The role of cilia for hydrocephalus formation

Julia Wallmeier | Marlene Dallmayer | Heymut Omran

Department of General Pediatrics, University Clinic Muenster, Muenster, Germany

Correspondence
Julia Wallmeier, Department of General Pediatrics, University Clinic Muenster, Albert Schweitzer Campus 1 Building A1, 48149 Muenster, Germany. Email: julia.wallmeier@ukmuenster.de

Abstract
Hydrocephalus is a common finding in newborns. In most cases, it is caused by intraventricular hemorrhage associated with prematurity, whereas in some patients the cause of hydrocephalus can be traced back to genetic changes, associated with disease syndromes such as RASopathies, lysosomal storage diseases, dystroglycanopathies, craniosynostosis but also ciliopathies. Ciliopathies are a group of diseases that can affect multiple organ systems due to dysfunction or the absence of cilia. Cilia are small organelles, extending from the cell surface. Nonmotile monocilia are ubiquitously present during cell development fulfilling chemosensory functions, whereas specialized epithelia such as the ependyma, lining the inner surface of the brain ventricles, exhibit multiciliated cells propelling fluids along the cell surface. This review highlights ciliopathies and their pathophysiology in congenital hydrocephalus. While nonmotile ciliopathies are often associated with severe prenatal hydrocephalus combined with other severe congenital brain malformations, motile ciliopathies, especially those associated with defects in multiciliogenesis can cause hydrocephalus and chronic lung disease.

KEYWORDS
cilia, ciliopathies, hydrocephalus, motile ciliopathies

1 CEREBROSPINAL FLUID FLOW IN THE VENTRICULAR SYSTEM

The human brain is surrounded by cerebrospinal fluid (CSF), which protects the organ from physical damage but also ensures that metabolic waste products are removed (Veening & Barendregt, 2010). Furthermore, growth factors and hormones within the CSF play an important role in brain development and repair (Veening & Barendregt, 2010). Via passive filtration of plasma from the choroidal capillaries and active transport involving carbonic anhydrase as well as membrane ion carrier proteins, the majority of CSF is produced in the choroid plexus, granular structures floating in the ventricular cavities (lateral, third, and fourth ventricle; Figure 1). After production, the CSF travels unidirectionally through the ventricular system (Figure 1) and multidirectionally in the subarachnoid spaces toward their site of reabsorption at the arachnoid villi (Figure 1: Sakka, Coll, & Chazal, 2011). Additionally, about one-third bypasses this way using the perineural subarachnoid space of cranial nerves to enter the lymphatic system (Leinonen, Vanninen, & Rauramaa, 2018). The movement of the CSF is evoked by pulsatile waves driven by the choroid arteries. In addition, a coordinated, asymmetrical, planar beating of motile cilia lining the ependyma creates a constant flow of CSF through the cerebral ventricles (Ibañez-Tallon et al., 2004; Meunier, Sawamoto, & Spassky, 2020).

2 CILIATED CELLS IN THE VENTRICULAR SYSTEM

Studies in mice revealed two major subtypes of ependymal cells: cuboidal multiciliated E1 cells (Figure 1, +2), which extend about 50 motile cilia with a 9 + 2 ultrastructure into the ventricular cavity,
as well as E2 cells (Figure 1, +2). E2 cells are often biciliated acting as mechanical or chemical sensors of cerebrospinal fluid flow or composition. E1 and E2 cells form rosette-like clusters, known as pinwheels, surrounding so-called type B1 cells, which are adult neural stem cells derived from radial glia (Mirzadeh et al., 2008; Figure 1, +2). Choroid plexus epithelia are also known to exhibit cilia, but there are conflicting reports on the number of cilia exposed on their surface: in mice, it was shown that some choroid plexus epithelial cells harbor a tufted group of cilia, while others only expose one singular cilium (Banizs et al., 2005). Other authors describe that all choroid plexus epithelial cells of domestic pigs exhibit groups of about 20 cilia on their surface (Narita, Kawate, Kakinuma, & Takeda, 2010). Nevertheless, the current agreement is that in general the cilia on the surface of the choroid plexus epithelial cells exhibit a $9 \pm 0$ ultrastructure and play chemosensory tasks, involved in the regulation of CSF production (Figure 1, +2; Damkier et al., 2013; Narita et al., 2010).

3 | HYDROCEPHALUS AND UNDERLYING GENETIC DEFECTS

Within a day, about 500 ml of CSF are produced (Damkier et al., 2013). The whole capacity of the ventricular system is only about 150 ml (ventricles: 25 ml, subarachnoid space: 125 ml; Sakka et al., 2011). Therefore, production and resorption need to be tightly regulated. “The dynamic imbalance between the production and absorption of cerebrospinal fluid leading to enlarged ventricles” is referred to as hydrocephalus (Tully & Dobyns, 2014). This definition includes hydrocephalus as a progressive process. Nonetheless, it is possible that clinical symptoms stay absent and surgical intervention due to intracranial pressure is not required. Hydrocephalus is a common finding with a variable prevalence of 1–35 in 10,000 individuals depending on the definition and population studied (Tully & Dobyns, 2014). Most cases are of acquired nature (secondary) caused by prenatal or postnatal intraventricular hemorrhage, infections with pathogens such as enterovirus, cytomegalovirus, or toxoplasmosis, or brain tumors such as posterior fossa, cerebellar astrocytoma, brainstem glioma, and ependymoma, being the most common brain tumors observed in children (Kahle, Kulkami, Limbrick, & Warf, 2016; Tully & Dobyns, 2014).

The clinical presentation depends on the age of onset. In some cases, ventriculomegaly is diagnosed by prenatal ultrasound at 18–20 weeks of gestation (Garne et al., 2010; Kahle et al., 2016). Newborns with hydrocephalus exhibit increased head circumference, irritability, vomiting, and bulging of the fontanel. Due to the closed fontanelle, older children and adults exhibit slightly different symptoms: a combination of headache, nausea and vomiting, loss of developmental milestones, diplopia, or papilledema. Papilledema can progress to optic nerve atrophy associated with loss of vision (Kahle et al., 2016). Imaging is the most important clinical investigation, whereas in young children with open fontanelles ultrasound can be offered, MRI imaging is the gold standard diagnostics (Raybaum, 2018). Enlarged ventricles are observed, which might be associated with other congenital brain malformations such as corpus
callosum hypoplasia/aplasia, Dandy-Walker malformation, and Pachygyria. In some cases, brain atrophy might be depicted referred to as hydrocephalus evacuo. So far, treatment is usually performed using shunt insertions. In the majority of cases, shunting from the ventricles to the peritoneal cavity (ventriculoperitoneal shunt) or rarely into the right atrium of the heart or pleural cavity. This surgical treatment faces potential long-term complications such as mechanical obstruction and shunt infection (Bayston, 2018; Tanrikulu & Özek, 2019).

In some cases, hydrocephalus is caused by genetic defects. Until today, pathogenic variants in more than 100 genes have been associated with hydrocephalus, but a large proportion is still unknown (Kousi & Katsanis, 2016). While in the majority of cases hydrocephalus is part of a defined syndrome, a few genes have been described for isolated hydrocephalus. Pathogenic variants in \textit{L1CAM} represent the most common genetic defect and account for about 10% of males with X-linked isolated hydrocephalus (Adle-Biassette et al., 2013). \textit{L1CAM} encodes a transmembrane glycoprotein that belongs to the immunoglobulin superfamily of cell adhesion molecules. Furthermore, genes associated with neural stem cell fate (\textit{TRIM71}, \textit{PTCH1}, and \textit{SMARCC1}; Furey et al., 2018; Jin et al., 2020) as well as genes involved in the Wnt signaling pathway (\textit{CCDC88C}; Ruggeri et al., 2018) or vesicular trafficking (\textit{AP1S2}; Cacciagli et al., 2014) have been associated with hydrocephalus (Kundishora et al., 2021). Besides, diseases such as RASopathies, lysosomal storage diseases, dystroglycanopathies, and craniosynostosis but also ciliopathies have been linked to hydrocephalus formation (Ibañez-Tallon et al., 2004; Kahle et al., 2016; Kousi & Katsanis, 2016).

### FIGURE 2

Ependymal cells as well as choroid plexus cells are covered by multiple cilia. \textit{Upper panel:} Choroid plexus epithelial cells exhibit about 20 nonmotile cilia. Cilia cross-section depicts the 9 + 0 structure of choroid plexus cilia (Narita et al., 2010). \textit{Lower panel:} There are two types of ciliated ependymal cells: cuboidal E1 cells harboring \~50 motile cilia per cell measuring 8–15 μm in length. The cilia are localized in <35% of the total apical surface and are positioned downstream of the direction of the ciliary beating (Mirzadeh et al., 2008; Mirzadeh, Han, Soriano-Navarro, García-Verdugo, & Alvarez-Buylla, 2010). E2 cells are often biciliated. It has been proposed that E2 cells serve as mechanical or chemical sensors of CSF flow or composition (Bruni, 1998; Mirzadeh et al., 2008). Axonemal cross-sections of E1 and E2 cell cilia depict a 9 + 2 structure. Nine outer microtubule doublets surround a central pair. The cilia present a slightly asymmetrical beating similar to airway cilia.

Ciliopathies are inborn defects of cilia comprising a wide spectrum of diseases (Fliegauf, Benzing, & Omran, 2007; Reiter & Leroux, 2017). Cilia are nearly ubiquitous cellular organelles. Since nonmotile cilia play crucial roles in physiology, like signaling and development, the so-called nonmotile/primary ciliopathies affect the function of various organs including the heart, kidney, skeleton, and brain (Reiter & Leroux, 2017). Some epithelia, such as the human airways, the male and female reproductive system but also the ependyma lining the brain ventricles exhibit multiciliated cells (Wallmeier et al., 2020). These cilia show a 9 + 2 ultrastructure (Figure 2) and produce a regular coordinated beat to move fluids along the cell surface. Defects in structure, function, or number of these motile cilia are referred to as...
motile ciliopathy. Dysfunction of airway cilia results in impaired mucociliary clearance and chronic destructive airway disease. Since sperm flagella resemble the structure of a motile cilium and furthermore the efferent ducts are covered with multiple motile cilia, motile ciliopathies can affect male fertility. In addition, female fertility can be affected since the fallopian tubes also exhibit multiciliated cells to propel the oocyte. Because nodal cilia function is crucial for the determination of the left–right axis, dysfunction of motile cilia might result in left–right anomalies such as situs inversus (Wallmeier et al., 2020).

5 PRIMARY CILIOPATHIES ASSOCIATED WITH HYDROCEPHALUS

Primary ciliopathies, caused by cilia with 9 + 0 ultrastructure (Figure 3), can be associated with hydrocephalus (Thomas, Boutaud, Reilly, & Benmerah, 2019). Hydrocephalus has been reported in human individuals with pathogenic variants in genes encoding primary cilia proteins such as TMEM216, TMEM67, CC2D2A, HYLS1, KIF7, GLI3, KIAA0586, IFT172, CEP83 as well as OFD1 (Figure 3; Kousi & Katsanis, 2016). While many primary ciliopathies have been regarded as distinct disease entities by symptomatic classifications, genetic analyses revealed that some should be regarded as a spectrum of the same disease such as the Meckel–Gruber/Joubert syndrome and Hydrocephalus/Acrosallosal syndrome.

The Meckel–Gruber syndrome (MIM: 249000) has a severe phenotype, which is often intrauterine lethal due to pulmonary hypoplasia. The clinical phenotype comprises hepatic developmental defects, bilaterally enlarged kidneys as well as postaxial polydactyly in ~80%. Furthermore, posterior fossa anomalies are present in most cases, most frequently occipital encephalocele (Hartill, Szymanska, Sharif, Wheway, & Johnson, 2017). Encephalocele subsequently leading to enlarged ventricles can be caused by autosomal recessive pathogenic variants in the gene TMEM216 encoding a tetraspanin-like transmembrane protein and component of the ciliary transition zone and also by pathogenic variants in the frizzled-like receptor TMEM67 (Table 1; Iannicelli et al., 2010). Moreover, autosomal recessive loss of function pathogenic variants in the transition zone component CC2D2A (Table 1) result in Meckel–Gruber syndrome, whereas autosomal recessive missense variants in the same gene cause a Joubert syndrome phenotype (MIM: 213300; Bachmann-Gagescu et al., 2012; Hartill et al., 2017). The molar tooth sign, which is characterized by cerebellar vermis hypoplasia, thickened, misoriented superior cerebellar peduncles, and an abnormally deep interpeduncular fossa is a characteristic finding in Joubert syndrome. The affected patients suffer from muscular hypotension, ataxia, developmental delay, abnormal eye movements, and abnormal respiratory control (Al-Qattan, Shamseldin, Salih, & Alkuraiya, 2017). Enlarged ventricles were reported in 13 out of 17 patients harboring autosomal recessive pathogenic variants in CC2D2A (Bachmann-Gagescu et al., 2012).

The acrosallosal (MIM: 209990) and hydrocephalus (MIM: 236680) syndrome share polydactyly and midline brain and facial abnormalities as the main clinical features. The hydrocephalus syndrome is a lethal syndrome leading to stillbirth or death shortly after birth. It is characterized by hydrocephaly, micrognathia, and polydactyly. Pathogenic variants in the basal body associated gene HYLS1 were the first to be identified in fetus with hydrocephalus syndrome (Mee et al., 2005; Paetau et al., 2008). The autosomal recessive disorder acrosallosal syndrome typically comprises corpus callosum agenesis, occasional anencephaly and/or Dandy–Walker malformations, hypertelorism, postaxial polydactyly of the hands, and preaxial polydactyly of the feet (Putoux et al., 2011). Autosomal recessive pathogenic variants in the genes encoding the hedgehog-associated proteins KIF7 (Table 1) and GLI3 (Table 1; McDonald-McGinn et al., 2010; Speksnijder et al., 2013) are associated with both disease entities, acrosallosal as well as hydrocephalus syndrome. Furthermore, patients with autosomal recessive pathogenic variants in KIAA0586 (Table 1), the human ortholog to the centriole component TALPID3, exhibit symptoms consistent with hydrocephalus and acrosallosal syndrome. In addition, some patients also develop hydrocephalus (Alby et al., 2015).

Some patients with autosomal recessive pathogenic variants in the intraflagellar transport motor protein complex component IFT172
TABLE 1  Clinical findings in ciliopathies associated with hydrocephalus

| Gene     | Inheritance | Syndrome                       | Primary cilia | Motile cilia | Occipital Encephalocele/Meningozele | Ventricleomegaly | Anencephaly | Arachnoidal cyst | Corpus callosum malformation | Molar tooth sign | Dandy Walker malformation | Facial dysmorphism (e.g., Micrognathia, cleft lip) | Situs inversus | Retinal anomalies (e.g., retinitis pigmentosa) | Airway disease | Kidney cysts/enlargement | Hepatic fibrosis/cysts | Polydactyly | Skeletal abnormalities (narrow thorax, short ribs, trident acetabulum, cone shaped epiphyses) | Shortening of long bones | Hypogonadism | Subfertility |
|----------|-------------|--------------------------------|---------------|-------------|-------------------------------------|------------------|-------------|-----------------|-------------------------------|-----------------|-----------------------------|-----------------------------------------------|--------------|-----------------------------------------------|----------------|--------------------------|------------------|------------|-------------------------------------------------|-------------------|-------------|-------------|
| TMEM67   | Aut-rec.    | Meckel-Gruber/Joubert         | +             | +           | +                                   | +                | +           | +               | +                             | +               | +                           | +                                             | +            | +                                             |       | +          | +                                             | +           | +                                             | +            | +          | +                                             |
| TMEM216  | Aut-rec.    | Meckel-Gruber/Barait/Joubert  | +             | +           | +                                   | +                | +           | +               | +                             | +               | +                           | +                                             | +            | +                                             | +            | +          | +                                             | +           | +                                             | +            | +          | +                                             |
| CC2D2A   | Aut-rec.    | Meckel-Gruber/Barait/Joubert  | +             | +           | +                                   | +                | +           | +               | +                             | +               | +                           | +                                             | +            | +                                             | +            | +          | +                                             | +           | +                                             | +            | +          | +                                             |
| HYLS1    | Aut-rec.    | Hydroethalus/Accalosal         | +             | +           | +                                   | +                | +           | +               | +                             | +               | +                           | +                                             | +            | +                                             | +            | +          | +                                             | +           | +                                             | +            | +          | +                                             |
| KIF7     | Aut-rec.    | Hydroethalus/Accalosal         | +             | +           | +                                   | +                | +           | +               | +                             | +               | +                           | +                                             | +            | +                                             | +            | +          | +                                             | +           | +                                             | +            | +          | +                                             |
| GLI3     | Aut-rec.    | Hydroethalus/Accalosal         | +             | +           | +                                   | +                | +           | +               | +                             | +               | +                           | +                                             | +            | +                                             | +            | +          | +                                             | +           | +                                             | +            | +          | +                                             |
| KIAA0586 | Aut-rec.    | Hydroethalus/Accalosal         | +             | +           | +                                   | +                | +           | +               | +                             | +               | +                           | +                                             | +            | +                                             | +            | +          | +                                             | +           | +                                             | +            | +          | +                                             |
| IFT172   | Aut-rec.    | Jeune/Mainzer Saldino/BBS      | +             | +           | +                                   | +                | +           | +               | +                             | +               | +                           | +                                             | +            | +                                             | +            | +          | +                                             | +           | +                                             | +            | +          | +                                             |
| CEP83    | Aut-rec.    | Nephronophthisis               | +             | +           | +                                   | +                | +           | +               | +                             | +               | +                           | +                                             | +            | +                                             | +            | +          | +                                             | +           | +                                             | +            | +          | +                                             |
| OFD1     | X-dominant  | Orofaciodigital                | +             | +           | +                                   | +                | +           | +               | +                             | +               | +                           | +                                             | +            | +                                             | +            | +          | +                                             | +           | +                                             | +            | +          | +                                             |
| MCIDAS   | Aut-rec.    | RGMC                           | +             | +           | +                                   | +                | +           | +               | +                             | +               | +                           | +                                             | +            | +                                             | +            | +          | +                                             | +           | +                                             | +            | +          | +                                             |
| CCNO     | Aut-rec.    | RGMC                           | +             | +           | +                                   | +                | +           | +               | +                             | +               | +                           | +                                             | +            | +                                             | +            | +          | +                                             | +           | +                                             | +            | +          | +                                             |
| FOXJ1    | Dominant    | RGMC                           | +             | +           | +                                   | +                | +           | +               | +                             | +               | +                           | +                                             | +            | +                                             | +            | +          | +                                             | +           | +                                             | +            | +          | +                                             |
Pathogenic variants in genes encoding components of the intraflagellar transport (IFT) motor protein complex usually cause chondrodysplasias with variable extraskeletal involvement such as the Jeune syndrome, characterized by narrow thorax with short ribs, trident acetabulum, cone-shaped epiphyses, and polycystically. If the kidney is also affected, it is referred to as Mainzer-Saldino syndrome (Halbritter et al., 2013). Pathogenic variants in IFT172 are associated with both Jeune syndrome and Mainzer Saldino syndrome. Furthermore, the phenotypic spectrum associated with IFT172 variants can also be categorized as Bardet–Biedl-like ciliopathy with postaxial polydactyly, retinitis pigmentosa, obesity, and hypogonadism (Schaefer et al., 2016).

Autosomal recessive pathogenic variants in CEP83 (Table 1), encoding a component of the ciliary distal appendage, result in nephronophthisis a chronic tubulointerstitial nephritis, the most frequent genetic cause of chronic renal failure in children. Affected patients with defects in CEP83 also exhibit extrarenal symptoms such as liver alterations and retinitis but also hydrocephalus in some cases (Faller et al., 2014).

X-linked dominant oro-facio-digital syndrome is caused by pathogenic variants in OFD1 (Table 1; Macca & Franco, 2009). Patients suffer from malformations with a broad clinical spectrum. In most cases, the patients present with facial dysmorphism, tongue anomalies, anomalies of the oral frenula, or skeletal abnormalities, but symptoms can also affect the kidneys or the brain. Malformations of the brain include agenesis of the corpus callosum, single or multiple intracerebral or arachnoidal cysts, porencephaly, gray matter heterotopia, abnormal gyration, microcephaly, cerebellar malformations, but also Dandy–Walker malformation have been reported (Bruel et al., 2017).

Depending on the localization of the respective variants, some patients with pathogenic variants in OFD1 are known to also exhibit respiratory symptoms consistent with a motile ciliopathy (Bukowy-Bierylko et al., 2019). These findings indicate that ciliopathy-associated proteins, which are localized in the ciliary transition zone or at the basal body (Figure 3) might result in a disease spectrum consistent with both defects in nonmotile and motile cilia (Wallmeier et al., 2020).

5.1 Pathophysiology of primary ciliopathies leading to hydrocephalus

Knowledge of a cilia-related pathophysiology leading to hydrocephalus is still scarce. The congenital brain malformation present in primary ciliopathies, such as corpus callosum defects, cerebellar malformation, and heterotopias and/or polymicrogyria, as well as the prenatal development of severe hydrocephalus, might indicate that primary cilia not only have crucial roles in cell differentiation but also affect cerebrospinal fluid homeostasis. Nonmotile monocilia function and especially sonic hedgehog signaling are crucial components during various processes of human brain development (Andreu-Cervera, Catala, & Schneider-Maunoury, 2021).

Dandy–Walker malformations, defined by large accumulation of CSF in the posterior fossa and cerebellar vermis agenesis or hypoplasia, are associated with hydrocephalus in 90% of cases at diagnosis (Thomas et al., 2019). For example, the acrocallosal syndrome caused by autosomal recessive pathogenic variants in the hedgehog signaling associated proteins KIF7 and GLI3 (Thomas et al., 2019) is associated with these findings. Consistently, sonic hedgehog signaling as well as primary cilia defects have been shown to be important for cerebellar development in mice by promoting the expansion of the cerebellar granule cell precursors, suggesting, that this process might be affected in the respective ciliopathies (Thomas et al., 2019; Wechsler-Reya & Scott, 1999).

Neural tube defects are almost always accompanied by hydrocephalus internus (Copp & Greene, 2010). This is most probably caused by tethering, which pulls the cerebellum into the foramen magnum as the vertebral column lengthens obstructing CSF flow (Kousi & Katsanis, 2016). During the third and fourth week of gestation, the neural tube forms. While early defects during primary neuration result in more severe phenotypes such as anencephaly or open spina bifida, defects in secondary neuration result in closed neural tube defects such as spina bifida occulta. Neural tube development is closely connected to the Planar cell Polarity Pathway (PCP), a noncanonical Wnt signaling cascade, which is associated with the primary cilium (Greene & Copp, 2014). More than 200 genes have been identified in mouse models to cause defects in neural tube closure, whereas only little is known in humans. Heterozygous pathogenic variants in the genes encoding PCP components such as VANGL1 coding for VANGL Planar Cell Polarity Protein 1 and its paralogue VANGL2 (Kibar et al., 2007) as well as FZD coding for Fuzzy Cell Polarity Protein (Seo et al., 2011) have been associated with neural tube defects in association studies.

The development of encephalocele present in Meckel–Gruber syndrome is not directly associated with neural tube closure. However, according to one of the most accepted theories, there is a deficiency in the separation of the germ layers after neural tube closure resulting in a defect in the formation of skull bone and meninges (Greene & Copp, 2014).

Besides their function in human brain development, nonmotile cilia, present on the choroid plexus epithelial cells, were proposed to function as a mechanical or chemical sensor of the CSF flow or composition comparable to the function of cilia in the human kidney (Meunier et al., 2020).
structural ciliary defects caused by abnormalities of structural components or assembly factors and impaired ciliary motility.

Interestingly, in the group of affected individuals with reduced generation of multiple motile cilia (RGMC), the number of patients with hydrocephalus is much higher (Amirav et al., 2016). RGMC is a ciliopathy caused by decreased number of cilia or a defect in cell differentiation and/or proliferation of multiciliated cells (Wallmeier et al., 2021). Autosomal recessive pathogenic variants in the gene CCNO (Table 1; Amirav et al., 2016; Wallmeier et al., 2014) encoding cyclin O, in MCIDAS encoding multicilin a transcription factor co-activator (Table 1; Boon et al., 2014; Robson et al., 2020), in TP73, member of the TP53 family of transcription factors (Wallmeier et al., 2021) as well as autosomal dominant de novo pathogenic variants in the transcription factor FOXJ1 (Table 1; Wallmeier et al., 2019) are known to cause RGMC.

Pathogenic variants in CCNO were the first report of a defect of multiciligenesis in humans (Wallmeier et al., 2014). Next to severe chronic destructive airway disease, MRI studies revealed enlarged ventricles in some of the affected (Amirav et al., 2016). In addition patients with autosomal recessive pathogenic variants in MCIDAS exhibit enlarged ventricles, an increased size of the choroid plexus, and arachnoid cysts (Boon et al., 2014; Robson et al., 2020). All individuals presented with a normal development and without any signs of neurologic deficits. Surgical intervention was not required in any of the cases. In contrast, in all reported FOXJ1 affected patients, surgical relief of intracranial pressure was necessary (Shapiro et al., 2021; Wallmeier et al., 2019). De novo loss of function pathogenic variants in FOXJ1 cause RGMC with obstructive hydrocephalus and aqueduct stenosis, next to respiratory symptoms, subcutility, and situs anomalies in some (Shapiro et al., 2021; Wallmeier et al., 2019).

6.1 Pathophysiology of motile ciliopathies leading to hydrocephalus

Mice deficient in ciliary motility-associated proteins such as DNAH5 exhibit severe hydrocephalus. Studies in DNAH5 mutant mice have revealed aqueduct stenosis and consecutively hydrocephalus occlusus. This leads to the assumption that ciliary flow maintained by ependymal cilia is crucial for the patency of the aqueduct (Ibañez-Tallon, Gorokhova, & Heintz, 2002). As the prevalence of hydrocephalus in humans affected with motile ciliopathies is much lower than in mice, this might suggest that this mechanism is less important in humans.

Interestingly, in the subgroup of affected individuals with defect in multiciligenesis (RGMC), the prevalence of hydrocephalus compared to the healthy population is severely increased (Amirav et al., 2016). So far, all reported individuals with de novo loss of function variants in FOXJ1 suffer from hydrocephalus (Shapiro et al., 2021; Wallmeier et al., 2019). MCIDAS mutant individuals also exhibit enlarged ventricles. A study by Robson et al. has shown that individuals with autosomal recessive variants in MCIDAS show an increased choroid plexus size (Robson et al., 2020). Because studies, modeling posthemorrhagic hydrocephalus in rats, revealed overproduction of CSF secondary to activation of toll-like receptor 4 (TLR4); it was furthermore speculated that motile cilia dysfunction might cause overproduction of cerebrospinal fluid and subsequent closure of the aqueduct of sylvius due to increased pressure (Robson et al., 2020). Furthermore, studies in mice have indicated that the absence of cilia might affect ion transport across the choroid plexus epithelium, consecutively leading to a disequilibrium of fluid (Banizs et al., 2005).

In addition, the proteins MCIDAS and FOXJ1 have been shown to be crucial in the differentiation of ependymal cells (Kyrousi, Lygerou, & Taraviras, 2017). Ependymal cells along the neural tube appear by the 25th week of gestation (Meunier et al., 2020). Radial glia cells are specified to enter the ependymal cell lineage by inhibition of the Notch pathway (Figure 4; Kyrousi et al., 2017; Spassky, 2013). Subsequently, Geminin coiled-coil domain-containing protein 1 (GMNC) and multicilin (MCIDAS) interact with E2F transcription factors and activate the multiciliated cell transcription program (Kyrousi et al., 2017; Meunier et al., 2020). Within about a week, radial glia cells go through intermediate stages (Figure 4) until they transform into multiciliated ependymal cells (Kyrousi et al., 2017; Meunier et al., 2020). FOXJ1 was shown to be crucial for the process of maturation of radial glia cells to multiciliated cells. Fox1 mutant mice

![Figure 4: Mcidas and Fox1 are important during the differentiation of ependymal cells from Radial glia. The RGMC-associated proteins Mcidas, Fox1, p73 and Ccno have been associated with ependymal cell differentiation and multiciligenesis in ependymal cells in mice. Mutations in MCIDAS, FOX1, and CCNO as well as TP73 cause RGMC, which are associated with Hydrocephalus internus in all, except for TP73 deficiency. Pathway and Schematic adapted from Kyrousi et al. (2017) and Meunier et al. (2020).]
specify ependymal cells, which finally fail to differentiate (Jacquet et al., 2009). These data indicate that enlarged ventricles in MCIDAS and FOXJ1 mutant individuals might be related to a defect in differentiation in the ependymal cell lineage and consecutively impaired ependymal function. Interestingly, patients with autosomal recessive pathogenic variants in the tumor protein TP73, which has been shown to be crucial for cell differentiation and proliferation of multiciliated respiratory epithelia (Wallmeier et al., 2021) and also for centriole docking and ciliation of ependymal cells in mice (Gonzalez-Cano et al., 2016), do not exhibit enlarged ventricles but congenital brain malformation consistent with lissencephaly.

It is important to note that some of the proteins associated with primary cilia also play a role in multiple motile cilia and multiciliogenesis. Consistent with a mucociliary clearance disorder, OFD1 mutant respiratory epithelia exhibit mislocalized basal bodies, reduced numbers, length of cilia, and a dyskinetic beating pattern (Bukowy-Bierylo et al., 2019). Besides, the distal appendage protein CEP83 was detected as a component of the basal bodies of multiciliated cells, indicating that these proteins might be important beyond their function in primary cilia. Furthermore, HYLS1 (Mee et al., 2005), CC2D2A, and KIAA0586 (Alby et al., 2015) have been associated with the ciliogenesis of multiple motile cilia in model organisms. To better understand these functions, ciliopathies affecting both primary and motile cilia, need to be further studied.

7 | CONCLUSION

Two distinct clinical phenotypes can occur in ciliopathies associated with hydrocephalus. Primary ciliopathies often lead to severe hydrocephalus, which is associated with other brain malformations and primary intellectual deficits. Due to the severity of the disease, these children might die in the womb or shortly after birth (e.g., Meckel–Gruber syndrome). However, the disease can also have a more subtle disease course. Hydrocephalus in motile ciliopathies usually develops after birth. While in some genetic defects, only enlarged ventricles are diagnosed, in FOXJ1 mutants, surgical intervention is required due to increased intracranial pressure. The disease usually also comprises chronic destructive airflow disease and situs anomalies in some (Wallmeier et al., 2019). Therefore, motile cilia-based pathology should be especially considered in those affected with hydrocephalus and chronic lung disease.

This review highlights the importance of cilia and especially multiple motile cilia in the development of hydrocephalus interns. Identification of new cilia-associated hydrocephalus genes as well as identification of overlapping syndromes affecting both primary as well as multiple motile cilia will help to understand the role of cilia in hydrocephalus development.

ACKNOWLEDGMENTS

The authors thank Heike Olbrich for carefully proofreading the manuscript. Julia Wallmeier received funding from the DFG (WA 4283/1-1) and Interdisziplinären Zentrum für Klinische Forschung Muenster (IZKF) SEED/017/21.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Julia Wallmeier https://orcid.org/0000-0002-3958-9065
Marlene Dallmayer https://orcid.org/0000-0002-6877-7268
Heymut Omran https://orcid.org/0000-0003-0282-6765

REFERENCES

Adle-Biassette, H., Saugier-Weber, P., Fallet-Bianco, C., Delezoide, A.-L., Razavi, F., Drouot, N., … Laquerrière, A. (2013). Neuropathological review of 138 cases genetically tested for X-linked hydrocephalus: Evidence for closely related clinical entities of unknown molecular bases. Acta Neuropathologica, 126(3), 427–442. https://doi.org/10.1007/s00401-013-1146-1
Alby, C., Piquand, K., Huber, C., Megarbané, A., Ichikou, A., Legendre, M., … Thomas, S. (2015). Mutations in KIAA0586 cause lethal ciliopathies ranging from a Hydrocephalus phenotype to short-rib polydactyly syndrome. American Journal of Human Genetics, 97(2), 311–318. https://doi.org/10.1016/j.ajhg.2015.06.003
Al-Qattan, M. M., Shamseldin, H. E., Salih, M. A., & Alkuraya, F. S. (2017). GLI3-related polydactyly: A review. Clinical Genetics, 92(5), 457–466. https://doi.org/10.1111/cge.12952
Amirav, I., Wallmeier, J., Loges, N. T., Menchen, T., Pennekamp, P., Mussaffi, H., … Soferman, R. (2016). Systematic analysis of CCNO variants in a defined population: Implications for clinical phenotype and differential diagnosis. Human Mutation, 37(4), 396–405. https://doi.org/10.1002/humu.22957
Andreu-Cervera, A., Catala, M., & Schneider-Maunoury, S. (2021). Cilia, ciliopathies and hedgehog-related forebrain developmental disorders. Neurobiology of Disease, 150, 105236. https://doi.org/10.1016/j.nbd.2020.105236
Bachmann-Gagescu, R., Ishak, G. E., Dempsey, J. C., Adkins, J., O’Day, D., Phelps, I. G., … Doherty, D. (2012). Genotype-phenotype correlation in CC2D2A-related Joubert syndrome reveals an association with ventiliculomegaly and seizures. Journal of Medical Genetics, 49(2), 126–137. https://doi.org/10.1136/jmedgenet-2011-100552
Bangs, F., & Anderson, K. V. (2017). Primary cilia and mammalian hedgehog signaling. Cold Spring Harbor Perspectives in Biology, 9(5), a028175. https://doi.org/10.1101/cshperspect.a028175
Banitz, B., Pike, M. M., Millican, C. L., Ferguson, W. B., Komlosi, P., Sheetz, J., … Yoder, B. K. (2005). Dysfunctional cilia lead to altered ependyma and chordoid pus function, and result in the formation of hydrocephalus. Development, 132(23), 5329–5339. https://doi.org/10.1242/dev.02115
Bayston, R. (2018). Cerebrospinal fluid shunt infection. In G. Cinalli, M. M. Ozek, & C. Sainte-Rose (Eds.), Pediatric Hydrocephalus (pp. 1–19). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-31889-9_7-1
Boon, M., Wallmeier, J., Ma, L., Loges, N. T., Jaspers, M., Olbrich, H., … Omran, H. (2014). MCIDAS mutations result in a mucolipidosis clearance disorder with reduced generation of multiple motile cilia. Nature Communications, 5(1), 4418. https://doi.org/10.1038/ncomms5418
Brueg, A.-L., Franco, B., Duffourd, Y., Thevenon, J., Jego, L., Lopez, E., … Thouvin-Robinet, C. (2017). Fifteen years of research on oral–facial–digital syndromes: From 1 to 16 causal genes. Journal of Medical Genetics, 54(6), 371–380. https://doi.org/10.1136/jmedgenet-2016-104436
confirmed by HYLS1 gene mutation analysis. Journal of Neuropathology & Experimental Neurology, 67(8), 750–762.

Putoux, A., Thomas, S., Coene, K. L., Davis, E. E., Alanay, Y., Ogur, G., ... Attié-Bitach, T. (2011). KIF7 mutations cause fetal hydrocephalus and acrocallosal syndromes. Nature Genetics, 43, 601–606. https://doi.org/10.1038/n.826

Raybaud, C. (2018). Radiology of hydrocephalus. In G. Cinali, M. M. Ozek, & C. Sainte-Rose (Eds.), Pediatric hydrocephalus (pp. 1–122). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-31889-9_44-1

Reiter, J. F., & Leroux, M. R. (2017). Genes and molecular pathways underpinning ciliopathies. Nature Reviews. Molecular Cell Biology, 18(9), 533–547. https://doi.org/10.1038/nrmm.2017.60

Robson, E. A., Dixon, L., Causon, L., Dawes, W., Benenati, M., Fassad, M., ... O’Callaghan, C. (2020). Hydrocephalus and diffuse choroid plexus hyperplasia in primary ciliary dyskinesia-related MCIDAS mutation. Neurology Genetics, 6(4), 1–7. https://doi.org/10.1212/NXG.0000000000482

Ruggeri, G., Timms, A. E., Cheng, C., Weiss, A., Kollros, P., Chapman, T., ... Mirzaa, G. M. (2018). Bi-allelic mutations of CCDC88C are a rare cause of severe congenital hydrocephalus. American Journal of Medical Genetics Part A, 176(3), 676–681. https://doi.org/10.1002/ajmg.a.38592

Sakka, L., Coll, G., & Chazal, J. (2011). Anatomy and physiology of cerebrospinal fluid. European Annals of OtoroIlinaryngology, Head and Neck Diseases, 128(6), 309–316. https://doi.org/10.1016/j.anorl.2011.03.002

Schaefer, E., Stoetzel, C., Scheidecker, S., Geoffroy, V., Prasad, M. K., Redin, C., ... Dollfus, H. (2016). Identification of a novel mutation confirms the implication of IFT172 (BBS20) in Bardet-Biedl syndrome. Journal of Human Genetics, 61(5), 447–450. https://doi.org/10.1038/jhg.2015.162

Seo, J. H., Zilber, Y., Babayeva, S., Liu, J., Kyriakopoulos, P., De Marco, P., ... Torban, E. (2011). Mutations in the planar cell polarity gene, Fuzzy, are associated with neural tube defects in humans. Human Molecular Genetics, 20, 4324–4333. https://doi.org/10.1093/hmg/ddr359

Shapiro, A. J., Kaspy, K., Daniels, M. L. A., Stonebraker, J. R., Nguyen, V. H., Joyal, L., ... Zariwala, M. A. (2021). Autosomal dominant variants in FOXJ1 causing primary ciliary dyskinesia in two patients with obstructive hydrocephalus. Molecular Genetics & Genomic Medicine, 9(7), 1–7. https://doi.org/10.1002/mgg3.1726

Spassky, N. (2013). Motile cilia and brain function: Ependymal motile cilia development, organization, function and their associated pathologies. In K. Tucker & T. Caspary (Eds.), Cilia and nervous system development and function (pp. 193–207). Dordrecht: Springer.

Speksnijder, L., Cohen-Overbeek, T. E., Knapen, M. F. C. M., Lunshof, S. M., Hoogeboom, A. J. M., van den Ouweland, A. M. C., ... Wessels, M. W. (2013). A de novo GLI3 mutation in a patient with acrocallosal syndrome. American Journal of Medical Genetics, Part A, 161(6), 1394–1400. https://doi.org/10.1002/ajmg.a.35874

Tanrikulu, B., & Özek, M. M. (2019). Mechanical shunt complications. In G. Cinali, M. M. Özek, & C. Sainte-Rose (Eds.), Pediatric hydrocephalus (pp. 1289–1307). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-27250-4_75

Thomas, S., Boutaud, L., Reilly, M. L., & Benmerah, A. (2019). Cilia in hereditary cerebral anomalies. Biology of the Cell, 111(9), 217–231. https://doi.org/10.1111/boc.201900012

Tully, H. M., & Dobyns, W. B. (2014). Infantile hydrocephalus: A review of epidemiology, classification and causes. European Journal of Medical Genetics, 57(8), 359–368. https://doi.org/10.1016/j.ejmg.2014.06.002

Veening, J. G., & Barendregt, H. P. (2010). The regulation of brain states by neuroactive substances distributed via the cerebrospinal fluid; a review. Cerebrospinal Fluid Research, 7, 1–16. https://doi.org/10.1186/1743-8454-7-1

Wallmeier, J., Al-Mutairi, D. A., Chen, C. T. C.-T., Loges, N. T., Pennekamp, P., Menchen, T., ... Omran, H. (2014). Mutations in CCNO result in congenital mucociliary clearance disorder with reduced generation of multiple motile cilia. Nature Genetics, 46(6), 646–651. https://doi.org/10.1038/ng.2961

Wallmeier, J., Bracht, D., Alsiafi, H. S., Dougherty, G. W., Olbrich, H., Cindric, S., ... Omran, H. (2021). Mutations in TP73 cause impaired mucociliary clearance and lissencephaly. The American Journal of Human Genetics, 1–12, 1318–1329. https://doi.org/10.1016/j.ajhg.2021.05.002

Wallmeier, J., Frank, D., Shoemark, A., Nöthe-Menchten, T., Cindric, S., Olbrich, H., ... Omran, H. (2019). De Novo mutations in FOXJ1 result in a motile ciliopathy with hydrocephalus and randomization of left/right body asymmetry. American Journal of Human Genetics, 105(5), 1030–1039. https://doi.org/10.1016/j.ajhg.2019.09.022

Wallmeier, J., Nielsen, K. G., Kuehni, C. E., Lucas, J. S., Leigh, M. W., Zariwala, M. A., & Omran, H. (2020). Motile ciliopathies. Nature Reviews. Disease Primers, 6(1), 77. https://doi.org/10.1038/s41572-020-0209-6

Wechsler-Reya, R. J., & Scott, M. P. (1999). Control of neuronal precursor proliferation in the cerebellum by sonic hedgehog. Neuron, 22(1), 103–114. https://doi.org/10.1016/S0896-6273(00)80682-0

How to cite this article: Wallmeier, J., Dallmayer, M., & Omran, H. (2022). The role of cilia for hydrocephalus formation. American Journal of Medical Genetics Part C: Seminars in Medical Genetics, 190C:47–56. https://doi.org/10.1002/ajmg.c.31972