Published as: *J Am Soc Nephrol*. 2009 January ; 20(1): 23–35.

Nephronophthisis: Disease Mechanisms of a Ciliopathy

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Abstract

Nephronophthisis (NPHP), a recessive cystic kidney disease, is the most frequent genetic cause of end-stage kidney disease in children and young adults. Positional cloning of nine genes (*NPHP1-9*) and functional characterization of their encoded proteins (nephrocystins) has contributed to a unifying theory that defines cystic kidney diseases as “ciliopathies”. The theory is based on the finding that all proteins mutated in cystic kidney diseases of humans or animal models are expressed in primary cilia or centrosomes of renal epithelial cells. Primary cilia are sensory organelles that connect mechanosensory, visual, and other stimuli to mechanisms of epithelial cell polarity and cell cycle control. Mutations in *NPHP* genes cause defects in signaling mechanisms that involve the non-canonical Wnt signaling pathway and the sonic hedgehog signaling pathway, resulting in defects of planar cell polarity and tissue maintenance. The ciliary theory explains the multiple organ involvement in NPHP, which includes retinal degeneration, cerebellar hypoplasia, liver fibrosis, *situs inversus*, and mental retardation. Positional cloning of dozens of unknown genes that cause NPHP will elucidate further signaling mechanisms involved. Nephrocystins are highly conserved in evolution, thus allowing the use of animal models to develop future therapeutic approaches.

Subjects

nephronophthisis; cystic kidney disease; planar cell polarity; wnt signaling; hedgehog signaling; ciliopathies

Clinical Features of Nephronophthisis

Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease that constitutes the most frequent genetic cause for end-stage kidney disease (ESKD) in the first 3 decades of life[1–4]. ESKD manifests at a median age 13 years. Initial symptoms are relatively mild, start around age 6 years, and consist of polyuria, polydipsia, secondary enuresis, and anemia 5. Regular fluid intake at nighttime is a characteristic feature of the patients’ history. Renal ultrasound reveals normal kidney size, increased echogenicity and corticomedullary cysts (Figure 1, A)[6]. Renal histology exhibits a characteristic triad of corticomedullary cysts, tubular basement membrane disruption, and tubulointerstitial nephropathy (Figure 1, B)[7,8]. Disease recurrence has never been reported in kidneys transplanted to NPHP patients[9]. NPHP is inherited in an autosomal recessive mode[4]. It was first described by Smith and Graham in 1945[10] and by Fanconi *et al.*[11], who introduced the term "familial juvenile nephronophthisis".

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In NPHP, ESKD develops within the first 3 decades of life[12–14]. In contrast, infantile NPHP, which is characterized by mutations in *NPHP2/inversin*, leads to ESKD between birth and age 3 years14,15. In more than 10% of cases NPHP can be associated with extrarenal involvement, primarily including retinal degeneration (Senior-Loken syndrome), cerebellar vermis aplasia (Joubert syndrome), liver fibrosis, and cone-shaped epiphyses. These clinical features will be discussed below in light of the cilia/centrosome theory of NPHP. Over 300 cases of NPHP have been published in the literature[7]. It has been reported from virtually all regions of the world16. The incidence of the disease has been estimated as 9 patients/8.3 million17 in the United States or 1 in 50,000 live births in Canada7,18. In the North American pediatric end-stage renal disease population pooled data indicate a prevalence of approximately 5% of all children with ESKD[19,20].

Cilia and Centrosomes: A Unifying Theory for Cystic Kidney Disease

Positional cloning has revealed nine genes that cause nephronophthisis if mutated[21–32]. These are monogenic recessive genes, implying that mutations in each single one is sufficient in itself to cause NPHP in a patient bearing mutations, indicating that their gene products are necessary for normal kidney function. Positional cloning thereby generated new insights into disease mechanisms of NPHP, and revealed that they are related to signaling mechanisms of primary cilia, centrosomes, and planar cell polarity[1,25,33,34]. The demonstration that nephrocystin-1 and inversin/NPHP-2 localize to primary cilia of renal tubular cells25 was among the first findings to support a new unifying theory of renal cystogenesis[33]. This theory states that proteins (“cystoproteins”) that are mutat[35]ed in renal cystic disease in humans, in mice, or zebrafish, are expressed in primary cilia, basal bodies, or centrosomes33,36. Basal bodies are the foundations from which cilia are assembled (Figure 2). After mitosis and cell division are completed, basal bodies derive from the *mother* centriole of the centriole pair that had organized the mitotic spindle in cell division. As cilia are formed from the basal body, the *daughter* centriole is placed on the side of the nucleus opposite to the basal body, thus specifying cell polarity. The structure and function of primary cilia and basal bodies is delineated in Figure 2.

It is becoming apparent that primary cilia are highly conserved structures that sense of a wide variety of extracellular cues in a broad spectrum of epithelial tissues. There is a wide range of cues that can be received by specific ciliary receptors, including photosensation, mechanosensation, osmosensation, and olfactory sensation. In general, it seems that the pathogenesis of ciliopathies is based on an inability of epithelial cells to sense or process extracellular cues[37].

Positional Cloning Reveals Nephronophthisis as a “Ciliopathy”

By positional cloning we and others have identified recessive mutations in nine different novel genes as causing NPHP: *NPHP1*[21,22], *NPHP2/inversin*[25], *NPHP3*[24], *NPHP4*[23,30], *NPHP5*[38], *NPHP6/CEP290*[27,39], *NPHP7/GLIS2*[31], *NPHP8/RPGRIP1L*[32,40,41], and *NPHP9/NEK8*[42], defining NPHP types 1 through 9, respectively. This has made molecular genetic diagnostics possible (www.renalgenes.org). Homozygous deletions in the *NPHP1* gene account for approximately 21% of all NPHP cases, whereas the other genes contribute less than 3% each (Figure 3). As determined in more than 1,000 families with NPHP, the causative genes are still unknown in about 70% of cases, indicating that further genes are involved in the pathogenesis of NPHP (Figure 3). Recently, evidence has been generated that more than one recessive gene may be mutated in individual patients with NPHP as has been proposed for the related disorder Bardet-Biedl syndrome (BBS)[43]. In this situation mutations in an additional NPHP gene may modify the clinical picture of NPHP in the direction of a more severe extrarenal involvement44,45. In the following, disease mechanisms of NPHP will be discussed in the context of the discovery of each of the *NPHP1* through *NPHP9* genes. We will describe how

the resulting insights into the function of their gene products, the nephrocystins, helped refine the cilia/centrosome theory of renal cystic diseases.

NPHP1 locates to Cell Contacts and the Cilia Transition Zone

Mutations in *NPHP1* were identified as causing juvenile nephronophthisis type 1[21,22]. *NPHP1* encodes nephrocystin-1, a protein that interacts with components of cell-cell and cell-matrix signaling, including p130Cas[46], focal adhesion kinase 2[47], tensin, filamin A and B[48,49]. It is located at adherens junctions and focal adhesions of renal epithelial cells[48,49], which are involved in cell-cell and cell-basement membrane contacts, respectively (Figure 4). It also interacts with the product of other nephronophthisis genes such as nephrocystin-2/inversin25, nephrocystin-324 and nephrocystin-423,[50]. More recently, it was shown that nephrocystin-1 is targeted to the transition zone of motile and primary cilia by the protein PACS-1 (phosphofurin acidic cluster sorting protein-1)[51]. This is initiated by casein kinase 2-mediated phosphorylation of three critical serine residues within a cluster of acidic amino acids in nephrocystin, leading to PACS-1 binding, and to colocalization of nephrocystin with PACS-1 at the base of cilia52.

When *NPHP1* was first identified[21], we proposed a pathogenic hypothesis that tied nephrocystin-1 in with defects of cell-cell and cell-matrix signaling[53,54]. This was based on the finding that nephrocystin-1 contains an SH3 domain, localizes to adherens junctions and focal adhesions of renal epithelial cells, and interacts with integral components of these structures, such as p130CAS[48,49]. This “adherens junction/focal adhesion hypothesis” of NPHP pathogenesis[53,54] has recently been partially reconciled with the “cilia/centrosome” hypothesis in an integrative hypothesis by showing that nephrocystin-4 in polarized epithelial cells colocalizes with β-catenin at cell-cell contact sites and to primary cilia, whereas in dividing cells it localizes to centrosomes[50] (Figure 4).

NPHP2/Inversin Implicates Cilia and Planar Cell Polarity in Cystogenesis

On the basis of positional cloning[14] and candidate gene data[55,56] we identified mutations inhuman inversin (*INVS*) as the cause of infantile NPHP (type 2) with and without *situs inversus*[25]. The renal cystic changes of infantile nephronophthisis (NPHP type 2) combine clinical features of NPHP and of PKD57. We demonstrated that nephrocystin-1 and NPHP2/inversin interact with β-tubulin, which constitutes the microtubule axoneme of primary cilia, and that they are localized at primary cilia of renal tubular cells25. This was one of the first findings to support a unifying theory of renal cystogenesis1,33,36,[58] which states that proteins (“cystoproteins”) which are mutated in renal cystic disease in humans, mice or zebrafish, are expressed in primary cilia, basal bodies, or centrosomes[25,33]. The interaction and co-localization to cilia and basal bodies of nephrocystin-1, inversin, and β-tubulin provided a functional link between the pathogenesis of NPHP, the pathogenesis of PKD, primary cilia function, and left-right axis determination25. Okada et al. had previously demonstrated that inversin is needed to position the cilia in cells of the ventral node59. Inversin was then shown to localize to different subcellular locations, in a cell cycle dependent manner (Figure 5). Specifically, it is found at the mitotic spindle in mitosis, at the midbody in cytokinesis, and in cilia, at the basal body and centrosome in interphase (Figure 5). All of these subcellular organelles are involved in regulation of planar cell polarity or the cell cycle (see below).

A major breakthrough was made for the understanding of the pathogenesis of renal cystic diseases when Simons et al. demonstrated a role of inversin/NPHP2 in signaling mechanisms of planar cell polarity necessary to maintain normal tubular development and morphology[60] as outlined in Figure 634,[60]. As a consequence of this model, when inversin is defective (as in NPHP type 2) the canonical Wnt pathway will prevail and disrupt apical-basolateral polarity of the renal epithelium[34]. Since planar cell polarity signaling is important for oriented cell

division, it seems logical that Fisher *et al.* recently have been able to demonstrate abnormal orientation of the mitotic spindle in two different rodent models of cystic kidney disease[61].

NPHP3: A Doorstep to Treatment?

By positional cloning we identified mutations in *NPHP3* as responsible for adolescent nephronophthisis in a large Venezuelan kindred[13,24]. We demonstrated that mutations in the murine ortholog *Nphp3* cause the renal cystic mouse mutant *pcy*[24], which was demonstrated to be responsive to treatment with a vasopressin receptor antagonist[62]. Recently, it was shown that complete loss of *Nphp3* function results in *situs inversus*, congenital heart defects, and embryonic lethality in mice[63]. In addition, truncating mutations of *NPHP3* in humans can cause a broad clinical spectrum of early embryonic patterning defects that resembles Meckel syndrome. This included *situs inversus*, polydactyly, central nervous system malformations, structural heart defects, preauricular fistulas, and a wide range of congenital anomalies of the kidney and urinary tract[63].

NPHP4 is Conserved in C. Elegans

Mutations in the novel gene *NPHP4* were identified by homozygosity mapping and total genome search for linkage[23,64,65]. Nephrocystin-4, like inversin, localizes to primary cilia, basal bodies, centrosomes, and the cortical actin cytoskeleton[50] (Figure 4). Nephrocystin-4 is conserved in *C. elegans* and expressed in ciliated head and tail neurons of the nematode[66]. Upon knockdown it exhibits a male mating phenotype, similar to the phenotype found upon knockdown of the polycystin-1 and polycystin-2 orthologs[66]. Localization of nphp-1 and nphp-4 to some of these ciliated neurons also overlaps with localization of the cystoprotein orthologs polycystin-1 (*lov-1*), polycystin-2 (*pkd-2*), and with many orthologs of Bardet-Biedl syndrome (BBS) proteins[66,67] similar to what has been described for *lov-1* and *pkd-2* mutants[68]. These data have been recently refined for specific neuronal cell type[69,70] and the necessity of *nphp-1* and *nphp-4* for morphologic integrity of ciliated neurons in *C. elegans* was demonstrated[71,72]. In addition, a role for *nphp-4* in life span of the worm has been demonstrated[73].

Evolutionary conservation of cystoproteins goes even further: Some cystoproteins have been conserved over more than 1.5 billion years of evolution from the unicellular organism *Chlamydomonas Reinhardtii* to vertebrates. *Ch. Reinhardtii* uses two motor cilia (flagella) for locomotion. Strikingly, nephrocystin-4 and at least six proteins mutated in BBS are conserved in *Ch. Reinhardtii* where they are part of its basal body proteome[67,74]. Defects of cystoprotein orthologs in *Ch. Reinhardtii* have deficient IFT and flagellar propulsion[75].

NPHP5: The Senior-Loken Syndrome Gene

When the novel gene *NPHP5* was identified as mutated in nephronophthisis type 526 all mutations detected were truncations of the encoded protein nephrocystin-5, and all patients had early-onset retinitis pigmentosa (Senior-Loken syndrome, SLSN). Nephrocystin-5 contains an IQ domain, which directly interacts with calmodulin[38], and is in a complex with the retinitis pigmentosa GTPase regulator (RPGR), which when defective causes X-linked retinitis pigmentosa. Both, nephrocystin-5 and RPGR are localized in connecting cilia of photoreceptors and in primary cilia of renal epithelial cells26 (Figure 4). The fact that connecting cilia of photoreceptors are the structural equivalents of primary cilia of renal epithelial cells rendered an explanation for retinal involvement in the retinal-renal syndrome Senior-Loken syndrome.

Cystogenesis: Defective Regulation of Planar Cell Polarity?

NPHP6/CEP290: Centrosomes as a Central Hub for Planar Cell Polarity Regulation

We identified by positional cloning recessive truncating mutations in a novel gene *NPHP6/CEP290* as the cause of NPHP type 6 and Joubert syndrome type 5[27]. Its gene product nephrocystin-6/Cep290[76] is part of the centrosomal proteome[77]. Like NPHP2/inversin (Figure 5) and NPHP4, NPHP6/CEP290 is expressed in centrosomes and the mitotic spindle in a cell-cycle dependent manner. We demonstrated that abrogation of *NPHP6* function in zebrafish causes planar cell polarity defects and recapitulates the human phenotype of NPHP type 6, including renal cysts, retinal degeneration, and cerebellar defects[27]. Nephrocystin-6 modulates the activity of ATF4/CREB2, a transcription factor that may be implicated in cAMP-dependent renal cyst formation[62]. Interestingly, a 300-amino acid in-frame deletion of *NPHP6/CEP290* caused retinal degeneration only, without renal or cerebellar involvement in the *rds16* mouse model[78]. This is in accordance with the recent finding that a hypomorphic mutation of *Nphp6/Cep290* represents the most frequent cause of Leber's congenital amaurosis[79]. Mutations in *NPHP6/CEP290* have been confirmed as causing JBTS with and without renal involvement [39]. Furthermore, truncating mutations in *NPHP6* were shown to cause Meckel-Gruber syndrome[80].

Correct orientation of the mitotic spindle and centrosomes with respect to the longitudinal axis of the tubule is critical for proper apical-basolateral polarity (Figure 7). Non-canonical Wnt signaling (see Figure 6) is involved in these processes in renal tubular morphogenesis, when in rodents postnatally renal tubules still elongate. The structure that would result from disruption of the longitudinal growth would be a dilated tubule or cyst. Recently, evidence was generated for a role of planar cell polarity in renal cystic diseases[61] by measuring orientation of the mitotic spindle through 3-D imaging of renal tubules. Comparison of the distribution of the mitotic angles in wild-type animals and rodent cystic kidney disease models revealed that mitotic angles of two rodent models of cystic kidneys, the HNF1 β-deficient mouse model and the *pck* rat model where clearly different from wild-type littermates[61].

NPHP7/GLIS2: The link to Hedgehog Signaling

Recently, we identified in a Cree Indian kindred mutations in the *NPHP7/GLIS2* gene, encoding the transcription factor Gli-similar protein 2 as the cause of NPHP type 7 (Figure 4) [31]. Starting at 8 weeks of age, *Glis2* mutant mice showed severe renal atrophy and fibrosis resembling human nephronophthisis[31]. Differential gene expression studies on *Glis2* mutant kidneys demonstrated that genes promoting epithelial-to-mesenchymal transition and fibrosis are upregulated in the absence of *Glis2*[31]. Strikingly, there was also prominent apoptosis present in distal tubular segments of the kidney, which might provide an explanation why in PKD kidneys are enlarged with hyperproliferation prevailing, whereas in NPHP kidney size is reduced. GLIS2 is related to the GLI transcription factor these findings implicated the hedgehog pathway in the pathogenesis of cystic kidney diseases (Figure 8). It is a signaling pathway that controls, cell determination and tissue patterning during embryogenesis. The other known role of Hh is the maintenance of stem cells pools in post-embryonic tissues.

NPHP8: A Clinical Spectrum from Meckel Syndrome to Joubert Syndrome

Recently, missense and truncating mutations in the *RPGRIP1L* gene were identified by positional cloning as the cause of a Joubert syndrome-like phenotype (cerebro-oculo-renal syndrome, CORS) and Meckel syndrome (Figure 4)[32]. It was shown that defects in the mouse ortholog *Rpgrip1l* (*Ftm*) recapitulate the cerebral, renal and hepatic defects of CORS and Meckel syndrome. RPGRIP1L colocalized at the basal body and centrosomes with the protein products of both *NPHP6* and *NPHP4*32. RPGRIP1L missense mutations found in CORS individuals diminished the interaction between RPGRIP1L and nephrocystin-4. Missense

mutations were seen in patients with Joubert syndrome32,[40]. These findings confirmed that there is a continuum for the multiorgan phenotypic abnormalities found in Meckel syndrome, Joubert syndrome/CORS, and nephronophthsisis on the basis of distinct mutations of identical genes (multiple allelism).

NPHP9: The Link From Cilia to Cell Cycle Control

We recently identified 3 different highly conserved amino acid changes in the gene *NEK8* (never in mitosis kinase 8) as causing NPHP type 9[81]. One of the mutations identified is positioned in the same RCC1 domain, in which the missense mutation causing the renal cystic mouse model *jck* is positioned[82,83]. Upon expression in medullary collecting duct cells all three mutant forms of *NEK8* showed defects in ciliary and centrosomal localization to varying degrees, supporting the notion that mutations in *NEK8* cause nephronophthisis (type 9)[42] (Figure 4). As NEK8 plays a major role in cell cycle regulation, these data prove a direct link between a protein defective in renal cystic disease and the role of centrosomes for cell cycle regulation (Figure 4). In this context it is interesting that also for polycystin-1 and -2 signaling the renal cystic phenotype has been linked to cell growth regulation. Polycystin-1 expression activates the JAK-STAT pathway, thereby up regulating p21(waf1) and inducing cell cycle arrest in G0/G1[84]. Cell cycle arrest required polycystin-2. Involvement of polycystin-1/2 signaling in the JAK/STAT pathway might explain how mutations of either gene can result in dysregulated growth84. Very recently, involvement of cell cycle regulation in renal cystic disease has been confirmed by demonstration that two mouse models of polycystic kidney disease (*jck* and *cpk*) can be efficiently treated with the cyclin-dependent kinase inhibitor roscovitine[85].

The Ciliary Theory Explains Extrarenal Involvement of Eye, Brain, and Liver in NPHP

A prominent feature of NPHP is involvement of multiple organs (pleiotropy) outside the kidney. Defects in other organs are usually of degenerative or developmental nature (Figure 9). Specifically, NPHP may be associated with tapetoretinal degeneration (Senior-Loken syndrome86,87), cerebellar vermis aplasia (Joubert syndrome88,89), ocular motor apraxia type Cogan90, mental retardation27, liver fibrosis91, or cone-shaped epiphyses of the phalanges (Mainzer-Saldino syndrome92). Infantile NPHP type 225 can be associated with *situs inversus*[15], retinitis pigmentosa[93], or cardiac ventricular septal defect[25]. In some instances there appears to be a genotype/phenotype correlation regarding pleiotropy. For instance, there is involvement of the retina in all known cases with mutations of NPHP5 or NPHP6. In other instances, such as *NPHP1* mutations, the molecular basis of eye involvement is unknown. The pleiotropy of NPHP has now found a potential explanation in the ciliary hypothesis of cystic kidney diseases (Figure 9). The extrarenal organ involvement in NPHP is discussed by organ system as follows:

Retinal Involvement (Senior-Loken syndrome)—The renal-retinal involvement in Senior-Loken syndrome can be explained by the fact that the primary cilium of renal epithelial cells is a structural equivalent of the connecting cilium of photoreceptor cells in the retina[94]. We have shown that nephrocystin-5 and nephrocystin-6 are expressed in the connecting cilia of photoreceptors[38,78].

Cerebellar Vermis Aplasia (Joubert Syndrome)—In Joubert syndrome (JBTS) NPHP is associated with coloboma of the eye, with aplasia/hypoplasia of the cerebellar vermis causing ataxia, and with the inconstant symptoms of psychomotor retardation, and episodic neonatal tachy/dyspnea[88,89,95–97]. The radiographic feature of JBTS on axial magnetic resonance brain imaging is the so-called “molar tooth sign” of the midbrain-hindbrain junction97,[98]. It is due to abnormal axonal decussation (nerve tract crossing) in the corticospinal tract and the superior cerebellar peduncles, as the basis of the motor and behavioral abnormalities of

JBTS[28]. Ocular motor apraxia type Cogan, defined as the transient inability of horizontal eye movements in the first few years of life, may also be associated with JBTS. This symptom has been described in patients with mutations in the *NPHP1*90,[99] ("JBTS4") and *NPHP4*[23] genes. Three different recessive genes, *NPHP1*[89,97,98], *AHI*100,[101] (JBTS type 3), and *NPHP6*[39], 76, have been found mutated in JBTS. Three further loci for JBTS have been identified: *JBTS1* on chromosome 9q34.3[102], *JBTS2/CORS2* on chromosome 11p12-q13.3[103]. In addition, mutations of *NPHP8/RPGRIP1L* can cause JBTS if at least one mutation is not truncating[32,40].

Liver Fibrosis—NPHP and the related disorder Bardet-Biedl syndrome (BBS) can be associated with periductal liver fibrosis (LF)[91,104–106], as has been described for a patient with *NPHP3* mutation24. Patients develop hepatomegaly and moderate portal fibrosis with mild bile duct proliferation. This pattern differs from that of classical congenital hepatic fibrosis, where biliary dysgenesis is prominent, and from hepatic involvement in ARPKD, Arima syndrome (cerebro-oculo-hepato-renal syndrome)107–109, and Meckel syndrome, which appears as bile duct proliferation. Bile duct involvement in these cystic kidney diseases may be explained by the ciliary theory, as the epithelial cells lining bile ducts (cholangiocytes) possess primary cilia.

Brain Malformations (Meckel Syndrome)—Meckel syndrome (MKS) features the association of renal cystic dysplasia with occipital encephalocele, polydactyly, and biliary digenesis (Figure 4). Two recessive genes have been identified, *MKS1*[110] and *MKS3*[111], and another gene locus, *MKS2*[112], has been mapped. Recently, a Meckel-like phenotype has been described for truncating mutations of *NPHP3*[63], *NPHP6/CEP290*[80], and *NPHP8/RPGRIP1L*[32,40]. Within the spectrum of NPHP-associated ciliopathies, MKS is the most severe, leading to perinatal mortality. Consequently, MKS represents the ciliopathy of the group that encompasses defects in most organs, and involvement is most severe and of developmental rather than degenerative nature. For instance, organ defects reveal cystic dysplasia rather than NPHP in the kidneys, microphthalmia of the eyes, bile duct dysgenesis in the liver, the occipital encephalocele in the brain, and bones are involved by postaxial polydactyly[113]. The notion that MKS is at the most pronounced end of the clinical spectrum, is supported by the finding that the presence of two truncating mutations in *NPHP8/RPGRIP1L* causes MKS, whereas one "mild" mutation (missense rather than truncating) may cause the less severe phenotype of JBTS[32]. In addition, the presence of 2 truncating mutations in *NPHP6/CEP290* may cause an MKS-like phenotype (MKS4) [80].

Cardiac Defects and Situs inversus—In a patient with mutation of*NPHP2* we observed a ventricular septal defect as a congenital cardiac malformation[15]. Thus, the role of inversin for left-right axis specification which had been described in mice was confirmed in humans[55,56]. The patient with *situs inversus* also had a cardiac ventricular septal defect, which may be viewed as a "heterotaxy" (left-right orientation) phenotype caused by the same mechanism[114] that lead to *situs inversus* in this patient. We confirmed the phenotypic combination of cystic kidney disease, *situs inversus*, and cardiac septal defect on the basis of *inversin* mutations is observed in humans, mice, and zebrafish[25]. The *PKD2* gene, mutations in which cause autosomal dominant PKD was shown to also represent a gene that regulates left-right axis determination, acting upstream of *Nodal*, *Ebaf*, *Leftb* and *Pitx2*[114,115].

Skeletal Defects—Multiple disease variants that are associated with NPHP include skeletal defects, strongly suggesting a role of primary cilia function in skeletal development. These include Jeune syndrome (asphyxiating thoracic dysplasia)[116–119], Ellis van Creveld syndrome120, RHYNS syndrome (retinitis pigmentosa, hypopituitarism, NPHP, skeletal dysplasia)121, Meckel-Gruber syndrome110,111, and Sensenbrenner syndrome

(cranioectodermal dysplasia)[122,123]. The association of NPHP with cone-shaped epiphyses of the phalanges (type 28 and 28A) is known as *Mainzer-Saldino syndrome*, and occurred in patients who also had retinal degeneration and cerebellar ataxia[92]. Interestingly, mutations in the ortholog of the intraflagellar transport protein IFT80 of *Ch. Reinhardtii* was found to be the cause of Jeune syndrome[124] (Figure 4), which emphasized again the strong evolutionary conservation of "ciliopathy genes".

Bardet-Biedl and Alstrom Syndromes—Bardet-Biedl syndrome (BBS) exhibits renal histology similar to NPHP[125,126] (Figure 4). Positional cloning of recessive genes mutated in BBS has revealed that the molecular relation between NPHP and BBS may lie in coexpression of the respective gene products in primary cilia, basal bodies, or centrosomes of renal epithelial cells[33]. Alstrom syndrome exhibits some phenotypic overlap with BBS (NPHP, retinitis pigmentosa, deafness, obesity, and diabetes mellitus without mental defect, polydactyly, or hypogonadism)[127]. The single underlying recessive gene, *ALMS1*, encodes a novel protein that is a molecular component of the centrosome[77,128–130]. This finding, together with the finding that BBS proteins localize to centrosomes, confirms the role of centrosomal proteins in cystic kidney diseases that are associated with diabetes, obesity and retinitis pigmentosa[131,132]. Obesity, which is part of the clinical spectrum of the ciliopathies BBS and ALMS. Interestingly, in the *Bbs6* knockout mouse model obesity was associated with hyperphagia and decreased activity of the mice[133].

Therapeutic Approaches to NPHP

Currently there is no effective prophylaxis or treatment available for NPHP other than supportive treatment once chronic renal failure has developed, and dialysis and transplantation for terminal renal failure. Gattone *et al.* have recently shown that the renal cystic phenotype of *pcy* mice, which is the equivalent of human NPHP type 3 can be strongly mitigated or even reversed by treatment with the vasopressin V2 receptor antagonist OPC31260[62]. Similar results were obtained using a *pkd2* mouse model[134]. This effect is thought to be mediated by a reduction in intracellular cAMP levels[27]. An important future challenge will be the development of therapies that capitalizes on what we have learnt about the biology of NPHP and other cystic diseases of the kidney.

Acknowledgments

This research was supported by grants from the National Institutes of Health to F.H. (R01-DK69274, R01-DK68306, R01-DK064614). F.H. is the Frederick G. L. Huetwell Professor, a Doris Duke Distinguished Clinical Scientist, and an Investigator of the Howard Hughes Medical Institute.

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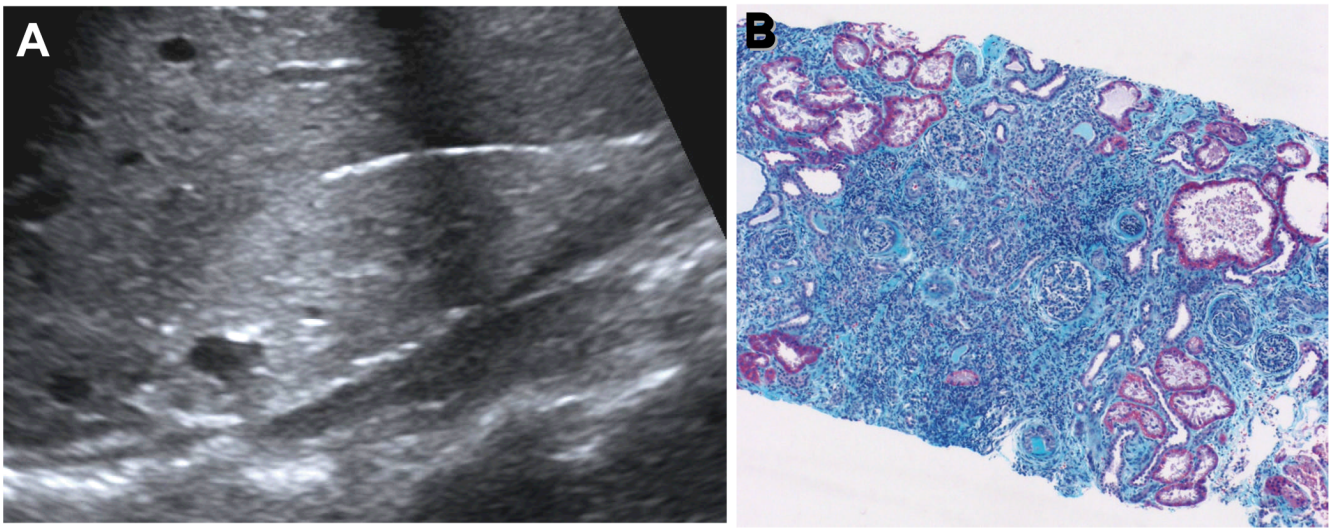


Figure 1. Morphology of nephronophthisis

(**A**) Renal ultrasound demonstrates increased echogenicity, loss of corticomedullary differentiation, and the presence of corticomedullary cysts. In contrast to polycystic kidney disease kidneys are not enlarged. (**B**) Renal histology in NPHP shows the characteristic triad of renal tubular cysts, tubular membrane disruption, and tubulointerstitial cell infiltrates with interstitial fibrosis and periglomerular fibrosis (B is courtesy of D. Bockenhauer, London).

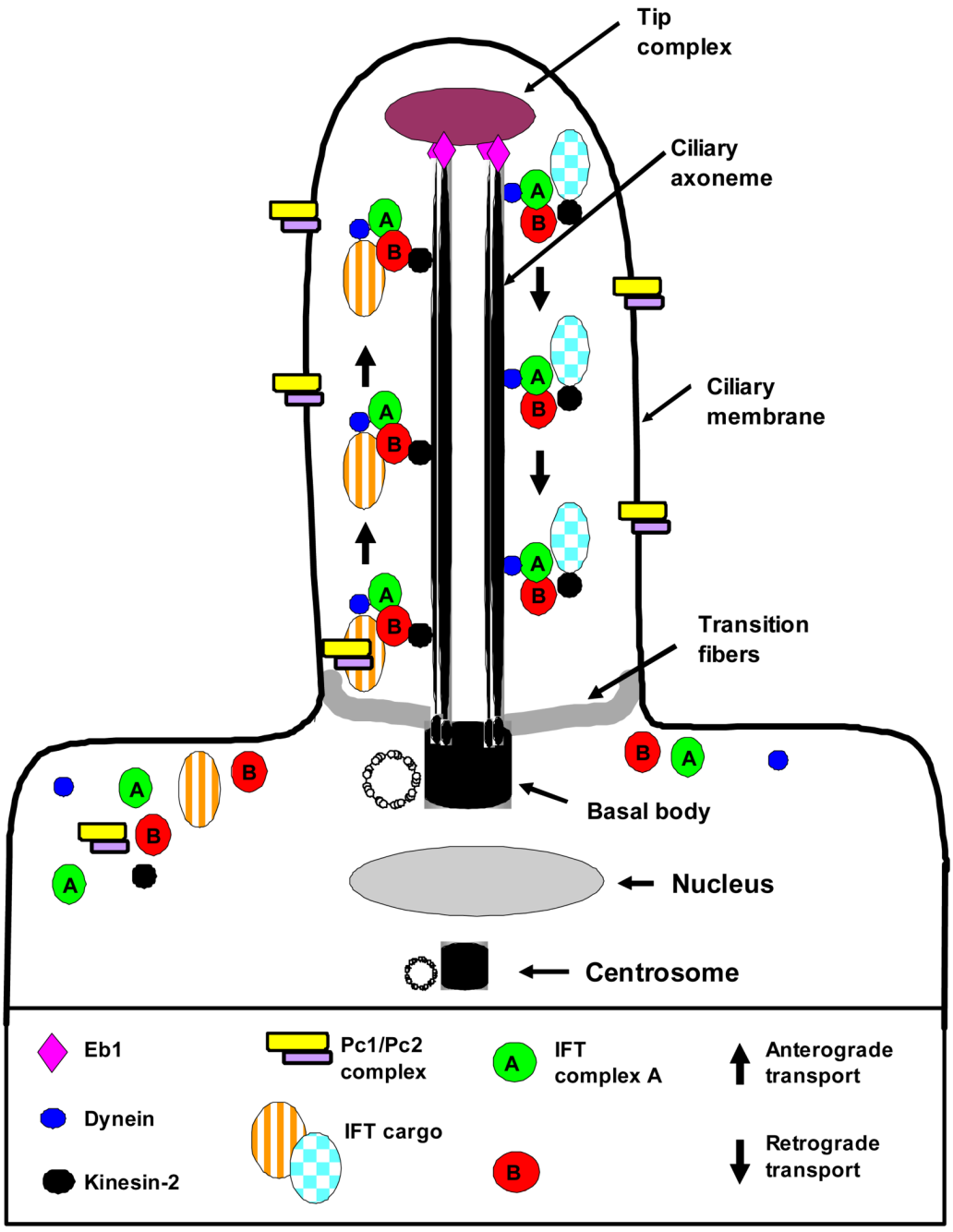


Figure 2. Cilia structure and intraflagellar transport

The cilium is a hair-like structure that extends from the cell surface into the extracellular space. Virtually all vertebrate cell types can produce cilia. Cilia consist of a microtubule-based axoneme covered by a specialized plasma membrane. The axoneme has nine peripheral microtubule doublets. There may be two central microtubules (9+2 *versus* 9+0 axoneme). 9+2 cilia usually have dynein arms that link the microtubule doublets and are motile, while most 9 +0 cilia lack dynein arms and are non-motile ("primary cilia") with a few exceptions. The ciliary axoneme is anchored in the basal body, a microtubule-organizing center derived from the mother centriole. The transition zone at the junction of the basal body acts as a filter for the molecules that can pass into or out of the cilium. Nephrocystin-1 is localized at the transition

zone of epithelial cells51. During ciliogenesis, cilia elongate from the basal body by the addition of new axonemal subunits to the distal tip, the plus end of the microtubules. Axonemal and membrane components are transported in raft macromolecular particles (complex A and B) by so-called intraflagellar transport (IFT) along the axonemal doublet microtubules135. Anterograde transport towards the tip is driven by heterotrimeric kinesin 2, which contains motor subunits Kif3a and Kif3b and a non-motor subunit. Mutations of Kif3a cause renal cysts and cerebellar vermis aplasia in mice136. Retrograde transport back to the cell body occurs via the motor protein cytoplasmic dynein 1B137 (modified from Bisgrove and Yost, 2006) 138.

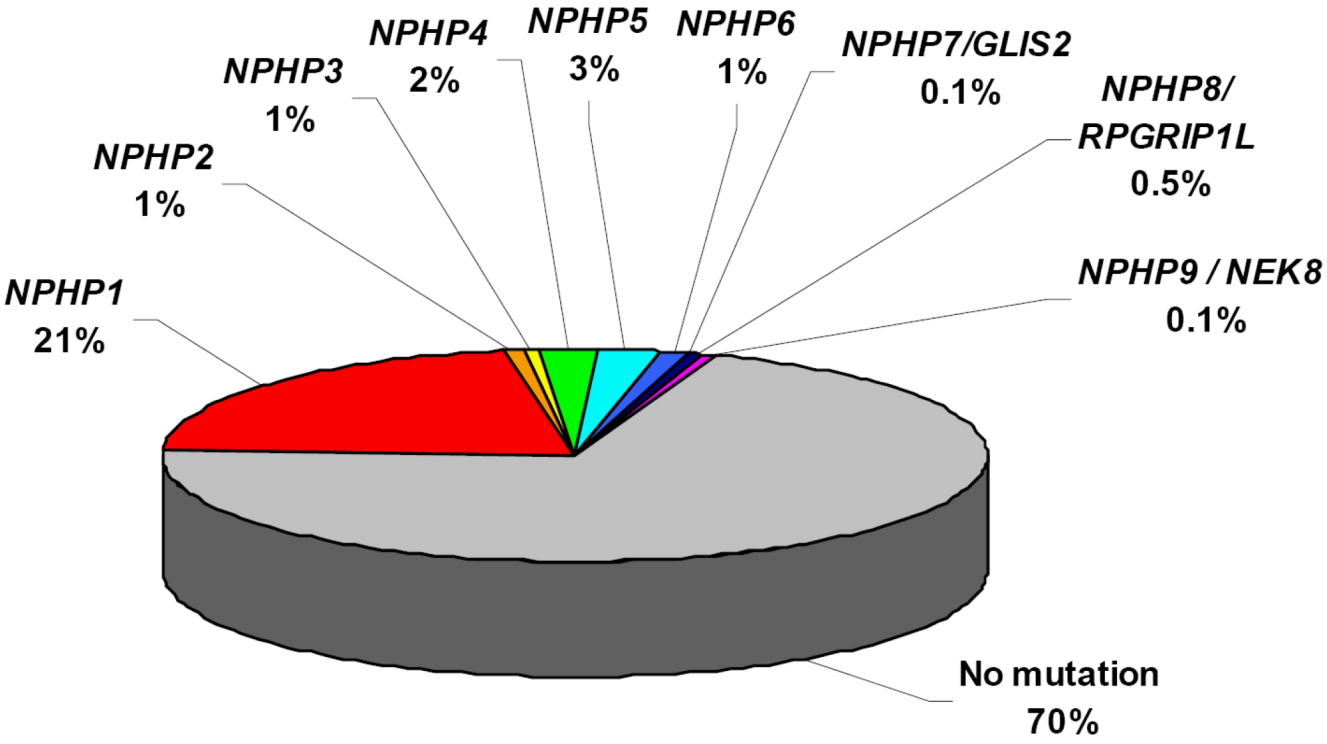


Figure 3. Distribution of causative mutations in the *NPHP1 – NPHP9* genes in a worldwide cohort of 1,079 families with NPHP

Note that, whereas mutations in *NPHP1* account for >21% of NPHP, all other genes are in the range of 3%.

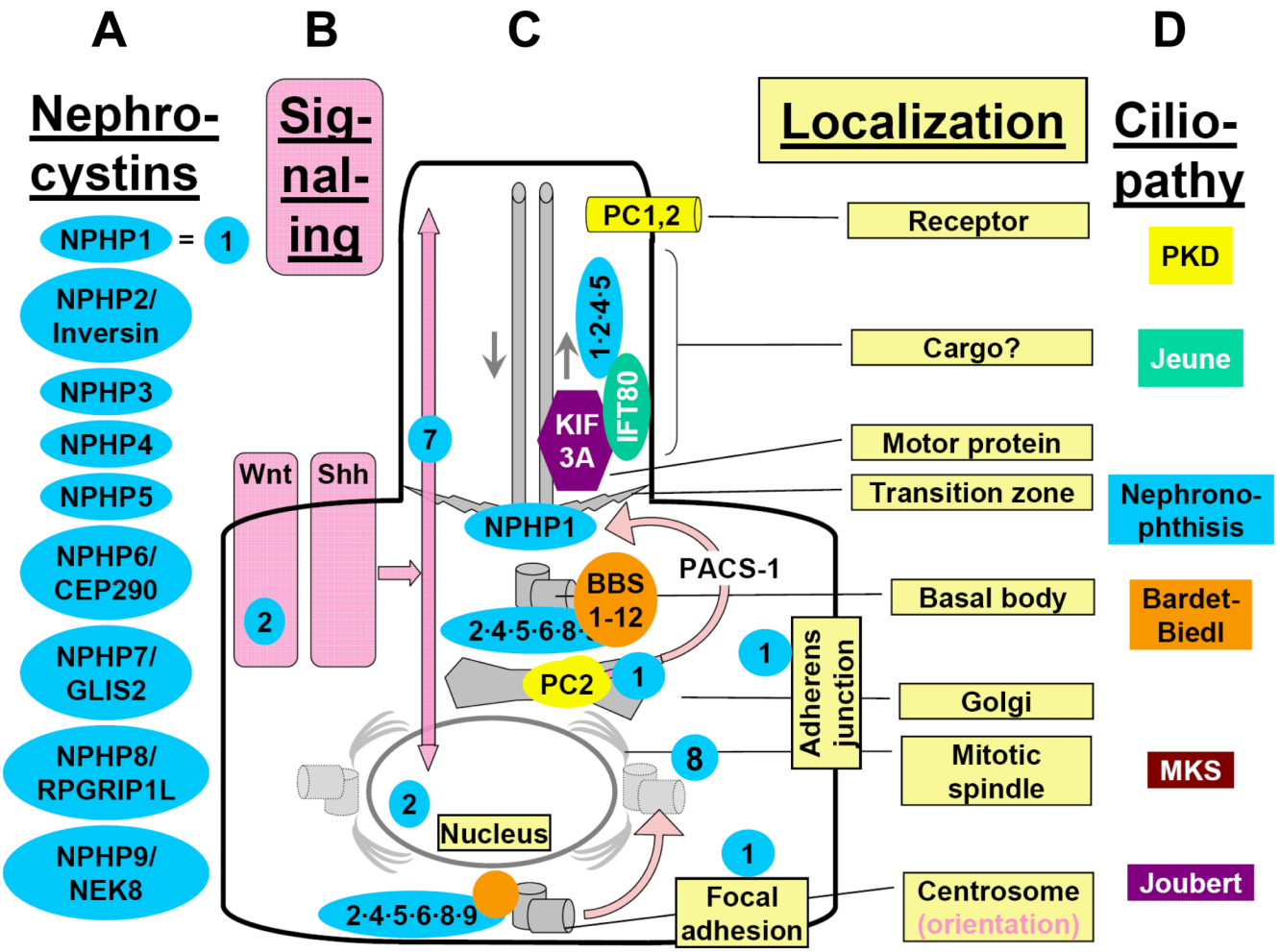


Figure 4. Subcellular localization of nephrocystins to primary cilia, basal bodies, the mitotic spindle, focal adhesions and adherens junctions, and functional interaction with other proteins mutated in renal "ciliopathies"

"Cystoproteins" are proteins of genes mutated in cystic kidney diseases of humans, mice, or zebrafish. Depending on cell cycle stage, cystoproteins are localized at different subcellular organelles (shown in grey)[27,139] including primary cilia, basal bodies, endoplasmic reticulum, the mitotic spindle, centrosomes, adherens junctions or focal adhesions. Arrows in the primary cilium indicate the direction of anterograde transport along the microtubule system mediated by kinesin-2 and retrograde transport by cytoplasmic dynein 1b. **A**) Most nephrocystins (blue) are located at cilia, the basal body, and centrosome in a cell cycle dependent manner. NPHP1 is also at the transition zone, focal adhesions and adherens junctions. **B**) Sensory cilia perceive and process cell external signals, and "cystoproteins" are involved in signaling mechanisms downstream of cilial signal recognition. Downstream of cilia (pink), Wnt signaling (Figure 6) and hedgehog signaling (Figure 8) play a role in planar cell polarity, which is mediated (**C**) partially through orientation of centrosomes and the mitotic spindle poles (Figure 7). **D**) Cilia-dependent mechanisms of planar cell polarity seem to be the central to the pathogenesis of the ciliopathies, the most prominent of which are listed on the right. Wht, the Wnti signaling pathway; Shh, the sonic hedgehog signaling pathway.

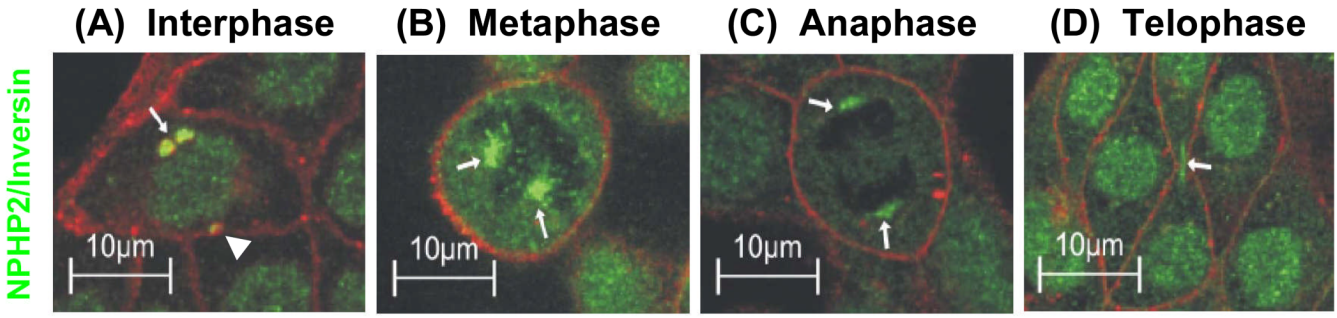


Figure 5. Inversin/NPHP2 localizes to cilia, centrosomes, and the mitotic spindle in a cell cycle dependent manner

Inversin/NPHP2 is found in interphase (**A**) in the cilial axoneme (not shown), close to the centrioles of the basal body complex (arrow) and at the centrosome (arrow head). In metaphase (**B**) and anaphase (**C**) it is at the mitotic spindle (arrows), and in telophase (**D**) at the midbody (arrow) of the separating cells and in the nucleus. This reflects a centriole-associated function of inversin/NPHP2 throughout the cell cycle (from Morgan et al. *Hum Mol Genet* 11:3345, 2002).

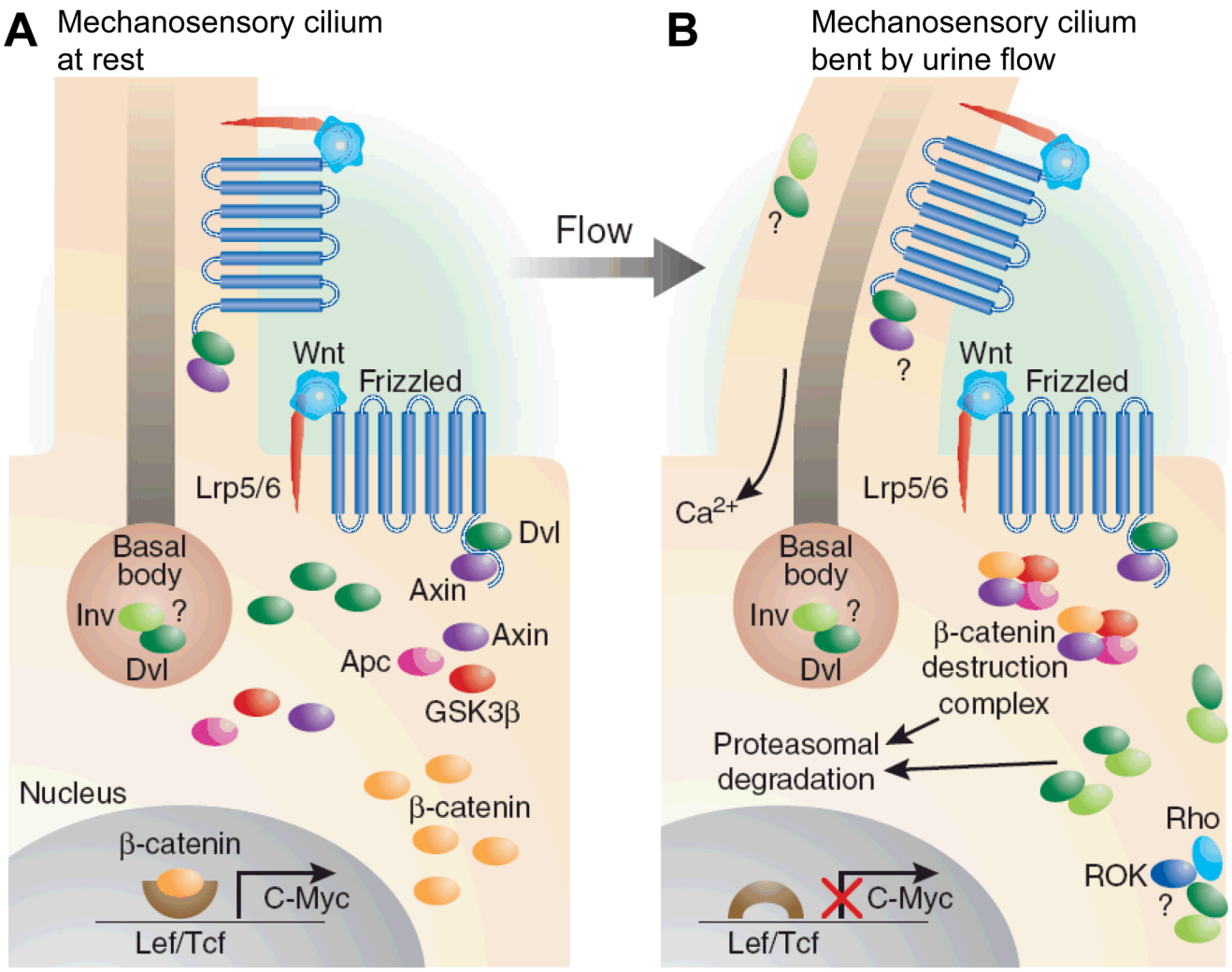


Figure 6. Inversin/NPHP2 mediates a switch from the canonical the non-canonical Wnt signaling pathway, which plays a role in planar cell polarity maintenance[60]

(**A**) This cartoon of arenal tubular epithelial cell shows how Wnt signaling occurs primarily through β-catenin–dependent pathways in the absence of urine flow. Ligand binding by the frizzled receptor results in inactivation of the β-catenin destruction complex through the presence of disheveled (Dvl), increased β-catenin levels, and upregulation of effector gene expression of the canonical Wnt signaling pathway. (**B**) Stimulation of the primary cilium, e.g. by urine flow, results in increased expression of inversin (Inv), which then reduces levels of cytoplasmic Dvl by increasing its proteasomal degradation. This allows reassembly and activation of the β-catenin destruction complex, thereby switching from the canonical to the non-canonical Wnt signaling pathway. The model is consistent with the finding that overexpression of β-catenin (equivalent to canonical Wnt signaling) leads to renal cysts in a mouse model[140] (from: Germino *Nature Genet* 37:455, 2005)[34].

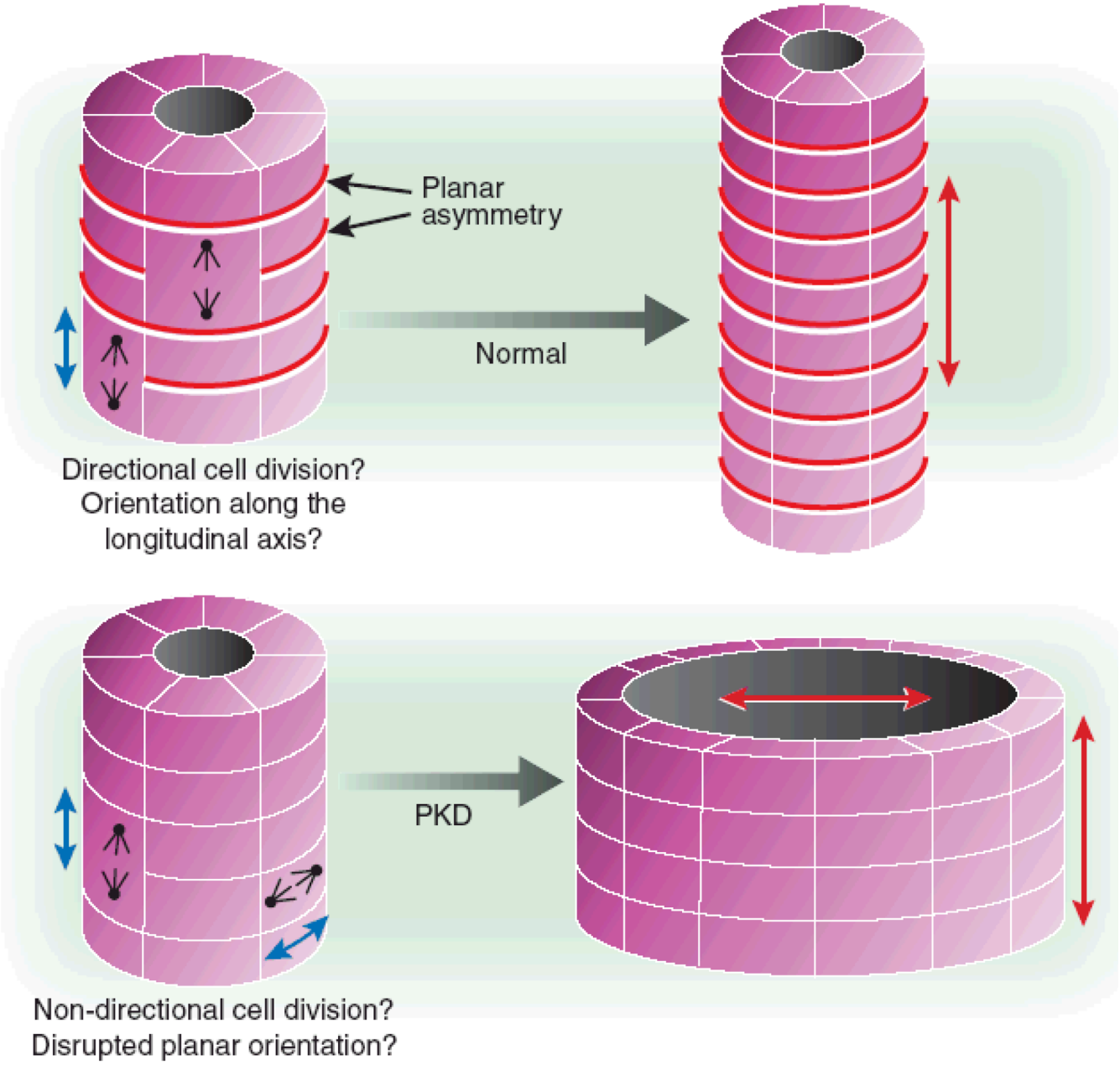


Figure 7. Defects of cystoproteins lead to disruption of planar cell polarity, and thereby to renal cysts through to malorientation of the centrosome or mitotic spindle complex

Correct orientation of the mitotic spindle and centrosomes with respect to the longitudinal axis of the tubule is critical for proper planar cell polarity (i.e., the orientation of an epithelial cell layer in 3-dimensional space). Non-canonical Wnt signaling (see Figure 6) is involved in regulation of planar cell polarity during renal tubular morphogenesis, when in rodents 2 weeks post partum the tubules still elongate. The structure that would result from disruption of this longitudinal orientation is a dilated tubule or cyst (from Germino *Nature Genet* 37:455, 2005).

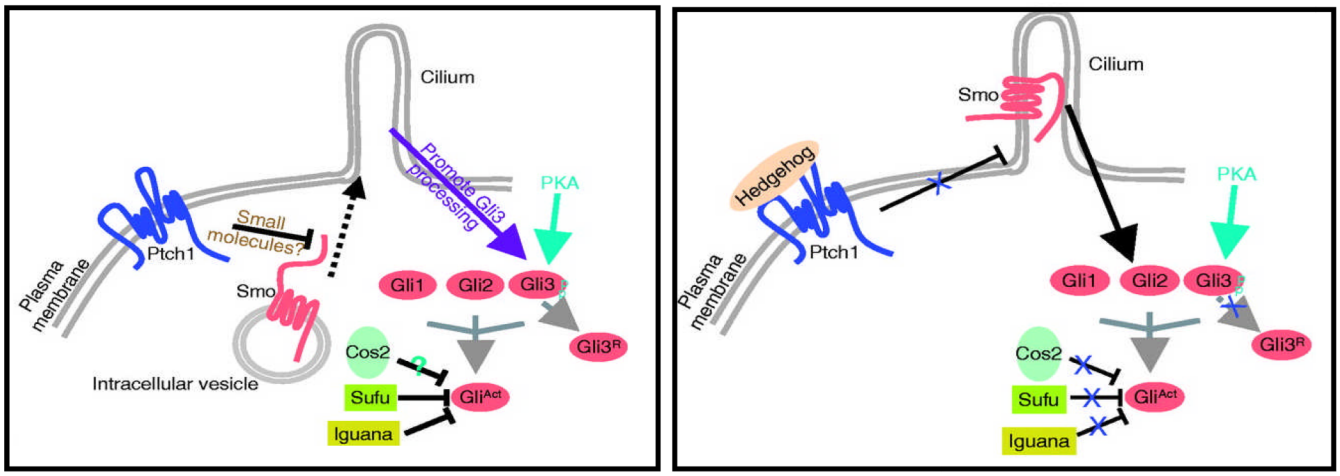
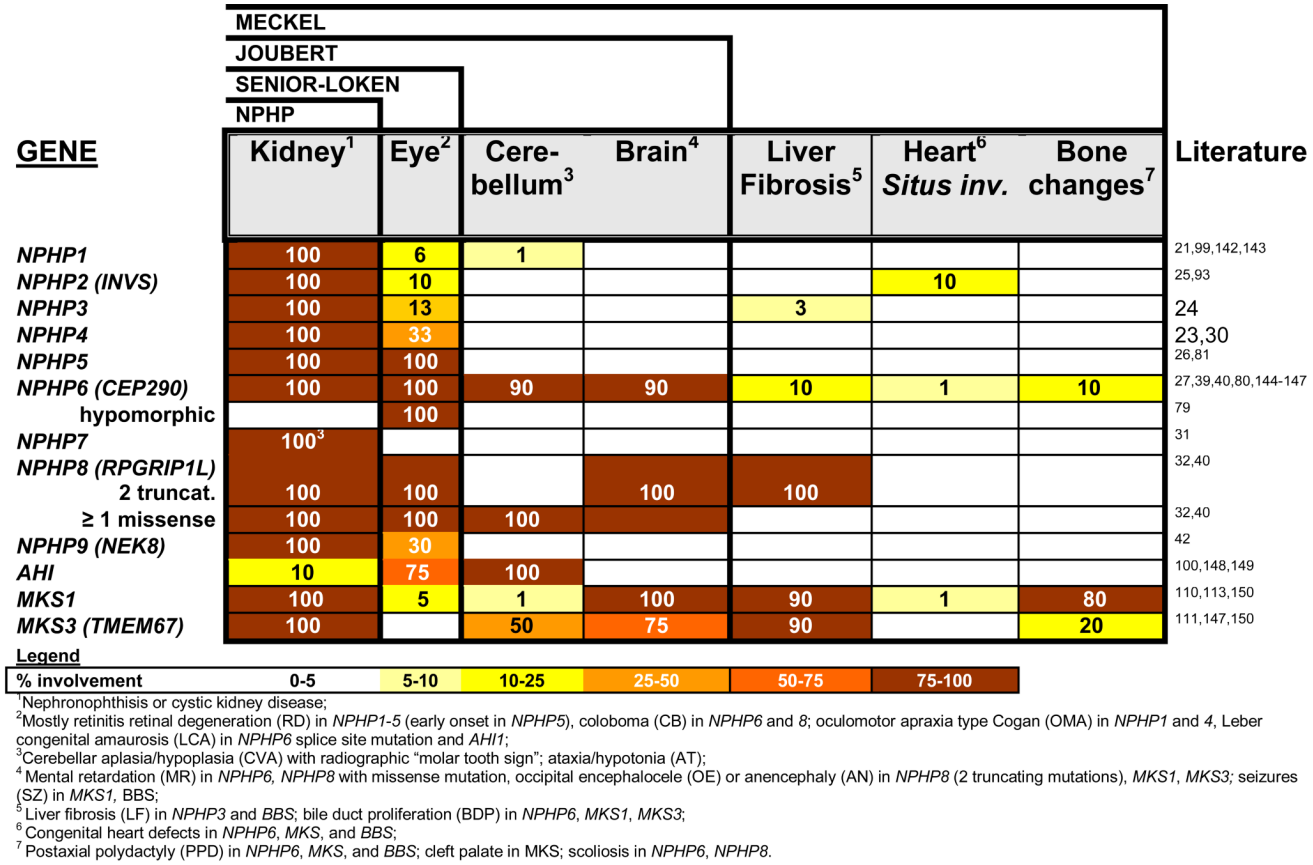


Figure 8. The hedgehog signaling pathway may be involved in renal cystogenesis

In the hedgehog pathway of vertebrates, upon binding of the Shh ligand to the patched (Ptch1) receptor, repression of the smoothened (Smo) receptor to be inserted into the cilium membrane is relieved, and posttranslational modification of the Gli transcription factors within the cilium induces both their activator (Gli^{Act}) and repressor (Gli^{R}) functions. Several studies in mice have demonstrated that ciliary proteins are needed for hedgehog signaling. Recently, the related transcription factor Gli-similar 2 (*GLIS2*) was found to be mutated in NPHP type 7 (from: Huangfu & Anderson *Development*, 2005)[141].



[1]Nephronophthisis or cystic kidney disease;
[2]Mostly retinitis retinal degeneration (RD) in *NPHP1-5* (early onset in *NPHP5*), coloboma (CB) in *NPHP6* and *8*; oculomotor apraxia type Cogan (OMA) in *NPHP1* and *4*, Leber congenital amaurosis (LCA) in *NPHP6* splice site mutation and *AHI1*;
[3]Cerebellar aplasia/hypoplasia (CVA) with radiographic "molar tooth sign"; ataxia/hypotonia (AT);
[4]Mental retardation (MR) in *NPHP6, NPHP8* with missense mutation, occipital encephalocele (OE) or anencephaly (AN) in *NPHP8* (2 truncating mutations), *MKS1*, *MKS3;* seizures (SZ) in *MKS1,* BBS;
[5]Liver fibrosis (LF) in *NPHP3* and *BBS*; bile duct proliferation (BDP) in *NPHP6*, *MKS1*, *MKS3*;
[6]Congenital heart defects in *NPHP6*, *MKS*, and *BBS*;
[7]Postaxial polydactyly (PPD) in *NPHP6*, *MKS*, and *BBS*; cleft palate in MKS; scoliosis in *NPHP6*, *NPHP8*.

Figure 9. Ciliopathies feature a broad spectrum of organ involvement, shown here for the nephronophthisis-related ciliopathies

There is overlap between different syndromes: Exlusive kidney involvement is called nephronophthisis. Associated retinal degeneration in known as Senior-Loken syndrome. Involvement of the cerebellum represents Joubert syndrome. In the most severe form, Meckel syndrome, there are brain malformations, liver fibrosis, heart defects, polydactyly and perinatal mortality associated. It has recently become evident that the spectrum can vary by at least 2 mechanisms: First, multiple allelism, in which a hypomorphic mutation may cause a milder phenotype. For example, a splice site mutation of *NPHP6* may cause Leber congenital optical atrophy (LCA) only. In another example, the presence of one non-truncating mutation in *NPHP8* can rescue the phenotype from Meckel syndrome to Joubert syndrome. Secondly, *NPHP* genes can modify each other. For instance, *NPHP6* and *AHI1* modify recessive *NPHP1* mutations to express a more severe phenotype[147].