Nephronophthisis (NPHP), an autosomal recessive cystic kidney disease, represents the most frequent genetic cause of end-stage kidney disease in the first three decades of life. Contrary to polycystic kidney disease, NPHP shows normal or diminished kidney size, cysts are concentrated at the corticomedullary junction, and tubulointerstitial fibrosis is dominant. NPHP can be associated with retinitis pigmentosa (Senior-Løken syndrome), liver fibrosis, and cerebellar vermis aplasia (Joubert syndrome) in approximately 10% of patients. Positional cloning of six novel genes (NPHP1 through 6) as mutated in NPHP and functional characterization of their encoded proteins have contributed to the concept of “ciliopathies.” It has helped advance a new unifying theory of cystic kidney diseases. This theory states that the products of all genes that are mutated in cystic kidney diseases in humans, mice, or zebrafish are expressed in primary cilia or centrosomes of renal epithelial cells. Primary cilia are sensory organelles that connect mechanosensory, visual, osmotic, and other stimuli to mechanisms of cell-cycle control and epithelial cell polarity. The ciliary theory explains the multiple organ involvement in NPHP regarding retinitis pigmentosa, liver fibrosis, ataxia, situs inversus, and mental retardation. Mutations in NPHP genes cause defects in signaling mechanisms, including the noncanonical Wnt signaling pathway. The “ciliopathy” NPHP thereby is caused by defects in tissue differentiation and maintenance as a result of impaired processing of extracellular cues. Nephrocystins, the proteins that are encoded by NPHP genes, are highly conserved in evolution. Positional cloning of additional causative genes of NPHP will elucidate further signaling mechanisms that are involved, thereby establishing therapeutic approaches using animal models in mouse, zebrafish, and Caenorhabditis elegans.

Nephronophthisis: A Frequent Genetic Cause of Kidney Failure in Children

Renal Involvement

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 three decades of life (1–4). Three clinical forms of NPHP have been distinguished by onset of ESKD: Infantile (5), juvenile (6), and adolescent NPHP (7), which manifest with ESKD at the median ages of 1, 13, and 19 yr, respectively. Initial symptoms are relatively mild (except in infantile NPHP type 2) and consist of polyuria, polydipsia with regular fluid intake at nighttime, secondary enuresis, and anemia (8). At an average age of 9 yr, a slightly raised serum creatinine is noted, before ESKD invariably develops within a few years. Renal ultrasound reveals increased echogenicity. Later, cysts appear at the corticomedullary junction within kidneys of normal or slightly reduced size (Figure 1, A and B) (9). Renal histology reveals a characteristic triad of tubular basement membrane disruption, tubulointerstitial nephropathy, and corticomedullary cysts (Figure 1C) (10,11). In NPHP, cysts arise from the corticomedullary junction of the kidneys (Figure 1, A and B). Because kidneys size is normal or slightly reduced (except in infantile NPHP type 2, in which there is moderate renal enlargement), cysts seem to develop e vacuo by loss of normal tissue. This is in contrast to polycystic kidney disease (PKD), in which cysts are evenly spread out over the entire organ and lead to gross enlargement of the kidneys (4).

NPHP is inherited in an autosomal recessive mode. This includes NPHP variants with extrarenal manifestations (4). NPHP has previously been grouped together with the clinical entity of medullary cystic kidney disease (MCKD) (6,10) because of similarities of clinical and pathologic features (12). Both NPHP and MCKD feature corticomedullary cysts in kidneys of normal or slightly reduced size. However, MCKD is clearly distinct from NPHP regarding multiple aspects: (1) MCKD follows autosomal dominant inheritance, (2) ESKD occurs in the fourth decade and later, and (3) in MCKD there is no extrarenal involvement other than hyperuricemia and gout.

NPHP was first described by Smith and Graham in 1945 (2) and by Fanconi et al. (3), who introduced the term “familial juvenile nephronophthisis.” Since then, >300 cases have been published in the literature (10). In NPHP, the earliest presenting symptoms are polyuria, polydipsia, decreased urinary concentrating ability, and secondary enuresis. They occur in >80% of cases (13) and start at approximately 6 yr of age. Anemia and growth retardation develop later in the course of the disease (8). Regular fluid intake at nighttime is a characteristic feature of the patient’s history and starts at approximately age 6 yr. Because of the mild nature of symptoms and the lack of edema, hypertension, or urinary tract infections, there is often a delay in the diagnosis of NPHP. This causes a risk for sudden death from fluid and electrolyte imbalance. Disease recurrence has
Renal histology in NPHP shows the characteristic triad of renal tubular and glomerular cysts, tubular membrane disruption, and tubulointerstitial cell infiltrates with interstitial fibrosis and periglomerular fibrosis. Adapted from Hildebrandt and courtesy of D. Bockenhauer.

never been reported in kidneys that were transplanted to patients with NPHP (14). By positional cloning, we and others have identified recessive mutations in six different novel genes as causing NPHP: NPHP1 (15,16), NPHP2/inversin (17), NPHP3 (18), NPHP4 (19), NPHP5 (20), and NPHP6 (21), defining NPHP types 1, 2, 3, 4, 5, and 6, respectively. This has made definite molecular genetic diagnostics possible (www.renalgenes.org). Homozygous deletions in the NPHP1 gene account for approximately 25% of all NPHP cases, whereas the other genes contribute <2% each. As expected in a recessive disease, penetrance of the renal phenotype seems to be 100%.

In NPHP, chronic renal failure develops within the first three decades of life (7,22,23). Infantile NPHP, which is characterized by mutations in NPHP2/inversin, leads to ESKD between birth and age 3 yr (17,23). In a study conducted in 46 children who had juvenile NPHP type 1 caused by mutations in the NPHP1 gene, a serum creatinine of 6 mg/dl was reached at a median age of 13 yr (range 4 to 20 yr) (6,22). Similarly, the median age of ESKD in patients with mutations in the NPHP5 gene was 13 yr (20). The median time lapse between a serum creatinine of 2 and 4 mg/dl was 32 mo and between 4 and 6 mg/dl was 10 mo (24). In patients with adolescent NPHP as a result of mutations in the NPHP3 gene, ESKD develops by 19 yr of age (18). If renal failure has not developed by the age of 25 yr, then the diagnosis of recessive NPHP should be questioned and autosomal dominant MCKD considered as a differential diagnosis. In MCKD, which follows autosomal dominant inheritance, ESKD occurs later in life. MCKD types 1 and 2 show a median onset of ESKD at 62 (25) and 32 yr (26), respectively. MCKD type 2 can be positively diagnosed by detection of mutations in the UMOD gene that encodes uromodulin/Tamm-Horsfall protein.

Epidemiology

NPHP has been reported from virtually all regions of the world (13). The incidence of the disease has been given as nine patients per 8.3 million (27) in the United States or one in 50,000 live births in Canada (10,28). Although a rare disorder, it represents the most frequent genetic cause of ESKD in the first three decades of life and is a major cause of ESKD in children, accounting for 10 to 25% of these patients in Europe (13,29–31). In the North American pediatric ESKD population, pooled data indicate a prevalence of approximately 5% of all children with ESKD (32,33).

Extrarenal Manifestations of Eye, Brain, and Liver

NPHP may be associated with tapetoretinal degeneration (Senior-Loken syndrome [SLSN] [34,35]), cerebellar vermis aplasia (Joubert syndrome [JBTS] [36,37]), ocular motor apraxia type Cogan (38), mental retardation (21,39), liver fibrosis (40), or cone-shaped epiphyses of the phalanges (Mainzer-Saldino syndrome [41]). Infantile NPHP type 2 (17) can be associated with situs inversus (17), retinitis pigmentosa (42), or cardiac ventricular septal defect (17). The ciliary theory of NPHP elucidates the pathogenic basis of extrarenal organ involvement regarding retinitis pigmentosa, liver fibrosis, ataxia, situs inversus, and mental retardation.

Retinal Involvement (SLSN)

SLSN, represented by the concomitant occurrence of NPHP with retinitis pigmentosa, was first described by Contreras (43), Senior (34), and Loken (35). Three different terms have been used in the literature to describe the retinal findings of SLSN: Retinitis pigmentosa, tapetoretinal degeneration, and retinal-renal dysplasia. This most likely reflects a spectrum within the pathogenesis that includes developmental defects (dysplasia) as well as defects of tissue maintenance (degeneration) (44). In children with recessive mutations in the NPHP1, 2, 3, and 4 genes, retinitis pigmentosa occurs in approximately 10% of all affected families, without any obvious genotype/phenotype correlation. Whether patients with retinal involvement carry an additional mutation in an unknown modifier gene is an open question. Early-onset and late-onset types of SLSN have been distinguished. The early-onset type seems to represent a form of Leber’s congenital amaurosis, because children exhibit coarse nystagmus and/or blindness at birth or develop these symptoms within the first 2 yr of life (45). It is interesting that patients with mutations in the NPHP5 or NPHP6 genes exhibit early-onset retinitis pigmentosa in all known cases (20,21). Fundoscopic alterations are present in all patients with late-onset SLSN by the age of 10 yr. The late-onset form manifests first with night blindness, followed by development of blindness during school age. Retinal degeneration is characterized by a constant and complete extinction of the electroretinogram, which precedes the development of visual and fundoscopic signs of retinitis pigmentosa (46). The kidney involvement in SLSN is
identical clinically to what is known from patients with NPHP without ocular involvement regarding age of onset, symptoms, and histology of renal disease.

Cerebellar Vermis Aplasia (JBTS)

In JBTS, a developmental disorder with multiple organ involvement, NPHP or cystic dysplasia occurs in association with coloboma of the eye (or retinal degeneration); with aplasia/hypoplasia of the cerebellar vermis causing ataxia; and with the facultative symptoms of psychomotor retardation, polydactyly, ocipital encephalocele, and episodic neonatal tachypnea/dyspnea (36,37,47–49). A pathognomonic diagnostic feature of JBTS on axial magnetic resonance imaging of the brain is the presence of prominent superior cerebellar peduncles, termed the “molar tooth sign” of the midbrain-hindbrain junction (49,50). In patients with the association of JBTS and NPHP, mutations have been described in three different genes, NPHP1 (37,49,50), AHI (51,52) (JBTS type 3), and NPHP4 (21,53). Patients with JBTS have abnormal axonal decussation (crossing in the brain) that affects the corticospinal tract and superior cerebellar peduncles, thereby explaining the motor and behavioral abnormalities (54). Some patients also have abnormal cerebral structure with cortical polymicrogyria (55). Occular motor apraxia type Cogan, defined as the transient inability of horizontal eye movements in the first few years of life, may be associated with JBTS. This symptom has been described in patients with mutations in the NPHP1 (38,56) (“JBTS4”) and NPHP4 (57) genes. It may be due to defects in the nuclei of the abducens nerve, which contain both ipsilaterally projecting motor neurons and contralaterally projecting interneurons, or supranuclear control regions such as the pontine paramedian reticular formation that projects to the abducens and oculomotor nuclei, which has been postulated for other forms of horizontal gaze palsy (58). Defects in axon guidance may be related to defects in renal tubule development by shared signaling pathways (see Signal Mechanisms Relevant for NPHP). Two additional loci for JBTS have been identified: JBTS1 on chromosome 9q34.3 (59) and JBTS2/CORS2 on chromosome 11p12-q13.3 (60).

Liver Fibrosis and Skeletal Changes

NPHP may be associated with liver fibrosis (40,61–63). Patients develop hepatomegaly and moderate portal fibrosis with mild bile duct proliferation. This pattern differs from that of classical congenital hepatic fibrosis, whereby biliary dysgenesis is prominent. A recessive mutation in the NPHP3 gene was recently described in a patient with NPHP and liver fibrosis (18). Hepatic involvement in NPHP type 2 (infantile NPHP) seems to involve only transient elevation of transaminases (23). The association of NPHP with cone-shaped epiphyses of the phalanges (type 28 and 28A), known as Mainzer-Saldino syndrome, was first published by Mainzer et al. (41) in patients who also had retinal degeneration and cerebellar ataxia.

Situs Inversus

The presence of situs inversus was shown in a patient with infantile NPHP and mutations in the NPHP2/inversin gene (17). Therefore, the role of inversin in left–right axis specification that had been described in mice was confirmed in humans (64,65). The patient with situs inversus also had a cardiac ventricular septal defect as a heterotaxy phenotype. This finding was analogous to the randomization of heart looping that was seen in nphp2/inversin knockout experiments in zebrafish. Recently, it has become apparent that products of other genes that are associated with renal cystic disease (in addition to inversin) are important for left–right axis determination of the body plan (66,67). The gene PKD2, mutations in which cause autosomal dominant PKD and that encodes the calcium release channel polycystin-2, had been shown in a Pkd2−/− mouse model to represent a gene that regulates left–right axis determination, acting upstream of Nodal, Ebf1, Left1, and Pitrk2 (68,69).

Other Syndromes with NPHP

Bardet-Biedl syndrome (BBS) exhibits renal histology that is similar to NPHP (70,71). Positional cloning of recessive genes that are mutated in BBS has revealed that the molecular relationship between NPHP and BBS may lie in coexpression of the respective gene products in primary cilia, basal bodies, and centrosomes of renal epithelial cells (72). For BBS, an oligogenic inheritance pattern has been described. This refers to the finding that mutations in more than one BBS gene may be required for full penetrance of some aspects of organ involvement (73).

Further disease variants have been described in association with NPHP, including Jeune syndrome (asphyxiating thoracic dysplasia) (74–77), Ellis van Creveld syndrome (78), RHYN syndrome (retinitis pigmentosa, hypopituitarism, NPHP, and skeletal dysplasia) (79), Alstrom syndrome (retinitis pigmentosa, deafness, obesity, and diabetes without mental defect, polydactyly, or hypogonadism) (80), and Meckel-Gruber syndrome (81,82), which in the case of MKS3 mutations can be allelic with JBTS (83). In Alstrom syndrome, the single underlying gene, ALMS1, encodes a novel protein that contains coiled-coil domains and a putative nuclear localization signal, as well as serine-rich and histidine-rich regions (84,85). ALMS1 forms a part of the centrosome (86,87). This, 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 (88,89). Additional NPHP-associated disorders are Sensenbrenner syndrome (cranioectodermal dysplasia) (90,91) and Arima syndrome (cerebro-oculo-hepato-renal syndrome) (92–94). NPHP has also been described in association with ulcerative colitis (95).

Positional Cloning Reveals Seven Causative Genes for NPHP

Since its first description in 1945 (2,3), the pathogenesis of NPHP had been elusive. Positional cloning revealed novel genes that cause NPHP when mutated. These are monogenic recessive genes, suggesting that mutations in each single one of these genes is sufficient to cause NPHP in a patient who bears these mutations, indicating that their products are necessary for normal kidney function. Positional cloning thereby generated new insights into disease mechanisms of NPHP and demonstrated that they are related to signaling mechanisms of sensory cilia, centrosomes, and planar cell polarity (1,72,96). Seven
NPHP-associated genes have been identified so far: NPHP1 through 6 and AHI1 (15–21,54,55,57) (Table 1).

**NPHP1**

We identified mutations in NPHP1 as causing juvenile NPHP type 1 (15,16). NPHP1 encodes nephrocystin-1, a protein that interacts with components of cell–cell and cell–matrix signaling, including p130Cas (97), focal adhesion kinase 2 (98), tensin, and filamin A and B (99,100). It also interacts with the products of other NPHP genes, such as nephrocystin-2/inversin (17), nephrocystin-3 (18), and nephrocystin-4 (57,101). Nephrocystin-1 localizes to adherens junctions and focal adhesions of renal epithelial cells (99,100), which are involved in cell–cell and cell–basement membrane communications, respectively (101,102) (Figure 2).

**NPHP2**

The renal cystic changes of infantile NPHP (NPHP type 2) combine clinical features of NPHP and of PKD (5). Guided by mapping of a locus for infantile NPHP to chromosome 9q21-q22 (23) and by the observation that a deletion in the inversin (Invs) gene causes renal cystic phenotype in the inv/inv mouse model (64,65), we identified mutations in human inversin (INVS) as the cause of infantile NPHP (type 2) with and without situs inversus (17). Inversin interacts with nephrocystin-1 and with β-tubulin, which constitutes the microtubule axoneme of primary cilium. We demonstrated that nephrocystin-1 and inversin localize to primary cilia of renal tubular cells (17)—the same subcellular compartment that was identified as central to the pathogenesis of PKD (1,66,103). This was one of the first findings to support a unifying theory of renal cystogenesis (1,72), which states that proteins that, when mutated, cause renal cystic disease in humans, mice, or zebrafish (“cystoproteins”) are expressed in primary cilia, basal bodies, or centrosomes (66,72). The interaction and co-localization to cilia 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 determination (17). The functional relationship between ciliary expression of these so-called “cystoproteins” (proteins mutated in cystic kidney disease) and the renal cystic phenotype, however, is still somewhat unclear. One of the first concepts for this relationship proposes that cilia may act as mechanosensors to sense fluid movement in the kidney tubule, where polycystin-1 transmits the signal to polycystin-2, which is a TRP type calcium channel. This would produce sufficient Ca2+ influx to induce Ca2+ release from intracellular storage, which then regulates numerous intracellular signaling activities that are linked to the regulation of cell cycle and planar cell polarity (103). In particular, inversin/NPHP2 function has been implicated in signaling mechanisms of planar cell polarity (see The Wnt Pathway) (104). Okada et al. (105) previously demonstrated that inversin is needed to position the cilia in cells of the ventral node.

**NPHP3**

By positional cloning in a large Venezuelan kindred (7), we identified mutations in NPHP3 as responsible for adolescent NPHP (18). We demonstrated that mutations in the murine ortholog Nphp3 cause the renal cystic mouse mutant pcy (18), which was recently shown to be responsive to treatment with a vasopressin receptor antagonist (106).

**NPHP4**

Mutations in NPHP4 were identified by homozygosity mapping and total genome search for linkage (19,57,107). The encoded protein, nephrocystin-4/nephroretinin, is in complex with other proteins that are involved in cell adhesion and actin cytoskeleton organization, such as nephrocystin-1, p130Cas, Pyk2, tensin, filamin, and α-tubulin. In polarized epithelial cells, nephrocystin-4 localizes to primary cilia, basal bodies, and the cortical actin cytoskeleton, whereas in dividing cells, it localizes to centrosomes (101). Nephrocystin-4 is conserved in Caenorhabditis elegans and expressed in ciliated head and tail neurons of the nematode (108). Upon knockdown, it exhibits a male mating phenotype, similar to orthologs of other genes that are mutated in cystic kidney disease (108).

**NPHP5**

Recently, we identified another novel gene (NPHP5) as being mutated in NPHP type 5 (20). It is interesting that all mutations found were truncations of the encoded protein nephrocystin-5, and all patients had early-onset retinitis pigmentosa (SLSN). Nephrocystin-5 contains two IQ domains, which directly interact with calmodulin (20) and is in complex with the retinitis pigmentosa GTPase regulator, which when defective causes

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**Table 1. Genetics and frequency of extrarenal associations in NPHP**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>AHI1</th>
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<td>NPHP4</td>
<td>NPHP5</td>
<td>NPHP6</td>
<td>AHI1</td>
</tr>
<tr>
<td>Frequency of gene mutation (%)&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1</td>
<td>2</td>
<td>2</td>
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<td>&lt;2</td>
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<tr>
<td>Kidney cysts (%)</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>?</td>
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<td>Retinal degeneration (%)</td>
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<td>15</td>
<td>100</td>
<td>100</td>
<td>?</td>
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<tr>
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<td>10</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>?</td>
</tr>
<tr>
<td>Oculomotor apraxia (Cogan) (%)</td>
<td>1</td>
<td>10</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>?</td>
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</table>

<sup>a</sup>NPHP, nephronophthisis.

<sup>b</sup>Percentage of 976 patients from different families with NPHP-associated disorders as evaluated in the authors’ worldwide cohort. Different frequencies have been reported by Saunier et al. (157).
X-linked retinitis pigmentosa. Both nephrocystin-5 and retinitis pigmentosa GTPase regulator localize to connecting cilia of photoreceptors and in primary cilia of renal epithelial cells (20). The fact that connecting cilia of photoreceptors are the structural equivalents of primary cilia of renal epithelial cells may explain retinal involvement in the retinal-renal syndrome SLSN.

**NPHP6**

Very recently, we identified by positional cloning of recessive truncating mutations in a novel gene NPHP6/CEP290, which encodes a centrosomal protein, as the cause of NPHP type 6 and JBTS type 5 (21). We demonstrated that abrogation of NPHP6 function in zebrafish causes planar cell polarity (convergent extension) defects and recapitulates the human phenotype of NPHP type 6, including renal cysts, retinitis pigmentosa, and cerebellar defects (21). In addition, a defect in cell size control and morphogenesis was found upon nphp6 knockdown in the nonvertebrate Ciona intestinalis. Nephrocystin-6 modulates the activity of ATF4/CREB2, a transcription factor that is implicated in cAMP-dependent renal cyst formation (106). Nephrocystin-6 is expressed in centrosomes and the mitotic spindle in a cell cycle–dependent manner. Its identification establishes a link between centrosome function and tissue architecture in the pathogenesis of cystic kidney disease, retinitis pigmentosa, and central nervous system development. Mutations in NPHP6/CEP290 have been confirmed as causing JBTS with and without renal involvement (21,53). It is interesting that a 300–amino acid in-frame deletion of NPHP6/CEP290 caused retinal degeneration only, without renal or cerebellar involvement in the rds16 mouse model (109). 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 (110).

**AHI1**

Finally, mutations in AHI1 have been detected in patients with JBTS with and without renal involvement (51,52,54,55). Taken together, these findings indicate that the nephrocystin proteins are involved in functions of sensory cilia, cell polarity, and cell division (101).

**Cilia: A Unifying Theory for Cystic Kidney Disease**

The demonstration that nephrocystin-1 and inversin/NPHP-2 localize to primary cilia of renal tubular cells (17) was...
among the first findings to support a new unifying theory of renal cystogenesis (72). This theory states that proteins that, when mutated, cause renal cystic disease in humans, mice, or zebrafish (“cystoproteins”) are expressed in primary cilia, basal bodies, or centrosomes (66,72) (Figure 3).

**Cilia Structure and Function**

The cilium is a hair-like structure that extends from the cell surface into the extracellular space. Virtually all vertebrate cell types have cilia in developing or mature tissue. Cilia consist of a microtubule-based axoneme covered by a specialized plasma membrane. The axoneme has nine peripheral microtubule doublets arranged around a central core. There may be two central microtubules (9 + 2 or 9 + 0 axoneme; Figure 3). 9 + 2 cilia usually have dynein arms that link the microtubule doublets and are motile, whereas most 9 + 0 cilia lack dynein arms and are nonmotile (“primary cilia”). The ciliary axoneme is anchored in the basal body, a microtubule-organizing center derived from the older of the two centrioles. 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 cells (111). During generation of cilia (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 microtubules (Figure 3) (112). Anterograde transport toward the tip is driven by heterotrimeric kinesin 2, which contains motor subunits Kif3a and Kif3b and a nonmotor subunit. Mutations of Kif3a cause renal cysts in mice (113). Kinesins also help form signaling complexes within the ciliary membrane. Retrograde transport back to the cell body occurs via the motor protein cytoplasmic dynein 1B (114).

The unexpected convergence in primary cilia of proteins that underlie cystic kidney diseases is supported by the following findings: (1) there is high evolutionary conservation of the proteins involved (Figure 4, A through D), (2) most of these proteins interact with each other (Figure 4C), and (3) clinical phenotypes are related to tissue-specific expression in the sensory cilia (Figure 4, E through G).

**Cilia: Conserved Modules to Sense Cell External Signals**

It is becoming apparent that primary cilia are highly conserved structures for sensing of a wide variety of extracellular cues by a broad variety of specialized tissues. A common “theme” conserved through evolution seems to suggest that many times when cells are to receive cues from the outside of the cell, they use a primary cilium. A broad range of cues can be received by specific ciliary receptors. These include photosensation (rhodopsin), mechanosensation (polycystin-1 and -2), osmosensation, and olfactory sensation (seven-membrane spanning olfactory receptors). The decision rules by which cells place a specific receptor molecule into the cilium are unknown. In general, it seems that the pathogenesis of ciliopathies is based on an inability of epithelial cells to sense or process extracellular cues (115).

**Figure 3.** Cilia structure and intraflagellar transport (IFT). (A) A typical cillum consists of an axoneme of nine doublet microtubules. Each doublet arises from the inner two microtubules of the basal body microtubule triplets. The axoneme is surrounded by a specialized ciliary membrane that is separated from the cell membrane by a zone of transition fibers. (B) A cross-section of 9 + 2 and 9 + 0 cilia. Cilia are broadly divided into two types on the basis of the presence or absence of a central pair of microtubule singlets in the axoneme (9 + 2 or 9 + 0 structure, respectively). Inner and outer dynein arms, which are usually associated with 9 + 2 cilia, can be present in either type of cilium and are important for ciliary motility. Ciliary assembly and maintenance is accomplished by IFT, which relies on the microtubule motor proteins kinesin 2 and cytoplasmic dynein to transport IFT protein complexes and their associated cargo up and down the length of the cillum (depicted in A). Eb1, end-binding protein 1. Adapted from Bisgrove and Yost (110a).
Evolutionary Conservation of Cystoproteins

For many “cystoproteins,” the renal cystic phenotype is conserved among vertebrates. For example, mutations in inversin lead to NPHP type 2 in humans, mice, and zebrafish (Figure 4) (17). At least two nephrocystins are conserved even in the nematode *C. elegans* by amino acid sequence, functional features, and their expression patterns and knockdown phenotypes: Nphp-1 and Nphp-4 are expressed in head (amphid) and tail (phasmid) neurons, which are ciliated osmosensor neurons of *C. elegans* (Figure 4A) (108,116). Localization of nephrocystin-1 and -4 to some of these ciliated neurons overlaps with localization of the cystoprotein orthologs polycystin-1 (*lov-1*) and polycystin-2 (*pkd-2*) and with many orthologs of BBS proteins (117). Knockdown of *nphp-1* and *nphp-4* leads to impaired male mating behavior (108), similar to what has been described for *lov-1* and *pkd-2* mutants (118). These data have been confirmed and refined for specific neuronal cell type (119,120). In addition, a role for *nphp-4* in the lifespan of the worm has been demonstrated (121). In *C. elegans*, *bbs-7* and *bbs-8* are required for the correct localization/motility of the IFT proteins osm-5/polaris and che-11 (122). A bioinformatics approach based on the ciliary/basal body hypothesis of BBS pathogenesis also helped to identify the cystoprotein BBS5 (123). Using bioinformatic screens for ciliary genes in combination with data from positional cloning, mutations in *ARL6* were identified as being responsible for BBS type 3 (124,125). ARL6, a small GTPase, is specifically expressed in ciliated cells and undergoes IFT.

Evolutionary conservation of cystoproteins goes even further: Some cystoproteins have been conserved over >1.5 billion years of evolution from the unicellular organism *Chlamydomonas reinhardtii* to vertebrates (Figure 4, A and B). *C. reinhardtii* uses two motor cilia (flagella) for locomotion. Strikingly, nephrocystin-4 and at least six proteins that are mutated in BBS are conserved in *C. reinhardtii* where they are part of the basal body components of motile cilia (flagella). A bioinformatics approach based on the ciliary/basal body hypothesis of BBS pathogenesis also helped to identify the cystoprotein BBS5 (123). Using bioinformatic screens for ciliary genes in combination with data from positional cloning, mutations in *ARL6* were identified as being responsible for BBS type 3 (124,125). ARL6, a small GTPase, is specifically expressed in ciliated cells and undergoes IFT.

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body proteome (117,126). Defects of cystoprotein orthologs in *C. reinhardtii* have deficient IFT and flagellar propulsion (127).

**Nephrocystin Complexes**

Many cystoproteins participate in protein–protein interaction complexes (Figure 4C). These interactions may partially explain why mutations in different *NPHP* genes lead to similar phenotypes. Some of the domains that occur in cystoproteins are shared between different proteins. Coiled-coil domains, for example, occur in six of eight proteins that are defective in NPHP-like diseases (nephrocystin-1, -2, -3, -5, and -6 and ALMS1). Nephrocystin-1 is targeted to the transition zone of motile and primary cilia (111), and casein kinase 2–mediated phosphorylation of three critical serine residues within a cluster of acidic amino acids in nephrocystin mediates phosphofurin acidic cluster sorting protein (PACS)-1 binding and is essential for co-localization of nephrocystin with PACS-1 at the base of cilia (128). The basal body/centrosomal expression of proteins that are involved in NPHP has led to identification of mutations in *NPHP6/CEP290* as the cause of NPHP type 6 (JBTS type 5). Its gene product nephrocystin-6/Cep290 (21) is part of the centrosomal proteome (86).

**Extrarenal Organ Involvement by Ciliary Dysfunction**

A prominent feature of NPHP is that in a certain percentage of cases, there can be involvement of multiple organs (pleiotropy) other than the kidney. In some instances, there seems 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 4, E through G). The extrarenal organ involvement in NPHP by organ system is discussed as follows.

**Retinal Degeneration.** The renal-retinal involvement in SLSN 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. We have shown that nephrocystin-5 and nephrocystin-6 are expressed in the connecting cilia of photoreceptors (20,21). In analogy to motor transport along the axoneme of primary cilia in kidney epithelial cells, in the connecting cilia of photoreceptors, cargo is trafficked along microtubule tracks from the photoreceptor inner segment to the outer segment via a motor protein complex that contains kinesin 2 and back to the cell body via a cytoplasmic dynein (Figure 5) (129). In this way, 10 billion molecules of the visual pigment rhodopsin are trafficked up and down the connecting cilia per human retina per day.

**Liver Fibrosis.** Both NPHP and BBS can be associated with liver fibrosis (40), whereas autosomal recessive PKD is associated with bile duct ectasia. Bile duct involvement in these cystic kidney diseases may be explained by the ciliary theory, because the epithelial cells lining bile ducts (cholangiocytes) possess primary cilia (Figure 4, F and G).

**Central Nervous System.** Recent findings suggest that oculomotor apraxia type Cogan (associated with *NPHP1* and *NPHP4* mutations), cerebellar vermis hypoplasia (in JBTS), and mental retardation (in NPHP type 6) may be due to defects in microtubule-associated functions during neurite outgrowth and axonal guidance. Mechanotransport along microtubules plays a role not only in intraciliary but also in axonal transport (130). An example is the motor protein KIF3A, which is mutated in a renal cystic mouse model and also plays a role in axonal transport (113). Because the malformations of the cerebellum that occur in JBTS (37) consist of abnormal “wiring” of decussating (crossing) neurons, impaired axonal outgrowth and axon guidance may be central to the neurologic defects in

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**Figure 5.** Primary (nonmotile) cilia of renal epithelial cells and connecting cilia of retinal photoreceptors are analogous structures. In the primary cilium (A) of renal epithelial cells (B), “cargo” proteins are trafficked along the microtubule tracks from the region of the Golgi stack to the tip of the cilia via the motorprotein kinesin 2 and back down via cytoplasmic dynein 1b. (C) In an analogous manner, approximately 10^9 molecules of the visual pigment rhodopsin are transferred up and down the connecting cilia per human retina per day. Adapted from references (66,129).
Signaling Mechanisms Relevant for NPHP

Nephrocystins and other cystoproteins are expressed in different subcellular compartments in a cell cycle–dependent manner (21,133). These subcellular compartments include focal adhesions, adherens junctions, cilia, basal bodies, centrosomes, and the mitotic spindle (Figure 6). It is still mostly unclear which of these subcellular localizations are most proximal to the pathogenesis of NPHP or other cystic kidney diseases. In relation to these subcellular localizations, many hypotheses on mechanisms of cystogenesis have been put forward. Among them are (1) the mechanosensory hypothesis of renal cilia function, (2) participation in signaling at focal adhesions and adherens junctions, (3) a role in the maintenance of planar cell polarity within the noncanonical Wnt signaling pathway, and (4) a role in centrosome-related functions of cell-cycle regulation. These hypotheses are discussed next (Figure 6).

The Mechanosensory Hypothesis

On the basis of the initial finding that bending the primary cilium elicits Ca\(^{2+}\) influx (134–136), it was shown that cilia can act as mechanosensors to sense fluid movement in the kidney tubule, in cooperation with polycystins. In this model, polycystin-1 transmits the signal to polycystin-2, which is a TRP calcium channel (103). This produces sufficient Ca\(^{2+}\) influx to induce Ca\(^{2+}\) release from intracellular stores, which then regulates numerous signaling activities inside the cell that are linked to cell-cycle regulation. It is thought that defects in cell-cycle regulation may be ultimately responsible for the development of kidney cysts (103). In support of the ciliary hypothesis of cystic kidney diseases, a motor protein of the kinesin II family, KIF3A, which is involved in intracellular transport, was shown to cause a murine renal cystic disease when mutated (113). Kramer-Zucker et al. (137) recently showed that cilia of larval zebrafish kidney tubules have a \(9 + 2\) configuration and are motile. Disruption of cilium structure or motility resulted in pronephric cyst formations, with left–right asymmetry defects. Because many data in support of the ciliary hypothesis are still hard to reconcile with this model; for example, the autosomal dominant variant of NPHP, MCKD2, is caused by mutations in \textit{uromodulin}, which has so far not been detected in cilia, basal bodies, or centrosomes.

Focal Adhesion Hypothesis

When NPHP1 was first identified (15), we proposed a pathogenetic hypothesis that tied in nephrocystin-1 with defects of cell–cell and cell–matrix signaling (102,138). 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 (99,100). This “adherens junction/focal adhesion hypothesis” of NPHP pathogenesis (102,138) recently was partially reconciled with the “cilia/centrosome” hypothesis in an integrative hypothesis by showing that nephrocystin-4/nephroretinin in polarized epithelial cells co-localizes with β-catenin at cell–cell contact sites and to primary cilia, whereas in dividing cells, it localizes to centrosomes (101) (Figure 6, A and B).

The Wnt Pathway

Recent results on inversin/NPHP2 shed light on the mechanosensory hypothesis of bending of primary cilia by tubular flow. They have provided data on downstream signaling events that are necessary to maintain normal tubular development and morphology (104): In this model (Figure 6, C, F, and H) the canonical Wnt signaling occurs primarily through β-catenin–dependent pathways in the absence of tubular flow. Stimulation of the primary cilium by flow, however, increases expression of inversin, which then reduces levels of cytoplasmic disheveled through proteosomal degradation and subsequently switches off the canonical pathway by allowing activation of the β-catenin destruction complex (96). When inversin is defective (as in NPHP type 2), the canonical Wnt pathway will prevail and disrupt apical-basolateral polarity of the renal epithelium (96). Because planar cell polarity signaling is important for oriented cell division, it seems logical that Fisher et al. (139) recently were able to demonstrate abnormal orientation of the mitotic spindle in two different rodent models of cystic kidney disease.

Centrosomes

Nephrocystin-6, which is mutated in JBTS, is a component of the centrosomal proteome (86). In addition, BBS4 is instrumental in recruiting proteins to the pericentrosomal matrix, implicating the centrosome and its relation to cell-cycle control in the pathogenesis of BBS (117,140). The products of the genes mutated in the ciliopathy Meckel-Gruber syndrome MKS1 (81) and MKS3 (82) recently were shown to localize to basal bodies and centrosomes (141) (Figure 6D).

Cell Cycle

A balance between hyperproliferation and apoptosis may play an important role in the pathogenesis of cystic kidney diseases (Figure 6, F and G). For example, whereas in PKD, kidneys are grossly enlarged, in NPHP and BBS, kidney size remains normal and cysts grow at the expense of normal tissue.
Nephrocystins localize to different subcellular compartments in a cell cycle–dependent manner and participate in multiple signaling pathways together with other “cystoproteins” (proteins that are mutated in cystic kidney disease). Functional complexes that play a role in the planar cell polarity (PCP) pathway are highlighted for focal adhesion (A), adherens junction (B), cilium (C), basal body (D), centrosome (E), nucleus (F), mitotic spindle (G), and the Wnt pathways (H). Cystoproteins are shown on colored background and in bold type using blue for nephrocystins (NPHP), orange for BBS proteins, green for polycystins (PKD) and fibrocystin (PKHD1), and yellow for cystoproteins of renal cystic mouse models. Proteins that are not bold have been described in the context of the pathogenesis of cystic kidney disease (e.g., as a binding partner to a bona fide cystoprotein). Associated proteins with no known role in cystic kidney diseases are shown on gray background. Black dots connect proteins that directly interact. (A) Focal adhesions. (1) Nephrocystin-1 directly interacts with the focal adhesion adapter protein p130Cas (“crk-associated substrate”) (99,100,102), which is a major mediator of focal adhesion assembly, binds to focal adhesion kinase, and mediates stress fiber formation (149). Nephrocystin-1 competes for binding to p130Cas with the proto-oncogene products Src and Fyn (99). (2) Nephrocystin-1 is in a protein complex with the focal adhesion proteins Pyk2/Fak2 (focal adhesion kinase 2), tensin (98), and filamin A and B. Its overexpression leads to activation of extracellular signal–regulated kinases 1 and 2 (ERK1 and ERK2) (98). (3) The knockout mouse models for tensin (151) and for the Rho GDia gene (152) both exhibit an NPHP-like phenotype, thereby implicating further proteins of the focal adhesion signaling cascade in the pathogenesis of NPHP-like diseases. (B) Adherens junctions. (1) Nephrocystin-1 co-localizes with E-cadherin and p130Cas to adherens junctions. (2) The C-terminal half of nephrocystin, the “nephrocystin homology domain,” is able to promote nephrocystin self-association and epithelial adherens junctional targeting (100). (3) Disruption of this targeting leads to reduced transepithelial resistance (100). (4) Nephrocystin-4 is in a protein complex with nephrocystin-1, NPHP2/inversin, p130Cas, and Pyk2/Fak2 and has been
(e vacuo). It seems that hyperproliferation may be the predominant mechanism in PKD-like diseases (142), whereas apoptosis is predominant in diseases of the NPHP and BBS group. A role of apoptosis was in fact confirmed in the Bbs2−/− and Bbs4−/− mouse models (143,144). Polycystin-1 and -2 signaling and the renal cystic phenotype may be linked by a function of these proteins in cell growth regulation. Polycystin-1 expression activates the JAK-STAT pathway, thereby upregulating p21(waf1) and inducing cell-cycle arrest in G0/G1 (145). The cell-cycle arrest requires polycystin-2. Involvement of polycystin-1/2 signaling in the JAK/STAT pathway might explain how mutations of either gene can result in dysregulated growth (145). Very recently, this hypothesis was confirmed by demonstration that two mouse models of PKD (jck and cpk) can be efficiently treated with the cyclin-dependent kinase inhibitor (R) roscovitine (146).

NPHP: Defects of Tissue Differentiation and Maintenance

It is striking that in renal cystic diseases (and in the associated extrarenal organ involvement), mutations of monogenic disease genes may lead to developmental defects (dysplasia), in which a structural organ defect is present at birth, but also to degenerative defects (degeneration), in which organ structure and function are normal at birth but deteriorate over time. Examples of this phenomenon from the side of cystic kidney disease are autosomal recessive PKD, in which there is a structural defect of the kidney present at birth, as opposed to NPHP, in which kidneys are normal at birth (with the exception of NPHP2) but degeneration leads to loss of renal function over the course of years. Regarding retinal involvement, NPHP can be associated with the developmental defect of retinal coloboma (lack of retinal tissue) in JBTs but also with the localization to adherens junctions in confluent MDCK cells (101). (C) Primary cilia. Recently, the development of a unifying hypothesis of renal cystogenesis was established (72). This hypothesis states that proteins that, when mutated, cause renal cystic disease in humans, mice, or zebrafish are part of a local subcellular localization to primary cilia, basal bodies, or centrosomes. This applies to polycystin-1 and -2; fibrocystin/polyductin; nephrocystin-1, -2 (inversin), -3, -4, and -5; BBS-associated proteins; cystin; polaris; ALMS1; and many others. Because nephrocystins interact, they are represented here as the “nephrocystin complex.” On the basis of positional cloning, mutations in the inversin gene were identified as causing infantile NPHP (type 2) (17). This established a link between the pathogenesis of NPHP and disease mechanisms of PKD (17), in which nephrocystin-1 interacts with both inversin and β-tubulin. Because β-tubulin is a major component of primary cilia, these led to demonstration of co-localization of all three proteins in the primary renal cilia of epithelial cells (17). The complex also contains NPHP3, which is mutated in adolescent NPHP (type 3) and in the renal cystic mouse model pcy. The ciliary hypothesis of cystic kidney disease was confirmed by revealing that Nphp3 mRNA was expressed in kidney, retinal connecting cilia, ciliated bile ducts, and the node, which regulates left–right body axis in mice (18), and by identifying mutations in nephrocystin-5 (IQCB1), which co-localizes with calmodulin to primary cilia of renal epithelial cells and retinal connecting cilia (20). (D) Basal bodies. Basal bodies are short cylindrical arrays of microtubules and other proteins that are found at the base of cilia and that organize the assembly of the ciliary axoneme. They are analogous to centrosomes. Nephrocystins localize to the transition zone of basal bodies (111). (E) Proteins that are mutated in BBS are components of the basal body transitional zone and are highly conserved in evolution. (E) Centrosomes. For a protein that is mutated in the related disease BBS4, it was shown that BBS4 is instrumental in recruiting proteins (e.g., PCM1) to the pericentrosomal matrix, confirming the role of centrosomal function in the pathogenesis of BBS (116). (F) Transcriptional programs. The transcription factor HNF1β regulates transcription of multiple genes that are mutated in cystic kidney disease–related genes (153). (G) Cell-cycle regulation. The hypotheses of functional involvement of the nephrocystin complex at focal adhesions and adherens junction on the one hand and the ciliary/centrosomal hypotheses on the other hand may be integrated by demonstration that different locations of the complex may predominate in a cell cycle–dependent manner. This is evidenced by the findings that inversin/nephrocystin-2 (17), nephrocystin-4 (101), and nephrocystin-6 (21) expression occurs in a cell cycle–dependent manner. Inversin exhibits a dynamic expression pattern in MDCK cells that show expression at centrosomes in early prophase, at spindle poles in metaphase and anaphase, and at the midbody in cells that undergo cytokinesis (133,154). Nephrocystin-4 was detected in MDCK cells at centrosomes of dividing cells and in polarized cells close at the centrosomes in early prophase, at spindle poles in metaphase and anaphase, and at the midbody in cells that undergo cytokinesis (145). Very recently, this hypothesis was confirmed by demonstration that two mouse models of PKD (jck and cpk) can be efficiently treated with the cyclin-dependent kinase inhibitor (R) roscovitine (146).
degenerative processes of tapetoretinal degeneration/retinitis pigmentosa (21,44,109). The joint occurrence of developmental and degenerative defect may be explained by the fact that many developmental transcriptional programs are reinitiated for mechanisms of tissue maintenance and repair. Because planar cell polarity plays an important role in developmental morphogenesis and also in the regeneration of differentiated tissue, a defect in planar cell polarity may explain both occurrence of cysts during organogenesis and degenerative cystogenesis as it occurs in NPHP.

**Therapeutic Approaches to NPHP**

No effective prophylaxis or treatment is available for NPHP. The only therapeutic options are supportive treatment once chronic renal failure has developed and dialysis and transplantation for terminal renal failure. An important future challenge will be the development of therapies that capitalize on what we have learned about the biology of NPHP and other cystic diseases of the kidney. Gattone et al. (106) recently showed 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. Similar results were obtained using a pdk2 mouse model (147). This effect is thought to be mediated by a reduction in intracellular cAMP levels, a finding that awaits reconciliation with the ciliary/centrosome hypothesis of NPHP. In this context, it is interesting that nephrocystin-6 directly interacts with the transcription factor ATF4/CREB2, which plays a role in the regulation of intracellular cAMP levels (21). Additional therapeutic approaches are being considered for PKD (148).

**Disclosures**

None.

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