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Nephronophthisis

Nephronophthisis (NPHP) is an autosomal recessive kidney disorder characterized by chronic tubulointerstitial nephritis and leading to end-stage renal failure. NPHP as a renal entity is often part of a multisystem disorder and has been associated with many syndromes including Joubert syndrome (and related disorders) and Senior–Loken syndrome. Recent molecular genetic advances have allowed identification of several genes underlying NPHP. Most of these genes express their protein products, named nephrocystins, in primary cilial/basal body structures. Some nephrocystins are part of adherens junction and focal adhesion kinase protein complexes. This shared localization suggests that common pathogenic mechanisms within the kidney underlie this disease. Functional studies implicate nephrocystins in planar cell polarity pathways, which may be crucial for renal development and maintenance of tubular architecture.

In brief

- Nephronophthisis (NPHP) is an autosomal recessive kidney disease leading to end-stage renal failure in children and young adults.
- Key histological findings in the kidney are tubulointerstitial fibrosis, tubular dilatation and cyst formation and tubular atrophy.
- NPHP is often a feature of a multisystem disease that may include retinal dystrophy (Senior–Loken Syndrome) and cerebello-ocular-renal syndromes (Joubert syndrome and related diseases (JSRD)).
- NPHP may present with an early decrease in urinary concentration
- End-stage renal failure (ESRF) typically occurs during early teenage years, with the exception of the rare infantile forms, where there is ESRF before 5 years of age.
- Molecular genetics now may allow easy detection of the most common mutations (involving *NPHP1* and accounting for 25% of all cases).
- NPHP is a 'ciliopathy' as evidence to date implicates the primary renal cilium and basal body apparatus in the pathogenesis of NPHP.
- Patients need regular monitoring of renal and liver function, eye examinations and preparation for renal transplantation, which is the treatment of choice for the renal failure that invariably ensues.

Introduction

Nephronophthisis (NPHP) is an autosomal recessively inherited renal disorder, which leads to progressive renal failure, usually within the first 3 decades of life.[1] Nephronophthisis literally means 'disappearance of nephrons'. Typical ultrasound features include normal or reduced renal size, loss of corticomedullary differentiation and corticomedullary cysts (Figure 1). Renal biopsy findings include tubular atrophy, interstitial fibrosis and tubular basement membrane defects, including abrupt transition between thickening and attenuation or disintegration.[2,3] A rare form of NPHP may lead to end-stage renal failure (ESRF) within 5 years of age and is termed infantile NPHP.[4] This differs from typical NPHP in that there is moderate renal enlargement, histological changes that include cortical microcysts, cystic dilatation of Bowman's spaces and lack of tubular basement membrane disruption.

NPHP is often part of a spectrum of multisystem disease and may not be detected unless appropriate investigations on relevant systems are performed. These disease associations form a very heterogeneous group (Table 1). The most

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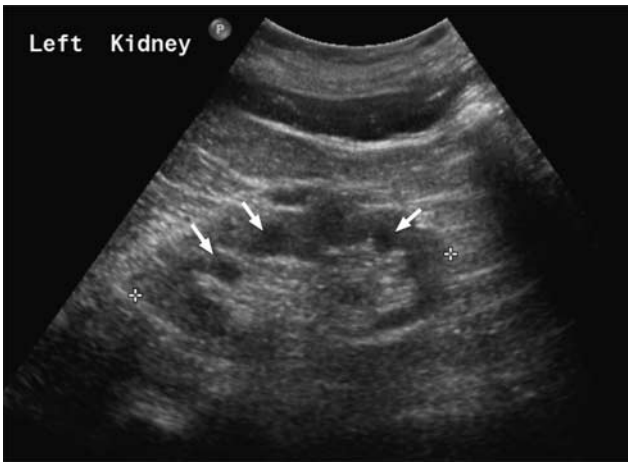

Figure 1 Ultrasound scan features of nephronophthisis. Renal ultrasound scan demonstrating corticomedullary cysts, some of which are arrowed.

commonly associated syndrome is retinal dystrophy and retinal degeneration leading to blindness (Senior–Loken syndrome).[1] Other associations include Joubert syndrome and related diseases (JSRD, reviewed in reference[5]), which often involves a cerebellar, retinal and renal phenotype referred to as CORS (cerebello-oculo-renal syndrome). Apart from these, a whole variety of syndromes have been reported in association with NPHP (Table 1).

NPHP has been reported worldwide, yet the incidence varies. A Canadian study reported an incidence of 1 in 50 000 live births,[6] whereas the incidence in the United States of America was estimated to be 9 per 8.3 million.[7] A more recent European study reported an incidence of NPHP as 1 in 61 800 live births.[8] However, as NPHP may present in adults with late enuresis and renal failure,[9] these figures may be an underestimate.

Clinical overview

Core diagnostic criteria

NPHP is genetically and clinically heterogeneous. Traditionally, NPHP has been subdivided into infantile, juvenile and adolescent forms, based on the age of onset of renal failure. It remains useful to distinguish the much rarer infantile NPHP from the more typical (non-infantile) forms of NPHP, to allow a targeted approach to diagnosis and molecular testing (Figure 2).

Infantile NPHP

1. Early onset ESRF (less than 5 years of age)
2. Possible antenatal presentation with fetal oliguria and oligohydramnios[10]
3. Renal USS – normal sized or enlarged kidneys
4. Renal biopsy – interstitial fibrosis, tubular atrophy, absence of tubular basement membrane irregularity, renal cortical microcysts
5. Associated extrarenal features peculiar to infantile NPHP include hypertension, situs inversus, ventricular septal defect.

Table 1 Syndromes which may exhibit nephronophthisis or are associated with mutations in NPHP genes

Syndrome	*Key features*
Joubert syndrome and related disorders	Cerebellar vermis aplasia/ hypoplasia
Cogan syndrome	Oculomotor apraxia
Senior–Loken syndrome	Retinitis pigmentosa
Meckel–Gruber syndrome	Occipital meningoencephalocoele, cystic kidneys and postaxial polydactyly
RHYNS syndrome	Retinitis pigmentosa, hypopituitarism, nephronophthisis, skeletal dysplasia
Boichis syndrome	Liver fibrosis, biliary duct proliferation
Mainzer–Saldino syndrome or conorenal syndrome	Cone-shaped epiphyses
Jeune syndrome or asphyxiating thoracic dystrophy syndrome	Short ribs
Sensenbrenner syndrome or cranioectodermal dysplasia	Skeletal dysplasia
Ellis van Creveld	Ectodermal dysplasia
Alstrom	Retinal dystrophy, hearing impairment, obesity, type 2 diabetes mellitus
Arima syndrome	Cerebro-oculo-hepato-renal syndrome

NPHP

1. Median onset of ESRF 12 years (may be beyond 25 years)[9]
2. Polyuria and polydipsia (and salt wasting) in early childhood (4–6 years of age)
3. Urinary concentration defect (<400 mosm/kg in early morning urine) that is not responsive to desmopressin
4. Growth retardation (secondary to salt wasting, dehydration and renal insufficiency)
5. Absence of (or minimal) haematuria and proteinuria
6. Renal USS – renal cortical hyperechogenicity, loss of corticomedullary differentiation, corticomedullary cysts[11]
7. Renal biopsy – microscopy typically shows interstitial nephritis, tubular atrophy and tubular dilatations. Typically there is both thickening and attenuation of the tubular basement membranes.
8. The clinical diagnosis of NPHP may be made (or looked for) following detection of an associated extrarenal disorder (see below and Table 2).

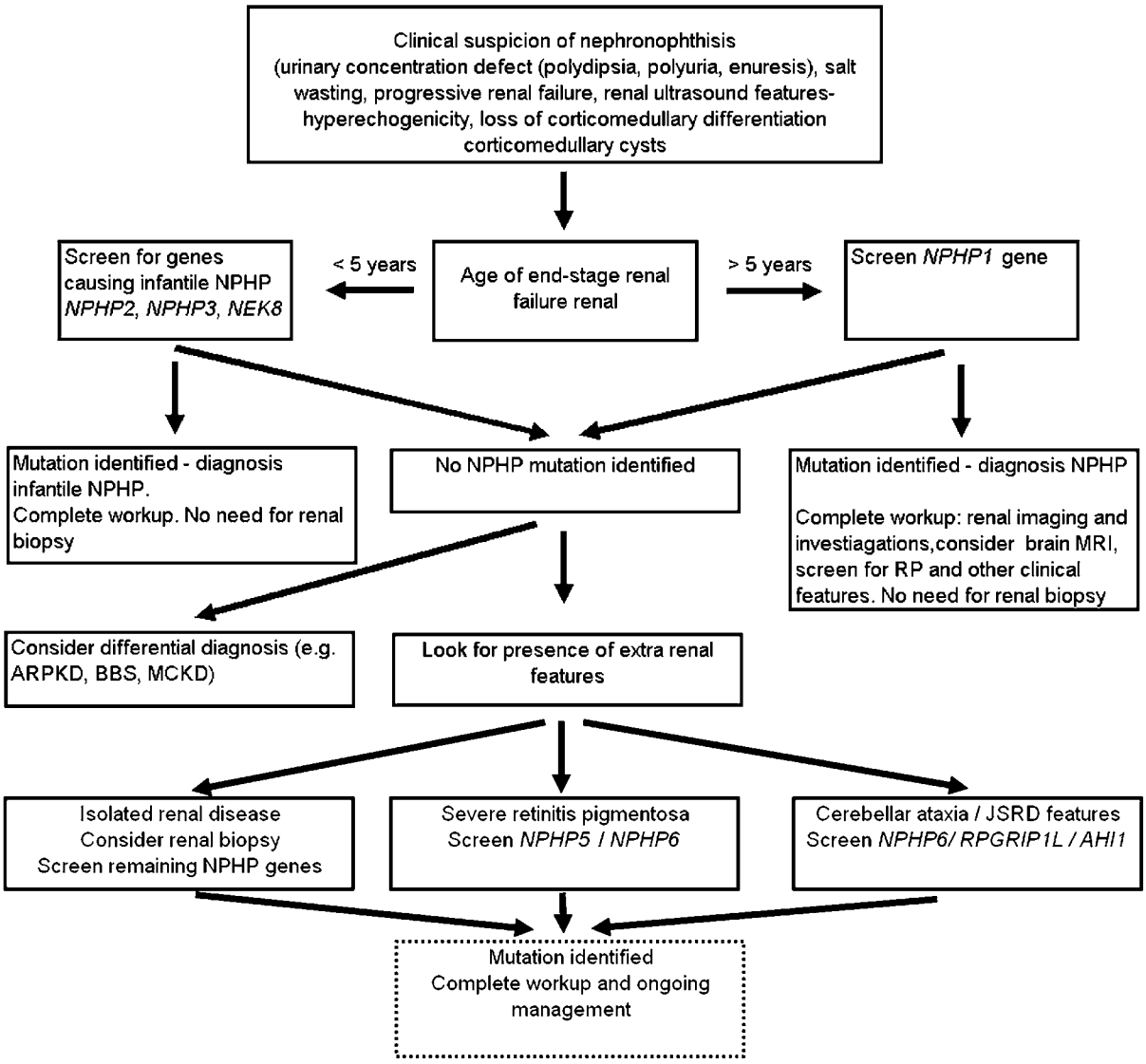

Figure 2 Diagnostic algorithm for NPHP. Where there is clinical or radiological suspicion of NPHP, the *NPHP1* gene should be screened first if onset of end-stage renal failure is greater than 5 years of age. NPHP1 mutations account for ~25% of cases of NPHP. Infantile NPHP is rare (<1% of cases) but should be suspected, and the known genes screened, if there are clinical and radiological features suggestive of NPHP and age of end-stage renal failure is less than 5 years of age. If no mutations are found additional NPHP genes should be screened depending on phenotype and a differential diagnosis of MCKD, ARPKD and BBS should be considered.

Nephronophthisis and disease associations

Many disorders have been described in which NPHP is a clinical feature. Such multisystem features and pleiotropy are typical of 'ciliopathies' such as NPHP. Extrarenal manifestations are seen in 10–20% of cases of NPHP.[12]

Senior–Loken syndrome

Here retinal dysplasia and degeneration (also known as tapetoretinal degeneration or retinitis pigmentosa) may lead to early and severe visual loss (within 2 years of age), resembling Leber's congenital amaurosis (LCA). Later onset forms present initially with night blindness, which progresses to visual loss by the age of 10 years. Diagnosis is made by performing an electroretinogram, which may show abnormalities before the physical signs of retinitis pigmentosa and visual loss. Molecular mechanisms of blindness are secondary to photoreceptor cell defects (reviewed in reference[13]).

Joubert syndrome and related disorders

Joubert syndrome and related disorders (JSRD) are characterized by cerebellar vermis hypoplasia and brainstem

Table 2 Extra renal manifestations associated with NPHP

Eye/retinal disease
Isolated oculomotor apraxia
Retinitis pigmentosa
Coloboma
Nystagmus
Ptosis
Neurological disease
Learning difficulties
Cerebellar ataxia with vermis hypoplasia
Hypopituitarism
Encephalocoele
Liver disease
Elevation of hepatic enzymes
Fibrosis, biliary duct proliferation
Skeletal
Phalangeal cone-shaped epiphyses
Short ribs
Postaxial polydactyly
Skeletal dysplasia
Others
Situs inversus
Cardiac malformations
Bronchiectasis

abnormalities.[5] Brain imaging (MRI) reveals a characteristic appearance of the brain stem known as the 'molar tooth sign'. Typically an affected child will have an irregular breathing pattern in the newborn period and often abnormal eye movements. During infancy hypotonia develops, with ataxia developing late in childhood. Other conditions associated with JSRD include CNS anomalies, ocular coloboma, retinal dystrophy, skeletal defects such as polydactyly, hepatic fibrosis and cystic dysplastic kidneys or NPHP.

Oculomotor apraxia

This is characterized by abnormal eye movements, which include nystagmus and difficulty with saccades (smooth visual pursuits). The transient inability to perform horizontal gaze eye movements in the first years of life is referred to as oculomotor apraxia (OMA) type Cogan and is associated with NPHP gene mutations.[14] Indeed, OMA may be a mild form of JSRD, as cerebellar vermis aplasia has been described in this condition.[15]

Skeletal defects

A variety of associated skeletal defects have been reported, the most frequent are cone-shaped epiphyses.[16,17] Scoliosis due to poor muscle tone (as part of a JSRD syndrome) and polydactyly (postaxial, most commonly) may also occur.

Cardiac defects

Situs inversus and other structural heart defects (cardiac ventricular sepal defect) have been reported in association with infantile NPHP.[18,19]

Other rare associations

Other syndromes that include NPHP have been described. These include Ellis van Creveld syndrome,[20] RHYNS (retinitis pigmentosa, hypopituitarism and skeletal dysplasia),[21] Alstrom syndrome, COACH syndrome, Jeune syndrome and Arima syndrome (Table 1).

Meckel–Gruber like syndrome

The association of occipital encephalocoele, polydactyly and ductal proliferation in the portal area of the liver and cystic kidney dysplasia is known as Meckel–Gruber syndrome (MKS). Recently, mutations in some of the genes implicated in NPHP/JSRD have been found in patients with MKS.[18,22–24] This broadens the phenotypic spectrum of diseases associated with NPHP gene defects and implies a common pathogenesis.

Diagnostic approaches

Initial evaluation

Diagnosis relies on a clinical suspicion of the disorder. NPHP should initially be investigated non-invasively.

Key features would include:

History

(i) polyuria and polydipsia, enuresis.
(ii) Complications of renal insufficiency/renal failure such as nausea, vomiting, itch, fatigue (anaemia), growth retardation.
(iii) Family history of renal disease (autosomal recessive pattern, consanguineous families)

Examination

(i) Blood pressure
(ii) Extrarenal manifestations such as retinal pigmentation, abnormal eye movements and polydactyly.

Investigations

(i) Urine dipstick (minimal proteinuria (<0.5 g/l) and minimal haematuria is typical).
(ii) Early morning urine to assess urinary concentration.
(iii) USS of abdomen and kidneys to assess renal size, to look for corticomedullary cysts, corticomedullary differentiation, to exclude renal tract dilatation and to examine for liver fibrosis/splenomegaly.
(iv) MRI scan and full neurological evaluation to assess cerebellar function if neurological symptoms.

(v) A baseline ophthalmological examination is essential to look for minor degrees of coloboma, retinopathy, OMA. Visual evoked potential studies may be performed in newborn children. ERG studies may be performed from 8 months of age.
(vi) Blood tests: renal function (urea, creatinine), liver function (albumin, transaminases, bilirubin), full blood count (to look for renal anaemia) and clotting studies (prothrombin time as a marker of liver function and before renal biopsy, if necessary). If renal failure is advanced, screening for renal osteodystrophy, hyperparathyroidism and metabolic acidosis should be performed.

Genetic testing Following appropriate genetic counselling, homozygous or heterozygous *NPHP1* deletion (found in around 25% of cases) can be screened easily by PCR. Other NPHP genes may be tested by direct sequencing (see http://www.orpha.net for a list of laboratories). A renal biopsy should not be necessary if a molecular genetic diagnosis can be made. If a molecular diagnosis is not available, a renal biopsy may be required to confirm or exclude NPHP (Figure 2).

ESRF and disease management Preparation for ESRF (renal replacement therapy) and consideration for renal transplantation should be undertaken during subsequent reviews of the patient, once a diagnosis has been made. NPHP does not recur in transplanted kidneys. Living-related kidney donation from unaffected family members, including heterozygous carriers (eg parents), is possible following clinical evaluation. Referral to the Joubert Syndrome Foundation (http://www.joubertsyndrome.org/) and other support organizations for families of children with disabilities (eg http://www.cafamily.org.uk/services.html or http://www.orpha.net) may be appropriate.

Differential diagnosis of NPHP

NPHP should not be confused with autosomal dominant polycystic kidney disease (ADPKD) which is characterized by bilateral, multiple renal cysts resulting in kidney enlargement over time, with extrarenal manifestations which include simple liver cysts, which arise from the biliary epithelium.

NPHP should be distinguished from medullary cystic kidney disease (MCKD), which shares pathological appearances at the macroscopic and microscopic level. However, unlike NPHP, MCKD is inherited in an autosomal dominant pattern, and the age of ESRF is usually later. Two different variants of MCKD are known, MCKD1 (gene remains unidentified) and MCKD2 (secondary to *UMOD* mutations), with a median onset of ESRD at 62 and 32 years,[25] respectively. In contrast to NPHP, the only extrarenal manifestation of MCKD is the occurrence of hyperuricaemia and gout.[25]

Given the antenatal/early childhood onset of renal disease in infantile NPHP, care must be taken to exclude autosomal recessive polycystic kidney disease (ARPKD; Figure 2). Like NPHP, ARPKD may present at a wide age distribution, from antenatally to adulthood. Antenatal ultrasound scanning may reveal markedly enlarged kidneys with increased echogencity. Kidney microcysts and fusiform dilation of collecting ducts are typical of ARPKD. Liver involvement is always present in ARPKD and may be the predominant clinical feature, with dilated intrahepatic bile ducts, liver fibrosis and portal hypertension. The gene defect is in the *PKHD1* gene, encoding its protein product fibrocystin (or polyductin).[26]

Finally, Bardet–Biedl syndrome (BBS) must be considered in the differential diagnosis of NPHP (Figure 2). BBS is another ciliopathy affecting multiple organ systems.[27] Clinical features may include obesity, learning difficulties, genitourinary tract malformations and limb deformities.[28] Renal lesions may include renal cysts, dysplasia, concentrating defects and progressive renal failure.[28] Histologically, cystic dilatation of the renal collecting ducts have been described,[29] reminiscent of infantile NPHP.

Molecular and genetic basis of NPHP

There are a growing number of genes implicated in NPHP. These will be briefly reviewed in terms of their phenotype, frequency and most common disease associations. NPHP is largely inherited as an autosomal recessive disease with homozygous single gene mutations/deletions or compound heterozygous mutations occurring in a single NPHP gene. This usually allows a molecular diagnosis and accurate genetic counselling to be performed. However oligogenicity, where allelic variants at multiple loci contribute to disease, has been documented for NPHP.[30] Likewise, additional NPHP gene mutations may modulate the phenotype in an epistatic way.[31] Thus a wide spectrum of clinical variants with any mutant gene(s) is possible (Table 3). The encoded NPHP proteins, called nephrocystins, typically posses multiple domains (Figure 3).

NPHP1 and nephrocystin-1

NPHP1 was the first NPHP gene identified, using positional cloning strategies in consanguineous families.[32,33] Homozygous deletions of ~250 kb DNA in the region 2q13 are the most frequent genetic abnormality found.[34] Other mutations include compound heterozygosity for the *NPHP1* gene deletion combined with a single point mutation in the *NPHP1* gene. *NPHP1* mutations account for about 25% of cases of NPHP. *NPHP1* mutations may be associated with congenital OMA type Cogan[14] and Senior–Loken syndrome[35] and also give rise to JSRD phenotypes.[31,36]

NPHP1 encodes a protein product named nephrocystin-1. Nephrocystin-1 has been localized to the primary renal cilium[19] and to epithelia cell adherens junctions.[37,38] More recently, the primary cilial localization has been refined to the transition zone (at the ciliary base) in renal and respiratory epithelia and to the connecting cilia in photoreceptor cells.[39] Targeting of nephrocystin-1 to the transition zone of the cilia is dependent on casein kinase 2 phosphorylation and an interaction with PACS-1.[40] Nephrocystin-1 also interacts with other nephrocystins (Nephrocystin-2, -3, -4 and Jouberin[16,41–44]) and there is evidence that this complex of proteins may function in multiple intracellular locations including the cilium, cell–cell adherens junctions and at focal adhesions.[19,37,38,44,45] Within the human kidney nephrocystin-1 is expressed in renal collecting ducts.[44]

INVS/NPHP2 and inversin

Mutations in *INVS/NPHP2* give rise to infantile NPHP.[19] These mutations are rare and account for $<1\%$ of all cases of NPHP worldwide. The gene encodes the protein named inversin, which has a dynamic distribution during cell cycle[46] and is expressed in renal cilia.[19,46,47] *INVS* mutations may cause situs inversus in affected patients, and knockout animals mimic the human disease, with large cystic kidneys at an early age, situs inversus and hepatobiliary malformations.[48] Retinitis pigmentosa is an uncommon but reported association with *INVS* mutations.[49] Inversin seems to play a crucial role in Wnt signalling, acting as a switch between canonical and non-canonical Wnt signalling pathways[50,51] and is required for convergent extension movements.[50] This suggests that inversin plays a role in the developing nephron and in maintenance of the tubular architecture. This coordinated ability of epithelial cells to divide and reorganize themselves to form and maintain tubular structures relies on planar cell polarity (PCP) signalling. PCP signalling is mediated via proteins associated with the primary cilia/basal body complex, such as inversin[50] and its disruption may underlie the pathophysiology of cyst development.[51]

NPHP3 and nephrocystin-3

Mutations in *NPHP3* can produce diverse phenotypes. Mutations were originally identified in a large Venezuelan kindred who exhibited NPHP.[16] Mutations in *NPHP3* were associated with hepatic fibrosis and retinal degeneration in some affected individuals.[16] Recently the phenotype of *NPHP3* mutations has been expanded to include Meckel–Gruber like syndrome.[18]

NPHP3 encodes nephrocystin-3, which interacts with nephrocystin-1[16] and inversin,[18] and can inhibit canonical Wnt signalling. A mouse model of NPHP type 3, named *pcy*, displays cystic kidney disease which responds to treatment with the aquaretic agents/vasopressin-2-receptor antagonists.[52]

NPHP4 and nephrocystin-4 (alias nephroretinin)

NPHP4 encodes nephrocystin-4 (alias nephroretinin), a highly conserved protein which interacts with nephrocystin-1.[42] Nephrocytsin-4 complexes with α-tubulin and localizes to the primary cilium and basal bodies.[41] *NPHP4* mutations may cause isolated NPHP, NPHP with RP and NPHP with OMA.[53] Recently, nephrocystin-4 has been reported to interact with RPGRIP1L.[24,54]

NPHP5 and nephrocystin-5

The *NPHP5/IQCB1* gene encodes nephrocystin-5. This protein contains two IQ calmodulin binding sites, which surround a coiled-coil domain. Similar to inversin, nephrocystin-5 interacts directly with calmodulin via its

Table 3 Genetics defects underlying NPHP, associated features and other clinical phenotypes

Gene (protein)	*Chromosome*	*NPHP type*	*Clinical features associated with NPHP*	*Other clinical phenotypes*
NPHP1 (nephrocystin-1)	2q13	NPHP	Mild JS, mild RP, Cogan	JS
NPHP2/INVS (inversin)	9q31	Infantile NPHP	RP, liver fibrosis, situs inversus, hypertension, VSD	
NPHP3 (nephrocystin-3)	3q22	NPHP, Infantile NPHP	Liver fibrosis, RP, situs inversus	MKS
NPHP4 (nephrocystin-4 or nephroretinin)	1p36	NPHP	Cogan, RP	
NPHP5/IQCB1 (nephrocystin-5)	3q21	NPHP	Severe RP	
NPHP6/CEP290 (nephrocystin-6/CEP290)	12q21	NPHP	JS, severe RP	Isolated RP, JS, MKS, BBS
NPHP7/GLIS2 (GLIS2)	16p	NPHP		
NPHP8/RPGRIP1L (RPGRIP1L)	16q	NPHP	JS	JS, MKS
NPHP9/NEK8 (NEK8)	17q11	NPHP, Infantile NPHP		
AHI1 (Jouberin/AHI1)	6q23	NPHP	JS, RP	JS

JS, Joubert syndrome type B; RP, retinitis pigmentosa; Cogan, oculomotor apraxia type Cogan; MKS, Meckel–Gruber syndrome; VSD, ventricular septal defect.

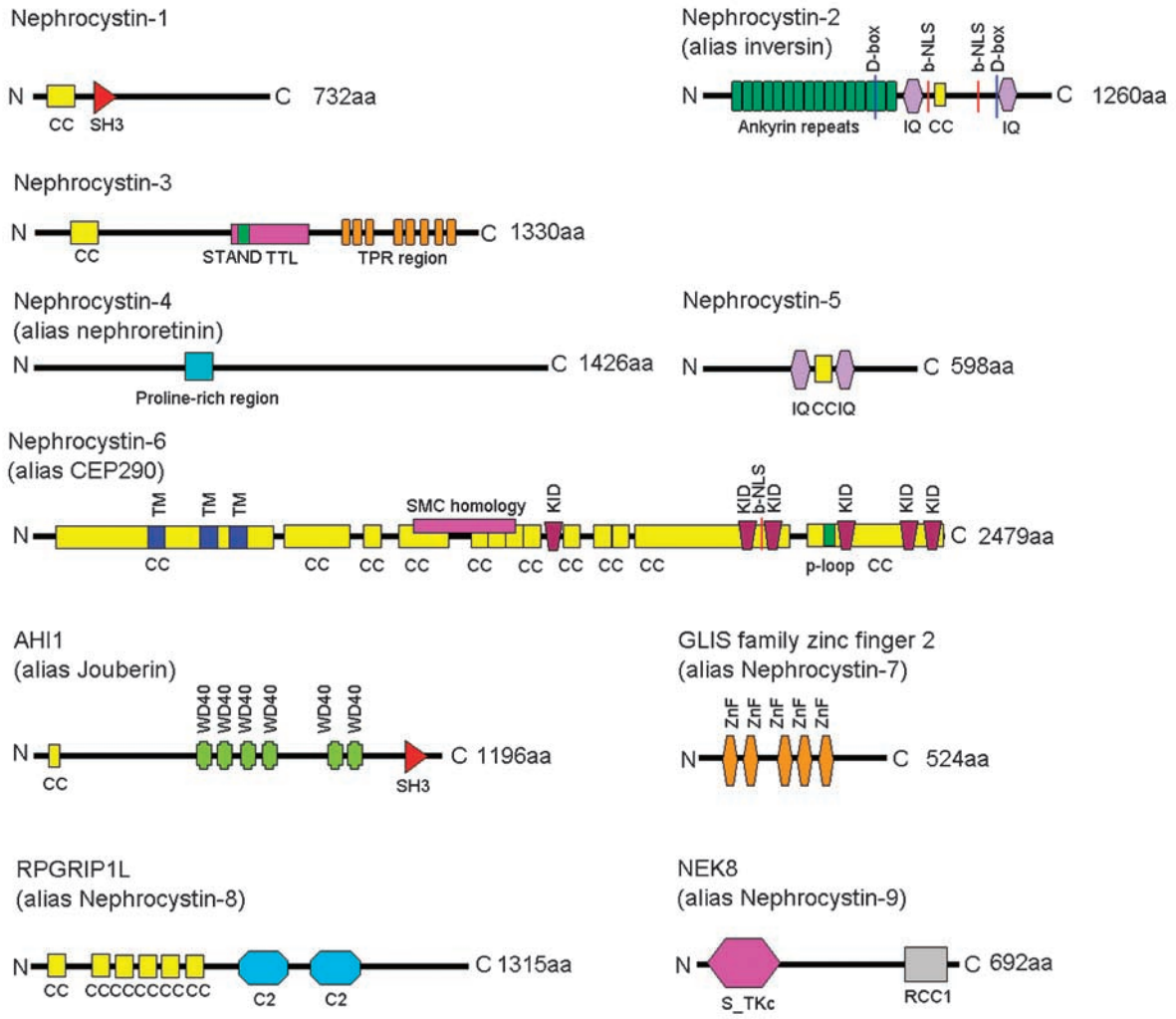

Figure 3 Nephrocystin proteins and their protein domains. Domain structure of the nephrocystin proteins. Nephrocystin proteins contain a diverse variety of protein domains and no common pattern can be identified. Nephrocystin-1, encoded by *NPHP1*, is a 732 amino acid (aa) protein, which possesses an N-terminal coiled-coil domain (CC) and a Src-homology 3 domain (SH3). Nephrocystin-2 (alias inversin), encoded by *NPHP2/INVS*, is a 1260 aa protein with 16 tandem ankyrin repeats, two IQ calmodulin binding domains (IQ). There are two destruction-box (D-box) regions (the first of which is Apc2 binding) and a bipartite nuclear localization signal (b-NLS) and a putative coiled-coil (CC) domain. Nephrocystin-3, encoded by *NPHP3*, is a 1330 aa protein, with a coiled-coil domain (CC), a tetratricopeptide-repeat domain (TPR) and a tubulin tyrosine ligase domain (TTL). Within the TTL a STAND (signal transduction ATPases with numerous domains) domain, which may be found in P-loop NTPases, is located. Nephrocystin-4 (alias nephroretinin), encoded by *NPHP4* is a 1426 aa protein, which lacks any known domains. There is a central proline rich region. Nephrocystin-5, encoded by *NPHP5/IQCB1* is a 598 aa protein. This protein possesses two IQ calmodulin binding sites, which surround a putative coiled-coil (CC) domain. Nephrocystin-6 (alias CEP290) is encoded by *NPHP6/CEP290*. This is a 2479 aa protein with multiple domains which include 13 coiled-coil (CC) domains; 3 tropomyosin homology domains (TM); 6 RepA/Rep$^+$ protein KID motifs (KID); a bipartite nuclear localization signal (b-NLS); a ATP/GTP-binding site motif A (p-loop). The extent of homology with Structural Maintenance of Chromosomes proteins (SMC) is also indicated. AHI1 (alias Jouberin) is encoded by *AHI1* and is an 1196 aa protein, which contains a Src-homology 3 domain (SH3), 6 WD40 domains (WD40) and an N-terminal coiled-coil domain. GLIS family zinc finger 2 (alias nephrocystin-7) is a 524 aa protein encoded by *GLIS2*. It contains 5 zinc finger domains (ZnF). RPGRIP1L (alias nephrocystin-8) is a 1315 aa protein encoded by *RPGRIP1L*. Protein domains include 6 coiled-coil (CC) domains and two protein kinase C conserved region 2 (C2) domains. The C-terminal C2 domain mediates the interaction with nephrocystin-4. NEK8 (alias nephrocystin-9) is a 692 aa protein with a serine/threonine protein kinases, catalytic domain (S-TKc) and a regulator of chromosome condensation (RCC1) domain, which is highly conserved throughout evolution.

IQ domains, with which it colocalizes to the primary cilium, and forms a complex with RPGR.[55] The clinical phenotype of *NPHP5* mutations is always associated with severe retinal degeneration (early onset Senior–Loken syndrome).

NPHP6/CEP290 and nephrocystin-6

The *NPHP6* (alias *CEP290*) gene encodes the nephrocystin-6 protein. Mutations in *NPHP6* account for a growing spectrum of clinical phenotypes which include isolated NPHP, Senior–Loken syndrome, JSRD,[56,57] MKS[22,23] and

BBS.[58] Mutations in *NPHP6* have also been described in 21% patients with isolated LCA, making this the most common gene defect for isolated LCA.[59] A mouse model, named rd16 has an in-frame deletion in *Nphp6/Cep290* and mimics this phenotype, with early onset retinal degeneration, but no kidney or brain disease. Nephrocystin-6 directly interacts with and activates the cAMP related transcription factor, CREB2 (alias ATF4).[56] Interestingly, single heterozygous mutations in *NPHP6* have been described in individuals with NPHP who have *NPHP1* homozygous deletions.[31] Similarly, a heterozygous nonsense mutation in *NPHP6* was described together with a heterozygous *NPHP4* missense mutation in an individual affected with Senior–Loken syndrome.[57] This tendency towards digenic and oligogenicity has recently been reported for other NPHP genes.[30,60]

NPHP7/GLIS2 and GLIS2

The *NPHP7/GLIS2* gene encodes the Kruppel-like zinc-finger transcription factor GLIS2 that localizes to both the primary cilia and the nucleus.[61] Mutations were reported in a consanguineous Oji-Cree Canadian family with affected members having isolated NPHP and early onset renal failure (by 8 years of age) but remains a rare genetic cause of NPHP.[61] A mouse model of targeted *Glis2* disruption within the kidney reveals increased rates of apoptosis, with tubular atrophy and fibrosis.

NPHP8/RPGRIP1L and RPGRIP1L

The *RPGRIP1L* gene encodes a protein named retinitis pigmentosa GTPase regulator interacting protein 1-like protein (RPGRIP1L). Mutations were initially reported in fetuses affected with MKS and patients with JSRD.[24,62] Additional features in some patients included scoliosis, polydactyly, pituitary agenesis and partial growth hormone deficiency, reminiscent of RHYNS syndrome.[62] Regarding *RPGRIP1L* mutations, some phenotype–genotype correlations can be drawn as homozygous truncating mutations seem to cause MKS[24,62] whereas a heterozygous truncating mutation or a homozygous missense mutation causes JSRD. RPGRIP1L is a centrosomal protein, which interacts with nephrocystin-4. JSRD causing mutations in *RPGRIP1L* confer loss off interaction with nephrocystin-4.[24]

A mouse model *Ftm−/−* (Fantom or fused-toe mouse) represents inactivation of the mouse ortholog *Rpgrip1l* (*Ftm*) and recapitulates the cerebral, renal and hepatic defects of JSRD and MKS.

NPHP9/NEK8 and NEK8

The *NEK8* gene encodes the NEK8 protein (never in mitosis A-related kinase 8). Mutations have been described in two families with NPHP and one consanguineous family with infantile NPHP. In one NPHP family with a homozygous *NPHP5* mutation, which accounts for the disease phenotype, a single heterozygous *NEK8* mutation was found.[60] These findings demonstrate firstly, the rarity of *NEK8* mutations and secondly, that *NEK8* mutations may contribute to oligogenicity in patients with NPHP. The *jck* mouse model of cystic kidney disease contains a missense mutation (G448V) in *Nek8*. Nek8 and polycystin-2 form a protein complex together, which adds weight to the argument that there are common mechanisms underlying NPHP and ADPKD.[63,64]

AHI1 and AHI1/Jouberin protein

The *AHI1* (*Abelson helper integration site 1*) gene encodes the AHI1 protein, which is also known as Jouberin. Mutations in *AHI1* were initially described in individuals with a JSRD phenotype, with no renal disease.[65,66] Subsequently, *AHI1* mutations were found in individuals with NPHP[67] and with retinal degeneration.[68] Jouberin is localized to adherens junctions, basal bodies and primary cilia.[69] Jouberin interacts with nephrocystin-1, and has been localized to the renal collecting duct.[69]

Other NPHP genes

NPHP1 gene mutations account for around 25% of all cases of NPHP. The remaining nine genes are each found in 0.05–3% of cases, and collectively probably only account for another 25% of cases of NPHP, meaning that many cases remain 'unsolved'. For JSRD, at least two additional loci have been reported. These are *JBTS1* on chromosome 9q34[70] and *JBTS2* (*CORS2*) on chromosome 11 (a large pericentromeric region).[71] Patients linked to the *JBTS2* locus often have renal disease as part of their disease spectrum. Very recently, mutations in *ARL13B*, which encodes a cilial protein, were found in patients with classical JS, with no renal phenotype.[72]

The role of the primary cilia in NPHP

The identification of genetic causes of NPHP has highlighted the paradigm, that all protein products of cystic kidney diseases are expressed in the primary renal cilium/basal body complex.[73] The primary cilium is present on nearly every cell in the human body and is a cell surface projection which acts as an 'antenna'. This organelle extends from the basal body and consists of an axoneme comprising nine microtubular doublets. Assembly of the axoneme occurs via a process called IFT where proteins are moved up and down the cilium.[73] Nephrocystins are located within this cilial subcellular domain, where they form complexes with themselves and other related proteins, probably to facilitate signalling cascades. Primary cilia are thought to sense tubular luminal flow (of urine) and regulate calcium entry (mediated by polycystin-2 channels).[74] Nephrocystins are expressed in the connecting cilium of the photoreceptor cell of the retina and defects here correlate with retinal defects and degeneration, often associated with *NPHP* gene mutations. Related

syndromes such as Jeune syndrome and Ellis van Creveld syndrome (EVC) have held true to the cilial paradigm. Jeune syndrome is secondary to mutations in the IFT protein IFT80[75] and *EVC* (together with EVC2) mutations underlie EVC, and encodes a cilial/basal body protein.[76]

Management

Clinical work up (see 'diagnostic approaches' section for details of initial evaluation)

Surveillance and management Given that the renal disease NPHP is often being managed in the context of extra-renal manifestations, ongoing surveillance of affected patients by appropriate specialists is important.

Regular evaluations

Patients with NPHP will invariably progress to end-stage renal failure. Management in a 'low clearance' setting is appropriate to allow time for consideration of renal replacement therapies. USS scans may detect renal cystic changes as the disease progresses. Growth, endocrine and sexual maturation and neurological evaluations should be regularly performed. Retinal disease may become progressive. Annual eye examinations commencing at the time of diagnosis is recommended.[5] Liver function tests should be performed regularly and liver ultrasound scan should be performed if suspicion of liver disease.

Genetic testing for NPHP

NPHP is a genetically heterogeneous disorder, however testing for the most common gene defect, a homozygous deletion of *NPHP1* (Figure 2), is readily available (see http://www.ukgtn.nhs.uk/gtn/Home; http://www.orpha.net and http://www.genetests.org/). Direct sequencing of other NPHP genes may also be performed (see http://autozygosity.org/diagnostic; http://www.renalgenes.org/ and http://www.orpha.net). Technologies are however changing rapidly and given that the genomic regions covered by all known NPHP genes is less than 1 mb (Table 4) a gene capture service followed by use of high throughput sequencing platforms may allow an efficient way of screening patients with NPHP in the near future. Indeed, with the recent descriptions of oligogenicity[30] and epistasis[31] in NPHP, testing of all NPHP associated genes may be important to understand this complex disorder.

Genetic counselling Genetic testing should not be performed before appropriate consent and genetic counselling. NPHP is inherited in an autosomal recessive manner, however in some affected individuals more than one associated gene may contribute to disease.[30] Such oligogenicity has also been reported in BBS.[77] In general, NPHP is a moderately severe disorder with major impacts on renal function and other aspects of health and development. The variable severity of the disorder in different families and even between individuals within families makes predicting outcome difficult.

Table 4 Coding exon numbers and genomic sequence length in NPHP genes

Gene	*Coding exons*	*Genomic DNA (bp)*
NPHP1	20	82 691
INVS/NPHP2	16	201 916
NPHP3	27	41 823
NPHP4	29	129 662
NPHP5	13	65 009
NPHP6/CEP290	53	93 204
NPHP7/GLIS2	6	7383
NPHP8/RPGRIP1L	26	103 954
NPHP9/NEK8	15	13 953
AHI1	23	109 962
Total	228	849 557

Prenatal diagnosis For families with a genetic diagnosis of NPHP, prenatal testing is possible. Prenatal imaging may reveal cystic kidney disease and other abnormalities (such as structural CNS lesions and polydactyly) in at-risk pregnancies. *INVS/NPHP2* mutations typically produce a prenatal cystic phenotype, as may the genes which have been reported to give a MKS-like phenotype (Table 3).

Treatment and care At present there are no proven treatments for NPHP. Treatment must centre on the progressive renal failure, which leads to ESRF and the need for dialysis and transplantation. Potential treatments, targeted towards the collecting duct may be available for future use. These include vasopressin V2 receptor antagonists, which may alter cystogenesis and progression of disease. The *pcy* mouse model of NPHP type 3 responded to treatment with OPC31260.[52] Evidence is also growing for use of rapamycin, an mTOR inhibitor, for reducing renal cystogenesis.[78,79]

Conclusion

During the past decade significant insight has been made in the molecular genetics of NPHP. This disease has moved from being a pathological description to an inherited ciliopathy, whereby the most common form may be readily detected by gene testing, without the need for a renal biopsy. Additional genes will no doubt be found, and high throughput technologies show promise for providing a screen of all currently known genes implicated in ciliopathies. The major challenge remains to understand the biological function of nephrocystin proteins, the molecular mechanisms that lead to renal failure and the potential treatments, which may prevent or reverse these changes. In practical terms, NPHP must be considered among the

differential diagnosis of any cause of renal failure of unknown origin. The recognition that NPHP is part of a ciliopathy, with a wide clinical spectrum of disease will allow earlier diagnosis to be made, allowing for time for genetic counselling, appropriate genetic testing and improved treatment planning for ESRF.

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