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Unveiling a New Link: Cholesterol Deficiency in Smith–Lemli–Opitz and Niemann–Pick C as a Driver of Ciliopathies

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ABSTRACT

The ciliopathies are a group of genetic disorders caused by defective function of either the primary cilia (a large number) or the motile cilia (a much smaller number). These have been defined as diseases with mutations in genes encoding individual ciliary or cilia-associated proteins. Recently, it has become apparent that the composition of the ciliary membrane influences its function. For instance, the ciliary membrane contains more cholesterol than other regions of the cell membrane and a variety of unique receptors and ion channels. Additionally, it appears that primary cilia have evolved to lower the threshold for activating signal transduction by establishing the environment essential for signaling pathways on a limited portion of the cell surface. By positioning receptors and downstream signaling components in this thin protrusion at a precise time and location within the plasma membrane, the cell can better orient its physiological response to external stimuli. Cholesterol deficiency can alter cilia formation and function with effects on Sonic hedgehog signaling. In this review, we discuss these new concepts and apply them to the developmental disorder Smith–Lemli–Opitz syndrome and the developmental and neurodegenerative disorder Niemann–Pick C disease, demonstrating that they are also ciliopathies.

1 | Introduction

Almost all our cells initially present a primary cilium, which is later lost in some tissues. This is in contrast to insects—*Drosophila* lack the embryonic signaling cilia—but have cilia in sensory neurons and motile sperm. In this species, Sonic hedgehog (Shh) signaling requires cytonemes (actin-based protrusions spanning several cell diameters) and the protein is localized in them and punctate bodies along them (Bischoff et al. 2013). Although most Shh signaling in vertebrates occurs via cilia, axonemes seem to be involved as well (Sanders et al. 2013; Fairchild and Barna 2014). In embryonic cells, the primary cilium transduces extracellular signals, and in other cells, such as the lung (where motile cilia clear mucus) and renal epithelium, there are multiple cilia. Motile cilia are found in the embryonic node and in sperm, while sensory neurons have immotile cilia.

In the following, we will present an overview of cilia function, focusing on the ciliary membrane composition, especially in Shh signaling. We will then present the defects in cholesterol metabolism in Smith–Lemli–Opitz (SLO) syndrome (with a severe cholesterol deficiency due to sterol delta-7-reductase deficiency) and Niemann–Pick C disease (a cholesterol lysosomal storage disease with partial cholesterol deficiencies at some sites, especially in developing cerebellar cells). We also point out that, since peroxisomes are essential for cholesterol delivery to the primary cilia, some features of Zellweger's syndrome are due to primary cilia dysfunction. The connection

between cholesterol and ciliopathies is well established, as statins cause ciliopathy-spectrum diseases, such as polycystic kidney disease and situs inversus, in zebrafish embryos by inhibiting the rate-limiting enzyme HMG-CoA reductase (Maerz et al. 2019).

While cholesterol in adequate amounts is important for cell membrane stability everywhere, our focus is on the role of cholesterol only in the ciliary membrane. We also do not discuss its many other important functions, including preventing adrenal insufficiency, serving as a precursor for bile acids, and myelinizing the central and peripheral nervous systems, nor do we discuss the damaging effects of increased oxysterols in SLO syndrome (Korade et al. 2010).

2 | Cilia Function and Membrane Composition

The ciliopathies and cilia structure have been manifoldly reviewed (Reiter and Leroux 2017; Derderian et al. 2023), and there are several book-length reviews (e.g., Kenny and Beales 2013) on the topic. The basic structure of the cilia is a basal body, sometimes known as the mother centriole, which consists of nine triplet microtubules from which a network of transition fibers extends to the base of the cilia. From the basal body, the axoneme with a skeleton of nine doublet microtubules is directed to the tip of the cilia. In motile cilia, this structure has the addition of axonemal inner and outer dynein arms and a central doublet of microtubules.

Despite being continuous with the cell's overall plasma membrane, the cilia's plasma membrane retains its own composition thanks to a transition zone that restricts which proteins can permeate into it. There is evidence that NPH4 (Awata et al. 2014) and septins (Hu et al. 2010; Chih et al. 2011) contribute to this barrier to free migration of proteins in the membrane. Therefore, primary cilia have likely evolved to concentrate relevant receptors on a small surface area of the cell, thereby reducing the threshold required to activate signal transduction. Placing receptors and downstream signaling components in this thin protrusion at a specific location on the plasma membrane at a specific time helps the cell orient its cellular response to external stimuli. Recent proteomic studies of cilia have surprisingly found a low overlap in shared proteins between different cell lines—only 45% between two mouse lines (IMCD3 kidney cells and NIH3T3 embryonic fibroblasts; Sroka et al. 2025). Another proteomic study of primary cilia found only a 31% sharing among (out of 715 proteins—91 of which are unique to primary cilia) three human cell lines (HTERT-RPE1 embryonic retinal cells, RPTEC-TERT1 epithelial kidney cells, and ASC52telo stromal adipose cells; Hansen et al. 2025).

The primary cilium is also a unique calcium cation (Ca^{2+}) compartment that can influence Wnt/β-catenin and Shh signaling (DeCaen et al. 2013), and a sodium gradient is needed to release lipidated hedgehog (Wang et al. 2021). A mutation in the gene encoding the calcium-binding domain-containing ciliary protein, CCD2A, causes Meckel syndrome, a ciliopathy with encephalocele, polycystic kidneys, and preaxial polydactyly (Tallila et al. 2008). Finally, in the extensive list of conditions affecting cilia, mutations in mitochondrial-associated proteins cause cilia elongation and ciliopathy-like findings (Burkhalter et al. 2019).

3 | Role of Cholesterol in Shh Function

Shh (as well as Wnt/β-catenin) is covalently linked to lipids and associates with lipoprotein particles. The current view posits that Shh monomers associate with lipoprotein particles via palmitoyl and cholesterol moieties, forming multimeric complexes (reviewed by Hu and Song 2019). Shh is transcribed as a longer peptide, which is cleaved to a shorter form as a cholesterol is added to the C-terminus (Porter et al. 1996), while a palmitoyl or other fatty acid (Long et al. 2015) moiety is frequently added to the N-terminus (Pepinsky et al. 1998; Chamoun et al. 2001). Mutations in this C-terminal domain generate Shh isoforms that are not suitable for cholesteroylation and are rapidly degraded in the ER, leading to no active ligand secretion (Chen et al. 2011). These mutations cause congenital brain malformations, such as holoprosencephaly (Roessler et al. 1997; Traiffort et al. 2004). Thus, a relative limit in available cholesterol may decrease Shh activation. Additionally, it has been found that the extracellular cysteine-rich domain (CRD) of Smoothened (Smo) binds cholesterol and that targeted mutations of key binding residues prevent Shh signaling (Huang et al. 2016). Cholesterol modification of Smo is required for its translocation to the primary cilium plasma membrane (Xiao et al. 2017; Hu and Song 2019), which provides the environment for interactions among various Shh signaling actors (Corbit et al. 2005; Rohatgi et al. 2007). Patched (Ptch), the Shh receptor, localizes on the plasma membrane of the cilium base in cholesterol-rich microdomains associated with caveolin-1 (Karpen et al. 2001), which is upregulated in NPC1 (Garver et al. 1997). Here, Ptch mediates cholesterol efflux from cells (Bidet et al. 2011), consistent with its structural similarity to NPC1 (Gong et al. 2018). A sodium gradient is involved in Ptch transporter activity, as extracellular sodium is required (Myers et al. 2017). The Ptch cholesterol transporter activity prevents Smo activation, retaining it within intracellular vesicles. After binding to the C-terminal cholesterol and the N-terminal palmitoyl-modified Shh, the activation of Ptch limits its cholesterol transport function (Qian et al. 2019). The Shh-Ptch complex is internalized and degraded by lysosomes (Incardona et al. 2002; reviewed in Ho and Scott 2002; Goetz and Anderson 2010). Then, plasma membrane cholesterol can activate Smo, allowing Smo to localize at the cilium membrane (Huang et al. 2016; Kinnebrew et al. 2019) (Figure 1). In line with this, cholesterol loading synergizes with Shh to activate Smo (Huang et al. 2016) and leads to Smo activation even in the absence of Shh ligand (Luchetti et al. 2016). A noncanonical Shh activation pathway (not involving Smo) results in actin reorganization and is more important for Shh-driven cancer than for its role in development (Faria et al. 2019).

In addition to metabolically active cholesterol, which is readily used for covalent modification of cholesterol-regulated proteins (Das et al. 2014), plasma membrane cholesterol participates in signaling pathways via a distinct mechanism. For instance, the enrichment of cholesterol in lipid rafts is being redefined in light of the discovery of a high-affinity cholesterol-binding motif in the intracellular juxta membrane domain of receptor proteins (Di Scala et al. 2017). The precise arrangement of this

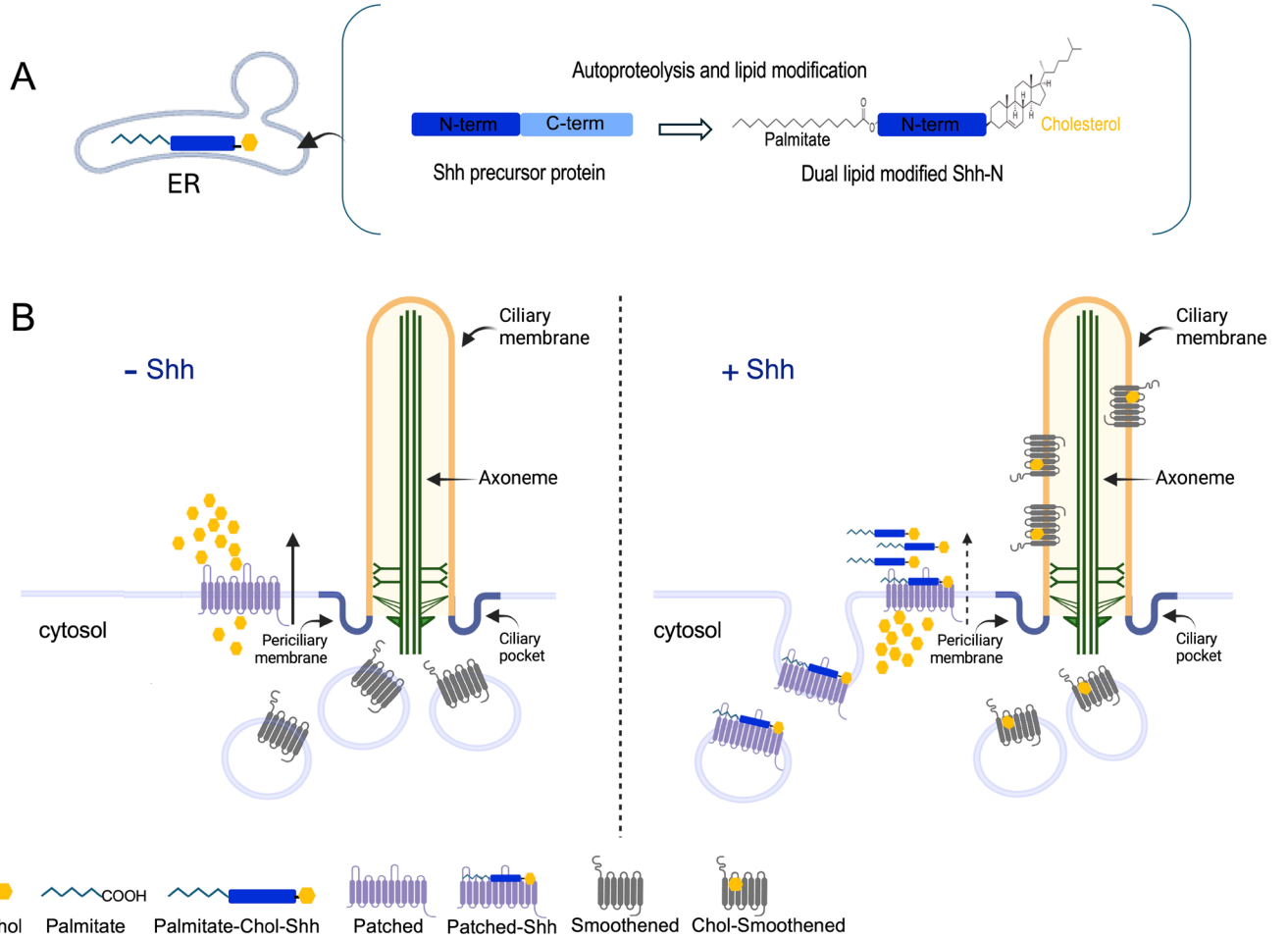

FIGURE 1 | A scheme of Shh precursor processing and cholesterol (Chol) Shh interplay at the primary cilium. (A) Following autoproteolysis of the Shh precursor protein in the endoplasmic reticulum (ER), a palmitate moiety and a Chol moiety are covalently bound to the N-terminal and C-terminal of Shh, respectively. Dual lipid modification ensures the assembly of secreted Shh into homomultimers, which creates a protein gradient essential for spatiotemporal responses to the Shh morphogen (not shown). (B) In the absence of Shh, Ptch facilitates the removal of Chol from the inner leaflet of the plasma membrane, thus preventing Chol modification of Smoothened (Smo); Smo traffics between endosomes and the ciliary plasma membrane. Chol becomes accessible for Smo modification and trafficking to the primary cilium membrane when Shh binds to Ptch, causing Ptch internalization and blocking Chol efflux. This figure was created in BioRender.

motif, called the cholesterol recognition amino acid consensus (CRAC), and its mirror motif, CARC, in the inner and outer leaflets of the plasma membrane, respectively, suggest a direct role for cholesterol in activation processes.

It has long been known that protein-lipid vesicles were delivered to the base of the cilium (Papermaster et al. 1985). More recent data has shown that Rab8 associates with ciliary-targeted vesicles at the Golgi body and is involved in their trafficking to the cilia (Moritz et al. 2001) but also other Rabs are involved (reviewed in Emmer et al. 2010). In tissue cultures of human NPC1 fibroblasts, delivery of Rab7 by a plasmid decreases cholesterol storage, i.e., ameliorating a major pathological feature (Choudhury et al. 2002; Ganley and Pfeffer 2006), whereas in a Npc1-deficient mouse model, transgenic expression of Rab9 extends lifespan (Kaptzan et al. 2009).

A key role in controlling Shh signaling is played by the kinesin Kif7, an anterograde motor protein that was recently shown to localize to the cilium tip, where it controls cilium length and structure (He et al. 2014). Kif7 mediates the activation and translocation of Gli (glioma-associated) proteins to the cilium tip by physically interacting with Gli transcription factors and influencing their processing (Cheung et al. 2009). In vertebrates, the zinc-finger transcription factors, Gli1 to an extent (Ruiz i Altaba 1998) but mostly Gli2 and Gli3, are the effectors of Shh signaling (Jiang and Hui 2008), controlling the expression of genes involved in cell proliferation and cell cycle progression (Tuson et al. 2011). Gli1 and Gli2 usually act as transcriptional activators and full-length or a truncated form of Gli3, are transcriptional activators or repressors, respectively (Huangfu and Anderson 2006; Haldipur et al. 2012). Shh controls Gli2 levels by suppressing both its processing and degradation (Pan et al. 2006). Transcriptional sequence analyses of total embryo nuclei from Day 13, five *Gli2* knockout embryos found both depleted (anterior) and enriched (dorsal) cell populations of roof plate derivatives (Huang et al. 2023), partially explaining the dorsalization of the embryos (Ding et al. 1998). While we focus on the role of Shh in embryonic development, it is also important to note that Shh signaling has an important negative role in autophagy. The studies demonstrating this have been performed in a large variety of cultured cells, using serum deprivation as a

model of nutrient deprivation, but suffer partial oxygen toxicity due to the use of 20% instead of tissue levels of 5% oxygen. Activation of Shh signaling inhibits the autophagy pathway (Jimenez-Sanchez et al. 2012) and inhibition of ciliogenesis partially inhibits autophagy (Pampliega et al. 2013). There are autophagosome deficiencies in many neurodegenerative diseases where lipid composition influences on the process are especially important (Hernández-Cáceres et al. 2024); increasing autophagosome activity has been shown to ameliorate symptoms in some rodent models of these diseases (Nixon 2013). Accumulation of autophagosomal vesicles has been observed in cultured NPC1 cells (Meske et al. 2014). On the other hand, autophagy stimulates ciliogenesis. Mutations in the Oral-Facial-Digital Syndrome I (*OFD1*) gene result in a ciliopathy. OFD1, located at the ciliary centriolar satellites, is degraded when autophagy is induced, and ciliogenesis is stimulated (Tang et al. 2013).

While primary cilia stimulate oligodendrocyte proliferation after hypoxic brain injury, they do not involve Shh signaling. In contrast, in the aftermath of hypoxic ischemia injury in newborns (humans) or lysolecithin-induced demyelination (mice), while Shh signaling is not activated (Hoi et al. 2023), an alternative pathway using a GPCR/cAMP/CRED is used instead.

4 | Alteration of Shh Function in SLO Syndrome

Smith, Lemli, and Opitz described their eponymous genetic disease (SLO) in 1964. They found three unrelated males with microcephaly, developmental delay, underdevelopment of the male genitalia, and a characteristic short nose and anteverted nostrils, while two had pyloric stenosis (Smith et al. 1964). Confirmatory cases were in girls as well as boys, suggesting autosomal recessive inheritance. Syndactyly of toes 2/3 was additionally reported as a feature (Pinsky and DiGeorge 1965; Dallaire and Fraser 1966). As younger, especially infant, cases were described (at first as a separate syndrome; Lowry et al. 1968), a more severe phenotype emerged with dwarfism, cerebellar hypoplasia, ambiguous genitalia (even sex reversal), congenital heart defects, renal and/or lung hypoplasia, and polydactyly (Rutledge et al. 1984; Donnai et al. 1986). Curry et al. (1987) reviewed 19 cases of which 18 had polydactyly (post-axial), 16 had congenital heart defects, 13 had cleft palate, and 10 had cataracts. Mesomelic dwarfism was also frequent. They suggested that these cases be designated as SLO II. It soon became apparent that SLO II was the infantile form of juvenile SLO with the discovery of severe cholesterol deficiency (Irons et al. 1993) due to 7-dehydrocholesterol reductase (DHCR7) deficiency (Shefer et al. 1995). SLO II was due to a greater severity of the deficiency (Tint et al. 1994; Cunniff et al. 1997; Neklason et al. 1999).

In 1996, it was suggested that there was defective modification of Shh in SLO (Porter et al. 1996). At that time, it was only the lack of the C-terminal cholesterol modification that was discussed. These authors further highlighted the implications of this defect for development, especially in SLO, pointing out the role of Shh in facial processes, where cholesterol deficiency results in abnormal facial bone development (Iwaya et al. 2024), as well as in the limb and lung buds (Cooper et al. 2003). Indian hedgehog, important in endochondral bone growth (explaining the dwarfism in SLO), and Desert hedgehog important for male gonadal development (explaining defects in male genitalia development) share the C-terminal cholesterol modification of Shh and were thus implicated in causation (Cooper et al. 2003). The authors used cyclodextrin to deplete cholesterol from developing chick embryo membranes (thus affecting ciliary membrane cholesterol as well) and found loss of midline tissues, replicating the anteverted nostrils of SLO (Cooper et al. 2003). The most severe phenotype was that seen in mouse embryos with no Shh (Chiang et al. 1996). While an alternative interpretation of the findings could be that the excess of 7HDC inhibited Shh function, studies on fibroblasts from *Dhcr7*$^{-Ex8}$ homozygous mice demonstrated that it is the lack of cholesterol that inhibits ciliary localization of Smo (Blassberg et al. 2016).

Shh is important in brain function (reviewed by Ruat et al. 2012). Many of the mutations affecting brain Shh function are those of ciliopathies, especially Kif7 (Joubert syndrome, hydrocephaly, acrocallosal syndrome), which is surprisingly increased (perhaps compensatory) in the Npc1 mouse cerebellum (Canterini et al. 2017). Other ciliary deficiencies (especially anterograde ciliary transport proteins: Ift172, Ift52, Ift57, and Ift88) cause microcephaly. Shh's role in stem cell proliferation especially affects the hippocampus, a major destination for newly generated neural stem cells (Ahn and Joyner 2005). The cerebellum appears to be particularly sensitive to cholesterol deficiency and shows major neurodevelopmental defects in NPC1 (see below) and in many ciliopathies. Shh from Purkinje cells, which have a prominent cilium (Del Cerro and Snider 1969) is a potent mitogen for the proliferation of granule cells and for their migration from the external to the internal granule cell layer (Traiffort et al. 2010). The MRI findings in SLO were initially described as cerebellar atrophy without details on the lobules involved (Garcia et al. 1973; Lee et al. 2013). Neuropathological examination of the cerebellum in a 3-year-old case showed patchy loss of Purkinje cells in the lateral lobes, but the internal granular layer did not appear abnormal. There was also mild hyperplasia of Bergmann's glia layer. Newborn mice without Dhcr7 (the mouse model of SLO) had poor sucking and swallowing (leading to their death), which suggests probable cerebellar dysfunction (Fitzky et al. 2001; Wassif et al. 2001).

Cerebellar underdevelopment is a feature of many ciliopathies (Reiter and Leroux 2017). Joubert syndrome is the first well-described syndrome of the modern era with cerebellar vermal hypoplasia or aplasia (Joubert et al. 1968). The classical radiographic finding, known as the “molar tooth sign” (MTI), is visible on axial brain magnetic resonance imaging (MRI) at the level of the mid-brain–hindbrain junction, indicates the absence of the vermis and thickening and deformation of the superior cerebellar peduncles with accompanying deformation of the fourth ventricle (Maria et al. 1999). Two genes have been implicated in its causation (Alazami et al. 2015), but up to seven variants with associated genes have been classified as forms of Joubert. They include cerebellar–oculo–renal syndrome, where a hypoplastic cerebellar vermis with the MTS is accompanied by notable ocular and renal involvement (Satran et al. 1999) and is associated with mutations in three different ciliary protein genes (Arts et al. 2007; Brancati et al. 2007). The lethal Mickel–Gruber syndrome of occipital encephalocele, polycystic kidney disease, and post- and (occasionally) preaxial polydactyly (Meckel 1822) is associated with mutations in up to 10 genes associated with ciliary

functions, but many of these are considered variants of Joubert syndrome (Consugar et al. 2007; Valente et al. 2010). Bardet–Biedl syndrome is a complex ciliopathy sometimes associated with cerebellar hypoplasia and associated with truncal obesity, male hypogonadotropic hypogonadism, female genitourinary malformation, retinal dystrophy, intellectual impairment, and renal abnormalities (Baskin et al. 2002). It is associated with mutations in up to 22 different ciliary protein genes, but again with overlap with Joubert and Meckel syndromes (Muller et al. 2010; Kousi et al. 2020). Thus, most ciliopathies have central cerebellar defects rather than the progressive degeneration seen in NPC1 disease due to defective Shh signaling. This may be due to early defects of Shh signaling causing dorsalization of the embryo (see above) compared to a later, lesser defect in Shh signaling in the developing cerebellum.

Outside the cerebellum, in the choroid plexus, ciliary Shh signaling is an important negative regulator of CSF fluid generation (Mao et al. 2025). Inhibiting this function results in increased CSF production and hydrocephalus (Mao et al. 2025).

Shh is required for normal limb development, and its complete absence results in a single digit (digit one; Chiang et al. 2001). It is interesting that the abnormalities of limb development vary with different ciliary/cholesterol disorders being preaxial in some (e.g., Meckel syndrome; Tallila et al. 2008) but postaxial in SLO (Rutledge et al. 1984). The complex, usually sporadic but overlapping with some genetic syndromes, birth defect association of VACTERL (vertebral anomalies, anal atresia, cardiac malformation, trachea-esophageal fistula, renal and limb [radial dysplasia, with either thumb hypoplasia or pre-axial polydactyly] abnormalities) has also been suggested as being due to deficient Shh signaling (Kim et al. 2001). Cleft palate can be caused by deficient primary cilia dysfunction (Ashe et al. 2012) while Wnt/β-catenin signaling from the primary cilia is important for early kidney development (Bai et al. 2022). The primary cilium also has important roles in the development of the enteric nervous system, especially neural crest precursor development (Bondurand and Southard-Smith 2016), and the developing heart (Willaredt et al. 2012). Table 1 lists the main characteristics of SLO syndrome.

5 | Defects in Shh and Primary Cilium Cause Cerebellar Hypoplasia in Niemann–Pick C1 Disease

It is becoming increasingly clear that Niemann–Pick C disease has two phases: a juvenile one of altered development, and a

TABLE 1 | Probable etiologies of features of Smith–Lemli–Opitz syndrome (partial to complete 7-dehydrocholesterol reductase [DHCR7] deficiency).

Microcephaly, delayed development, hypotonia, abnormal sleeping patterns	Deficient Sonic hedgehog signaling (with many roles in brain development, especially cell proliferation, e.g., in the hippocampus and cerebellum) due to deficient C-terminal cholesterol and decreased primary ciliary membrane cholesterol.
Short nose with anteverted nostrils, Blepharoptosis	Deficient Sonic hedgehog signaling (important for midfacial development) due to deficient C-terminal cholesterol and decreased primary ciliary membrane cholesterol.
Cleft palate	Possible primary ciliary defects due to decreased ciliary membrane cholesterol.
Hypospadias or lack of male genitalia	Deficient Indian hedgehog signaling (important for external male genitalia growth) due to deficient C-terminal cholesterol and decreased primary ciliary membrane cholesterol.
Postaxial polydactyly, syndactyly toes 2/3	Deficient Sonic hedgehog signaling (important for limb patterning) due to deficient C-terminal cholesterol and decreased primary ciliary membrane cholesterol.
Congenital heart defects	Probable primary cilia defects, including of Sonic hedgehog signaling.
Hirschsprung disease	Possible primary ciliary defects causing deficient neural crest precursors for enteric neurons due to decreased ciliary membrane cholesterol.
Unilobular lung	Deficient Sonic hedgehog signaling due to deficient C-terminal cholesterol and decreased primary ciliary membrane cholesterol.
Oligopapillary renal dysplasia	Possible primary ciliary defects, especially of Wnt signaling, due to decreased ciliary membrane cholesterol.
Dwarfism	Deficient Indian hedgehog signaling (important for endochondral bone growth) due to deficient C-terminal cholesterol and decreased primary ciliary membrane cholesterol.

Note: Symptoms from Donnai et al. 1986 and Curry et al. 1987 as attributed above.

later one of neurodegeneration. The early phase seems mostly due to a partial deficiency of cholesterol, affecting the primary cilia and Shh signaling, leading to abnormal development of the cerebellum. The later phase seems to be caused by mitochondrial dysfunction, leading to neural inflammation and neurodegeneration. It is the earlier phase that may be referred to as a ciliopathy.

This was first suggested by a study performed on skin fibroblasts from five patients, which showed alterations in the primary cilium in NPC1 disease. The authors found the primary cilium to be shortened an average of about 50% and that Ptch levels decreased in parallel with the decrease in NPC1 protein in these fibroblasts (Formichi et al. 2018). A study from our group, published concomitantly, reported similar findings by showing the presence of shorter cilia and a lower number of ciliated cells in *Npc1* mice and NPC patient fibroblasts, along with the downregulation of Shh signaling effectors (Canterini et al. 2017). This evidence provided a highly reasonable explanation for our earlier finding that the cerebellum of NPC1 disease mouse models has a smaller size, which recalls cerebellar hypoplasia typical of the ciliopathies discussed above that exhibit cerebellar abnormalities or deficiencies in genes involved in the formation and function of primary cilia. Indeed, both Purkinje cells (Del Cerro and Snider 1969) and granule cell progenitors (GCPs) in the external granule cell layer (EGL) of the developing cerebellum have been found to possess primary cilia (Del Cerro and Snider 1972), and more recently their presence has been also demonstrated in the cerebellar-specific astrocyte type -Bergmann glia (Cheng et al. 2018; Marazziti et al. 2013). A great number of conditional mouse models carrying hypomorphic alleles of cilia genes, including various intraflagellar transport (IFT) or IFT-associated proteins such as IFT88 and Kif3a, have elegantly demonstrated that the formation and function of primary cilia on these cells is required for normal cerebellar morphogenesis (Chizhikov et al. 2007; Spassky et al. 2008; Breunig et al. 2008; Han et al. 2008). This process requires a coordinated control of progenitor proliferation, neuronal differentiation, and migration (Goldowitz and Hamre 1998; Chizhikov and Millen 2003), which encompasses the two post-natal weeks and involves the three main cerebellar cell types—GCPs, Purkinje cells, and Bergmann glia. At birth, the surface of the developing cerebellum anlage is populated by GCPs that form a germinal neuroepithelium—the external granule layer, EGL—wherein the expansion of this progenitor pool is dictated by the mitogenic activity of Shh released by Purkinje cells. Specifically, there is a glial-neuronal interplay through the primary cilium that is crucial, since the ablation of Shh signaling in Bergmann glia leads to a decrease of GCP proliferation due to early cell cycle exit and migration to the internal granule layer (IGL). We have reported a 25% reduction in cerebellar size in *Npc1* null mutant mice caused by the disruption of GCP expansion dynamics in the EGL with premature cell cycle exit, and decreased differentiation and migration of granule neurons (Nusca et al. 2014). Thereafter, we uncovered additional anomalies of the developing cerebellum of *Npc1* hypomorphic mice, including a poorer differentiation/maturation of Bergmann glia (Caporali et al. 2016). All these findings are consistent with defective Shh signaling, as indicated by the presence of shorter cilia and the reduction of ciliated cells in *Npc1* mice and NPC patient fibroblasts (Canterini et al. 2017; Formichi et al. 2018) discussed above. The finding that β-hydroxyl cyclodextrin reverses these abnormalities when given shortly after birth strengthens the connection between cerebellar hypoplasia, primary cilium shortening, and cholesterol dyshomeostasis (Nusca et al. 2014; Caporali et al. 2016; Canterini et al. 2017).

Obviously, disrupted cerebellar morphogenesis translates to functional deficits in both mouse models and patients. Granule neurons generated in the EGL are crucial for a number of cerebellar-controlled functions, including the processing of motor and sensory information and pruning of ascending climbing fibers that connect with Purkinje cells (Huang et al. 2013; Lackey et al. 2018; Manzini et al. 2006; Watanabe and Kano 2011). The latter is a major milestone in normal cerebellar development, as it resolves the redundancy that arises when a few climbing fibers connect to the same Purkinje cell at earlier developmental stages. Specifically, granule neurons contribute to conditioned responses to stimuli (Giovannucci et al. 2017) and make synapses with mossy fibers (Huang et al. 2013), both of which are important for motor learning. Table 2 lists the main characteristics of NPC1/2 disease.

6 | Primary Cilia Distribution in the Brain and Its Relationship With Clinical Features of SLO and NPC1 Disorders

In the postnatal brain, the primary cilium controls the proliferation of neural stem cells (Breunig et al. 2008; Han et al. 2008;

TABLE 2 | Probable causes of features of Niemann–Pick C1/2 disease.

Potentially lethal cholestatic liver disease in infants	Massive storage of cholesterol in liver and spleen.
Gradually decreasing hepatomegaly and splenomegaly during childhood	An increase in mitochondrial membrane cholesterol leading to normal function.
Ataxia, spasticity, myoclonic jerks, paralysis of upward gaze	Defective cerebellar development due to decreased Sonic hedgehog signaling.
Loss of speech, dystonia akinesis, grand mal seizures	Neurodegeneration primarily due to inflammation with defects in autophagy, mitochondrial function, and altered mTOR.
Adulthood psychosis, dystonia, preserved intellectual function, paralysis of upward gaze	Presumably slower neurodegeneration due to inflammation with defects in autophagy, mitochondrial function, and altered mTOR.

Note: Symptoms from Stampfer et al. (2013) and Rego et al. (2019).

Amador-Arjona et al. 2011; Tong et al. 2014) and the maturation of neurons (Kumamoto et al. 2012; Luo et al. 2015). Cultured cerebellar granule neurons and interneurons produced from the medial ganglionic eminence (MGE) have been reported to assemble a primary cilium (Baudoin et al. 2012; Trivedi et al. 2014). Defective connectivity, morphogenesis, and migration of MGE-derived interneurons were observed in KO mice for the ciliary small GTPase Arl13b or the ciliary IFT molecules Kif3a or Ift88 (Baudoin et al. 2012; Higginbotham et al. 2012; Guo et al. 2017). Additionally, the migration and differentiation of embryonic pyramidal neurons were impaired by Kif3a knockdown (KD) (Chen et al. 2019).

Primary cilia are found on neocortical, hippocampal, dentate gyrus, and olfactory bulb (OB) neurons (Berbari et al. 2008; Stanić et al. 2009). Both glutamatergic pyramidal neurons and GABAergic inhibitory neocortical neurons are provided with primary cilia. GABAergic interneurons assemble a short primary cilium of about 0.2–0.5 μm, named procilium, by docking of the mother centriole to the cell membrane (Baudoin et al. 2012) after they have completed their migration from MGE. The procilium grows slowly and reaches 0.5–2 μm length by P0–P4. Thereafter, the elongation of neocortical cilia is a remarkably long process that takes weeks to complete, follows varying temporal patterns, and attains lengths that differ across layers. Cilia length peaks and stabilizes between P60 and P90, with average lengths of about 5 μm in all layers, except for Layer 5, which has longer cilia (6 μm on average and up to 11 μm) (Arellano et al. 2012).

Primary cilia are also found in astrocyte-like neural precursors in the hippocampus that express Shh signaling components and react to secreted Shh to control neurogenesis and differentiation (Breunig et al. 2008; Han et al. 2008). These cells form the neurogenic hippocampal niche, which generates dentate gyrus granule neurons throughout life. Young adult-born neurons have been demonstrated to lack a primary cilium, but when they get closer to their destination, they assemble one, further elongate their dendrites, and form synapses with projections from the entorhinal cortex. This suggests that cilia assembly is essential for the dendritic refinement and synaptic integration of adult-born neurons (Kumamoto et al. 2012). Therefore, through increased Wnt/β-catenin signaling, conditional loss of cilia from adult-born neurons caused severe abnormalities in dendritic refinement and synapse formation.

Apart from the hippocampal neurogenic niche, it has been reported that primary cilia are required for proper proliferation of a subset of Shh-responsive neural stem cells in the major postnatal germinal niche, the lateral ventricle subventricular zone (SVZ) (Angot et al. 2008; Tong et al. 2014). These neuroblasts are the precursors of neurons that migrate along the rostral migratory stream (RMS) in chain-like aggregates toward the OB (Luskin 1993). Their primary cilium enables immature adult-generated neurons migrating to the OB to finely respond to chemoattractive cues provided by Shh-expressing cells above the RMS (Angot et al. 2008; Hor and Tang 2010). Neural progenitors at the hippocampus dentate gyrus and the SVZ decreased when Shh and Smo were conditionally knocked out (Balordi and Fishell 2007). Nonetheless, the lifelong supply of newly generated neurons to the hippocampus and OB is fundamental for proper cognitive, behavioral, and neurosensory abilities. Adult hippocampal neurogenesis is an intricate process that includes the migration, positioning, maturation, and integration of new excitatory neurons within existing circuits. This neuroplasticity is crucial for cognitive functions, emotional regulation, and adaptation. Similarly, SVZ-derived neurons integrate into the granule and periglomerular cell layers of the OB, where they fully mature and provide GABAergic inhibitory inputs to the dendrites of mitral and tufted cells. Perceptual learning, a type of learning in which experience improves the ability to discriminate between olfactory stimuli, requires the integration of these newborn neurons in the OB networks (Moreno et al. 2009; Mouret et al. 2009; Gheusi et al. 2000). In particular, the activation of post-natally born neurons has been associated with improved odor discrimination learning when rodents were challenged with difficult odor discrimination tasks (Alonso et al. 2012).

The cognitive, behavioral, psychiatric and neurosensory impairments that are probably caused by ciliary anomalies discussed above are common to ciliopathies, including SLO and NPC1 syndromes. For example, MRI revealed that 42.31% of a group of 34 patients with BBB syndrome had hippocampal dysgenesis. Slow information processing, attention problems, and obsessive-compulsive behaviors were associated with neuroanatomical abnormalities (Bennouna-Greene et al. 2011). Patients with SLO have been found to have severe hippocampal hypoplasia and incomplete inversion, which results in a more rounded, vertical, and medially positioned hippocampus compared to normal (Grynspan et al. 2013). Stunted and thicker CA3–CA1 subfields, poorly defined boundaries between the pyramidal layer and the molecular layer and the *stratum radiatum*, the dispersion of immature cells in the pyramidal and polymorph layers of the dentate gyrus, and the complete lack of emerging pyramidal cells in the deeper layer of the CA1 were among the abnormalities. Sensory hyperreactivity, irritability, language impairment, sleep cycle disruption, self-harming behavior, autism spectrum behaviors, and cognitive abilities ranging from borderline intellectual functioning to profound mental retardation are all characteristics of the behavioral phenotype of SLO syndrome (Tierney et al. 2000).

Although it is not the main characteristic, olfactory impairment has been documented in mouse models of NPC1 disease and can be present in people with SLOS (Hovakimyan et al. 2013; Rava et al. 2022). Neuroblast loss and impaired neuronal maturation (Seo et al. 2014) as well as a reduction in the number of SVZ-derived neuroblasts (Dragotto et al. 2019) are the causes of olfactory impairments in NPC1 animals.

7 | Zellweger's Syndrome

Zellweger's syndrome is a group of peroxisome-deficient disorders caused by mutations in peroxisomal biogenesis (*PEX* mutations) or in specific peroxisomal enzymes. Of the many clinical features described (Wanders 2004), polycystic kidneys are prominent, while retinitis pigmentosa and hypospadias

occasionally occur, thus showing clinical overlaps with ciliopathies. These defects are explicable as due to primary ciliary dysfunction. Cultured cells of several different peroxisomal mutant patients accumulate large amounts of cholesterol, and ciliary cholesterol is deficient, with shortened cilia due to a lack of peroxisome-mediated transport along microtubules to the cilia (Miyamoto et al. 2020). Furthermore, it has recently been shown that the ciliary localization of polycystins is regulated by cholesterol and that polycystin-2 physically interacts with cholesterol (Itabashi et al. 2025). At the primary cilium, PC2 and polycystin-1 (PC1) form a heteromeric cation channel complex that perceives chemical ligands or mechanical shear stress to regulate the proliferation and differentiation of renal epithelial cells. A similar role is exerted by the PC1–PC2 complex at the primary cilium of neural progenitor cells (Winokurow and Shumacher 2019).

The primary cilium acts as a signaling center in the growth plate during bone development, directing the formation of cartilage and endochondral ossification. Therefore, aberrant epiphyseal alterations, such as stippling (calcified patches on bones, especially hips/knees), a typical radiological sign of Zellweger syndrome, might be caused by defective primary cilia. Epiphyseal stippling is unclear, despite the fact that skeletal problems are very frequently associated with SLO syndrome.

8 | Conclusion

One has to look beyond just proteins of the primary cilia to fully understand the ciliopathies. The ionic composition of the ciliary internal milieu and the lipid composition of the ciliary membrane are also very important. In this review, we have discussed two genetic disorders whose clinical manifestations are well explained by a cholesterol deficiency on the ciliary membrane. Therefore, it is our opinion that SLO and NPC1 are both ciliopathies. This viewpoint can aid in a thorough understanding of their complicated and multifaceted range of developmental anomalies and symptoms.

Author Contributions

R.P.E. and M.T.F. conceived the conceptual ideas and wrote the manuscript. M.T.F. prepared the figure; both authors prepared the tables.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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