the development of retinal disease or neurological involvement in patients with renal ciliopathies caused by homozygous deletions within the gene encoding nephrocystin-1, *NPHP1*.^{6,7} In support of this multiallelic hypothesis, large genetic screening studies examining mutations in ciliary genes and whole-exome sequencing efforts in cohorts of patients with ciliopathy have often disclosed several heterozygous variants of unclear pathogenic significance,^{8–12} which might represent genetic modifiers of the disease phenotype.

In this Review, we focus on ciliopathies with major neurological involvement, describe their clinical features and known pathogenetic mechanisms, and discuss the possible aetiologies of associated brain malformations. Representative clinical findings and brain MRI images of the CNS defects associated with these ciliopathies are also summarized (Figure 2 and Figure 3, respectively).

Neurological involvement in ciliopathies

Neurological defects are a common finding in many ciliopathies, highlighting a critical role for primary cilia in brain development. Primary neuronal cilia were first identified over 50 years ago,^{13,14} but their function remained largely unexplored until the past decade. Recognition of the involvement of cilia-associated gene products in diverse neurological syndromes has improved our understanding of the critical functions of cilia in the CNS, as well as in the pathogenesis of CNS defects in ciliopathies. Ciliopathies with neurological involvement are discussed in more detail in the sections that follow.

Joubert syndrome

Joubert syndrome (JS; Mendelian Inheritance in Man [MIM] 213300) is an autosomal or X-linked recessive condition characterized by neonatal hypotonia, oculomotor apraxia, intellectual disability of variable severity, ataxia, and neonatal breathing abnormalities. The hallmark feature of JS is a distinctive midbrain and hindbrain malformation known as the ‘molar tooth sign’ (MTS). Resembling a tooth on axial brain MRI sections, this radiological feature reflects thickened and maloriented superior cerebellar peduncles, hypoplasia of the cerebellar vermis, and a deep interpeduncular fossa (Figure 3a,b). In association with the MTS, other CNS defects can be observed in patients with JS, including occipital meningoencephalocoele, ventriculomegaly, an enlarged posterior fossa, polymicrogyria and other neuronal migration defects, hypothalamic hamartoma, and corpus callosum abnormalities.¹⁵

Patients with JS have a wide spectrum of phenotypic severity, and extraneurological features variably accompany the pathognomonic finding of MTS and define specific phenotypic subgroups.¹⁶ These extraneurological features include facial dysmorphisms (Figure 2a), retinal dystrophy, juvenile nephronophthisis, polydactyly (Figure 2b,c), congenital hepatic fibrosis, and chorioretinal coloboma.¹⁶

As well as being clinically heterogeneous, JS is now known to be genetically heterogeneous, with causative mutations in 22 genes identified to date (Table 1). However, these genes only account for about half of JS cases, suggesting that a number of causative genes remain unidentified. All known JS-associated genes encode proteins found within primary cilia, mainly within the transition zone, and more than half have also been implicated in other ciliopathies.

The prevalence of JS, similar to that of the other ciliopathies with CNS involvement, is low and often remains undetermined; nevertheless, many authors report an estimated prevalence of JS—which is probably the commonest of this group of disorders—of between 1/80,000 and 1/100,000 live births.¹⁶ A higher prevalence has been reported for some of these ciliopathies in isolated populations (such as Ashkenazi Jewish, Hutterite, French Canadian or Finnish populations) due to founder effects of specific gene mutations.^{16,17}

Meckel syndrome

Meckel syndrome (MKS; MIM 249000) is a severe perinatal lethal disorder that phenotypically overlaps with JS. MKS is classically diagnosed by a triad of features consisting of occipital encephalocoele, cystic kidneys (Figure 2d), and postaxial polydactyly. However, MKS can present with a wide spectrum of developmental abnormalities including liver fibrosis, pulmonary hypoplasia, skeletal abnormalities, and additional CNS defects, including agenesis of the corpus callosum, hydrocephalus, cerebellar hypoplasia and other posterior fossa abnormalities, total anencephaly or holoprosencephaly.¹⁸

Autosomal recessive mutations within at least 15 genes have been shown to cause MKS, of which 11 are also known to also cause JS (Table 1). Indeed, exhibition of both MKS and JS phenotypes among siblings is not uncommon.^{19,20}

Bardet–Biedl syndrome

Bardet–Biedl syndrome (BBS; MIM 209900) is a heterogeneous autosomal recessive disorder that typically manifests with obesity, postaxial polydactyly (Figure 2e), renal abnormalities and retinal dystrophy. BBS phenotypes can also encompass other common features of ciliopathies, such as hepatic abnormalities, situs inversus, male infertility and olfactory deficits. In fact, most patients with BBS are infertile and commonly develop diabetes mellitus.^{21,22}

Mild-to-moderate intellectual disability is common in BBS, and children with this disorder can have delayed motor milestones and speech acquisition.²¹ Other neurological signs include ataxia and poor motor coordination, although structural abnormalities in the cerebellum have only occasionally been reported (Figure 3c,d).^{23,24} Several studies have

reported the occurrence of hydrocephalus and a reduction in hippocampal volume in patients with BBS, defects that are also seen in several mouse models of BBS.^{25–31}

To date, mutations in 17 genes have been reported to cause BBS (Table 1). Many of these genes encode components of a specific basal body structure, called the BBSome (Figure 1), which is involved in assembly of primary cilia. BBS was the first ciliopathy for which a triallelic or oligogenic mode of inheritance was proposed; autosomal recessive mutations in one BBS-associated gene were not fully penetrant in some BBS families, and only the co-occurrence of a third heterozygous mutation in a different BBS-associated gene resulted in clinical manifestation of the disease.³² Despite limited overlap between the causative genes for BBS and those responsible for other ciliopathies, heterozygous mutations in some BBS-related genes have been found to modify the phenotypic expression of other ciliopathies, such as those caused by *CEP290* mutations;¹¹ conversely, heterozygous *CEP290* mutations have been detected in some patients with BBS, supporting the multiallelic aetiology in distinct ciliopathies.³³

Orofaciodigital syndromes

Orofaciodigital syndromes (OFDs) are a group of ciliopathies characterized by orofacial and digital abnormalities. Distinctive oral and facial anomalies in OFD include tongue nodules (hamartomas [Figure 2f] or lipomas), multiple or hyperplastic frenula (Figure 2g), cleft palate or lip (Figure 2h), hypertelorism, wide nasal bridge, bifid tongue, or dental irregularities such as missing teeth.³⁴ Abnormalities of the digits can include polydactyly (most typically preaxial or mesoaxial with Y-shaped metacarpals), brachydactyly and/or syndactyly (Figure 2i,j).

Intellectual disability is frequently associated with OFD, and a broad spectrum of pathological neurological findings have been reported, including agenesis of the corpus callosum, hydrocephalus, intracerebellar cysts, and cerebellar agenesis.^{35–37} A subgroup of OFD, termed OFD syndrome type VI (OFD6; MIM 277170), is characterized by the presence of the MTS (Figure 3e), and is thus part of the JS spectrum. In this form, hypothalamic hamartomas have also been commonly reported (Figure 3e,f).^{38,39}

At least 13 different forms of OFDs have been described, although the genetic underpinnings of most of these disorders remain elusive.^{40,41} The most extensively studied subtype is OFD syndrome type I (OFD1; MIM 311200), which is an X-linked disorder caused by disruptions in *OFD1*, which encodes a centriolar protein involved in cilia biogenesis. *OFD1* mutations are often lethal in males, and heterozygous females can present with a spectrum of OFD-associated defects and poly cystic kidney disease.^{40,42–44} Interestingly, mutations in *OFD1* are also known to be a rare cause of JS, variably associated with polymicrogyria, hydrocephalus and polycystic kidney disease.^{45,46} Mutations in the ciliary gene *TCTN3* were shown to cause OFD syndrome type IV or Mohr–Majewski syndrome (OFD4; MIM 258860), a severe OFD subtype characterized by skeletal dysplasia.⁴¹ Finally, the JS-related OFD6 is principally caused by mutations in the *C5orf42* gene,⁴⁷ whereas the ciliary genes *TMEM216* and *KIF7* are only rarely mutated in this form.^{48,49}

Acrocallosal syndrome

Acrocallosal syndrome (ACLS; MIM 200990) is a rare disorder characterized by a variety of developmental anomalies including agenesis of the corpus callosum (Figure 3g,h), craniofacial abnormalities, intellectual disability, and mainly preaxial polydactyly. Facial dysmorphisms observed in patients with ACLS include hypertelorism and a prominent forehead (Figure 2k). Patients with the major features of ACLS can also have the MTS, eliciting a diagnosis of JS. ACLS is usually caused by mutations in *KIF7* (Table 1);^{49,50} however, rare cases in which the disease is attributable to mutations in *GLI3*, which encodes a cilia-related transcriptional regulator with roles in developmental signalling, have been reported.^{51,52}

Hydrolethalus syndrome

Hydrolethalus syndrome (HLS; MIM 236680) is a rare, lethal, recessive genetic syndrome that can present with a diverse spectrum of malformations, including hydrocephalus, postaxial and preaxial polydactyly, club feet, hypothalamic hamartoma, cerebellar malformation, heart and lung defects, cleft palate, and micrognathia.^{53,54} Midline defects are a prominent brain malformation in fetuses with HLS, and a distinctive ‘keyhole-shaped’ foramen magnum has been described in many cases.^{54,55}

A single founder mutation in *HYLS1*, which encodes a centriolar protein that is required for ciliogenesis, was found in Finnish patients with HLS.^{56,57} More recently, mutations in *KIF7* have also been shown to cause HLS.⁴⁹

Pallister–Hall and Greig syndromes

Pallister–Hall syndrome (PHS; MIM 146510) and Greig cephalopolysyndactyly syndrome (GCPS; MIM 175700) are autosomal dominant allelic disorders that share many clinical features. PHS presents with postaxial or central polydactyly, bifid epiglottis, imperforate anus, and lung or kidney dysplasia. A hallmark feature of this disorder is hypothalamic hamartoma, and patients might have associated pituitary defects, visual problems or gelastic seizures.

GCPS is also characterized by polydactyly, as well as cranio facial abnormalities including macrocephaly, prominent forehead and hypertelorism.⁵⁸ Although CNS involvement is not always observed in patients with GCPS, intellectual disability, hydrocephalus and epilepsy have been reported in some cases.⁵⁹ As mutations in *GLI3* cause both PHS and GCPS (Table 1),^{60–62} as well as non-syndromic forms of autosomal dominant polydactyly, some individuals have suggested that these distinct but overlapping syndromes be viewed as ‘GLI3 morphopathies’.^{58,63}

Ciliary structure–disease relationships

Cilia are dynamically assembled and disassembled as cells progress through the cell cycle, with studies implicating the autophagy pathway as a key mechanism regulating cilio genesis.^{64,65} The establishment and maintenance of the primary cilium is highly dependent on efficient protein transport into and out of the organelle, which is accomplished via a

microtubule-based transport mechanism called intraflagellar transport (IFT). Anterograde (up the ciliary axoneme to the tip region of the cilium) and retrograde (down the axoneme to the cell body) transport of proteins along the cilium are mediated by two IFT complexes (IFT-B and IFT-A), which make use of kinesin and dynein motor proteins, respectively (Figure 1).⁶⁶

Besides the IFT machinery, other protein complexes cluster at the basal body and at the transition zone, forming functional networks that are essential for ciliary assembly and function. Indeed, defects in many proteins of these complexes are associated with a variety of ciliopathies. The physical interaction of multiple different proteins within each complex and their overlapping functions explain why mutations in the distinct genes that encode them result in similar ciliopathy phenotypes. In particular, mutations affecting proteins comprising the transition zone protein network are mostly responsible for JS and MKS, whereas genes encoding proteins of the basal body BBSome complex are usually mutated in BBS. The functions of these two important complexes are briefly discussed in the next two sections.

The B9/tectonic-like complex

Although the ciliary membrane is contiguous with the plasma membrane, studies have shown that the transition zone at the base of the cilium represents a diffusion barrier, which is essential for selective localization of certain transmembrane proteins within the cilia. Specific Y-shaped protein structures serve to connect the ciliary axoneme and the plasma membrane, forming a 'gating system' that actively regulates movement of proteins into and out of the cilium, in order to maintain the cilium as a highly compartmentalized organelle.⁶⁷ Interestingly, a network of about 15 proteins, nearly all of which are involved in JS and MKS pathogenesis, was found to localize at these transition zone structures.⁶⁸ This complex is termed the B9 or tectonic-like complex, as it contains all known B9-domain proteins and tectonic proteins, as well as several transmembrane family (TMEM) proteins, coiled-coil and C2 domain-containing protein 2A (CC2D2A), centrosomal protein of 290 kDa (CEP290), and jouberin (also known as abelson helper integration site 1 protein homologue [AHI1]). Specifically, the B9/tectonic-like complex prevents the rapid diffusion of unselected proteins across the transition zone, at the same time promoting the access of selected proteins and receptors (such as ADP-ribosylation factor-like protein 13B [ARL13B] and smoothened homologue [SMO]) to the cilium.⁶⁹

Converging data have shown that disruption of the B9/tectonic-like complex by silencing or mutation of one of its components *in vitro* and *in vivo* resulted in reduced cilia formation, significant reduction of ciliary receptor expression, and defective SHH signalling in the remaining cilia.^{69,70} These abnormalities also had dramatic effects on the many developmental processes in which primary cilia are involved.^{69,70} These findings highlight the normal roles of cilia and specific ciliary proteins, and provide insight into the pathological mechanisms underlying ciliopathies such as JS and MKS.

The BBSome

Most of the proteins encoded by genes mutated in patients with BBS comprise the eponymous BBSome complex that localizes both at the basal body and at the ciliary tip. This structure mediates and regulates microtubule-based intracellular transport processes that are crucial for ciliary biogenesis and signalling.⁷¹ Data have revealed that the BBSome is first responsible for assembling the IFT complexes at the ciliary base, then moves along the ciliary axoneme using the IFT-B machinery to reach the ciliary tip, where it reorganizes the IFT complex for retrograde transport, forming the IFT-A.⁷² Correct assembly of the IFT complexes is essential for movement of receptors, structural proteins and signalling molecules along the cilium and, as a consequence, the lack of functional BBS proteins results in defective ciliogenesis, aberrant signalling and, thus, altered developmental pathways.^{71,73} In particular, research has focused on the mechanisms regulating the trafficking of several G-protein-coupled receptors (GPCRs) to primary cilia. GPCRs comprise a large group of proteins that have critical roles in neural signalling, as well as in the correct functioning of other ciliopathy-affected organs such as the retina and kidneys.^{74,75} Increasing evidence suggests that components of the BBSome and members of the tubby protein family (which are mutated in a spontaneous mouse model of BBS) interplay with IFT proteins to promote the ciliary localization of specific GPCRs such as rhodopsin, somatostatin receptor 3 and melanin-concentrating hormone receptor 1, and that disruption of this trafficking process leads to ciliary dysfunction.^{76,77} These results inform our understanding of the specific biological processes that are affected in BBS.

Cilia in key developmental pathways

Primary cilia are essential for the normal development of embryos, and perturbations in cilium formation or function can lead to profound defects in embryogenesis, as are manifest in the described ciliopathies. In fact, key morphogenic signals that are active during embryonic development are among the extracellular signals that the cilium is able to sense and integrate. Here we will discuss the involvement of primary cilia in the three pathways that are most relevant to neural development: SHH, and canonical and non-canonical Wnt signalling. Importantly, primary cilia are also implicated in the correct functioning of several other signalling cascades, including the platelet-derived growth factor receptor α (PDGFR α), fibroblast growth factor (FGF), mTOR, Notch and Hippo pathways.⁷⁸

Primary cilia and the SHH pathway

One of the best-studied functions of primary cilia during vertebrate development is their involvement in SHH signal transduction, a pathway with critical roles in neuronal specification and CNS patterning.⁷⁹ Mice with genetic mutations resulting in defects in IFT-associated proteins were identified in a forward genetic screen that examined SHH signalling, demonstrating a key role for ciliary transport processes in this pathway.^{80,81} Indeed, multiple additional lines of evidence suggest that the primary cilium serves as a signalling platform that spatially and temporally regulates components of the SHH cascade.

In the absence of SHH, the regulatory receptor Patched (PTC) is localized to the base of the cilium, where it represses the activity of SMO by preventing its localization to the cilium. In

the presence of SHH, however, PTC is removed while active SMO accumulates in the cilium, promoting activation of GLI transcription factors.⁸² Several ciliopathy-associated genes are well-characterized components of this pathway. For instance, mutations in *GLI3* can cause either GPCS or PHS (and rarely ACLS; Table 1), and are associated with dysregulation of SHH pathway signalling.^{83,84} *KIF7*, which can be mutated in JS, ACLS and hydrolethrus syndrome, encodes a ciliary kinesin that seems to mediate anterograde transport and thus regulate the activity of GLI transcription factors; although mammalian KIF7 was initially linked to hedgehog signalling only in invertebrates, the subsequent finding that KIF7 localizes to the base of the cilium and translocates to the ciliary tip following SHH pathway activation clarifies its role in mammalian SHH signalling.⁸⁵ Consistent with this role, disease-associated mutations in *KIF7* lead to dysregulation of GLI transcription factors.⁵⁰ Of particular note, craniofacial abnormalities are a major feature of both *KIF7* and *GLI3* disorders,^{49,58} and genetic or pharmacological disruption of SHH signalling is also associated with severe craniofacial defects.^{86,87}

In addition to genes with known roles in the SHH pathway, mounting evidence suggests that other ciliopathy-associated genes have either a direct or an indirect role in the regulation of SHH signalling. As an example, genes encoding various members of the B9/tectonic-like complex that are mutated in JS or MKS (such as *TCTN1*, *TCTN2* and *TCTN3*, *ARL13B*, *RPGRIP1L* and *MKS1*) have all been associated with perturbations of SHH signaling.^{41,69,73,88–93}

Primary cilia and Wnt pathways

The canonical Wnt pathway—Primary cilia have been implicated in the correct functioning of another major developmental pathway, the canonical Wnt signalling cascade, which eventually leads to the stabilization and nuclear localization of β -catenin, with subsequent activation of target genes. In 2011, we demonstrated that joubertin, which is encoded by the *AHI1* gene that can be mutated in patients with JS,⁹⁴ and is a component of the transition zone B9/tectoniclike complex that can also localize to the nucleus, positively regulates canonical Wnt signalling by facilitating β -catenin translocation to the nucleus.⁹⁵ Furthermore, our study showed that the primary cilium served to modulate Wnt pathway responsiveness by sequestering joubertin and β -catenin in the cilium, thus limiting their nuclear entry.⁹⁵ As the canonical Wnt pathway has important roles in neurodevelopment,⁹⁶ disruption of this pathway could partly explain the neurological manifestations in JS.

The noncanonical Wnt pathway—The noncanonical Wnt (or planar cell polarity [PCP]) pathway, which is implicated in several developmental processes such as left–right patterning and neural tube closure,⁹⁷ is tightly integrated with correct functioning of primary cilia. Indeed, mutations in several ciliopathy-related genes, such as *TMEM237*, *TMEM216* or *TMEM67*, resulted in abnormal PCP cascade activity and, conversely, mutations in proteins with key roles in the PCP pathway, such as inturned, fuzzy or dishevelled, were found to alter ciliogenesis.^{98,99} Inversin, a protein mutated in various ciliopathies with occasional CNS involvement, was found to play a key part in activation of PCP by recruiting dishevelled to the plasma membrane in response to activated frizzled, and antagonizing dishevelled-stimulated canonical Wnt signalling.¹⁰⁰ Again, these relationships

suggest that correct ciliary function is required for normal development, and they focus attention on developmental pathways that might explain the malformations observed in ciliopathies, including neurological manifestations.

Ciliary functioning and CNS development

Notably, all the pathways discussed above have key roles in the embryonic development of the CNS, which might contribute towards explaining most of the neurological symptoms associated with many ciliopathies. For example, studies have shown that primary cilia are important in the regulation of neuronal progenitor cell proliferation and the generation of neurons both in the cerebral cortex and the hippocampus,^{101,102} as well as in orchestrating the coordinated migration and placement of postmitotic inter neurons in the developing cerebral cortex.¹⁰³ In particular, ARL13B, which is encoded by a JS-related gene and is enriched in cilia,¹⁰⁴ was found to have a critical function in the initial processes regulating formation of polarized radial glial cells at early stages of CNS development in mice.¹⁰⁵ The radial glia are essential prenatally, ensuring balanced production of neurons and neuronal progenitors. Postnatally, they provide an instructive structural scaffold informing oriented migration and placement of different groups of neurons. Moreover, selective disruption of normal ciliogenesis induced by mutations in BBS-associated genes or those encoding IFT proteins was found to markedly impair dendrite outgrowth and branching, as well as their synaptic integration, both in developing cortical neurons and in the adult hippocampus.^{106,107}

Cerebellar defects in ciliopathies

During cerebellar development, granule cell precursors (GCPs) originating from the rhombic lip, a major germinal zone, migrate to the external germinal layer where they proliferate to form granule neurons, an abundant cell type that accounts for nearly half of the neurons in the adult brain.¹⁰⁸ GCPs are among the most important determinants in the processes of cerebellar development, growth and foliation: for instance, *Zic1*-knockout mice, in which GCPs demonstrated defective proliferation and migration, developed a smaller cerebellum with reduced foliation.¹⁰⁹ SHH signalling is a key regulator of growth and patterning in the cerebellum and, thus, disruptions in this pathway has been hypothesized to account for cerebellar malformations and associated ataxia seen in JS and other ciliopathies.^{110,111} In fact, the proliferation of GCPs is highly dependent on SHH secreted locally by mature Purkinje cells under the control of a para crine feedback loop,¹¹² and this pathway has been found to be disrupted in several models of ciliary dysfunction. For example, *Kif3a*-knockout^{110,113} and *Ift88*-knockout¹¹³ mice present defective primary cilia in cells of the developing CNS, and exhibit cerebellar hypoplasia and defects in foliation accompanied by decreased SHH signalling. Furthermore, in 2012, Aguilar *et al.*¹¹⁴ demonstrated an overall defect in GCP proliferation in the cerebellum in fetuses with JS or MKS, a finding that closely correlated with impairment of SHH signalling. However, the observed deficiency in GCP proliferation involved both the cerebellar vermis and hemispheres, and thus does not fully explain the selective vermian hypoplasia that is a typical neurological feature in JS (Figure 3b).

The canonical Wnt signalling cascade is also crucial for the correct development of the cerebellum, highlighting another pathway through which ciliary malfunction might lead to the cerebellar defects that are characteristic of certain ciliopathies. Activation of this pathway by ectopic overexpression of activated β -catenin impaired the proliferation of GCPs while promoting the growth of another class of progenitors, multipotent neural stem cells.¹¹⁵ Similarly, in a conditional *Apc*-knockout mouse model, lack of adenomatous polyposis coli—a negative regulator of canonical Wnt signalling—and resultant constitutive overexpression of activated β -catenin in GCP cells was associated with severe impairments in the structure of the internal granular layer, with premature differentiation of GCP and disorganization of the Purkinje layer.¹¹⁶ In line with these findings, we have shown that mice lacking either joubertin or CEP290, two ciliary proteins that can be mutated in JS, present with dysregulation of Wnt– β -catenin signalling.¹¹⁷ In these mice, abnormal Wnt signalling was associated with cerebellar hypoplasia and a midline fusion defect similar to that seen in patients with JS, which could be partially rescued by treatment with lithium, a Wnt pathway modulator.¹¹⁷ Intriguingly, prior to our study, a similar phenotype comprising cerebellar vermian hypoplasia and a midline fusion defect had been observed in mice on selective ablation of β -catenin in precursor cells of the developing CNS at mid-gestation,¹¹⁸ supporting a key role for canonical Wnt signalling in cerebellar development, potentially mediated by primary cilia.

Primary cilia in adult neurogenesis

Primary cilia have also been implicated in adult neurogenesis, raising the intriguing possibility that ongoing defects in neuronal proliferation and maturation could contribute to the cognitive impairment seen in many patients with ciliopathies. Several studies have shown that cilia are required for normal progenitor-cell proliferation in the hippocampal dentate gyrus, during an SHH-dependent postnatal growth spurt that expands this structure.^{119,120} Although malformations of the hippocampus have not commonly been reported in ciliopathies, several studies have, nevertheless, described hippocampal abnormalities in patients with JS,^{15,121} *ACLS*⁴⁹ or *HLS*.^{49,54}

In addition to deleterious effects on postnatal development, lack of primary cilia in adult progenitor cells resulted in a reduction in hippocampal neurogenesis and a deficit in spatial learning in mice.¹⁰¹ Interestingly, primary cilia have also been demonstrated to be critical for synapse formation in adult-born hippocampal neurons from newborn mice,¹⁰⁷ indicating that these organelles are required for successful integration of adult-born neurons into existing brain circuitry. Interestingly, deletion of primary cilia was associated with increased Wnt– β -catenin signalling in this study,¹⁰⁷ suggesting that regulation of this pathway by cilia might explain these observations. Although these findings support a potential pathomechanism for intellectual disability associated with ciliary dysfunction, further work will be necessary to determine whether this pathway has a role in human ciliopathies.

Neural defects and motile cilia

The ciliopathies discussed in this Review result from dysfunctions relating to primary cilia that have an axonome structure comprising a ring of nine microtubule doublets (9+0 arrangement), but lack the additional central doublet of microtubules found in motile cilia

(9+2 arrangement), which usually makes them immotile. However, motile cilia are also found in the CNS, and their dysfunction is related to specific neurological phenotypes. In motile cilia, the 9+2 axonemal structure, along with the presence of inner and outer dynein arms on the axonemal microtubules, enables the organelles to beat regularly, supporting their mechanical functions in specific tissues such as the respiratory epithelium and the oviduct, or as flagella propellers on sperm. In humans, a defect in the structure or function of motile cilia and flagella causes a condition known as primary ciliary dyskinesia (PCD; also known as Kartagener syndrome), characterized by chronic sinusitis and bronchitis, otitis media, infertility, situs inversus and, occasionally, hydrocephalus.¹²²

In the CNS, motile cilia are found on ependymal cells throughout the brain, and their coordinated beating facilitates directional flow of the cerebrospinal fluid through the ventricular system. As a consequence, defective functioning of ependymal motile cilia results in hydrocephalus without obstruction, although this manifestation is observed much more frequently in several different mouse models than in patients with PCD.¹²³ Interestingly, however, the boundaries between motile ciliopathies and primary ciliopathies seems to be blurred, as hydrocephalus can also be observed in a number of disorders of primary cilia, such as OFD syndromes, BBS, HLS, and GCPS (Table 1). Indeed, some BBS-related proteins are known to be expressed not only in primary cilia, but also in conjunction with motile cilia, and deficiency in these proteins causes structural and functional defects, with reduced ciliary beating, in motile cilia.¹²⁴ Mutations in such proteins might, therefore, affect the function of both primary and motile cilia, with the defects in motile cilia explaining hydrocephalus. Nevertheless, in a hydrocephalic mouse model of BBS caused by knock-in mutations in the *Bbs1* gene,²⁹ equivalent to the commonest *BBS1* mutation found in human BBS, mortality was markedly increased and was associated with reduced proliferation of neuronal precursor cells in the periventricular regions. These outcomes were attributed to aberrant PDGFR α signalling in primary cilia, whereas motile cilia were found to be functioning normally.²⁹ In this model, a therapeutic approach using lithium, which targeted two downstream effectors of the PDGFR α pathway, was able to increase neuronal precursor cell proliferation and to partly rescue the hydrocephalic phenotype.²⁹

Conclusions

A wealth of data generated over the past decade has highlighted the central role of primary cilia in a wide spectrum of human diseases. Although we still do not fully understand the mechanisms that underlie neurological malformations associated with these ciliopathies, increasing knowledge of the molecular basis and pathogenetic mechanisms of these disorders has clarified a common aetiology, and has facilitated elucidation of potential mechanisms that lead to brain malformations. Now that we have started to better understand the complexities of ciliary structure and function, the time has come to push research further in exploration of potential avenues for early therapeutic strategies in cilia-related disorders. The partial rescue of neurological defects seen in *Bbs1*-mutant and *Ahi1*-mutant mice after treatment with lithium represents an encouraging finding, indicating that modulators of Wnt, PDGFR α or SHH signalling pathways could represent promising approaches to the development of effective treatments for ciliopathies.

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Key points

- Primary cilia are single hair-like, non-motile sensory organelles that are found on the surface of almost all cells in vertebrates
- Physiological roles of primary cilia include chemical and mechanical sensation, signal transduction, and control of cell growth
- Mutations in genes relating to the structure or function of primary cilia are responsible for a clinically and genetically heterogeneous class of disorders known as ciliopathies
- A subset of ciliopathies are commonly associated with intellectual disability and brain malformations that can include midbrain and/or hindbrain malformations, agenesis of the corpus callosum, and encephalocele
- Primary cilia have key roles in mediating morphogenic and mitogenic signals during development, and perturbations in these pathways probably contribute to the neurological features of ciliopathies

Review criteria

PubMed was searched for English-language articles published up to October 2013 using the search terms “ciliopathies” and “primary cilium”. Relevant papers were also identified through searches of the authors’ own files. The final reference list was generated on the basis of originality and relevance to the broad scope of this Review.

Figure 1. Structure and organization of the primary cilium

The primary cilium is a hair-like structure that protrudes from the cell surface. Microtubules form the core structure of the cilium, the axoneme. Protein cargo is transported up and down the cilium via anterograde and retrograde IFT mediated by kinesin and dynein motor proteins, respectively, which travel along the axoneme. Several proteins implicated in Joubert syndrome and Meckel syndrome form a large complex at the transition zone, which is involved in the regulation of ciliogenesis and in the control of the traffic of specific molecules into and out of the cilium. Proteins mutated in BBS cluster in the BBSome

complex. This complex is relevant for ciliogenesis and regulates the correct assembly and functioning of the IFT machinery. Abbreviations: BBS, Bardet–Biedl syndrome; IFT, intraflagellar transport.

Figure 2. Typical clinical features in ciliopathies with CNS involvement

a | Facial dysmorphisms in JS, including prominent forehead, high and rounded eyebrows, upturned nose and anteverted nostrils. **b,c** | Postaxial polydactyly type B (*digitus minimus*) in patients with JS. **d** | Abdominal protrusion in a medically aborted fetus with Meckel syndrome; on autopsy, the swelling was found to be due to cystic and enlarged kidneys. **e** | Postaxial polydactyly type A of the foot in Bardet-Biedl syndrome. **f** | Hamartomatous mass of the tongue in OFD6. **g** | Hyperplasia of the frenula in a patient with OFD6. **h** | Midline cleft of the upper lip in OFD6. **i** | Polysyndactyly in a fibularly bent hallux in OFD6. **j** | Mesoaxial polysyndactyly in a patient with OFD6. **k** | Characteristic facies in acrocallosal syndrome. Abbreviations: JS, Joubert syndrome; OFD6, orofacioidigital syndrome type VI. Written consent for publication was obtained from the parents of the children shown in panels a and k.

Figure 3. Common MRI findings in ciliopathies with CNS involvement

Axial (upper panels) and midline sagittal (lower panels) brain MRI scans. **a,b** | Joubert syndrome. Molar tooth sign (arrows in a), with horizontally oriented and thickened superior cerebellar peduncles (arrows in a and b), and hypoplasia and dysplasia of the cerebellar vermis (arrowheads in b). **c,d** | Bardet-Biedl syndrome. Minimally enlarged interfoliar spaces in the superior vermis (arrow in d), with otherwise normal size and morphology of the brainstem, vermis and cerebellar hemispheres. **e,f** | Orofaciodigital syndrome type VI. Molar tooth sign with thickened and elongated superior cerebellar peduncles (horizontal arrows in e), hypoplasia and dysplasia of the cerebellar vermis (black arrow in f) and both cerebellar hemispheres, enlarged fourth ventricle and posterior fossa (black arrowheads in f), hypothalamic hamartoma (vertical arrow in e, white arrow in f), elongated midbrain with thin pons (not shown), and abnormal prominence of the posterior lower brainstem (white arrowhead in f). **g,h** | Acrocallosal syndrome. Agenesis of corpus callosum (arrow in h) with secondary abnormal configuration of the lateral ventricles; brainstem and cerebellum seem morphologically normal. Panels e and f reprinted with permission from the American Society of Neuroradiology © Poretti, A. *et al. Am. J. Neuroradiol.* **29**, 1090–1091 (2008).

Table 1

Genetic basis and neurological features of ciliopathies involving the CNS

Ciliopathy	Disease-related genes	Neurological features
Joubert syndrome	<i>AHI1, ARL13B, C5orf42, CC2D2A, CEP41, CEP290, EXOC8, INPP5E, KIF7, NPHP1, OFD1, PDE6D, RPGRIP1L, TCTN1, TCTN2, TCTN3, TMEM67, TMEM138, TMEM216, TMEM231, TMEM237, TTC21B[‡], ZNF423</i>	Molar tooth sign Oculomotor apraxia Encephalocele Intellectual disability Ataxia Retinal dystrophy Corpus callosum hypoplasia
Meckel syndrome	<i>B9D1, B9D2, CC2D2A, CEP290, MKS1, NPHP3, RPGRIP1L, TMEM67, TMEM216, TMEM237, TCTN2</i>	Encephalocele Posterior fossa defects Hydrocephalus Corpus callosum hypoplasia Perinatal lethality
Orofaciodigital syndromes	<i>C5orf42, KIF7, OFD1, TCTN3, TMEM216</i>	Cerebellar defects Hydrocephalus Intellectual disability Hypothalamic hamartoma* Molar tooth sign*
Bardet–Biedl syndrome	<i>ARL6, BBS1, BBS2, BBS4, BBS5, BBS7, BBS10, BBS12, CCDC28B[‡], CEP290, MKKS, MKS1, LZTFL1, PTHB1, SDCCAG8, TMEM67[‡], TRIM32, TTC8, WDPCP</i>	Intellectual disability Retinal dystrophy Hydrocephalus
Acrocallosal syndrome	<i>KIF7, GLI3</i>	Intellectual disability Corpus callosum agenesis
Hydroletharus syndrome	<i>HYLS1, KIF7</i>	Hydrocephalus Midline cerebral defects Cerebellar defects Abnormal foramen magnum Hypothalamic hamartoma Perinatal lethality
Pallister–Hall syndrome	<i>GLI3</i>	Hypothalamic hamartoma
Greig cephalopolysyndactyly syndrome	<i>GLI3</i>	Intellectual disability Hydrocephalus

* Only in orofacioidigital syndrome type VI.

[‡] Reported as a genetic modifier only.