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REVIEW

Nephronophthisis: a pathological and genetic perspective

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Abstract

Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease and is one of the most frequent genetic causes for kidney failure (KF) in children and adolescents. Over 20 genes cause NPHP and over 90 genes contribute to renal ciliopathies often involving multiple organs. About 15–20% of NPHP patients have additional extrarenal symptoms affecting other organs than the kidneys. The involvement of additional organ systems in syndromic forms of NPHP is explained by shared expression of most *NPHP* gene products in centrosomes and primary cilia, a sensory organelle present in most mammalian cells. This finding resulted in the classification of NPHP as a ciliopathy. If extrarenal symptoms are present in addition to NPHP, these disorders are defined as NPHP-related ciliopathies (NPHP-RC) and can involve the retina (e.g., with Senior-Løken syndrome), CNS (central nervous system) (e.g., with Joubert syndrome), liver (e.g., Boichis and Arima syndromes), or bone (e.g., Mainzer-Saldino and Sensenbrenner syndromes). This review focuses on the pathological findings and the recent genetic advances in NPHP and NPHP-RC. Different mechanisms and signaling pathways are involved in NPHP ranging from planar cell polarity, sonic hedgehog signaling (Shh), DNA damage response pathway, Hippo, mTOR, and cAMP signaling. A number of therapeutic interventions appear to be promising, ranging from vasopressin receptor 2 antagonists such as tolvaptan, cyclin-dependent kinase inhibitors such as roscovitine, Hh agonists such as purmorphamine, and mTOR inhibitors such as rapamycin.

Keywords Nephronophthisis · Inherited nephropathy · Ciliopathy · Joubert syndrome · Senior-Løken syndrome · Liver fibrosis · Skeletal abnormalities

Introduction

Nephronophthisis (NPHP) is an autosomal recessive, progressive tubulointerstitial kidney disease which results in kidney cyst development and kidney failure (KF). NPHP is one of the most frequent monogenetic causes for KF in children and adolescents [1, 2]. In 1951, Fanconi described this disease as "familial juvenile nephronophthisis" which relates to the Greek and means "disaggregation of the nephron" [3]. Over 1500 NPHP cases have been reported worldwide [4–6]. NPHP exhibits geographic variation with an incidence of 1 in 50,000 in Canada, 1 in 61,800 in Finland, and one in one million in the USA [7–10].

Historically, NPHP has been categorized based on the age of onset of KF in three clinically different forms of NPHP: infantile, juvenile, and adolescent NPHP. The most frequent and classical form of NPHP is the juvenile form, which has a mean age of 13 years for development of KF [11]. The infantile form is rare and is characterized by onset of KF before the age of 4 years. Adolescent NPHP results in KF at the median age of 19 years [12]. Although NPHP affects mostly children, NPHP is also diagnosed in adults [13]. The clinical symptoms of NPHP are subtle, may start several years before onset of KF, and include anemia and incapability to concentrate urine resulting in polyuria, secondary enuresis, growth retardation, and polydipsia [4]. Initially, imaging by kidney ultrasound shows normal kidney

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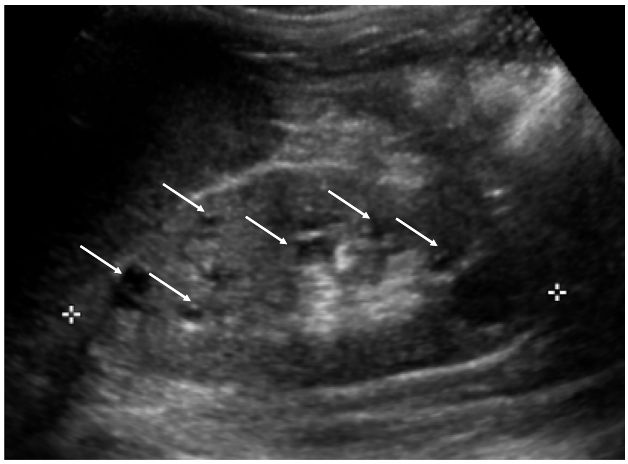


Fig. 1 Kidney ultrasound findings in a patient with nephronophthisis (NPHP). Multiple cysts (white arrows) along the cortico-medullary border; decreased cortico-medullary differentiation and increased echogenicity can be detected by kidney ultrasound

size, poor cortico-medullary differentiation, increased echogenicity, and in some patients cortico-medullary cysts. In patients with infantile NPHP, a kidney ultrasound may show enlarged kidneys. In the later stages of NPHP small, atrophic kidneys, increased echogenicity, and prominent cyst development are detected by kidney ultrasound [14] (Fig. 1).

In 15–20% of NPHP patients, additional extrarenal symptoms are found which include retinal degeneration (Senior-Løken syndrome [SLSN], 10–15%), cerebellar vermis aplasia (Joubert syndrome [JBTS]) (Fig. 2), liver fibrosis, *situs inversus,* oculomotor apraxia (OMA) (e.g., Cogan syndrome), and bone-related phenotypes (e.g., Mainzer-Saldino, Jeune, and Sensenbrenner syndromes) (Fig. 3).

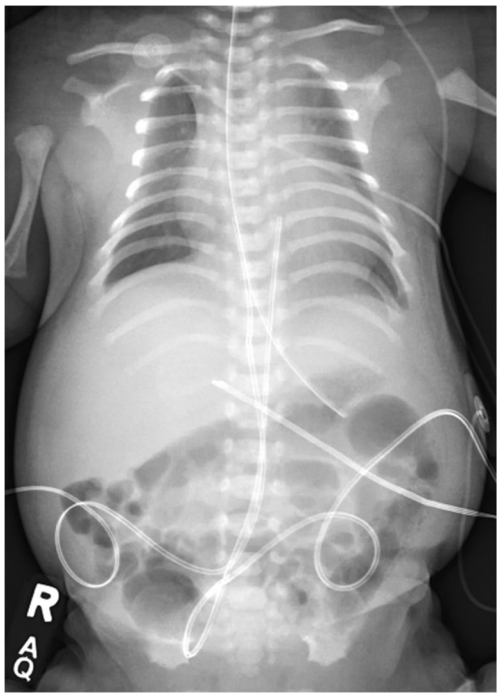


Fig. 3 This chest x-ray of a patient with Jeune syndrome shows the relatively small chest compared to the distended abdomen

NPHP has been described in association with a broad range of different syndromes (Table 1). An extreme form of the nephronophthisis-related ciliopathy (NPHP-RC) spectrum is Meckel-Gruber syndrome (MKS) which is characterized by occipital encephalocele, liver fibrosis, microphthalmia, and polydactyly and is often lethal [15].

Since the first description of a gene for NPHP 25 years ago, over 20 genes responsible for NPHP (*NPHP*) were

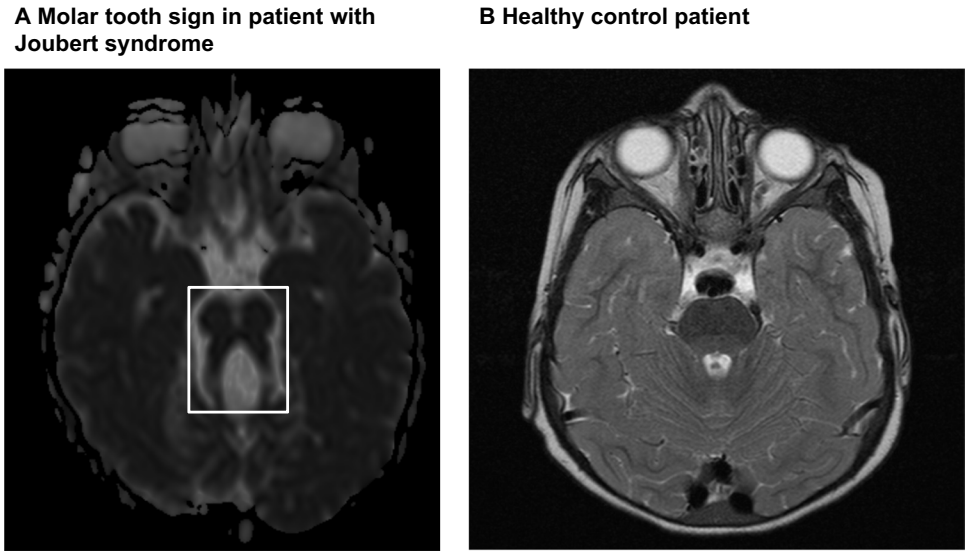


Fig. 2 The left image (**A**) shows a magnetic resonance imaging (MRI) of the brain in a patient with Joubert syndrome (JBTS). At the center of the image (white box), the classical neuroradiological sign of JBTS called the "molar tooth sign" (MTS) is shown. MTS is characterized by cerebellar vermis aplasia, thickened and elongated superior cerebellar peduncles, and a deepened interpeduncular fossa. The right image shows CNS imaging of a healthy control patient (**B**)

Table 1 Extrarenal manifestations associated with NPHP and resulting syndromes associated with *NPHP* pathogenic variants

	Syndrome
Ophthalmologic disorder	
Retinitis pigmentosa	Senior-Løken syndrome (SLSN)
	Arima syndrome (cerebro-oculo-hepato-renal syndrome)
	Alstrom (RP, obesity, DM type 2, hearing impairment)
	RHYNS (RP, hypopituitarism, skeletal dysplasia)
Oculomotor apraxia	Cogan syndrome
Nystagmus	Joubert syndrome/Joubert syndrome–related disorders
Coloboma	Joubert syndrome/Joubert syndrome–related disorders
Neurological disorder	
Encephalocele	Meckel-Gruber syndrome (occipital encephalocele, NPHP)
Vermis aplasia	Joubert syndrome/Joubert syndrome–related disorders
Hypopituitarism	RHYNS (RP, hypopituitarism, skeletal dysplasia)
Hepatic disorder	
Liver fibrosis	Boichis syndrome
	Meckel-Gruber syndrome (occipital encephalocele, NPHP)
	Arima syndrome (cerebro-oculo-hepato-renal syndrome)
	Joubert syndrome/Joubert syndrome–related disorders
Skeletal disorder	
Short ribs	Jeune syndrome/asphyxiating thoracic dystrophy
Cone-shaped epiphysis	Mainzer-Saldino syndrome
Postaxial polydactyly	Joubert syndrome/Joubert syndrome–related disorders
	Bardet-Biedl syndrome (NPHP, RP, obesity, deafness)
	Ellis-van Creveld
Skeletal dysplasia	Sensenbrenner syndrome/cranioectodermal dysplasia
	Ellis-van Creveld
Others	
Situs inversus	
Cardiac malformation	
Bronchiectasis	
Ulcerative colitis	

RP, retinitis pigmentosa/retinal degeneration; *DM*, diabetes mellitus; *NPHP*, nephronophthisis

identified (Table 2). The corresponding proteins are called nephrocystins and are mostly expressed in the centrosome and cilia, which represent antenna-like protrusions from cellular surfaces [1]. Pathogenic variants in these genes explain up to 50–70% of NPHP patients [2]. Currently, NPHP is most reliably diagnosed by genetic testing. Commercial testing includes the use of NPHP panels applying high-quality, clinical-grade next-generation sequencing (NGS) which detects deletions and duplications and analyzes the entire coding region and can be completed in 2 to 4 weeks. Prenatal findings for NPHP relate mostly to the infantile form of NPHP with oligohydramnios and bilaterally enlarged kidneys [16]. The identification of these genes has inspired novel concepts in NPHP and cystic kidney disease regarding novel signaling pathways, sensory cilia, centrosomes, planar cell polarity, and modular protein networks.

Kidney pathology features of nephronophthisis

The recurring morphologic theme among the different *NPHP* pathogenic variants is development of a chronic tubulointerstitial nephropathy (CTIN) with the potential for cyst formation [9, 17–22] (Table 3). At birth, the kidneys are grossly and microscopically normally developed. Although there is little morphologic information on the earliest stages of disease, on occasion, cortico-medullary cysts may be present [17] (Fig. 4). Kidney size in the juvenile and adolescent forms is usually normal or slightly decreased at presentation [9]. The histological hallmark of NPHP is a nonspecific CTIN with tubular atrophy, thick replicated tubular basement membranes, interstitial fibrosis, periglomerular fibrosis, and variably dense lymphocytic inflammation (Fig. 5A–D) [9]. Cysts develop in

Table 2 Summary of *NPHP1-NPHP21* and *NHPH1L* and *NPHP2L* genes, gene products, chromosomal localization, phenotypes, extrarenal symptoms, and interaction partners. Frequency of *NPHP* pathogenic variants is based on Halbritter et al. (2013) Hum Genet 132:865–884

Gene (protein)	Chromosome	Phenotype (mean age at KF)/frequency	Extrarenal symptoms	Interaction partners
NPHP1 (nephrocystin-1)	2q13	NPHP (13 years)/19.7%	RP (10%), OMA (2%), JBTS, and LF (rarely)	Inversin, nephrocystins-3–5, nephrocystin-8/RPGRIP1L, RPGR, polycystin-1, filamin A and B, tensin, PALS1/PATJ, Par6, β-tubulin, PTK2B, p130(Cas), Pyk2
NPHP2/INVS (inversin)	9q31	Infantile NPHP (<4 years)/1.1%	RP (10%), LF, *situs inversus*, CHD	Nephrocystin-1, 3, 5, 9, and 16, calmodulin, catenins, β-tubulin, APC2, RPGR
NPHP3 (nephrocystin-3)	3q22	Infantile and adolescent NPHP/1.6%	LF, RP (10%), situs inversus, MKS, CHD	Nephrocystin-1, 9, 16, Inversin
NPHP4 (nephrocystin-4)	1p36	NPHP (21 years)/3.2%	RP (10%), OMA, LF	Nephrocystin-1, 6, 8, 9, BCAR1, PALS1/PATJ, Par6, p130(Cas), Pyk2, PTK2B, Jade-1, Lats1, TAZ, α-tubulin, RPGR, RPGRIP1, TMEM107, 237
NPHP5/IQCB1 (nephrocystin-5)	3q21	NPHP (13 years)/2.9%	Early-onset RP, SLNS	Calmodulin, RPGR, nephrocystin-6
NPHP6/CEP290 (nephrocystin-6/CEP290)	12q21	NPHP/2.6%	JBTS, MKS, RP, LF, LCA	ATF4, nephrocystin-5, Rab8a, Rkip, MKKS, FAM161A, RPGR, CC2D2A, Tectin1
NPHP7/GLIS2 (nephrocystin-7/GLIS2)	16p	NPHP/0.1%	_	TRIM32, p120 catenin, β catenin CtBP1, HDAC3
NPHP8/RPGRIP1L (nephrocystin-8/RPGRIP1L)	16q	NPHP/0.5%	JBTS, MKS, LCA	Nephrocystin-1, 4, 6, RPGR, CSPP, Nek4, Pmsd2
NPHP9/NEK8 (nephrocystin-9/NEK8)	17q11	Infantile NPHP/0.1%	LF, CHD	Nephrocystin-1, 3, 4, 16, Inversin, TAZ, Anks3
NPHP10/SDCCAG8 (nephrocystin-10/SDCCAG8)	1q43	Juvenile NPHP/0.6%	RP (SLS), BBS-like	OFD1, FAM161A, AZI1, RABEP2
NPHP11/TMEM67/MKS3 (nephrocystin-11/meckelin)	8q22.1	NPHP/2.6%	JBTS, MKS, LF	MKS1, nephrocystin-1, 4, 6, nesprin-2, TMEM216, filamin A
NPHP12/TTC21B/ /JBTS11 (nephrocystin-12/IFT139)	2q24.3	Early onset NPHP, juvenile NPHP/0.7%	JATD, MKS, JBTS, BBS-like	IFT121, NPHP13/IFT144, ciliopathy modifier
NPHP13/WDR19 (nephrocystin-13/IFT144)	4p14	NPHP/0.5%	JATD, SBS, CED, RP, Caroli, BBS-like	Presumed IFT139 and IFT140 interaction
NPHP14/ZNF423 (nephrocystin-14/ZNF423)	16q12.1	Infantile NPHP, PKD	JBTS, *situs inversus*	PARP1, nephrocystin-6, ZNF521
NPHP15/CEP164 (nephrocystin-15 centrosomal protein 164 kDa)	11q23.3	NPHP (8 years)	RP, JBTS, LF, obesity	Nephrocystin-3, 4, 18, ATRIP, CCDC92, TTBK2, Dvl3, INPP5, Chibby
NPHP16/ANKS6 (nephrocystin-16/ANKS6)	9q22.33	Infantile and juvenile NPHP	LF, *situs inversus*, CHD cardiovascular abnormalities	INVS, nephrocystin-3, 9, HIF1AN, BICC1, ANKS3
NPHP17/IFT172 (nephrocystin-17/IFT172)	2p23.3	NPHP	JATD, MZSDS, JBTS	IFT140, IFT38, IFT57, IFT80, MKS1
NPHP18/CEP83 (nephrocystin-18/centrosomal protein 83 kDa)	12q22	Early-onset NPHP (3 years)	Learning disability, hydrocephalus, LF	Nephrocystin-15, IFT20
NPHP19/DCDC2 (nephrocystin-19/doublecortin domain-containing 2)	6p22	NPHP	LF	Dishevelled-3

Table 2 (continued)

Gene (protein)	Chromosome	Phenotype (mean age at KF)/frequency	Extrarenal symptoms	Interaction partners
NPHP20/MAPKBP1 (nephrocystin-20/ mitogen activated protein kinase binding protein 1)	15q1	NPHP		JNK2, WDR62
NPHP21/ADAMTS9 (nephrocystin-21/a disintegrin and metalloproteinase with thrombospondin type 1 motif)	3p14.1	NPHP	JBTS, CHD, deafness, coloboma, short stature, hepatosplenomegaly	
NPHP1L/XPNPEP3 (nephrocystin-1L/ XPNPEP3)	22q13	NPHP	Cardiomyopathy, seizures	Cleaves LRRC50, ALMS1, nephrocystin-6
NPHP2L/SLC41A1 (nephrocystin-2L/ SLC41A1)	1q32.1	NPHP	Bronchiectasis	

ATF4, activating transcription factor 4; *APC2*, anaphase-promoting complex 2; *BCAR1*, breast cancer anti-estrogen resistance 1; *CAD*, cranioectodermal dysplasia; *CC2D2A*, coiled-coil and C2 domain-containing 2A; *CHD*, congenital heart disease; *JATD*, Jeune asphyxiating thoracic dysplasia; *JBTS*, Joubert syndrome; *LCA*, Leber congenital amaurosis; *LF*, liver fibrosis; *MKS*, Meckel-Gruber syndrome; *OMA*, oculomotor apraxia; *PTK2B*, protein tyrosine kinase 2B; *RP*, retinitis pigmentosa; *RPGR*, retinitis pigmentosa GTPase regulator; *SBS*, Sensenbrenner syndrome

approximately 70% of patients by the final stage of kidney disease but are not required for diagnosis (Fig. 6A–D) [20]. Cysts develop initially as microcysts with tubular ectasia and diverticular outpouchings that affect distal tubules and collecting ducts (Fig. 7) [21]. With disease progression, secondary glomerulosclerosis supervenes, and cysts may become more frequent and macroscopic at the cortico-medullary junction or may replace the entire kidney medulla (Fig. 6B–D).

Few kidney genotypic-histologic phenotypic correlations exist for NPHP. Most NPHP pathogenic variants (e.g., *NPHP1*, *NPHP 3–13*, *NPHP 15*, *NPHP 17*, *NPHP19-21*, *NPHP1L*, and *NPHP2L*) result in the juvenile and adolescent forms, and kidney biopsy shows the aforementioned CTIN [19]. However, kidney biopsies in patients with *NPHP1* pathogenic variants have 3 distinctive features—floret-shaped tubules, tubular diverticuli, and macula-densa-like epithelium [22] (Fig. 8A and B). Multiple other pathogenic variants are responsible for the infantile form of NPHP that include *NPHP1*, *NPHP3*, *NPHP6/RPGRIP1L*, *NPHP9/NEK8*, *NPHP12/TTC21B*, *NPHP14/ZNF423*, and *NPHP18/CEP83* genes. The infantile form of NPHP is notable for kidney enlargement due to more widespread cyst development affecting both cortex and medulla, clinically suggestive of a polycystic kidney disease [18]. The cortical cysts may involve any nephron segment, including glomeruli (Fig. 9). Furthermore, in the infantile form, interstitial inflammation is less conspicuous, and tubular basement membranes are usually thin, lacking the irregular basement membrane multi-layering of the juvenile and adolescent forms.

NPHP-related phenotypes and their genotype–phenotype correlation

The clinical spectrum of NPHP-RC can present with a wide range of symptoms. The gene products involved in NPHP-RC, called nephrocystins, are almost all expressed in centrosomes and primary kidney cilia with the exception of the *NPHP20/MAPKBP1*, *NPHP1L/XPNPEP3*, and *NPHP2L/SLC41A1* gene products (Fig. 10A) [23–25]. Most of the nephrocystins show an overlapping expression pattern with other gene products responsible for other cystic kidney diseases, e.g., ADPKD, ARPKD, JBTS, SLSN, and Bardet-Biedl syndrome (BBS) [19, 26]. Therefore, these disorders are categorized as ciliopathies [19, 27]. NPHP can occur isolated or in association with additional extrarenal phenotypes. These multisystem characteristics are due to the fact that NPHP-RC is a ciliopathy and that nephrocystins are expressed in cilia/centrosomes of multiple tissues. The pleiotropy seen in NPHP-RC is explained by the finding that almost every

Table 3 Pathology characteristics of the different forms of NPHP

Pathology characteristics of juvenile and adolescent NPHP
Initially normal kidney size, later smaller kidneys
Non-specific signs of chronic tubulointerstitial nephritis (e.g., with tubulointerstitial fibrosis, tubular atrophy), thickened (multi-layering) or thinned tubular basement membranes, and inflammatory cell infiltrates
Potential cyst formation (in 70% of patients), tubules develop diverticula or tubular ectasia (not true cysts but rather tubular dilatation) in the collecting duct or distal tubules (often along the cortico-medullary border in a radial distribution)
Cysts can attain considerable size from one to several centimeters
Periglomerular fibrosis, collapse of glomeruli, glomerulosclerosis
Vascular fibrointimal thickening of arteries and medial hypertrophy
Per pathology criteria no differentiation possible between juvenile and adolescent forms of NPHP
Pathology characteristics of infantile NPHP
Infantile forms of NPHP result often in larger kidney size
Tubular basement membrane is usually thin, lacks multi-layering of juvenile and adolescent forms
As in juvenile and adolescent forms dilatations/cysts of collecting ducts and distal tubules and tubular atrophy
Glomeruli may develop microcysts, Bowman capsule can be 2–3 × enlarged, normal glomerular tuft
Less interstitial inflammation compared to juvenile or adolescent forms

cell is ciliated [26]. Therefore, NPHP-RC can involve the retina, CNS, liver, and bones. Tissue can be affected either by dysplasia during the prenatal period or organ degeneration in the postnatal period. Conditions that occur together with NPHP are outlined below.

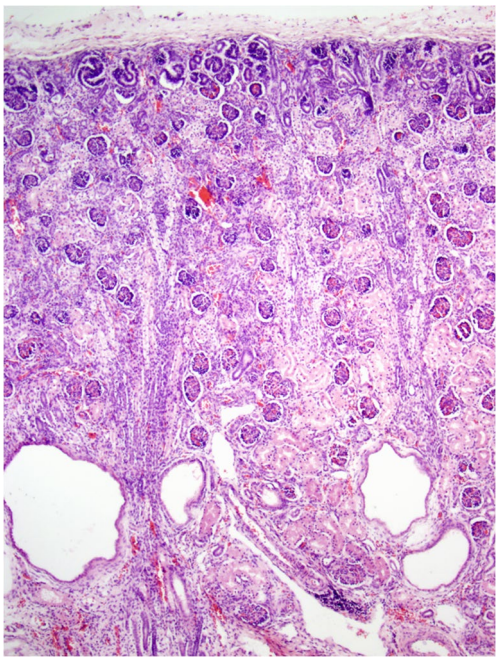


Fig. 4 This fetal kidney contains a row of cysts at the cortico-medullary junction. A sibling had developed juvenile NPHP. The cortex shows normal development with a thin subcapsular nephrogenic zone typical of an incompletely formed kidney. At this early stage of disease, there is no sign of a chronic tubulointerstitial nephropathy

Senior-Løken syndrome (SLSN)

The disease association of NPHP and RP is named Senior-Løken syndrome (SLSN) (OMIM#2669,000) [28]. Approximately 10–15% of NPHP patients have retinitis pigmentosa (RP), which is a form of retinal degeneration, and can cause early and significant visual impairment [28] (Fig. 11A). Early onset of RP is reminiscent of Leber congenital amaurosis (LCA). Late onset of RP results in night blindness and progressive visual loss. Fundoscopy and electroretinography help to diagnose RP. While the etiology of RP is not entirely resolved, there are indications that RP also may be caused by a ciliary defect. The photoreceptor consists of the rod outer (ROS) and rod inner segments (RIS) which are linked by the connecting cilium (Fig. 11B). Specific nephrocystins are expressed in the connecting cilium, which if impaired possibly interferes with the transport of photo-transducing substances (e.g., rhodopsin) [26, 29]. Dependent on the *NPHP* pathogenic variant, RP occurs between 6 and 100% in frequency (6% with *NPHP1*, 10% with *NPHP2/INV*, 90% with *NPHP10/SDCCAG8,* 100% with *NPHP5* and *NPHP6* pathogenic variants, respectively). Pathogenic variants in *NPHP13/WDR19* and *NPHP15/CEP164* also cause RP [30, 31]. Several gene products, which cause NPHP (*NPHP1*, *NPHP6/CEP290*, *NPHP8/RPGRIP1L*, *NPHP10/SDCCAG8*) and NPHP-related ciliopathies (*AH1* in JBTS), are crucial in photoreceptor development [32, 33].

Joubert syndrome (JBTS)

Joubert syndrome (JBTS) (OMIM%213300) is characterized by cerebellar malformations such as mid-hindbrain

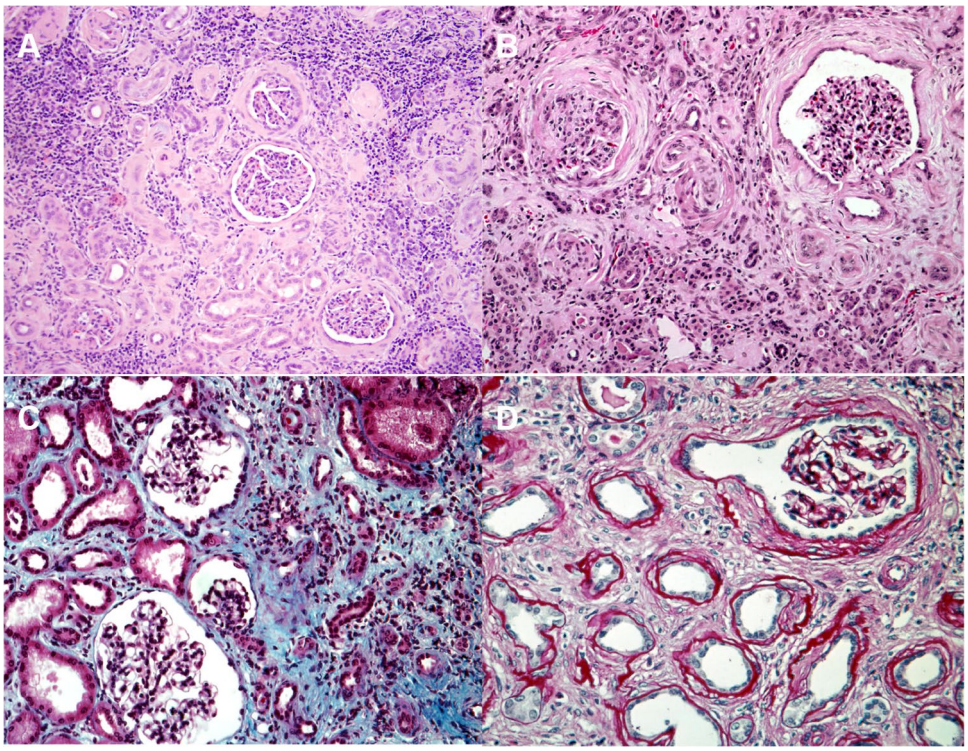


Fig. 5 **A** This biopsy shows features typical of a nonspecific chronic tubulointerstitial nephropathy. There is diffuse tubulointerstitial disease with relative glomerular preservation. The glomeruli appear largely intact within a field of atrophic tubules and chronic inflammation. **B** The glomeruli in this biopsy show periglomerular fibrosis and early glomerulosclerosis in the left glomerulus. The background shows chronic tubulointerstitial disease. **C** This biopsy shows three intact glomeruli with extensive tubular atrophy and interstitial fibrosis highlighted by the trichrome stain. **D** This biopsy shows periglomerular fibrosis and prominent tubular basement membrane replication. Although tubular basement membrane replication is a common finding in NPHP, it is present in a variety of chronic kidney diseases and lacks diagnostic utility

malformation and cerebellar vermis hypoplasia (CVH) (diagnosed as "molar tooth sign" by brain imaging) (Fig. 2A), developmental delay, mental retardation, cerebellar ataxia, hypotonia, oculomotor apraxia, nystagmus, and neonatal tachypnea [34]. Other possible symptoms related to JBTS are liver fibrosis, ocular coloboma, and polydactyly [34]. A synonym for JBTS is cerebello-oculo-renal syndrome (CORS). JBTS is caused by pathogenic variants in *NPHP6/CEP290*, *NPHP8/RPGRIP1L*, *NPHP11/TMEM67*, *NPHP14/ZNF423*, *NPHP15/CEP164*, *NPHP17/IFT172*, and *NPHP21/ADAMTS9* [31, 35–40]. Patients with cerebellar vermis hypoplasia, oligophrenia, ataxia, coloboma, and hepatic fibrosis (COACH syndrome), a JBTS-related condition with liver involvement, have mostly pathogenic variants in *NPHP11/TMEM67* and less frequent pathogenic variants of *NPHP8/RPGRIP1L* and *CC2DA2* [41]. Pathogenic variants in *NPHP6/CEP290*, *NPHP11/TMEM67*, and *AHI1* genes are the most common causes for kidney involvement in JBTS patients [42]. Overall, more than 20 genes have been published to cause JBTS, and about one-third of them can also cause NPHP [34]. A good indicator for development of NPHP in JBTS patients is the development of impaired urinary concentration [43].

Meckel-Gruber syndrome (MKS)

Meckel-Gruber syndrome (MKS) (OMIM#249000) is an autosomal recessive disorder characterized by renal cystic dysplasia, occipital encephalocele, microphthalmia, polydactyly, *situs inversus*, bile duct proliferation, and pulmonary hypoplasia. Usually, MKS is perinatally lethal. MKS is an example of allelism with two truncating pathogenic variants (due to nonsense, frameshift or splice-site pathogenic variants) in *MKS1*, *NPHP3*, *NPHP6/CEP290*, *NPHP8/RPGRIP1L*, and *NPHP11/TMEM67/MKS3* causing the severe MKS phenotype; if a patient has at least one missense pathogenic variant, usually, the milder phenotype of JBTS or SLSN will develop [15, 36, 44–46]. MKS is characterized by developmental

defects, whereas NPHP and SLSN are thought to display degenerative defects of the kidney and retina. The transition zone (TZ) of cilia appears to be crucial for the pathogenesis of MKS [47]. The TZ of cilia is a specific region at the base of all cilia characterized by a Y-shaped assemblage that links axoneme microtubules to the surrounding membrane [48].

Oculomotor apraxia type Cogan (OMA)

Oculomotor apraxia (OMA) type Cogan is characterized by abnormalities in the horizontal gaze. Affected individuals have nystagmus and have to move their head by jerky movements in order to track objects. OMA is relatively rare in patients with NPHP and is mostly found in patients with *NPHP1* and *NPHP4* pathogenic variants [26].

Liver fibrosis

Periportal liver fibrosis has become a more prominent characteristic of the NPHP-RC spectrum. Several case reports confirmed liver fibrosis with *NPHP3* pathogenic variants as part of renal-hepatic-pancreatic dysplasia and also MKS [46, 49]. Patients with *NPHP9/NEK8* pathogenic variants can present with early onset of cholestasis, paucity of bile ducts, cystic-dysplastic liver changes, and hepatic fibrosis [50, 51]. *NPHP11/MKS3/TMEM67* pathogenic variants are also a frequent cause of NPHP-RC with liver fibrosis [52]. Liver involvement has also been identified in a few NPHP patients with *NPHP15/CEP164*, *NPHP16/ANKS6*, *NPHP18/CEP83*, *NPHP19/DCDC2*, and *NPHP21/ADAMTS9* pathogenic variants [31, 39, 53–55].

Skeletal defects

Most of the gene products involved in skeletal symptoms and NPHP are members of the intraflagellar transport (IFT) complex within cilia (Fig. 10A and B). Because there is no protein synthesis inside cilia, they require import of proteins from the cytosol and intra-ciliary protein transport. Imported proteins are transported along microtubules into the cilium by anterograde IFT and

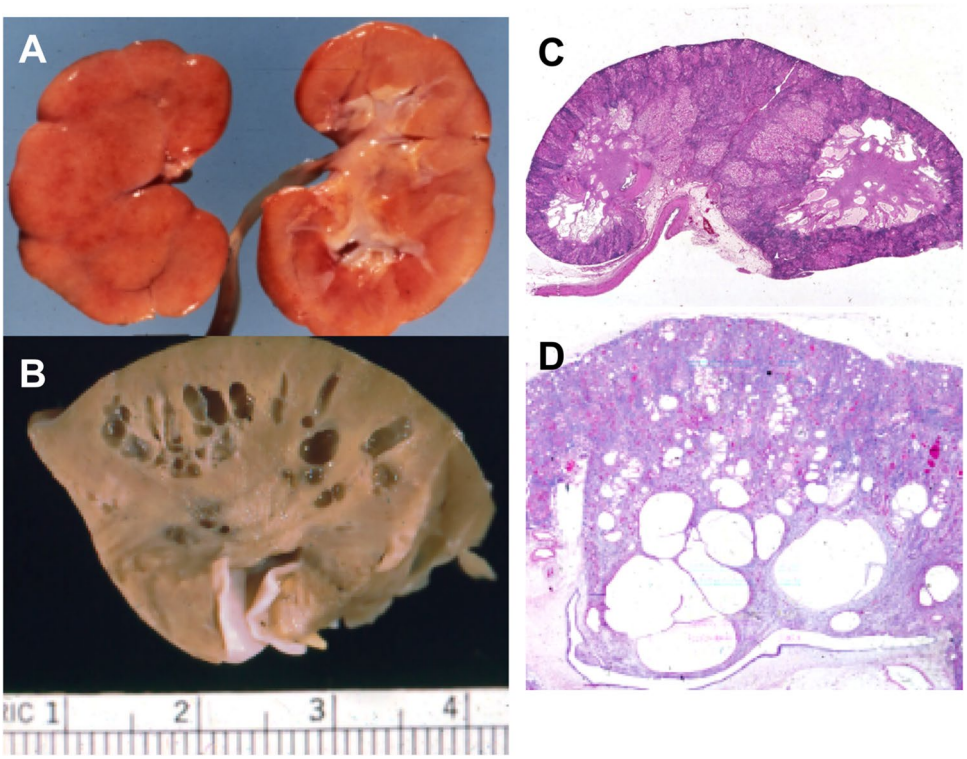


Fig. 6 **A** This autopsy kidney shows end-stage NPHP. There is advanced nephrosclerosis resulting in cortical thinning. The corticomedullary junction is indistinct due to interstitial fibrosis. No medullary cyst formation is present (courtesy of the Jay Bernstein, MD Consultative Collection). **B** This autopsy kidney also shows end-stage NPHP. In contrast to **A**, it shows numerous medullary cysts clustered along the cortico-medullary junction or replacing the entire renal pyramid (left side) (courtesy of the Jay Bernstein, MD Consultative Collection). **C** This autopsy whole mount section is from a patient with renal-retinal dysplasia. It shows numerous cysts that replace the upper and lower pole pyramids, while the mid-polar pyramids are free of cyst formation (courtesy of the Jay Bernstein, MD Consultative Collection). **D** This autopsy whole mount section shows numerous large medullary cysts and smaller cortical cysts. The cortical cysts are somewhat zonal reflecting medullary ray location (courtesy of the Jay Bernstein, MD Consultative Collection)

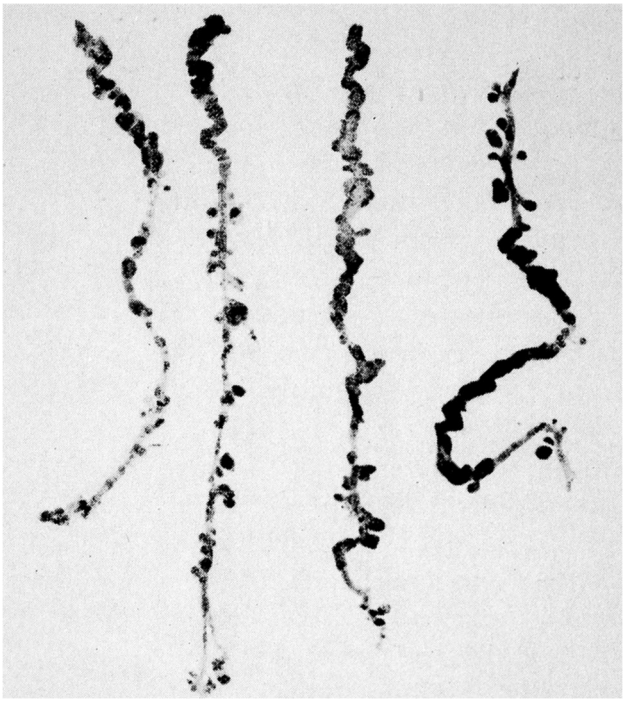


Fig. 7 Four descending limbs of Henle isolated by microdissection are altered by multiple diverticuli which vary in size and configuration. These cystic tubular enlargements were confined to collecting ducts and distal tubules and were not true cysts, but rather tubular dilations (with permission from Sherman FR, Studnicki FM, Fetterman GH (1971) Renal lesions of familial juvenile nephronophthisis examined by microdissection. Am J Clin Pathol 55:391–400)

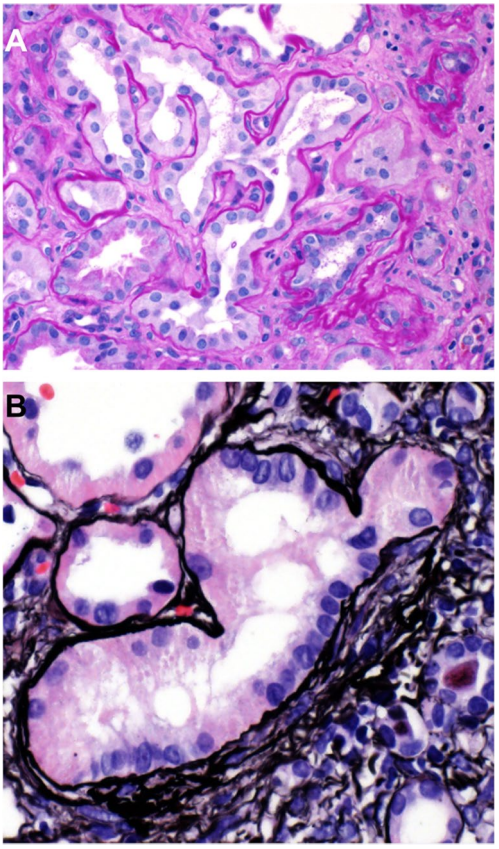


Fig. 8 **A** This biopsy from a patient with *NPHP1* deletion shows a floret-shaped tubule characterized by a complex branched architecture (periodic acid-Schiff stain). **B** This biopsy from a patient with *NPHP1* deletion shows a macula densa-like compact row of nuclei (right side), and a diverticulum (upper right) in which there is tubular cell outpouching with an attenuated tubular basement membrane (Jones methenamine silver stain)

exported by the retrograde IFT—similar to a conveyer belt (Fig. 10B) [1, 27]. Cone-shaped epiphyses of the phalanges (Mainzer-Saldino syndrome, OMIM#266920) represent the most common skeletal manifestation with NPHP [56]. Genetic variations in different genes contribute to Mainzer-Saldino syndrome (Fig. 10B) (Table 4). Other disorders affecting the skeletal system associated with NPHP are Jeune syndrome (asphyxiating thoracic dysplasia, OMIM #208500), which features short limbs and rib cage narrowing contributing to respiratory distress (Fig. 3) [57]. Ellis-van Creveld syndrome is characterized by short stature, short extremities, and polydactyly [58]. Cranioectodermal dysplasia (CED), which is also named Sensenbrenner syndrome (OMIM #218330), is characterized by rib cage narrowing, polydactyly, brachydactyly, dolichocephaly, pectus excavatum, and ectodermal involvement with delayed tooth eruption, skin laxity, and sparse hair. For more details, please see the section on nephrocystin-13 and nephrocystin-17 below. For a list of other members of the IFT causing skeletal defects, please see Table 4 [57–61].

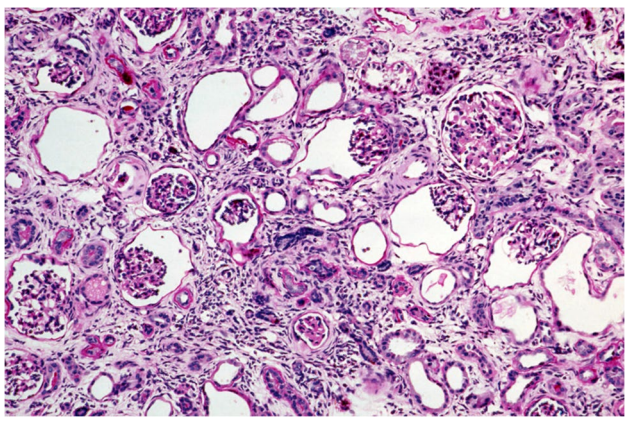
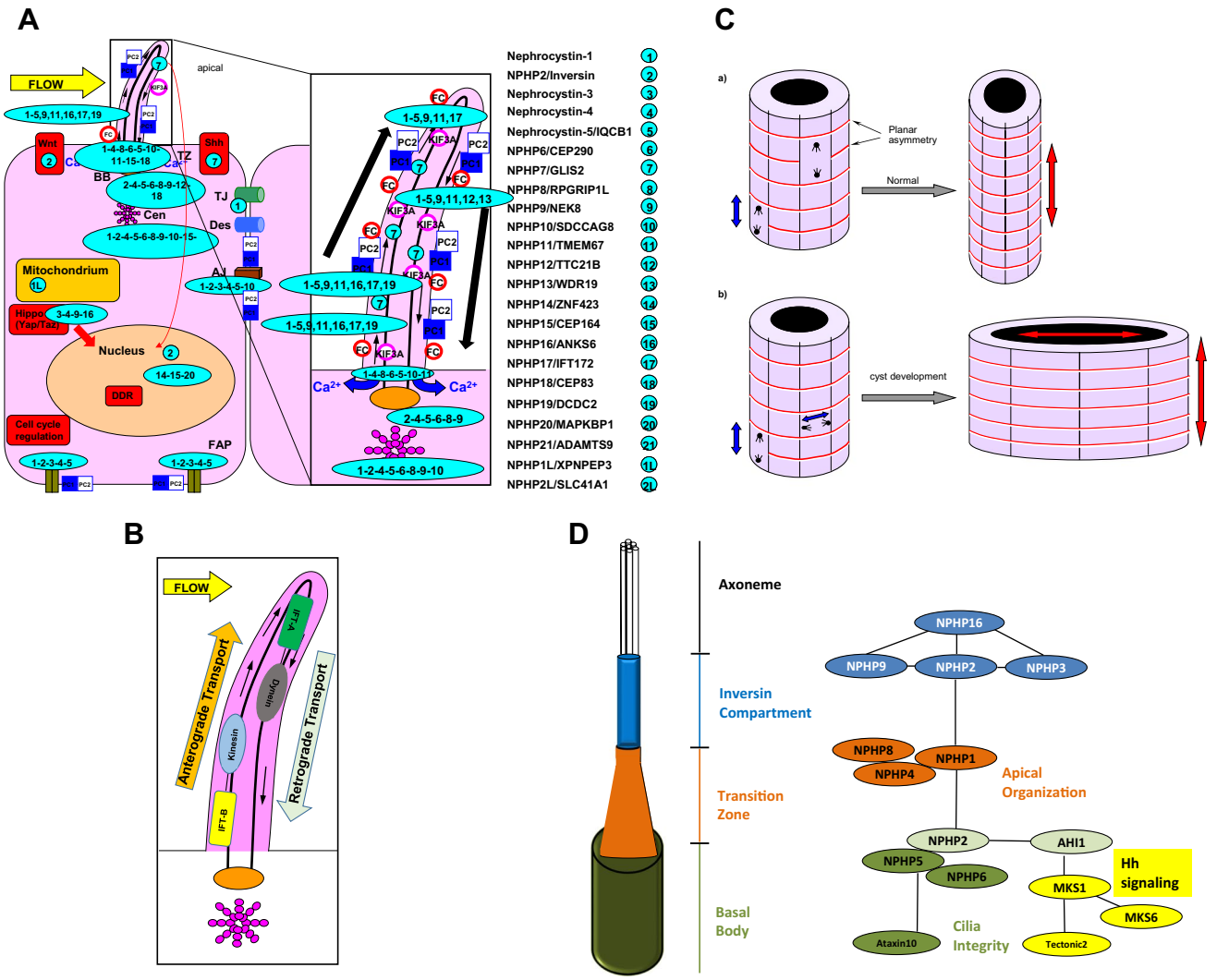


Fig. 9 This biopsy from a patient with infantile NPHP shows several glomerular microcysts with ectatic Bowman's capsules with a background of chronic tubulointerstitial nephropathy. The tubular basement membranes are thin, lacking the thick replication seen in the juvenile and adolescent forms of NPHP (courtesy of the Jay Bernstein, MD Consultative Collection)



Cardiac defects

Ventricular septal defects were published in association with infantile NPHP and pathogenic variants in *NPHP2/inversin* and *NPHP3* [46, 62]. Pathogenic variants in *NPHP9/NEK8* result in severe congenital heart defects including truncus arteriosus, unseptated atrium and ventricle, biventricular hypertrophy, aortic valve stenosis, and mitral and pulmonary valve stenosis [50, 51]. *NEK8* was found to be an interaction partner of the *NPHP16/ANKS6* gene product, and *NPHP16/ANKS6* truncating pathogenic variants also result in cardiac phenotypes such as hypertrophic obstructive cardiomyopathy, aortic stenosis, and pulmonary stenosis [53, 63]. Pathogenic variants in the *NPHP1L/XPNPEP3* gene resulted in hypertrophic cardiomyopathy and hypertension [23]. Vascular defects such as aneurysms were found with *NPHP19/DCDC2* pathogenic variants [55].

Genotype–phenotype correlation/oligogenicity in NPHP-RC

The genotype–phenotype correlation in NPHP-RC is influenced by gene locus heterogeneity, allelism, and modifier genes [27]. Gene locus heterogeneity refers to the existence of different genes, which, if mutated, can result in phenotype variability. Allelism is of detrimental importance for NPHP and was found for several *NPHP* genes where presentation of two null (e.g., nonsense or protein truncating) pathogenic variants causes more severe phenotypes with earlier onset, dysplastic, and multiorgan involvement compared to missense pathogenic variants which result in milder phenotypes with later onset, degenerative phenotypes and restricted organ involvement [4, 26]. Two pathogenic variants (either compound heterozygous or homozygous) in any of the over 20 *NPHP* genes are sufficient to cause NPHP. In some NPHP patients, a third affected allele was identified, which may contribute to oligogenicity and modulation of the phenotype in an epistatic way [64].

◂**Fig. 10** Different pathomechanism models for nephronophthisis. **A** Subcellular localization of the nephrocystins. Nephrocystins are located in primary cilia, basal bodies, the mitotic spindle, focal adhesions, and adherens junctions. Most nephrocystins are detected in the primary cilium (see enlarged box), the basal body (BB), and centrosomes (Cen) in a cell cycle–dependent manner. Nephrocystin-1 is expressed in the transition zone (TZ), focal adhesion plaques (FAP), adherens junctions (AJ), and tight junctions (TJ). The anterograde and retrograde transport along the microtubule transport is shown by arrows in the cilium. The intraflagellar transport is mediated by kinesin-2, a heterotrimeric protein that is composed of two motor units (Kif3a and Kif3b) and one nonmotor unit (KAP3). Sensory cilia transfer external stimuli. Wnt and hedgehog (Shh) signaling interfere with planar cell polarity by orientation of centrosomes and mitotic spindles. Other ciliary expressed proteins are polycystin-1 (PC1), polycystin-2 (PC2), and fibrocystin (FC). Adapted with permission from Watnick T, Germino G (2003) From Cilia to Cyst. Nat Genet 34:355–356. **B** Intraflagellar transport (IFT) moves proteins within the cilium required for ciliogenesis and signaling. Anterograde (base-to-tip) transport is provided by the IFT-B complex and functions together with the kinesin-2 motor. Retrograde (tip-to-base) transport is performed by the IFT-A complex which is regulated by the dynein-2 motor. The six gene products resulting in CED when mutated are involved in the retrograde transport. Figure is adapted from Tan W, Lin A, Keppler-Noreuil K (1993) Cranioectodermal Dysplasia. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A (eds) GeneReviews(®). University of Washington, Seattle. **C** Altered planar cell polarity. During development and maintenance of the tubular architecture, correct orientation of the mitotic spindle and centrosomes of renal tubular epithelial cells is important for proper growth of the longitudinal axis of the tubule (a). If the apical-basolateral polarity is disrupted, a dilated tubule or cyst may develop (b). Non-canonical Wnt signaling is involved in proper cell orientation. Urinary flow in the renal tubules may provide signaling via cilia about cellular orientation. Adapted from Germino G (2005) Linking Cilia to Wnts. Nat Genet 37:455–457. **D** Nephrocystin protein network. Summary of the three different NPHP-JBTS-MKS protein modules. Ciliary location of protein modules, involved proteins, and the function of the module are shown. The nephrocystin-1–4-8 module (orange) is strongly expressed in the TZ (orange) and accumulates at cell–cell contacts. This module may be involved in organization of apical structures. The protein module consisting of nephrocystin-5–6 (green) is strongly expressed in the basal body (green) and is critical for ciliogenesis. The third protein module contains MKS1-6/CC2D2A and Tectonic2 (yellow) and is involved in Hh signaling. All three modules are bridged by nephrocystin-2, nephrocystin-3, and nephrocystin-9, which are expressed along the entire cilium. Ellipse, protein; black line, interaction; touching ellipses, direct interactions validated by in vitro binding. Adapted from Sang L, Miller JJ, Corbit KC et al. (2011) Mapping the NPHP-JBTS-MKS protein network reveals ciliopathy disease genes and pathways. Cell 145:513–528 [87]

Molecular mechanisms of NPHP

The "ciliary hypothesis": ciliary expression of nephrocystins may explain the pleiotropy found in NPHP

The localization of nephrocystin-1 at adherens junctions and focal adhesions led to the first hypothesis that *NPHP1* pathogenic variants result in defective cell–cell and cell–matrix signaling, called the "adherens junction/focal adhesion hypothesis" [1]. Later, ciliary expression of nephrocystin-1 was detected in kidney and respiratory epithelial cells and the connecting cilium of the photoreceptor [62]. Subsequently, the "adherens junction/focal adhesion hypothesis" was connected to the "cilia hypothesis" by the finding that nephrocystin-4, an interaction partner of nephrocystin-1, colocalizes with β-catenin at cell–cell contact sites, but is also expressed in primary cilia and centrosomes of dividing cells [65]. The vast majority of genes, which, if mutated, result in cystic kidney disease, are expressed in the primary cilium, centrosomes, basal bodies, or the mitotic spindle in a cell cycle–dependent fashion, thus leading to the term "ciliopathy" [1, 27]. The primary cilium can be found in almost every cell and projects antenna-like into the lumen from the cellular surface (Fig. 10A). Primary cilia contain an axoneme, which consists of 9 + 0 microtubular doublets, in contrast to 9 + 2 microtubular doublets in motile cilia [1, 27]. Inside the cilium, IFT provides transport of proteins (Fig. 10B) (Table 4). Ciliary function is very diverse including photosensation; mechanosensation due to urinary flow; and osmotic, olfactory, and temperature sensation [1, 27]. At the root of the cilium, the basal body is located, which derives from the mother centriole and is required for cilia assembly. The transition zone (TZ) is localized between the basal body and the ciliary axoneme and is important for the pathogenesis of NPHP. Several nephrocystins can be found in multi-protein complexes at the TZ. The TZ is important for basal body anchoring and establishing a ciliary gate during ciliogenesis [66]. Protein entry into and exit from the primary cilium is controlled by this gate. The hedgehog signaling molecule Smoothened clusters in the TZ and pathogenic variants in *RPGRIP1L* disrupt this accumulation and signaling [67]. This points to the TZ as a crucial gatekeeper which, if dysfunctional, results in NPHP.

Ciliary function in NPHP is still incompletely understood. One hypothesis is that primary cilia may sense tubular flow of urine [68]. Extrarenal symptoms associated with NPHP can also be better understood with the ciliary hypothesis. The photoreceptor contains the connecting cilium, which is responsible for rhodopsin transport (Fig. 11B) [1, 27]. Pathogenic variants in *NPHP5/IQCB1*, *NPHP6/CEP290*, and *NPHP10/SDCCAG8* cause retinitis pigmentosa and the encoded proteins are all localized in the connecting cilium of the photoreceptor [33, 35, 69]. In addition, the CNS and the hepatic cholangiocytes also displayed ciliary expression of nephrocystins, which could explain the association of NPHP with JSTB and liver fibrosis, respectively [49, 52]. Finally, a ciliary defect was also found in a small number of patients with Jeune syndrome with pathogenic variants in either *IFT80* or *TTC21B*, which both encode proteins participating in IFT and skeletal phenotypes [57, 70]. The identification

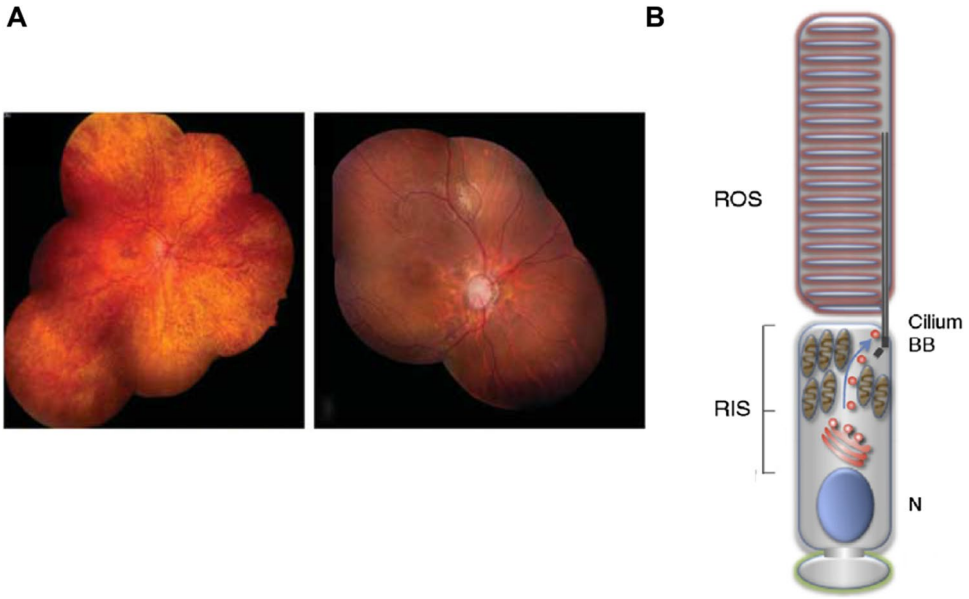


Fig. 11 Retinitis pigmentosa can be found in 10–15% of all NPHP patients. **A** The left image shows funduscopy findings of a patient with SLS revealing retinitis pigmentosa, and the right image shows diffuse retinal atrophy in a patient with NPHP. Images are taken from [4]. **B** Model of rod photoreceptor. This is the structural equivalent to the primary cilia in renal epithelial cells. Photo-transducing substances such as rhodopsin are synthesized in the RIS and are sequestered in the light-sensing membranes of the ROS. Image is taken from Wang J, Deretic D (2014) Molecular complexes that direct rhodopsin transport to primary cilia. Prog Retin Eye Res 38:1–19. RIS, rod inner segment; ROS, rod outer segment; N, nucleus; BB, basal body

of inversin in NPHP2 has linked ciliary expression with Wnt signaling and planar cell polarity [71].

Planar cell polarity

The term planar cell polarity (PCP) refers to the cellular orientation in a plane perpendicular to apico-basal polarity. Correct orientation of the mitotic spindle and centrosomes is required for correct PCP [72]. PCP is necessary for maintenance of normal tubular development, morphology, and recovery after cellular injury [72]. In case of abnormal PCP, the tubules do not extend longitudinally but at an angle to the longitudinal axis, which results in tubular dilatation and subsequently in a cystic structure [72] (Fig. 10C). Several gene products causing cystic kidney disease were shown to modify the Wnt signaling pathway, which is linked to PCP and the cilium [55, 71]. The Wnt signaling pathway consists of the canonical pathway, dependent on β-catenin, and the non-canonical pathway (which contributes to PCP) [71]. Nephrocystin-2/inversin appears to be the switch that determines which Wnt pathway is activated [71]. Nephrocystin-2/inversin, nephrocystin-3, nephrocystin-4, nephrocystin-7/GLIS2, nephrocystin-15/CEP164, and nephrocystin-19/DCDC2 interfere with Wnt signaling [31, 55, 71, 73, 74]. A recent review has focused on the relationship between Wnt signaling and cystic kidney disease [75].

Sonic hedgehog signaling (Shh)

Involvement of nephrocystins with Hh signaling was first noticed with the discovery of *NPHP7/Glis2* [76]. Shh is closely related to the primary cilium, and dysregulation of Shh signaling results in developmental defects and different cancers [77]. The secreted ligand Shh binds to the receptor Patched (Ptch1) which is localized at the primary cilium. This induces internalization of Ptch1 and permits translocation of Smoothened (Smo), a G-protein-coupled receptor into the primary cilium with its subsequent activation [78]. When Smo accumulates and is activated in the primary cilium, it converts Gli repressor (Gli3r) forms into Gli activator (Gli3a) forms, which activate expression of target genes.

DNA damage response pathway

The DNA damage response (DDR) signaling pathway enables cells to respond to DNA damage by arresting the cell cycle and promoting DNA repair. This pathway safeguards cells that DNA repair is complete before the cells move through the S phase of mitosis. Several of the *NPHP* gene products have been linked to this pathway including CEP290, NEK8, SDCCAG8, ZNF423, CEP164, and MAPKBP1, implying additional, non-ciliary function in the cellular nucleus [25, 31, 79–81]. It appears that during phases of high proliferation

Table 4 Summary of affected members of the IFT in ciliary skeletopathies including anterograde transport (complex B) and retrograde transport (complex A). Affected protein is shown, followed by corresponding gene name in parenthesis. Pathogenic variants for JADT, CED, and MZSDS are shown

	JADT	Other SRP	CED	MZSDS	Kidney involvement (NPHP)	References
Retrograde transport (IFT-A complex)						
IFT43			+		+	[59]
IFT121 (*WDR35*)			+		+	[61]
IFT122 (*WDR10*)			+		+	[60]
IFT139 (*TTC21B/NPHP12*)	+				+	[70]
IFT140	+			+	+	[107]
IFT144 (*WDR19/NPHP13*)	+				+	[58]
Anterograde transport (IFT-B complex)						
IFT80	+				-	[57]
IFT172 (*NPHP17*)	+			+	+	[40]

CED, cranioectodermal dysplasia; *JATD*, Jeune asphyxiating thoracic dysplasia; *MZSDS*, Mainzer-Saldino syndrome; *Other SRP*, other short-rib polydactyly syndromes except Jeune syndrome

such as morphogenesis, DDR signaling is crucial and causes tissue dysplasia when defective. On the contrary, during postnatal maintenance of tissue, replication stress is lower and would result only in a degenerative phenotype when defective. This may explain why certain loss-of-function pathogenic variants result in severe congenital dysplasia and malformation of the kidneys, brain, and eyes, while hypomorphic pathogenic variants in the same genes cause a milder phenotype characterized by NPHP and retinal degeneration.

Other signaling pathways involved in NPHP

Another signaling pathway linked to nephrocystin-3 and nephrocystin-9/NEK8 is the Hippo pathway [51, 82]. Both directly interact with the final effector of Hippo signaling TAZ, whereas nephrocystin-4 acts as a negative regulator of Hippo signaling. Nephrocystins and polycystins (causing ADPKD when mutated) were shown to modify the Hippo signaling pathway [82].

High levels of cAMP have been shown to enhance epithelial cell proliferation and fluid secretion as drivers of cyst formation in polycystic kidney disease [83]. A growing body of evidence implies elevated levels of cAMP with NPHP. In mIMCD3 cell lines with stable knockdown of *Nphp3*, *Nphp6*, or *Nphp8*, increased cAMP levels were detected. In 3D spheroid culture systems, these cells did not form a lumen and formed abnormal spheroids. Applying octreotide, an inhibitor of cAMP synthesis, improved these changes, linking elevated cAMP to polarity defects [84].

Enhanced mTOR (mechanistic target of rapamycin) activity has been linked to cystic kidney disease and especially to cyst-lining epithelium of different NPHP mouse models [85]. The mTOR signaling pathway is regulated by the primary cilium and it has been hypothesized that the flow sensing of the primary cilium modifies cellular size via mTOR regulation [86].

Different NPHP protein networks contribute to NPHP, JBTS, and MKS

Given the genetic heterogeneity of NPHP involving various signaling pathways, it is obvious that there is no single, unifying mechanism causing NPHP. So far, the following themes have emerged in NPHP:

1. NPHP is a kidney ciliopathy with almost all affected proteins being localized in primary cilia and regulating ciliary function and structural integrity. Dysfunctional ciliary signaling includes downstream Wnt and Shh signaling.
2. Each NPHP protein has a specific localization and role along the cilium, either along the axoneme, the TZ zone, or the centrosome. At least four different nephrocystin protein modules were identified along the cilium (Fig. 10D): the nephrocystin-1–4-8 module, the inversin-nephrocytin-3–9-16 module, the nephrocystin-5–6 module, and the MKS1-6-TCTN2 (pathogenic variants in *TCTN2* gene were identified in JBTS patients) module [87]. The protein network consisting of nephrocystins-1–4-8 is mostly expressed in the ciliary TZ which influences ciliary signaling. The protein complex consisting of nephrocystins-5–6 is expressed in the ciliary basal body. The module of nephrocystins-5–6 is critical for ciliogenesis while the other protein modules were not strictly required. A third module contained MKS1-6/CC2D2A and Tectonic2. Pathogenic variants in *MKS1* cause MKS, and *CC2D2A/MKS6* pathogenic variants are causative for JBTS and MKS by causing impaired ciliogenesis and neural tube defects [41, 45]. Nephrocystin-2, nephrocystin-3, nephrocystin-9, and nephrocystin-16 are bridging the three different modules [87]. The different localizations of the nephrocystin-1–4-8 and nephrocystin-5–6 modules and bridging proteins such

as nephrocystin-2, nephrocystin-3, and nephrocystin-9 (which are expressed along the entire axoneme) indicate that these complexes may have different functions regarding apical organization, hedgehog (Hh) signaling, and cilia integrity (Fig. 10D).

3. Increasing evidence points to a link between *NPHP* pathogenic variants and dysfunctional DDR signaling. It remains unclear if altered DDR signaling occurs independently or if loss of ciliary function occurs downstream of abnormal cell cycle progression during replication stress.

Genes mutated in NPHP-RC

The increasing number of newly discovered nephrocystins points to a variety of involved pathways, and many nephrocystins interact with each other or other proteins creating protein modules (Fig. 10D). We outline all the nephrocystins and summarize their impact on the pathomechanisms in NPHP.

NPHP1 is expressed at focal adhesions, adherens junctions, and in cilia

In 1997, two groups published homozygous deletions in the *NPHP1* gene on chromosome 2q13 as a cause for NPHP type 1 [88, 89]. *NPHP1* deletions can also cause retinal and ocular phenotypes resulting in SLSN and OMA [4]. CNS involvement ranges between 3 and 7% and the liver is affected in 0.8–8% of *NPHP1* pathogenic variants [4, 5]. A total of 23.4% of NPHP1 individuals have extrarenal manifestations [4]. *NPHP1* deletions represent the most common form of NPHP, which is diagnosed in approximately 20% of all NPHP patients. *NPHP1* encodes nephrocystin-1, which is primarily expressed in the collecting duct [90]. Interaction partners of nephrocystin-1 such as Pyk2 and p130(Cas) pointed to a role for nephrocystin-1 in the adherens junctions [91]. Moreover, interaction of nephrocystin-1 was also shown with other nephrocystins (see Table 2), suggesting a larger protein complex of nephrocystins [49, 62, 92]. Nephrocystin-1 and nephrocystin-4 physically interact and colocalize in mitotic spindles and primary cilia pointing to a role in cell division [65].

NPHP2/INVS pathogenic variants pointed to a ciliary defect by causing infantile NPHP, *situs inversus*, and cardiac defects

NPHP2/INVS, which encodes nephrocystin-2/inversin, was identified as the gene mutated in NPHP2 [62]. Autosomal recessive pathogenic variants in *NPHP2/INVS* were found mostly in children with age of onset of KF younger than 4 years of age and who had possible antenatal presentation with oligohydramnios [18]. Other extrarenal manifestations affect the eye (optic nerve atrophy, retinal degeneration) (16.3%), CNS (hydrocephalus) (8.3%), position of organs (e.g., *situs inversus*), and heart (e.g., ventricular septal defects, aortic coarctation) (24.9%) [4]. Nephrocystin-2/inversin was the first ciliary expressed nephrocystin and revealed coexpression with nephrocystin-1 in primary cilia of kidney epithelial cells [62]. Interaction was shown with nephrocystin-1, nephrocystin-3, calmodulin, catenins, anaphase-promoting complex 2, and β-tubulin (Table 2) [46, 62, 93–95]. β-Tubulin contributes to the microtubule axoneme of primary cilia. Nephrocystin-2/inversin also serves as an anchor protein for other nephrocystins (e.g., NPHP3, NPHP9/NEK8, and NPHP16/ANKS6) (Fig. 10D) [53, 87]. Moreover, nephrocystin-2/inversin expression was published in cell cycle–dependent fashion in the mitotic spindle in mitosis, the mid-body in cytokinesis, and in cilia, the basal body, and the centrosomes during interphase; nephrocystin-2/inversin is involved in Wnt signaling and planar cell polarity (PCP) (see above) [93].

NPHP3 pathogenic variants cause a diverse spectrum of phenotypes

NPHP3 pathogenic variants were identified in the kidney cystic mouse model *pcy* and in a Venezuelan kindred [49]. It encodes nephrocystin-3 which colocalizes and interacts with nephrocystin-1 and inversin, and was also found as a component of a protein network containing inversin, nephrocystin-9/NEK8, and nephrocystin-16/ANKS6 (Fig. 10D) [46, 49, 53]. Similar to inversin, nephrocystin-3 may also inhibit canonical Wnt signaling [46]. In humans, a genotype–phenotype correlation was not confirmed and the classification as "adolescent NPHP" appears arbitrary as *NPHP3* pathogenic variants result in a range of different phenotypes, ranging from adolescent NPHP, NPHP with RP, NPHP with liver fibrosis, infantile NPHP, to MKS [4, 46, 49].

Nephrocystin-4 links the nephrocystin "cell-junction" hypothesis with nephrocystin expression in primary cilia

NPHP4 pathogenic variants cause a spectrum of phenotypes ranging from isolated NPHP, NPHP with OMA, and SLSN [92]. Approximately 45% of patients had extrarenal symptoms including the eyes (coloboma and LCA) (35%), the CNS (mental retardation, developmental delay, deafness) (10%), and the liver (10%) [4]. The encoded protein nephrocystin-4 localizes to the TZ of primary cilia, basal bodies, centrosomes, and the actin cytoskeleton [92]. Nephrocystin-4 may work in concert with nephrocystin-1 at the TZ in

order to regulate entry and exit of ciliary cargo for IFT [96]. Moreover, nephrocystin-4 interacts with nephrocystin-1, nephrocystin-8/RPGRIP1L, p130(Cas), PALS1/PATJ, Par6, and α-tubulin [36, 65, 92] (Table 2). Finally, NPHP4 negatively regulates Hippo signaling, which controls cell proliferation and tumor suppression [82], and inhibits canonical Wnt signaling [73].

NPHP5 pathogenic variants result in Senior-Løken syndrome, a retinal-kidney phenotype

Truncating *NPHP5/IQCB1* pathogenic variants were found in patients with SLSN [69]. Nephrocystin-5 colocalizes with nephrocystin-1 and nephrocystin-4 in the primary cilia, adherens junctions, and focal adhesions [69]. Nephrocystin-5 and nephrocystin-6 both interact and are expressed in the connecting cilia of photoreceptors [35, 69, 97]. Nephrocystin-5 requires nephrocystin-6 for centrosomal localization and both are expressed in the connecting cilia of photoreceptors (Fig. 11B) [35, 87]. Nephrocystin-5 also interacts with calmodulin and the retinitis pigmentosa GTPase regulator (RPGR) underlining its role in the photoreceptor [69].

NPHP6/CEP290 pathogenic variants cause Joubert syndrome

Pathogenic variants in *NPHP6/CEP290*, which encodes nephrocystin-6, were initially published in patients with JBTS [35]. Nephrocystin-6 is localized at the centrosome and the mitotic spindle [35]. In a cohort of 19 families with *NPHP6/CEP290* pathogenic variants, all patients had extrarenal symptoms characterized by dysplastic phenotypes including the eye (LCA, coloboma) (87.4%), the CNS (CVH, mental retardation, hydrocephalus, microcephaly, occipital encephalocele) (72.9%), and the liver (liver fibrosis) (6.2%) [4]. Pathogenic variants in *NPHP6/CEP290* result in a wide variety of phenotypes, ranging from JBTS without kidney involvement, isolated NPHP, SLSN, JBTS to MKS, and BBS (Bardet-Biedl syndrome) [4, 35, 44, 98]. Interestingly, in patients with two null pathogenic variants, always, more than two organs were involved and patients had at least one dysplastic phenotype, whereas patients with less than two null pathogenic variants never had a dysplastic phenotype and almost never had more than two organs involved [4]. Nephrocystin-6/CEP290 interacts with and modifies ATF4 (activating transcription factor 4), which is a cAMP-regulated transcription factor that is involved in cyst formation. *NPHP6/CEP290* was the first nephrocystin to link altered cAMP levels with progression of kidney disease. Elevated cAMP levels are detected in epithelial cells from cystic kidneys and have become a target for therapy [84]. Nephrocystin-6/CEP290 also interacts with Tectonic family member 1 (TCTN1), which forms a protein complex with multiple MKS proteins at the TZ of cilia and modifies hedgehog (Hh) signaling [47]. Finally, *NPHP6/CEP290* pathogenic variants have been linked to abnormal DNA damage response, cell signaling, and kidney cystogenesis [79].

NPHP7 links NPHP with altered hedgehog signaling

Pathogenic variants in *NPHP7/GLIS2* were initially only identified in one large Cree Native American kindred [76]. Therefore, *NPHP7/GLIS2* pathogenic variants represent a very rare cause of NPHP. Affected patients developed KF prior to the age of 8 years. *NPHP7/GLIS2* encodes a Kruppel-like zinc finger transcription factor, Gli-similar protein 2 (Glis2), which is a member of the Hh pathway [76]. Nephrocystin-7/Glis2 localizes to primary cilia and the nucleus and maintains the mature tubular epithelial phenotype [76].

NPHP8/RPGRIP1L pathogenic variants result in JBTS and MKS

Pathogenic variants in *NPHP8/RPGRIP1L* were published in patients with a JBTS-like phenotype, called cerebro-oculo-renal syndrome (CORS) [36]. All patients had juvenile onset of KF independent from the nature of the pathogenic variant and the majority of patients had dysplastic phenotypes of the CNS (CVH, occipital encephalocele, developmental delay) (75%). No patients with two nonsense pathogenic variants were found, suggesting that this may be lethal [4]. The clinical spectrum of *NPHP8/RPGRIL1L* pathogenic variants ranges from LCA, isolated NPHP, JBTS, COACH syndrome (cerebellar vermis hypoplasia, oligophrenia [e.g., developmental delay and mental retardation], ataxia, coloboma, and hepatic fibrosis), to MKS, with overall more commonly extrarenal manifestations. *NPHP8/RPGRIL1L* encodes the retinitis pigmentosa GTPase regulator interacting protein 1-like (RPGRIP1L), which interacts with nephrocystin-4. *NPHP8/RPGRIL1L* colocalizes with nephrocystin-4 and nephrocystin-6 at centrosomes and basal bodies and was found in a protein complex with these two nephrocystins in photoreceptors of mammalian retina [32, 36].

Pathogenic variants in *NPHP9/NEK8* link NPHP with ADPKD and the Hippo pathway

Pathogenic variants in *NPHP9/NEK8* link PKD and NPHP in an intriguing way [27]. *NPHP9/NEK8* encodes the never in mitosis A-related kinase A (NEK8) protein, which is localized in centrosomes and cilia and is important in cell-cycle regulation [99]. In one study, homozygous *NPHP9/NEK8* nonsense pathogenic variants were found in three fetuses from a consanguineous kindred, resulting

in complete loss of NEK8 expression, and caused multiorgan involvement including enlarged cystic-dysplastic kidneys, congenital hepatic fibrosis, cystic-dysplastic pancreas, severe congenital heart defects, and hypoplastic lungs [51]. Functional studies of *NPHP9/NEK8* pathogenic variants showed altered ciliogenesis, epithelial morphogenesis, apoptosis, proliferation, DNA damage control, and Hippo signaling [50, 80]. Decreased PKD1 and PKD2 but elevated c-myc expression was demonstrated, and interaction between wild-type (WT) nephrocystin-3 and NEK8 was shown [51]. PKD and NPHP have significantly different histological characteristics but the involved gene products share common subcellular localization in primary cilia and centrosomes [27]. NEK8 provides an interesting link between both diseases by interacting with polycystin-2 and altering polycystin-2 phosphorylation [100]. Moreover, nephrocystin-2/inversin, which if mutated causes the PKD-like phenotype with enlarged cystic kidneys, is required for targeting of nephrocystin-9/NEK8 to the primary cilium [53, 101]. Nephrocystin-16/ANKS6, which, if mutated, also results in enlarged kidneys, liver fibrosis, and cardiac defects, also interacts with NEK8 [53, 63]. Inhibition of the Hippo effector YAP by Verteporfin improved *NPHP9/NEK8* pathogenic variant-induced changes in 3D spheroids, thus representing a potential therapy [50].

NPHP10/SDCCAG8 pathogenic variants link NPHP and Bardet-Biedl syndrome (BBS)

Homozygosity mapping and ciliopathy candidate exome capture identified truncating *NPHP10/SDCCAG8/BBS16* (serologically defined colon cancer antigen 8) pathogenic variants in 10 different families with NPHP and retinal degeneration (SLSN phenotype) [33]. Patients with *NPHP10/SDCCAG8* pathogenic variants mostly present with SLSN (9 out of 10 families) (accounting for 3.3% of SLSN patients) but few patients also have characteristics of BBS-like symptoms such as hypogonadism, obesity, or mild mental retardation [33]. Independent of the kind of pathogenic variant, all patients presented with juvenile onset KF [4]. Other extrarenal manifestations included degenerative lesions of the CNS (mental retardation, neuropathy, cystic brain lesion) (20%) and the eye (80%) [4]. *NPHP10/SDCCAG8* contains 8 coiled-coil domains (a feature shared by many proteins that are disrupted in NPHP) and localizes in both centrosomes and cell–cell junctions with nephrocystin-5 [33]. Moreover, SDCCAG8 and nephrocystin-5 colocalize in the TZ of photoreceptors, which may correlate with the phenotype of SLSN. The gene product of *NPHP10/SDCCAG8* also interacts with OFD1 (oral-facial-digital syndrome 1), which is associated with NPHP-related ciliopathies [33].

Pathogenic variants in *NPHP11/TMEM67/MKS3* are responsible for a majority of NPHP-related liver fibrosis

A wide spectrum of phenotypes including NPHP with liver disease, JBTS, Meckel syndrome, and BBS is caused by pathogenic variants in *NPHP11/TMEM67/MKS3* [15, 37, 52, 98]. Liver disease seems to be very prevalent (18 of 20 patients) in patients with *NPHP11/TMEM67/MKS3* pathogenic variants [41]. In a cohort of 20 families, no patients with two nonsense pathogenic variants were found, suggesting that this may cause a lethal phenotype [4]. Almost all patients presented with juvenile onset KF and all patients had extrarenal manifestations including dysplastic phenotypes of the CNS (CVH, brain atrophy, Dandy-Walker malformation, developmental delay) (88%), the eye (coloboma, optic nerve atrophy) (49.6%), and degenerative liver phenotypes (liver fibrosis, cholangiopathy, hepatomegaly) (77%) [4]. Patients with two missense pathogenic variants in *NPHP11/TMEM67/MKS3* almost always develop liver disease [4]. Missense pathogenic variants in *NPHP11/TMEM67/MKS3* cause a hypomorphic allele which results in a milder phenotype with NPHP and liver disease, whereas truncating pathogenic variants appear to cause a more severe phenotype [102]. *NPHP11/TMEM67/MKS3* encodes meckelin, and of special interest are *NPHP11/TMEM67/MKS3* exons 8 to 15, where even missense pathogenic variants can cause MKS if combined with another truncating pathogenic variant. The function of the protein region encoded by exons 8–15 remains unknown [103]. Most pathogenic variants in *NPHP11/TMEM67/MKS3* cause a JBTS-related phenotype, in particular COACH syndrome (cerebellar vermis hypoplasia, oligophrenia [e.g., developmental delay and mental retardation], ataxia, coloboma, and hepatic fibrosis) [37, 41]. Meckelin is expressed in primary cilia and the plasma membrane and interacts with MKS1, another gene product altered in MKS [104]. Meckelin is also found at the TZ together with other MKS proteins including MKS1 and requires Tctn1 for this localization to modulate Hh signaling [47]. In cilia, MKS proteins form complexes with several nephrocystins (e.g., nephrocystin-1, nephrocystin-4, nephrocystin-6/RPGRIP1L) to establish the basal body/TZ membrane attachment [66].

Intraflagellar transport protein 139 contributes to NPHP and Jeune syndrome and modifies disease severity

Disease-causing homozygous and compound heterozygous pathogenic variants in *NPHP12/TTC21B* were described in patients with Jeune syndrome and NPHP [70]. *NPHP12/TTC21B* encodes for IFT139 which is required for ciliary retrograde IFT. IFT139 localizes to the basal body,

specifically to the TZ of photoreceptors, and the axoneme [70]. In addition, IFT139 also regulates Hh signaling [105]. In humans, additional heterozygous modifier pathogenic variants were described in patients who already carried compound heterozygous pathogenic variants in *NPHP4* or other ciliopathy genes [70]. *NPHP12/TTC21B* may function as a genetic modifier of disease in approximately 5% of ciliopathy patients by increasing the pathogenic variant load, thus supporting the idea of oligogenicity and triallelic inheritance [64].

Nephrocystin-13 and nephrocystin-17 cause NPHP and skeletal dysplasias and are members of IFT

Over the last few years, skeletal disorders associated with NPHP provided exciting insight about ciliopathies and IFT [106]. A variety of skeletal disorders including Jeune syndrome (aka asphyxiating thoracic dysplasia) (JADT), cranioectodermal dysplasia (CED) (aka Sensenbrenner syndrome), and Mainzer-Saldino syndrome (MZSDS) can present with NPHP (Table 4).

The expression pattern of the genes identified in these multiorgan disorders are consistent with the ciliary hypothesis, and primary cilia were also found in chondrocytes [27]. Most genes involved in NPHP associated with skeletal dysplasia contribute to IFT (Fig. 10A and B) which is crucial for cilium assembly and maintenance. Pathogenic variants in two components of the ciliary anterograde IFT (complex B: IFT80, IFT172) and all six components of the retrograde transport (complex A: IFT43, IFT121, IFT122, IFT139, IFT140, IFT144) were identified in skeletal ciliopathies associated with NPHP (Table 4) (Fig. 10A and B) [30, 40, 57, 59–61, 70, 107]. The IFT-A complex provides retrograde transport from the tip to the base of the cilium, while the IFT-B complex is involved in the anterograde transport from base to tip (Fig. 10A and B). All components of the IFT-A complex contribute to skeletal ciliopathies as a distinct spectrum of NPHP-RC and five out of 14 IFT-B members are also associated with skeletal phenotypes (e.g., IFT27, IFT80, IFT81, IFT88, and IFT172).

JADT is part of the short-rib polydactyly group and is characterized by a narrow rib cage (Fig. 3) causing frequent respiratory failure, polydactyly, and brachydactyly, and can include extraskeletal features such as cystic kidney disease, liver disease, and retinal degeneration [106]. Pathogenic variants in the retrograde IFT-A components IFT139 (*TTC21B*) (see above), IFT140 (described with CED below), and IFT144 (*WDR19*) (described with MZSDS below) were described in JADT [106].

CED overlaps clinically with JADT but patients usually have milder rib cage narrowing, dolichocephaly and ectodermal involvement with delayed tooth eruption, skin laxity, sparse and fine hair, and slow-growing nails. Extraskeletal involvement includes cystic kidney disease, liver cirrhosis, and retinal dystrophy. Pathogenic variants in *IFT121/WDR35*, *IFT122/WDR10*, *IFT43*, and *NPHP13/IFT144/WDR19* contribute to CED [106]. Mainzer-Saldino syndrome (MZSDS) is characterized by phalangeal cone-shaped epiphyses, retinal dystrophy, and NPHP. Variable symptoms include cerebellar ataxia, narrow thorax, and hepatic fibrosis. Pathogenic variants in *IFT140* and *IFT172* result in either MZSDS or JADT [40, 107]. To expand on all involved IFTs is beyond the scope of this review. We will focus on NPHP13/IFT144 and NPHP17/IFT172 as examples for the proteins involved in anterograde and retrograde IFT. *NPHP13/ IFT144 (WDR19)* pathogenic variants cause isolated NPHP, JADT, or CED [30, 58]. IFT144 is a member of the retrograde IFT complex A and is expressed in cilia. IFT172 is a member of the anterograde transport complex B (Fig. 10A and B). Extraskeletal symptoms include NPHP, liver failure, retinal degeneration, and cerebellar vermis hypoplasia as seen in JBTS [40]. IFT172 is localized to the axoneme and the ciliary base. Surprisingly, IFT172 mutant cilia were longer than wild-type cilia and displayed reduced adenylyl cyclase III activity which may result in lower cAMP signaling and less PKA activity, which is a negative regulator of Shh signaling [108]. IFT172 forms a complex with IFT38, IFT57, and IFT80 and was found to interact genetically with MKS1 [109]. While Indian hedgehog (Ihh) is crucial for endochondral ossification, Shh is required for patterning of the forming skeleton [110]. Both IFT-A and IFT-B complexes are required for regulation of Shh signaling.

Pathogenic variants in *NPHP14* and *NPHP15* affect DNA damage response signaling, thereby linking cilia and centrosomes to DNA repair

Pathogenic variants in genes encoding members of the DNA damage response (DDR) signaling pathway were identified for NPHP14 and NPHP15 [31]. Pathogenic variants in *NPHP14/ZNF423* and *NPHP15/CEP164* resulted in JBTS and early kidney involvement. ZNF423 interacts with the DNA damage sensor PARP1, which recruits ATM (ataxia, telangiectasia mutated), an essential component of the DDR pathway. Nephrocystin-14/ZNF423 also interacts with nephrocystin-6 [31]. Pathogenic variants in *NPHP15/CEP164* were found in patients with retinitis pigmentosa, JBTS, juvenile NPHP, liver fibrosis, and obesity. While wild-type nephrocystin-15/CEP164 colocalized with the mother centriole and mitotic spindle poles, the mutant CEP164 proteins lacked centrosomal localization. Similar to ZNF423, CEP164 also plays a role in the DDR signaling pathway and in ciliogenesis. These studies provided the first link between DNA damage control and cilia/centrosomes by disturbing cell-cycle checkpoint control. This is detrimental

for survival of embryonic and adult progenitor cells [31]. In the meantime, *NPHP9/NEK8*, *NPHP10/SDCCAG8*, and *NPHP20/MAPKBP1* have also been linked to the DDR pathway [25, 80, 81].

ANKS6 encodes nephrocystin-16, which links nephrocystin-9 to inversin and nephrocystin-3

Different nephrocystin subnetworks exist (NPHP1-NPHP4-NPHP8; NPHP5-NPHP6, NPHP2-NPHP3-NPHP9; and the MKS module) (Fig. 10D) [87]. *ANKS6* was identified by studying interaction partners of NEK8/NPHP9 using mass spectrometry [53]. *ANKS6* is expressed in the proximal segment of the primary cilium. *ANKS6* pathogenic variants resulted in infantile onset of cystic kidney disease or juvenile NPHP [53]. Most *ANKS6* pathogenic variants were heterozygous with the identification of additional pathogenic variants in either *INVS*, or *NPHP1*, suggesting an oligogenic inheritance. *ANKS6* missense pathogenic variants cause enlarged cystic kidneys and no extrarenal symptoms, whereas truncating pathogenic variants result in enlarged kidneys, cardiac defects, *situs inversus*, and liver fibrosis. Consistent with the hypothesis that *ANKS6* may be part of the NPHP2-NPHP3-NPHP9 module, human *INVS* and *NPHP3* pathogenic variants also resulted in cardiac phenotypes. Coimmunoprecipitation studies confirmed physical interaction between *ANKS6*, NEK8/nephrocystin-9, inversin, and nephrocystin-3. *ANKS6* is critical as an activator of NEK8 kinase [63]. *ANKS6* also interacts with another regulator of Dishevelled called bicaudal 1 (BICC1) [111], thus linking *ANKS6* with Wnt signaling. Loss of *ANKS6* also affects the Hippo pathway and results in Yap deficiency and liver abnormalities [112].

The distal appendages at the mother centriole are required for ciliogenesis and contribute to NPHP-RC due to *NPHP18/CEP83* pathogenic variants

Ciliogenesis requires docking of the basal body to the plasma membrane [113]. This is mediated by the distal appendages (DAP) which are present at the mother centriole. Failler et al. performed targeted exon sequencing in NPHP patients to identify additional DAP components [54]. Biallelic pathogenic variants in *CEP83/NPHP18* which encodes the centrosomal protein CEP83 were identified in patients with early-onset NPHP, learning disabilities, and hydrocephalus [54]. Their fibroblasts and kidney tubular cells displayed ciliary defects and altered DAP composition. CEP83/nephrocystin-18 colocalizes with CEP164/nephrocystin-15 at DAPs [113]. Pathogenic variants in *CEP83/NPHP18* resulted in impaired interaction with CEP164/nephrocystin-15.

Pathogenic variants in *NPHP19/DCDC2* link defective Wnt signaling with kidney and hepatic disease

Homozygous and compound heterozygous pathogenic variants in *DCDC2* were found in two families with early liver fibrosis. One of the two affected individuals also had NPHP. The other patient may have been too young to develop the kidney phenotype yet [55]. No other NPHP-RC phenotypes were identified. Immunofluorescent studies revealed WT DCDC2 localization in the axoneme of primary cilia and the spindle microtubules in a cell cycle-dependent manner with mutant DCDC2 failing to localize to primary cilia. The WT DCDC2 protein interacts with Dishevelled-3, a mediator of Wnt signaling, while some of the DCDC2 pathogenic variants failed to interact. *DCDC2* overexpression inhibits β-catenin-dependent Wnt signaling, while DCDC2 down-regulation via siRNA enhanced β-catenin-induced activation of T cell factor (TCF)-dependent transcription. In a 3D cell culture system with IMCD3 cells, knockdown of *DCDC2* resulted in significantly fewer cilia and constitutively activates Wnt signaling. Treatment with the Wnt inhibitor iCRT14 rescued the effect of DCDC2 knockdown, thus underlying the significance of DCDC2 for Wnt pathway modulation and of Wnt inhibitors as possible future treatment options.

Late-onset NPHP independent from cilia is caused by *NPHP20/MAPKBP1*

One of the few rare cases of cilia-independent NPHP is due to recessive pathogenic variants in *MAPKBP1/NPHP20* [25]. This gene encodes a scaffolding protein required for JNK signaling. Unlike most other nephrocystins, MAPKBP1 is not localized in cilia and no ciliary defects were found in fibroblasts of affected individuals, and knockdown of MAPKBP1 in murine cell lines revealed increased DNA damage signaling [25].

Pathogenic variants in *NPHP21*/ADAMTS9 result in a syndromic appearance of NPHP

Homozygosity mapping identified two homozygous pathogenic variants in *ADMTS9*, a metalloproteinase [39]. Both children presented with NPHP-RC including a Joubert-like phenotype, deafness, and short stature. ADAMTS9 was found to be localized close to cilia and centrosomes.

Pathogenic variants in *NPHP1L1/XPNPEP3* may represent a phenocopy of NPHP

A nephronophthisis-like (NPHPL) phenotype was discovered in two consanguineous kindreds with a splice-site

pathogenic variant and a deletion in *NPHP1L/XPNPEP3* [23]. The associated phenotype was more complex including cardiomyopathy and seizures in addition to KF. The mutated gene product of *NPHP1L/XPNPEP3* causes a complex-I-defect mitochondriopathy with decreased NADH-CoQ-oxidoreductase activity [23]. *NPHP1L/XPNPEP3* was the first gene not consistent with the ciliary hypothesis and may result in a phenocopy of NPHP. Therefore, this condition has also been named NPHP1L.

NPHP-like phenotype is caused by a pathogenic variant in a magnesium transporter

Another gene causing nephronophthisis but lacking ciliary localization is *SLC41A1*, thus contributing to a nephronophthisis-like phenotype [24]. Hurd et al. discovered the magnesium channel SLC41A1 as the *NPHP2L* gene with a homozygous splice pathogenic variant resulted in skipping of exon 6 and caused an in-frame deletion of a transmembrane domain of SLC41A1. In cell culture models, the deletion of exon 6 inhibited magnesium transport. RT-PCR confirmed *SLC41A1* mRNA expression in TAL and DCT.

Therapeutic approaches

Given the different affected signaling pathways for NPHP, multiple therapeutic approaches have been developed:

Elevated cAMP levels are linked to cystic kidney disease such as in ADPKD and NPHP. Vasopressin 2 receptor (V2R) antagonists such as tolvaptan reduce cAMP synthesis by decreasing V2R downstream G protein signaling and subsequently reducing adenylate cyclase activity. In a mouse model of NPHP3 (*Pcy* mouse), V2R antagonists impaired kidney cAMP accumulation and rescued the cystic kidney phenotype [114]. The use of tolvaptan has now been successfully implemented in clinical trials of adult ADPKD patients.

CEP290/NPHP6 and *NEK8/NPHP9* are important regulators of DDR signaling because pathogenic variants in these genes result in DNA replication stress and elevated cyclin-dependent kinase (CDK) levels [79, 80]. Inhibition of CDK improves DNA damage caused by loss of function of CEP290/NPHP6 and NEK8/NPHP9, thus leading to the rationale that CDK inhibition may be a therapeutic strategy to treat NPHP. CDK inhibitors such as roscovitine and its analog S-CR8 reduced the disease progression of kidney cysts and loss of kidney function in a mouse model carrying an *NEK8* pathogenic variant (*jck* mouse) [80]. Roscovitine also improved the ciliary phenotype of primary kidney epithelial cells from a CEP290/NPHP6 patient and prevented cyst growth in collecting ducts of Cep164-deficient mouse kidneys [115, 116].

Manipulation of Hh signaling seemed promising given its crucial role in the primary cilium. Whereas deletion of Gli2 improved the kidney cystic phenotype in a mouse model for TTC21B/NPHP12, applying the Hh agonist purmorphamine restored the defects found in 3D cell cultures of CEP290/NPHP6 kidney epithelial cells [105, 117].

Increased activity of the mTOR pathway has been associated with cystic kidney disease. Treatment with rapamycin improved the kidney cystic phenotype in the *Pcy* mouse (model for NPHP3) and zebrafish models for pathogenic variants in *Inversin* (*inv*), *IQCB1/NPHP5* (*iqcb1*), and *CEP290/NPHP6* (*cep290*) [85, 118].

Gene therapy may provide some promising leads. For example, overexpression of Nphp5/Iqcb1 in *Nphp5*$^{-/-}$ mice using an adeno-associated virus improved retinal degeneration and ciliogenesis [119].

Despite these promising leads, so far, no clinical trial in NPHP patients has been undertaken. Therefore, treatment of NPHP at this point remains mostly supportive with therapy of anemia, secondary hyperparathyroidism, metabolic bone disease, and blood pressure. Kidney replacement therapy is needed once fluid overload and uremia become more pronounced. Currently, the best therapeutic option for NPHP patients is a kidney transplant as NPHP does not recur in a new organ.

Conclusion

It is important to recognize that many NPHP patients may have extrarenal symptoms and it is important to assemble clinical, pathological, and genetic information to form a holistic picture. Due to progress in gene identification, frequency of identified *NPHP* pathogenic variants increased to 50–70% of all NPHP/NPHP-RC cases [2]. More international consortiums have been founded to address this challenging condition [120]. As we learn more about the involved signaling pathways, individualized therapies may become more available.

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Declarations

Conflict of interest The authors declare no competing interests.

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