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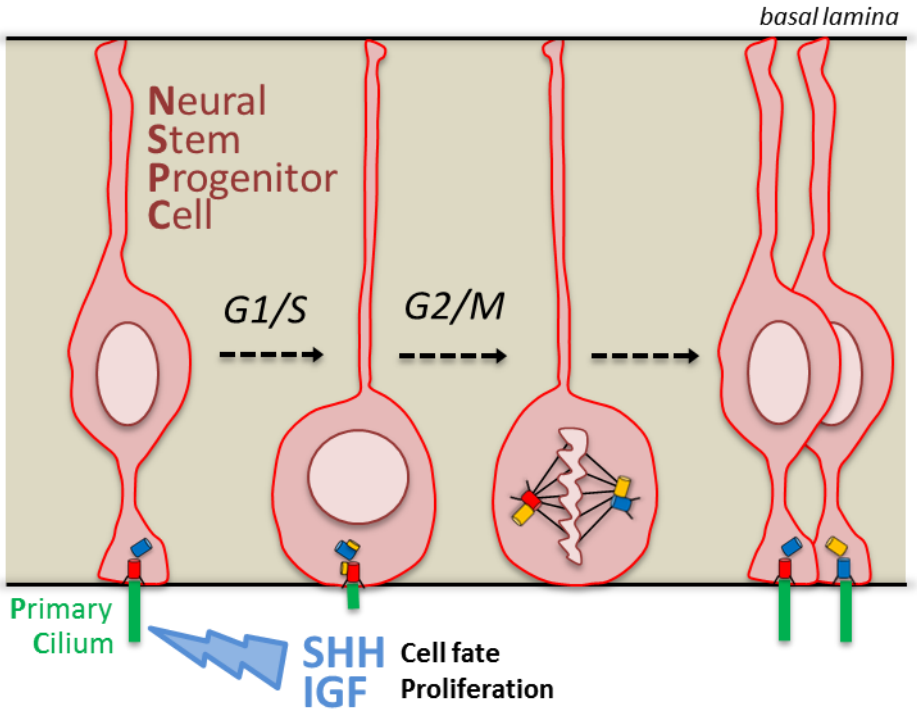
Abstract:

Ciliopathies are complex genetic multisystem disorders causally related to abnormal assembly or function of motile or non-motile cilia. While most human cells possess a non-motile sensory/primary cilium (PC) during development and/or in adult tissues, motile cilia are restricted to specialized cells. As a result, PC-associated ciliopathies are characterized by high phenotypic variability with extensive clinical and genetic overlaps. In the present review, we have focused on cerebral developmental anomalies which are commonly found in PC-associated ciliopathies and which have mostly been linked to Hedgehog signaling defects. In addition, we have reviewed emerging evidence that PC dysfunctions could be directly or indirectly involved in the mechanisms underlying malformations of cerebral cortical development including primary microcephaly.

Graphical Abstract:



Ciliopathies are complex genetic disorders linked to primary cilia dysfunction. Cerebral anomalies are one of the manifestations observed in ciliopathies which have mainly been linked to defective Hedgehog signaling. Additionally, in neuron progenitors, ciliary disassembly controls the duration of the G1/S transition and, therefore, proliferative versus neurogenic divisions. Increased cilium length or defects in disassembly may also contribute to microcephaly by a loss of progenitors.

Abbreviations

ACLS: acrocallosal syndrome

BB: basal body

BBS: Bardet Biedl Syndrome

CC: corpus callosum

CSF: cerebrospinal fluid

HLS: hydrolethalus syndrome

IFT: intraflagellar transport

JBTS: Joubert Syndrome

KIF: Kinesin family member

MCD: malformations of cerebral cortical development

MKS: Meckel-Grüber Syndrome

MPD: microcephalic primordial dwarfism

NTD: Neural Tube Defect

NSPC: neural stem and progenitor cells

OFD: orofaciodigital syndrome

PC: primary cilia

PCD: Primary ciliary dyskinesia

PCP: Planar Cell Polarity

SHH: Sonic Hedgehog

Introduction

Cilia are highly conserved organelles which can be motile or immotile (Satir and Christensen, 2007). All cilia are assembled from a basal body, which corresponds to the mother centriole of the centrosome for immotile sensory or primary cilia (PC), and to amplified centrioles for motile cilia in multiciliated cells. Ciliogenesis is a complex process involving the docking of centrioles to intracellular membranes (primary ciliary vesicles), through centriolar distal appendages (Figure 1A), and the subsequent elongation of the microtubule-based axoneme thanks to a highly conserved transport machinery, the intraflagellar transport (IFT), which mediates import and export of ciliary components inside and from the ciliary compartment (Figure 1B). Indeed, both the 'cilioplasm' and ciliary membrane are separated, respectively, from the cytoplasm and plasma membrane through the 'ciliary gate' based on two important structures, the transition fibers (centriolar distal appendages) and the transition zone. Ciliary components are efficiently transported through this barrier by the IFT machinery, IFT-A and IFT-B subcomplexes, associated with anterograde (Kinesin II, KIF3A/B) and retrograde (cytoplasmic dynein 2) motors, allowing the dynamic transport of ciliary receptors and signaling intermediates in and out of the PC (Figure 1B; (Lechtreck, 2015)). While motile cilia are formed only on terminally differentiated cells (Spassky and Meunier, 2017), PC are additionally also found on cells able to divide, including stem cells. In proliferating cells, PC are disassembled through a complex, cell cycle-linked process (Sánchez and Dynlacht, 2016) allowing the release of the duplicating centrosome from the plasma membrane to enable its function in mitotic spindle assembly. Interestingly, at least in some cell types (see below), a ciliary membrane remnant remains associated with the mother centriole during this process (Figure 1A). This ciliary-primed older mother centriole will be asymmetrically inherited by one of the daughter cells, which will then ciliate and respond to PC-dependent signals more rapidly (Anderson and Stearns, 2009; Paridaen *et al.*, 2013).

Motile cilia line the epithelial surfaces of the respiratory tract, testis efferent ducts, oviduct and brain ventricles, where they are required for the oriented movement of fluids or gametes (Spassky and Meunier, 2017). The characteristic disease of motile cilia is primary ciliary dyskinesia (PCD), a group of genetic diseases characterized by chronic airway infections, sinusitis and otitis media. Some patients can also present with situs inversus and congenital heart disease in the case of Kartagener syndrome (Mitchison and Valente, 2017; Reiter and Leroux, 2017). In the brain, defects in motile cilia can be associated with hydrocephalus characterized by the enlargement of brain ventricles resulting from an accumulation of cerebrospinal fluid (CSF) in the cerebral ventricles (Lee, 2013; Spassky and Meunier, 2017).

Primary cilia (PC) are sensory/signaling cilia present on most cell types during development where they control key signaling pathways. The most well characterized cilia-dependent signaling

pathway in vertebrates is Sonic Hedgehog (SHH), which plays a key role in vertebrate organogenesis (limbs, brain) controlling proliferation and specification. Most of the components of this pathway including Patched (SHH receptor, Ptch), Smoothened (Smo) and GLI transcription factors, were shown to dynamically localize to PC depending on SHH stimulation. Furthermore, SHH signaling culminates in the processing of the bifunctional GLI transcription factors to generate GLI activator or repressor forms in a cilium-dependent manner. Pathway activation results in the removal of Ptch from the ciliary membrane, while Smo accumulates preventing Gli processing (mainly GLI3R) and leading to the generation of GLI activator forms (mainly GLI2A; (Bangs and Anderson, 2017)). In addition to SHH, PC were also involved in other important signaling pathways during development (PDGFα, TGFβ) and tissue homeostasis/function, including sensing of molecules (photoreceptors outer segment (photons)) or of mechanical clues (Kidney epithelia, chondrocytes; (Nishimura *et al.*, 2019)). In the brain, PC play crucial roles during cerebral development, especially in early patterning of the neural tube, neural stem cell pool regulation, neural differentiation and migration (Guemez-Gamboa *et al.*, 2014; Youn and Han, 2018).

Impaired assembly or function of PC lead to a still growing group of highly heterogeneous inherited diseases collectively called (primary) ciliopathies which can affect almost all tissues/organs (Mitchison and Valente, 2017; Reiter and Leroux, 2017). The notion of a "ciliopathic" disorder was first attributed to Bardet–Biedl syndrome (MIM #209900, BBS; (Ansley *et al.*, 2003)), a condition characterized by retinal dystrophy, cystic kidneys, polydactyly, obesity and intellectual disabilities. Each of these classical ciliopathy-associated manifestations and others (hepatic fibrosis, skeletal dysplasia, etc…) can be found isolated or in specific associations in affected individuals, then defining syndromic forms. PC-associated ciliopathies are mostly recessively inherited and genetically highly heterogeneous, with both major phenotypic and genetic overlap. Indeed, allelism (a common causal gene for distinct disorders), has been shown between several ciliopathies, such as between Joubert (MIM #213300, JBTS) and Meckel (MIM #249000, MKS) syndromes where almost 13 common genes have so far been reported, supporting the notion that MKS represents the extreme lethal form of JBTS.

Developmental brain anomalies are among the characteristic and frequent ciliopathy-associated manifestations (Guemez-Gamboa *et al.*, 2014). Besides hydrocephalus, which could be linked to both motile and PC dysfunctions, cerebellar hypoplasia and corpus callosum malformations, together with neural tube closure defects, are manifestations classically associated with abnormal PC biogenesis or function. Microcephaly with or without malformations of cerebral cortical development (MCD) can also occasionally be attributed to PC-ciliopathies but are not commonly categorized among classical ciliopathy-associated manifestations. Recent data, however, indicate

that PC dysfunctions could participate, directly or indirectly, in the pathological mechanisms underlying microcephaly and/or MCD.

Here, we have summarized the known mechanisms involved in ciliopathy-associated brain anomalies in humans and emphasized the emerging evidence implicating PC dysfunctions in cerebral cortical disorders, particularly in primary microcephaly.

Ciliopathy-associated cerebral anomalies:

The key role of cilia during brain development in vertebrates was first demonstrated in a genetic mouse screen for mutations affecting dorso-ventral patterning of the neural tube, which showed that disruption of genes coding for IFT subunits or for the IFT anterograde motor protein *Kif3a* are required for SHH signaling (Huangfu *et al.*, 2003). This initial connection between SHH signaling and PC function in mammals, together with the delineation of the brain defects observed in various ciliopathies, largely contributed to the explosion of interest in PC. Here we have focused on the major ciliopathy-related, developmental brain disorders present in ciliopathy cases in humans and which have been largely linked to misregulation of the SHH pathway.

Neural tube defects:

Neural tube defects (NTDs) include a large variety of central nervous system malformations which may be classified in 1) open forms (e.g. anencephaly/exencephaly, spina bifida aperta) resulting from defects of primary neurulation, 2) closed forms (e.g. spina bifida occulta) resulting from defects of secondary neurulation and 3) herniation forms (e.g. encephalocele) due to post-neurulation defects (Greene and Copp, 2014). Anencephaly, as well as occipital encephalocele, can manifest in PC-ciliopathies, including MKS and Hydrolethalus syndrome (HLS, MIM #614120; see *Corpus callosum agenesis*).

In various vertebrate species, NTD have been closely linked to abnormal SHH signaling (Greene and Copp, 2014; Shimada and Mukhopadhyay, 2017), which orchestrates neural tube patterning (Ribes and Briscoe, 2009; Bangs and Anderson, 2017). SHH is initially secreted by the notochord and then by cells of the floor plate (Figure 2B). The subsequent ventral to dorsal gradient of SHH leads to the differentiation of neuron subtypes by the induction of specific sets of transcription factors. In addition, SHH also appears to act as a negative regulator of the dorsolateral bending of the neural tube which happens at mid spinal levels (where SHH is low; Figure 2B, red arrows) through coordinated apical domain contraction of neuroepithelial cells and is required for the closure of the neural tube (Nikolopoulou *et al.*, 2017). Accordingly, overactivation of the pathway is commonly associated with closure defects, as observed in *Ptch1*-/- (Goodrich *et al.*, 1997) or *Sufu* -

/- mice (Cooper *et al.*, 2005), which show an expansion of ventral markers and loss of dorsal markers and exhibit exencephaly. On the contrary, complete absence of SHH signaling in *SHH* deficient mice (Chiang *et al.*, 1996) or reduced SHH signaling in *Smo* -/- mice (Zhang *et al.*, 2001) leads to severe dorso-ventral patterning defects of the neural tube which is closed but lack forebrain separation leading to holoprosencephaly (HPE; MIM #236100), a spectrum of midline defects in which cyclopia is the most severe form (Dubourg *et al.*, 2018). Mouse models of the invalidation of PC-genes often present with NTD but the situation appears more complicated than a simple link between PC presence/absence or excess/reduced SHH activity and NTD versus HPE. In the ventral spinal cord, some IFT-A mutants with almost normal PC present a phenotype reminiscent of SHH overactivation including an expansion of ventral cell types at the expense of dorsal cell types and NTD. On the other hand, additional IFT-A mutants with abnormal PC (very short and bulged PC) show dorso-ventral patterning defects consistent with reduced SHH activity (loss of ventral cell fates) similar to those observed in IFT-B mutants which lack cilia but both can still present exencephaly (for review see Bangs and Anderson, 2017).

In conclusion, besides the apparently simple dichotomy between mutants with an invalidation of genes encoding direct components of SHH signaling leading either to overactivation or reduced activity of the pathway with subsequent NTD or not, ciliary mutants represent a complex group with varying consequences on neural tube closure. Importantly, emerging, and sometimes controversial, roles of PC in other signaling pathways have suggested that their disruption could also be involved in NTD. Among them, the planar cell polarity (PCP) pathway is largely associated with NTD, although the types of NTDs are distinct from those observed in ciliary mutants. Indeed, PCP mutants exhibit severe forms of NTD involving the entire neural tube (craniorachischisis) whereas NTD in cilia mutants are mostly restricted to the cranial region (exencephaly; (Nikolopoulou *et al.*, 2017)). Thus, in view of all those evidence in animal models, PC are clearly playing a key role in neural tube patterning and closure through regulation of SHH signaling, but further investigations are needed to improve our understanding on the involvement of PC in the process of neural tube closure, in particular to delineate the potential involvement of other PC-dependent signaling pathways.

Corpus callosum agenesis:

The corpus callosum (CC) is the largest axonal tract or commissure in the brain, consisting of over 190 million axons from callosal neurons connecting homologous cortical areas of the two cerebral hemispheres (Figure 3A,B). The CC influences higher cognition, social interaction and language (Edwards *et al.*, 2014). CC development is a highly complex process with several steps from early telencephalic patterning, callosal neuron expansion and specification, to the guidance of callosal

axons to cross the midline and reach specific contralateral regions. CC malformations represent the most frequent brain malformations observed at birth in humans and are highly heterogeneous, ranging from complete to partial agenesis or hypoplasia (Figure 3C,D,F). CC malformations can be isolated but are frequently part of congenital syndromes; including several ciliopathies. In particular, acrocallosal syndrome (ACLS, MIM #200990) is characterized by the association of CC agenesis or hypoplasia with intellectual deficiency, polydactyly and craniofacial features. We have previously identified *KIF7* mutations as responsible for this condition, as well as for an overlapping lethal ciliopathy, the hydrolethalus syndrome (HLS, MIM #614120; (Putoux *et al.*, 2011)).

KIF7 is a ciliary kinesin which plays a crucial and conserved role in the organization of a SHH signaling platform at the distal end of PC (Bangs and Anderson, 2017; Reilly and Benmerah, 2019). In both humans and mice, KIF7 depletion leads to overactivation of the SHH pathway associated to abnormal GLI3 processing (Liem *et al.*, 2009; Putoux *et al.*, 2011). Interestingly, *GLI3* mutations in humans lead to Greig syndrome (MIM #175700), an ACLS overlapping syndrome with frequent CC agenesis (Vortkamp *et al.*, 1991; Biesecker, 2008). Other ciliopathies are associated to CC malformations including the Orofaciodigital syndrome I (OFD1, MIM #311200; (Thauvin-Robinet *et al.*, 2013)) as well as in Orofaciodigital syndrome IV (OFD4, OMIM #258860) associated to mutations in *TCTN3* (Thomas *et al.*, 2012). OFD1 is playing a key role in centriole elongation and therefore in ciliogenesis (Ferrante *et al.*, 2006; Singla *et al.*, 2010), whereas TCTN3 is a key component of the transition zone which is implicated in SHH signaling (Garcia-Gonzalo *et al.*, 2011; Wang *et al.*, 2017). The growing number of ciliary genes identified as responsible for ciliopathies associating CC malformations, along with the use of murine models of ciliary gene depletion (Benadiba *et al.*, 2012; Laclef *et al.*, 2015; Putoux *et al.*, 2018), has revealed the role of cilia-dependent SHH signaling, especially through regulation of GLI3 processing, in CC development. Indeed, GLI3 was previously shown to play key roles during brain development and neural tube patterning (Theil *et al.*, 1999; Persson *et al.*, 2002) and the introduction of one or two alleles of $Gli3^{\Delta 699}$, which produces only the short isoform of Gli3 (repressive form; GLI3R) rescues several brain defects observed in ciliopathy models, including CC anomalies (Besse *et al.*, 2011; Laclef *et al.*, 2015). For instance, in *Kif7-/-* mice, which recapitulate major ACLS features, including CC agenesis, the introduction of the $Gli3^{\Delta 699}$ allele is sufficient to rescue CC anomalies as well as specific patterning defects of the cortical septum boundary responsible for the altered distribution of guidepost cells required to guide callosal axons through the midline (Putoux *et al.*, 2018).

Interestingly, CC malformations can also be found in association with malformations of cerebral cortical development (MCD) and primary microcephaly, notably in the case of mutations in *WDR62* (Bilgüvar *et al.*, 2010), *ASPM* (Passemard *et al.*, 2009), *RTTN* (Kheradmand Kia *et al.*, 2012; Chartier *et al.*, 2018) or *NDE1* (Alkuraya *et al.*, 2011; Bakircioglu *et al.*, 2011) all encoding proteins

more or less associated to PC dynamics and/or functions. In those cases, PC dysfunctions might affect callosal neuron generation, in addition to callosal axon guidance (see in 'malformations of cerebral cortical development').

Cerebellar hypoplasia:

Cerebellum function was historically restricted to sensory-motor processing but it has also long been associated to cognition. JBTS is the archetypal ciliopathy affecting cerebellar development. It was first described in 1969 (Joubert *et al.*, 1969, 1999) and is characterized by a cerebellar and brainstem malformation which results in the appearance of a molar tooth on axial brain magnetic resonance imaging and was thus called "the molar tooth sign" (Figure 3A, E, G). This anomaly arises from the combination of cerebellar vermis hypodysplasia, thick, elongated and horizontally oriented superior cerebellar peduncles and a deep interpeduncular fossa (Maria *et al.*, 1997; Valente *et al.*, 2013). SHH signaling (Wechsler-Reya and Scott, 1999) and PC (Chizhikov *et al.*, 2007; Spassky *et al.*, 2008) have been shown to play a key role during mouse cerebellar development by promoting the expansion of cerebellar granule cell precursors, suggesting that disruption of this pathway underlies the cerebellar malformations observed in ciliopathy cases. A defect in the SHH-dependent expansion of granule cell precursors was further demonstrated in human in both the cerebellar hemispheres and vermis in MKS and JBTS fetuses (Aguilar *et al.*, 2012), likely explaining the global cerebellar phenotype. The specific vermis dysplasia may be linked to another earlier mechanism potentially involving defective WNT signaling, another crucial pathway for cerebellar development (Hatten and Roussel, 2011), which was also linked to PC (Lancaster *et al.*, 2011). Of the mutations thus far identified in JBTS, most of the affected genes encode ciliary proteins which localize to the transition zone and play a key role in SHH signaling (Bangs and Anderson, 2017; Mitchison and Valente, 2017), while their contribution to WNT signaling in this context remains to be fully explored.

Hydrocephalus:

Hydrocephalus is characterized by an enlargement of the brain ventricles resulting from an excess of cerebrospinal fluid (CSF) secondary to impaired CSF flow, blockage of aqueducts connecting the brain ventricles, excess CSF production or a lack of CSF reabsorption. CSF is produced by the choroid plexus of the lateral, third and fourth ventricles and normally flows from the lateral ventricles to the third ventricle, through the aqueduct of Sylvius, into the fourth ventricle, and finally along the spinal channel and subarachnoid space, where the CSF is reabsorbed into the blood or lymphatic system (Lee, 2013). The coordinated and oriented beating of motile cilia at the apical surface of ependymal cells participates in establishing CSF flow (Spassky and Meunier, 2017). Defects in either the

specification of ependymal cells, the amplification/orientation of basal bodies or ciliary beating are associated to impaired CSF flow and subsequent hydrocephalus. Surprisingly, while hydrocephalus is frequently observed in many animal models of PCD, although with mouse strain variability, it is only occasionally present in individuals diagnosed with PCD (Mitchison and Valente, 2017). This difference suggests that other genetic mechanisms underlie distinct susceptibility to hydrocephalus (Lee, 2013; Spassky and Meunier, 2017).

Hydrocephalus is also one of the manifestations of severe PC ciliopathies including MKS, OFD and HLS. Most of the causal genes identified for those ciliopathies encode PC-associated proteins crucial for SHH signaling (see previous chapters), while *HYLS1*, the first gene identified in HLS, was involved in various steps of ciliogenesis including the assembly of transition fibers, the docking of basal bodies onto membranes (motile cilia) and transition zone organization (Dammermann *et al.*, 2009; Wei *et al.*, 2016). Whether the proteins implicated so far have potential functions either at motile cilia functions and/or the specification/differentiation of ependymal cells through their known function in the SHH pathway (Yu et al., 2013) remain to be investigated. Interestingly, it was shown that hydrocephalus in *Ift88* mutant mice develops before the appearance of motile cilia and has been attributed to the overproduction of CSF by the choroid plexus resulting from defects in PC (Banizs *et al.*, 2005) which then could also explain the hydrocephalus present in severe PC ciliopathies.

Could microcephaly be regarded as a ciliopathy-associated phenotype?

The cerebral cortex is a highly organized, six-layered structure that contains billions of neurons and glial cells. This region of the brain is responsible for higher-order cognition and reasoning, language, advanced motor skills, and social-emotional behavior and has undergone pronounced expansion during evolution from the small and smooth (lissencephalic) cortex of mice, to the large and profoundly folded (gyrencephalic) cortex of humans. In the last decade, substantial progress has been made in identifying the cellular and molecular events regulating cortical expansion and folding. In particular, multiple neural stem and progenitor cells (NSPC) have been identified and classified based on specific hallmarks including the mode of cell division, proliferative capacity, polarity and location of mitosis ((Hansen *et al.*, 2010; Lui *et al.*, 2011; Nonaka-Kinoshita *et al.*, 2013); Figure 4A). Primary microcephaly (MIM #251200) is characterized by a reduced brain size present at birth (or antenatal) and which can occur either isolated or in association with malformations of cortical development (MCD), including gyration defects. Primary microcephaly is highly genetically heterogeneous with several genes encoding proteins involved in the biology of the centrosome and/or microtubules dynamics (Naveed *et al.*, 2018; Romero *et al.*, 2018). While these proteins were shown to play essential roles in NSPC cell cycle dynamics and checkpoints through their roles in cytoplasmic microtubules and mitotic spindle organization/dynamics (Faheem *et al.*, 2015; Morris-

Rosendahl and Kaindl, 2015; Doobin *et al.*, 2017), recent evidence also stressed their involvement in PC dynamics and/or signaling properties.

The first evidence for a possible link between PC and microcephaly came from the identification of mutations in *PCNT* in patients with microcephalic osteodysplastic primordial dwarfism, type II (MOPDII, MIM #210720; (Rauch *et al.*, 2008)) belonging to the group of microcephalic primordial dwarfism (MPD) associating microcephaly and dwarfism (Bober and Jackson, 2017). Pericentrin, first described as an integral component of the pericentriolar matrix of the centrosome, involved in cytoplasmic microtubules dynamics and mitotic spindle organization/orientation (Delaval and Doxsey, 2010), is also required for ciliogenesis (Jurczyk *et al.*, 2004; Martinez-Campos *et al.*, 2004; Galati *et al.*, 2018). Subsequently, mutations in other MPD causal genes linked to PC biogenesis were identified, including in *PLK4* in patients with characteristic ciliopathy phenotypes (Martin *et al.*, 2014). However, mutations in both genes primarily affect centrosome/centriolar functions (*PCNT*) and number (*PLK4*), which could be the direct cause of the observed phenotypes, as in the cases of mutations in other genes encoding centrosome-associated proteins (Faheem *et al.*, 2015; Romero *et al.*, 2018).

Another similar and unexpected association of MPD and PC was evidenced in the context of the Meier-Gorlin syndrome (MGS, MIM #224690) for which mutations were identified in genes encoding subunits of the origin recognition complex (ORC), a key component of the DNA replication licensing machinery (Klingseisen and Jackson, 2011). Unexpectedly, the ORC subunit ORC1 was also shown to control centriole duplication through negative regulation of Cyclin E–CDK2 (Hossain and Stillman, 2012) and ORC mutations in affected individuals result in ciliogenesis defects as well as cell cycle progression delay, which likely contributes to both bone development defects and microcephaly (Stiff *et al.*, 2013). The role of ORC in ciliogenesis *in vivo* was further recently confirmed in zebrafish where knockdown of the expression of ORC subunits resulted in classical ciliopathy-associated phenotypes, in addition to microcephaly (Maerz *et al.*, 2019). However, and again, the relative contributions of centrosome, cell cycle (S phase progression) and PC defects to microcephaly were not directly investigated.

ASPM (Bond *et al.*, 2002) and *WDR62* (Bilgüvar *et al.*, 2010; Nicholas *et al.*, 2010) are the two major causal genes for primary microcephaly and for which the pathological mechanisms have also recently been associated to PC anomalies. Both proteins were previously involved in centrosome-related functions (centriole duplication) where they co-localize. Their disruption in mice leads to PC defects as well as to abnormal mother centriole/ciliary remnant asymmetric inheritance, subsequently resulting in abnormal NSPC fate determination as a result of the abnormal sensing of extracellular cues mediated by the PC (Jayaraman *et al.*, 2016). Similarly, loss of *Katnb1 in mice*, a gene involved in severe cases of primary microcephaly with gyration defects in humans (Hu *et al.*,

2014; Mishra-Gorur *et al.*, 2014), leads to abnormal SHH signaling, likely due to the presence of supernumerary centrioles and PC, thus suggesting possible cilia-dependent signaling defects in the mechanisms underlying microcephaly due to mutations in this gene. Finally, mutations in *STIL* have been reported in isolated microcephaly cases (Kumar *et al.*, 2009) as well as in microcephaly cases with HPE (Kakar *et al.*, 2015; Mouden *et al.*, 2015). STIL is required for centriolar duplication and its depletion resulted in both ciliogenesis (Vulprecht *et al.*, 2012) and PC/SHH signaling defects (David *et al.*, 2014), suggesting once more the possible involvement of PC-dependent signaling defects in the mechanisms underlying microcephaly.

The role of ciliary defects in microcephaly was more directly investigated in the case of mutations in *CPAP/CENPJ,* first identified in isolated microcephaly in humans (Bond *et al.*, 2005), but also in Seckel syndrome (MIM **#**210600), belonging to the group of MPD disorders (Al-Dosari *et al.*, 2010). CPAP has been shown to negatively regulate the length of centrioles and cilia (Kohlmaier *et al.*, 2009; Schmidt *et al.*, 2009; Tang *et al.*, 2009; Wu and Tang, 2012). Disease causing mutations in *CPAP/CENPJ* result in longer PC in NSPC, increasing the length of time required for disassembly prior to mitosis (Figure 1A). As a consequence, cell cycle (G_1/S transition) is delayed, leading to the premature switch of progenitors from a proliferative to a differentiating state (Gabriel *et al.*, 2016). Similar observations were initially made for Tctex-1 (or DNYLT1), a dynein-associated protein which negatively regulates ciliary disassembly (Li *et al.*, 2011), NDE1 (Kim *et al.*, 2011; Doobin *et al.*, 2016), a partner of CPAP involved in severe forms of microcephaly (Alkuraya *et al.*, 2011; Bakircioglu *et al.*, 2011), and more recently for KIF2A (Broix *et al.*, 2018), a kinesin involved in ciliary disassembly upon cell-cycle reentry (Miyamoto *et al.*, 2015) for which mutations were identified in MCD cases (Poirier *et al.*, 2013; Cavallin *et al.*, 2017).

Interestingly, PC in NSPC were also implicated in the signaling of Insulin-like growth factors (IGF-I and IGF-II) which are both secreted in the CSF by the choroid plexus. The IGF-I receptor (IGF1R) was localized at the ciliary membrane of NSPCs and its activation by IGF-I was implicated in cilia resorption through phosphorylation of Tctex-1, therefore allowing S-phase progression (Yeh *et al.*, 2013). It is therefore likely that IGF1 and IGF2 control NSPC proliferation of NSPC through PC-dependent IGF1R mediated signaling (Lehtinen *et al.*, 2011), in agreement with the fact that mutations of both *IGF1* (Woods *et al.*, 1996) and *IGF1R* (Abuzzahab *et al.*, 2003) or their invalidation in mice (Beck *et al.*, 1995; Lehtinen *et al.*, 2011) lead to microcephaly, in addition to global growth restriction.

Finally, among the “classical” ciliopathy spectrum, microcephaly is one of the features defining the OFD type XIV, a ciliopathy caused by mutations in *C2CD3* (Thauvin-Robinet *et al.*, 2014). C2CD3 is required for the elongation of the distal end of centrioles, a step essential for the docking and biogenesis of subdistal and distal appendages at the mother centriole (Thauvin-Robinet *et al.*, 2014;

Ye *et al.*, 2014). Impairing the assembly of distal appendages results in strong ciliogenesis defects (Graser *et al.*, 2007; Tanos *et al.*, 2013), likely explaining the ciliopathy phenotypes observed upon mutations in *C2CD3* (Thauvin-Robinet *et al.*, 2014). However, the direct contribution of the observed ciliogenesis defects to the etiology of microcephaly remains to be determined in this case as C2CD3 also possesses centriolar-specific functions, including a role in the biogenesis of subdistal appendages (Thauvin-Robinet et al., 2014), upon which cytoplasmic microtubules are anchored, which could also contribute to the observed microcephaly.

All these results based on both human genetics and murine model studies, strongly suggest PC anomalies as a potential pathological mechanisms underlying primary microcephaly. Accordingly, studies on developmental cell biology have extensively stressed the role of PC in cerebral cortical development. Firstly, all NSPC have been shown to harbor a PC which is localized at the apical membrane of apical progenitors and thus in a strategic location to sense and transduce signals from the CSF (Figure 4A,B). Besides its important role in SHH signaling, PC have now clearly been described as playing a key role in the control of cell cycle progression and, therefore, for the expansion/commitment of progenitor required for normal cerebral cortical size and folding (reviewed in (Youn and Han, 2018)). For instance, at the onset of cortical neurogenesis, increasing numbers of apical progenitors switch from symmetric to asymmetric divisions, resulting in the self-renewal of apical progenitors and the generation of basal progenitors located deeper in the developing cortex. Interestingly, the delamination of basal progenitors (loss of apical membrane and retraction toward the basal side) is preceded by the assembly of a basolateral rather than apical PC (Wilsch-Brauninger *et al.*, 2012). These basally localized cilia are thought to transduce specific signals, no longer from the CSF, but rather from neighboring cells or from molecules present in the intercellular space, potentially promoting the switch from apical to basal progenitors, including delamination. As another exciting example, during the asymmetric division of NSPC, the cilium is disassembled and the mother centriole of the older centrosome (basal body) remains associated to a ciliary membrane remnant (Figures 1A and 4B) that is therefore asymmetrically transmitted to one of the two daughter cells (Anderson and Stearns, 2009; Paridaen *et al.*, 2013). The inheritance of this pre-primed mother centriole allows the quicker assembly of a functional cilia at the apical surface which appears to dictate the progenitor state statue to the corresponding daughter cell (Paridaen *et al.*, 2013), suggesting that the asymmetric inheritance of the centriole-ciliary membrane remnant participates to the control of NSPC fate. Finally, PC were also involved in apico-basal polarity of radial glial cells scaffold supporting neuronal migration (Higginbotham *et al.*, 2013) or during interneurons tangential migration to the cortical plate (Baudoin *et al.*, 2012). All of these observations are indeed clearly pointing to a key role of PC during cortical development in addition to its well accepted role during early steps of neural tube patterning and closure.

Conclusion

Since the discovery of the link between cilia and SHH signaling in the neural tube in 2003, extensive progress has been made in the understanding of the mechanisms underlying cerebral defects in ciliopathy patients. Most of the evidence comes from studies in animal models, however, some studies conducted in human fetal tissues or cells confirmed similar underlying mechanisms. SHH signaling has been the focus of most studies whereas the involvement of other PC-dependent signaling pathways remains to be further explored. While hydrocephalus, corpus callosum malformations, cerebellar hypoplasia and NTD are classically considered as belonging to the clinical spectrum of ciliopathies, it was not the case for primary microcephaly, even in cases of mutations in genes encoding centrosome-associated proteins which were generally thought to primarily affect their functions in microtubules dynamics and/or spindle orientation/organization. However, the significance of PC function during cerebral cortical development now appears evident as PC dynamics and signaling defects have been demonstrated in the context of primary microcephaly, with or without MCD. It is also strengthened by the various neurological features exhibited almost constantly by individuals diagnosed with ciliopathies, including cognitive deficits, autism spectrum disorders and seizures, which also suggest more subtle cortical defects. Nevertheless, as pointed out in this review, it remains challenging to distinguish the relative contributions of the function of primary microcephaly gene products at the centrosome and/or PC since defects in centriolar duplication or the assembly of mother centriole appendages would have a dual impact on centriole-associated processes (spindle organization and orientation, nucleation and anchoring of microtubules) as well as on ciliogenesis.

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Conflict of interest:

None to declare.

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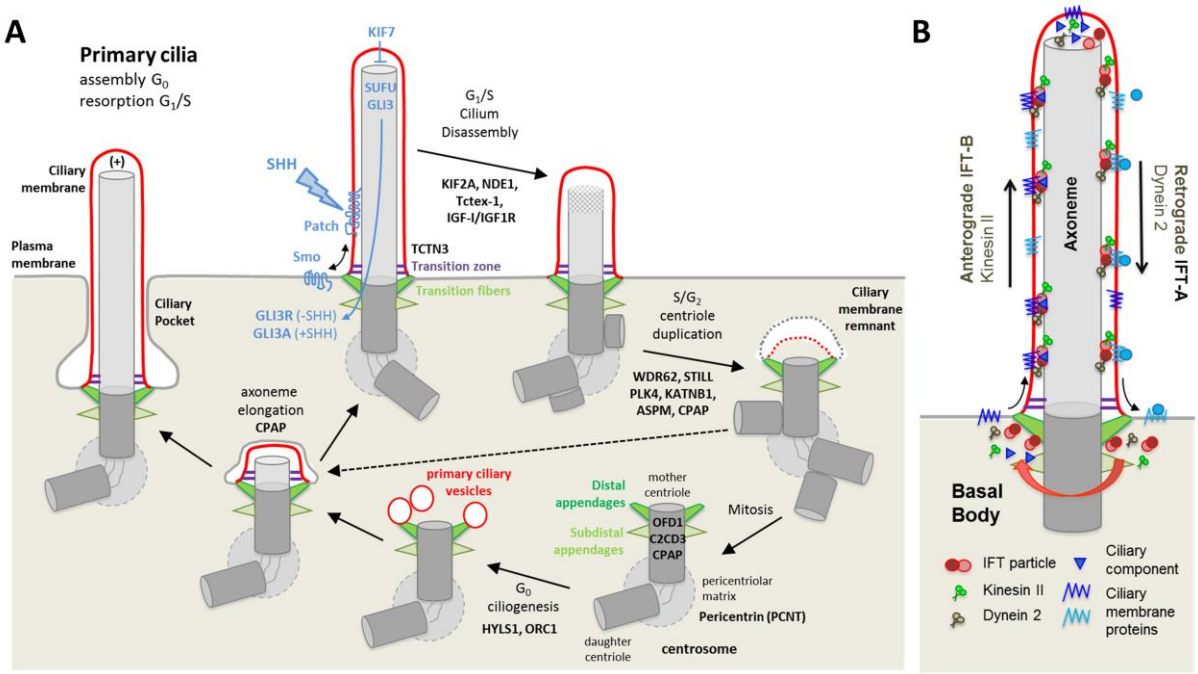

Figure 1: Assembly and disassembly of primary cilia (PC)

A. Primary cilia are formed through the elongation of the mother centriole. During ciliogenesis in quiescent cells, primary ciliary vesicles dock at the distal appendages of the mother centriole of the centrosome, incoming vesicles fuse and the axoneme elongates intracellularly before to be exported to the plasma membrane. During cell cycle reentry, the axoneme is disassembled while centrioles start to duplicate. The duplicated centrosomes then separate from the apical membrane. In the case of asymmetric divisions, a ciliary membrane remnant (CR) remains attached to the old mother centrioles only thus generating an asymmetry between daughter cells. The cell which inherits the mother centriole associated to the CR is able to reestablish a PC earlier than the other daughter cell, which has to undergo *de novo* early steps of ciliogenesis. **B**. Assembly and disassembly of PC involves the transport of proteins from the cytoplasm to the PC which is called intraflagellar transport (IFT). IFT is a bidirectional transport based on the loading of selected cargos in the cytoplasm and ciliary compartment to IFT particles powered by anterograde (Kinesin-II) and retrograde (Dynein 2) motors, allowing their transport along axonemal microtubules in and out from PC .

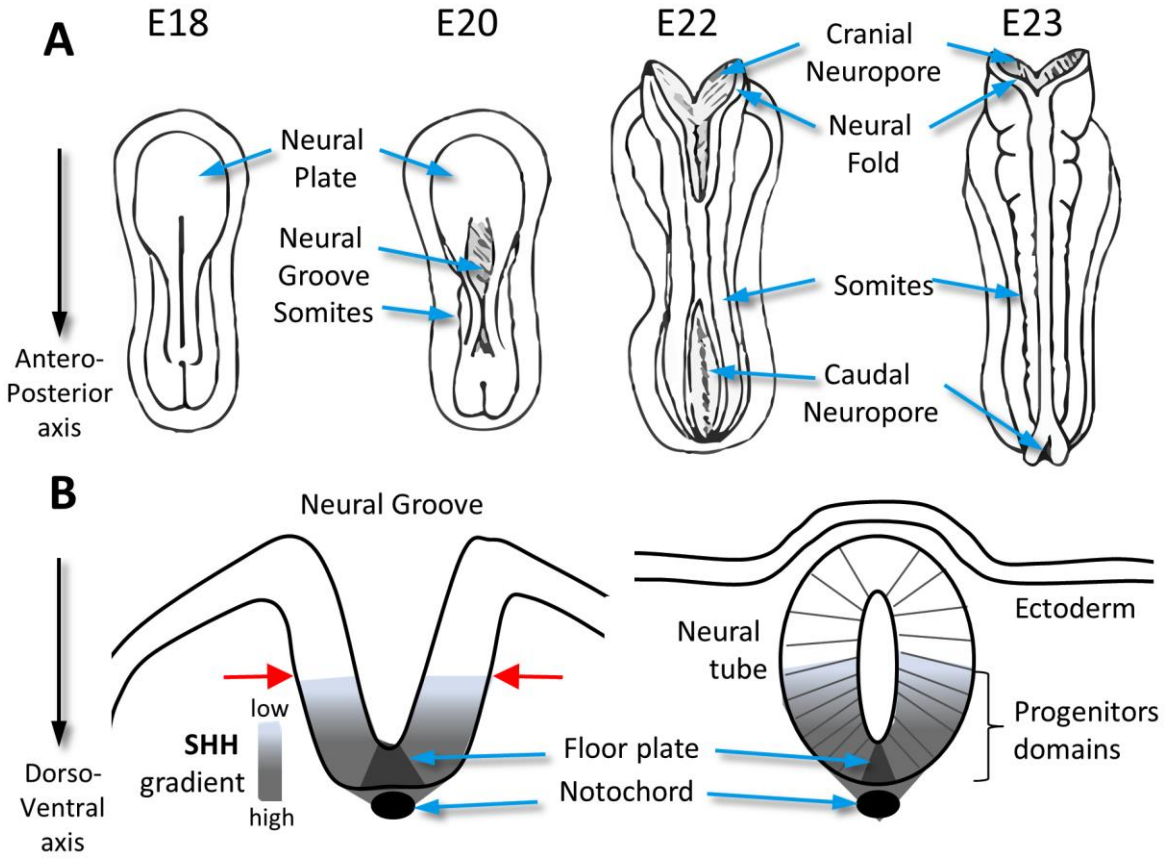

Figure 2: Neural tube closure and patterning.
A. Dorsal views of forming neural tube in the developing embryo. Day of development indicated above each scheme. **B.** The folding of the neural plate is induced by SHH which is secreted from the Notochord, and then by the floor plate, and forms a ventro-dorsal gradient. This SHH gradient dictates the localization of the bending events of the neural tube at the ventral midline (red arrows) where SHH is low. After neural tube closure, this SHH gradient generates various domains of progenitor cells which are arranged along the dorsal–ventral axis.

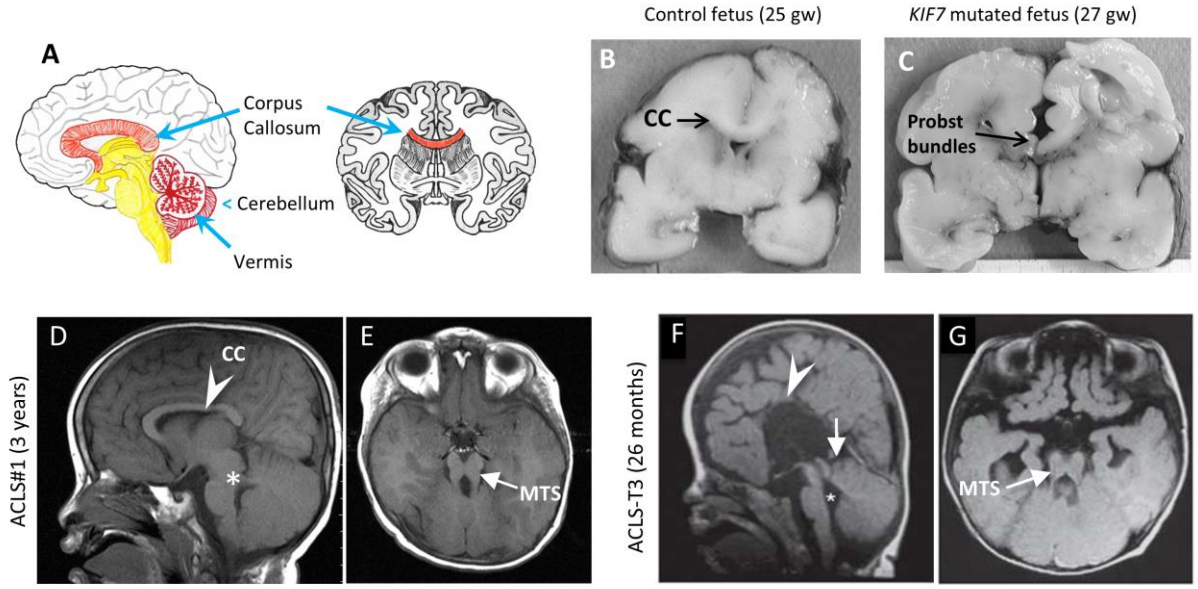

Figure 3: Brain anomalies in *KIF7* mutated patients.
A. Brain organization with corpus callosum and cerebellum highlighted. **B,C.** Coronal slice through fixed fetal brain of control (**B**) and *KIF7* mutated fetus (**C**) shows the normal appearance of corpus callosum in (**B**) which is absent in the mutated case with characteristic Probst bundles composed of the callosal axons that failed to cross the midline (**C**). **D, E**. Brain MRI of ACLS case 1 at 3 years from Putoux et al., 2012. **D**. Sagittal T1 view shows normal corpus callosum (arrowhead), and thickened superior cerebellar peduncles (white asterisk). **E.** Axial T1 view shows dysmorphism of the fourth ventricle with thickened and elongated superior cerebellar peduncles resulting in a molar tooth sign (arrow). **F, G.** Brain MRI sections in individual ACLS-T3 from Putoux et al., 2011. (**F**) Sagittal brain MRI showing the corpus callosum agenesis (arowhead), dysplastic superior vermis (arrows) or dilated lateral and fourth ventricle (asterisks). **G.** Axial view of brainstem abnormalities with deep interpeduncular fossa and stretched cerebellar peduncles. Panels **B-G** were used with permission from Tania Attié-Bitach and Ferechte Razavi.

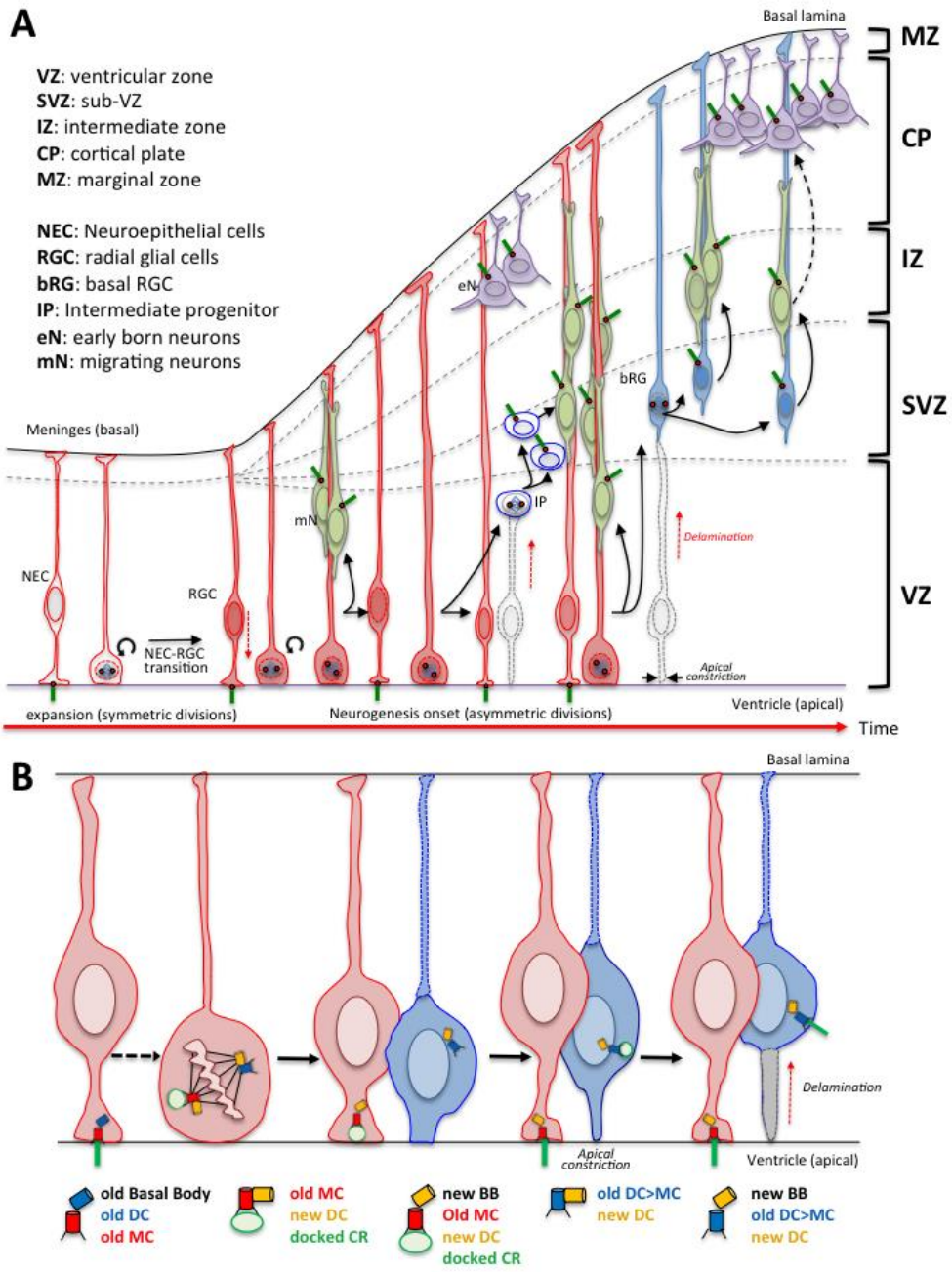

Figure 4: The developing cerebral cortex and primary cilia dynamics and location.
A. NSPC subtypes, their mode of division and their progeny are schematized. Primary cilia are present on all NSPC subtypes as well as on neurons; being apically localized on apical progenitors**. B.** Apical progenitors harbor a cilium (green) which is assembled from the mother centriole (basal body, red) at the apical membrane and then protrudes into the brain ventricle to sense and transduce signals from the CSF. During asymmetric division, the mother centriole retains a ciliary membrane remnant (CR, green vesicle) throughout mitosis. This CR-primed mother centriole is inherited by a daughter cell which rapidly assembles an apical PC and remains a progenitor. The second daughter cell inherits a mother centriole (blue) which was the daughter centriole in the mother cell. This new mother centriole is not pre-associated with a ciliary vesicle and therefore ciliogenesis is delayed. In addition, the newly formed cilium in this cell is not apical but basolateral, thus sensing different signals than from the CSF inducing apical constriction and delamination to finally give rise to basal progenitors (IP or bRG) or directly to neurons.