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Review Article

Nephronophthisis: A Genetically Diverse Ciliopathy

Roslyn J. Simms,[1,2] Ann Marie Hynes,[1] Lorraine Eley,[1] and John A. Sayer[1,2]

[1] *Institute of Human Genetics, International Centre for Life, Newcastle University, Central Parkway, Newcastle upon Tyne NE1 3BZ, UK*
[2] *Renal Services, Freeman Hospital, The Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle Upon Tyne NE7 7DN, UK*

Correspondence should be addressed to John A. Sayer, j.a.sayer@ncl.ac.uk

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Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease and a leading genetic cause of established renal failure (ERF) in children and young adults. Early presenting symptoms in children with NPHP include polyuria, nocturia, or secondary enuresis, pointing to a urinary concentrating defect. Renal ultrasound typically shows normal kidney size with increased echogenicity and corticomedullary cysts. Importantly, NPHP is associated with extra renal manifestations in 10–15% of patients. The most frequent extrarenal association is retinal degeneration, leading to blindness. Increasingly, molecular genetic testing is being utilised to diagnose NPHP and avoid the need for a renal biopsy. In this paper, we discuss the latest understanding in the molecular and cellular pathogenesis of NPHP. We suggest an appropriate clinical management plan and screening programme for individuals with NPHP and their families.

1. Introduction

Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease and a leading genetic cause of established renal failure (ERF) in children and young adults [1]. NPHP literally means "disappearance of nephrons," which alludes to its histopathology, with interstitial fibrosis and corticomedullary cysts replacing normal renal tissue. The median age of an affected child with ERF is 13 years [2]. The incidence of NPHP varies worldwide; it was previously identified to range from 1 in 50,000 to 1 in 900,000 [3–5]; however, these figures are likely to underrepresent the true frequency, since molecular testing has diagnosed NPHP in adults presenting with advanced chronic kidney disease (CKD) [6, 7]. The prevalence of NPHP amongst the paediatric population with ERF is 5% in the USA [2] and 6.5% in the UK [8, 9].

Early presenting symptoms in children with NPHP usually develop at around 6 years of age and include polyuria, nocturia or secondary enuresis, polydipsia, and lethargy (secondary to anaemia) [10]. These features are a consequence of salt wasting and an inability to concentrate urine (<400 mosm/kg early morning urine), implicating dysfunction of the renal cortical collecting duct [11]. Renal ultrasound identifies normal or reduced kidney size, with increased echogenicity and corticomedullary cysts [2]. There is a less common infantile variant of NPHP in which children reach ERF by 3 years of age and have enlarged cystic kidneys on renal ultrasound [12]. Infantile NPHP is distinct from autosomal recessive polycystic kidney disease (ARPKD). There is a diffuse distribution of cysts within the kidneys of children with ARPKD, and it is more often associated with liver cysts and fibrosis [13]. A diagnostic renal biopsy of NPHP reveals a characteristic triad of tubular basement membrane disruption, tubulointerstitial nephropathy/fibrosis, and corticomedullary cysts [4, 14]. Increasingly, molecular genetic testing [15] is being utilised to diagnose NPHP and avoid the need for a renal biopsy [16].

NPHP is associated with extra renal manifestations in 10–15% of patients [1]. The most frequent anomaly is retinal degeneration; other associated features and disorders include cerebellar vermis hypoplasia (Joubert Syndrome (JS)), occipital encephalocele (Meckel-Gruber syndrome

(MKS)), hepatic fibrosis, situs inversus, bronchiectasis, and skeletal defects [1]. In addition to this apparent variability in the spectrum and severity of phenotype, NPHP is genetically heterogenous. To date mutations have been identified in 13 genes (Table 1) which collectively account for approximately 30% of patients [17]. The protein products of all of these genes localise on primary cilia and related structures (basal bodies, centrosomes), resulting in a unifying hypothesis that cystic kidney diseases are ciliopathies [18]. In this paper, we will discuss the latest understanding in the molecular and cellular pathogenesis of NPHP and suggest an appropriate management plan/screening programme for individuals and their families, particularly in view of the considerable clinical heterogeneity.

2. Molecular and Genetic Pathogenesis

NPHP is a recessive monogenic disorder [19], meaning that two mutations (homozygous or compound heterozygous) in a single gene are sufficient to cause disease [20]. Thirteen genes have been identified in affected families with NPHP (Table 1), and these genes currently allow 30% of patients with NPHP to be "solved" in terms of a molecular diagnosis. These genes have been identified using positional cloning strategies and homozygosity mapping in consanguineous families [20]. Subsequent localisation of all these encoded proteins, termed "nephrocystins," to the primary cilium/basal body led to recognition of NPHP as a ciliopathy [17]. Primary cilia are highly conserved, microtubule-based hair-like structures which extend from the apical surface of almost every epithelial cell. They function in order to detect extracellular cues and mediate cellular signalling pathways (discussed below) [13]. Ciliary genes are currently recognised as attractive candidates to evaluate when attempting to define the molecular cause of NPHP in the presently undiagnosed 70% of patients. With this in mind, combined homozygosity mapping, ciliopathy candidate exome capture, and parallel sequencing have recently been performed resulting in the successful identification of pathological mutations in the gene, serologically defined colon cancer antigen 8 (*SDCCAG8*), in families with NPHP [21]. *SDCCAG8* is synonymous with *NPHP10*. Also recently, the targeted screen of the ciliary gene *TTC21B* revealed that its protein product, IFT139, is essential for retrograde intraflagellar transport. IFT139 interacts with ciliopathy proteins BBS4 and BBS8, and pathogenic mutations in *TTC21B* were identified in patients with NPHP and more severe related ciliopathies [22].

Although the majority of currently known NPHP genes produce proteins which localise to primary cilia/basal bodies/centrosomes, recent identification of an NPHP-like locus in two affected families suggests that NPHP genes may not be exclusively ciliary [35]. Genome-wide homozygosity mapping identified pathogenic mutations in X-prolyl aminopeptidase 3 (*XPNPEP3*), or *NPHPL1* (NPHP like 1 gene), of which the protein product localises to mitochondria [35]. Although not currently identified in the primary cilium, XPNPEP3 may influence cilia function through enzymatic cleavage of associated ciliary proteins [35].

Whilst homozygous mutations in the *NPHP* genes can cause isolated NPHP, mutations in the same gene can be pleiotropic inducing a spectrum and variable severity of phenotypes. Similarly, it would appear logical that the type of mutation may influence the phenotype. For example, a missense mutation may cause isolated NPHP or Senior-Loken syndrome (SLS, retinitis pigmentosa), whilst a truncating mutation could cause MKS. Recently the effect of different mutations in *NPHP6* on clinical presentation has been eloquently reviewed [36]. Furthermore, some *NPHP* genes are more likely to be associated with certain extrarenal features. The concept of "modifier genes" has been recognised in patients with the related ciliopathy, Bardet-Biedl syndrome (BBS), where pathogenic mutations in more than one gene have been detected, implicating a role for oligogenicity [37]. Oligogenicity or triallelism, whereby a mutation in a third allele may exert an epistatic effect and modify the phenotype, has been described in NPHP [38]. A brief description of each of the NPHP genes, their encoded nephrocystin proteins, and any interacting protein partners is given below.

2.1. NPHP1 and Nephrocystin-1. *NPHP1* was the first NPHP gene identified and accounts for the majority (20–25%) of known cases of isolated NPHP [23, 24]. Recently adults presenting with signs of NPHP and ERF in two generations of a Turkish family with no known consanguinity suggested a diagnosis of a dominant cystic kidney disease such as medullary cystic kidney disease (MCKD) or perhaps a novel variant of dominant NPHP [7]. However, identification of homozygous mutations in *NPHP1* in all affected family members revealed a pseudodominant inheritance of unknown cause as a consequence of unidentified consanguineous relationships. The increased age at presentation with ERF is atypical and is hypothesised to be a consequence of currently unknown modifier genes [7]. *NPHP1* mutations can also cause retinal and cerebellar phenotypes leading to SLS and JS. Oligogenicity has been reported in families with NPHP, where mutations in both *NPHP1* and *NPHP3*, *NPHP1* and *NPHP4* [38], and *NPHP1* and the ciliopathy gene *Abelson helper integration site-1* (*AHI1*, the most frequently mutated gene in JS [39]) have been detected [40]. Mutations in both *NPHP1* and *NPHP6* have been identified in patients with NPHP-related ciliopathies including SLS and JS [41]. This evidence of genetic interaction, known protein-protein interactions between various nephrocystins, combined with an awareness of other ciliary proteins such as BBSome functioning as a complex [13], makes it highly likely that several of the nephrocystins form a functional supramolecular complex within cells [17, 20]. Nephrocystin-1 localises at cell-cell contacts including tight junctions, adherens junctions, and focal adhesions [42, 43]. Nephrocystin-1 has also been identified at the transition zone/base of the primary cilium [44]. Nephrocystin-1 interacts with various other proteins important in maintaining the cellular scaffolding or cytoskeleton including jouberin [45], ack1 [46], filamin A and B, tensin (actin binding), β-tubulin (microtubule structure), and protein tyrosine kinase 2B (PTK2B) [20].

Table 1: Mutated genes in nephronophthisis and associated extrarenal manifestations.

Locus	Gene	Chromosome	Protein	Mutation frequency [20]	Extrarenal features	Ref.
NPHP1	*NPHP1*	2q13	Nephrocystin-1	23%	SLS, JS,	[23, 24]
NPHP2	*INV*	9q31	Inversin	1-2%	SLS, HF VSD, situs inversus	[25]
NPHP3	*NPHP3*	3q22.1	Nephrocystin-3	<1%	SLS, HF, MKS, situs inversus	[26]
NPHP4	*NPHP4*	1p36.22	Nephrocystin-4 or nephroretinin	2-3%	SLS	[27, 28]
NPHP5	*IQCB1*	3q21.1	Nephrocystin-5 or IQ motif containing B1	3-4%	SLS	[29]
NPHP6	*CEP290*	12q21.32	Centrosomal protein 290	1%	LCA, SLS, JS, MKS, BBS	[30]
NPHP7	*GLIS2*	16p13.3	GLI similar 2	<0.5%		[31]
NPHP8	*RPGRIP1L*	16q12.2	RPGRIP1-like	0.5%	SLS, JS, MKS	[32]
NPHP9	*NEK8*	17q11.1	NIMA-related kinase 8	<0.5%	SLS	[33]
NPHP10	*SDCCAG8*	1q44	Serologically defined colon cancer antigen 8	<0.5%	SLS, BBS-like	[21]
NPHP11	*TMEM67*	8q22.1	Transmembrane protein 67	<0.5%	JS, HF, MKS	[34]
NPHPL1	*XPNPEP3*	22q13	X-prolyl aminopeptidase 3	<0.5%	cardiomyopathy, seizures	[35]
	TTC21B	2q24.3	Intraflagellar transport protein 139	<1%	JS, MKS, BBS, JATD	[22]

BBS: Bardet-Biedl syndrome; HF: hepatic fibrosis; JATD: Jeune asphyxiating thoracic dystrophy; JS: Joubert syndrome; LCA: Leber's congenital amaurosis; MKS: Meckel-Gruber syndrome; SLS: Senior-Loken syndrome; VSD: ventricular septal defect.

2.2. NPHP2/INVS and Inversin. Mutations in *NPHP2* are distinct because they cause infantile NPHP, characterised by an earlier presentation of ERF (at approximately 3 years of age), with enlarged kidneys on ultrasound. Additional clinical features include cardiac anomalies (situs inversus and ventricular septal defects (VSD)) [47]. Although *NPHP2* mutations are a rare cause of NPHP compared to *NPHP1*, there has been intense research regarding the molecular/cellular pathogenesis of inversin. Inversin is located in the primary cilium and other subcellular sites in a cell-cycle dependent manner [48]. A previous work has suggested that inversin acts as a switch between canonical and non-canonical (planar cell polarity) Wnt signalling [49]. When inversin is lost after *NPHP2* mutation, it was proposed that sustained canonical Wnt signalling led to cell proliferation and random oriented cell division [49]. However, recent experiments in the *inv* mutant mouse model of NPHP showed no difference in canonical Wnt signalling compared to controls [50]. In addition to nephrocystin-1, inversin interacts with calmodulin, catenins, β-tubulin [25], and anaphase-promoting complex 2 [48, 51].

2.3. NPHP3 and Nephrocystin-3. Mutations in *NPHP3* are again a rare cause of isolated NPHP; however, they can cause a broad spectrum of phenotypes as shown in Table 1. Nephrocystin-3 colocalises with nephrocystin-1 [26] and inversin [52] in primary cilia, adherens junctions, and focal adhesions [20]. The *pcy* mouse model of NPHP displays cystic kidneys and responded to treatment with aquaretic agents/vasopressin-2-receptor antagonists [53].

2.4. NPHP4 and Nephrocystin-4/Nephroretinin. Individuals with mutations in *NPHP4* most frequently have an associated retinal phenotype [54]. Nephrocystin-4 colocalises and interacts with nephrocystins 1, 3 and inversin in primary cilia and associated appendages, adherens junctions, and focal adhesions [20, 27]. Nephrocystin-4 also interacts with nephrocystin-8 [55, 56], α-tubulin, breast cancer antioestrogen resistance 1 (BCAR1), PTK2B [20], and the tight junction proteins PALS1/PATJ/Crb3 which are required for epithelial morphogenesis [57].

2.5. NPHP5/IQCB1 and Nephrocystin-5. NPHP5 mutations are associated with early onset retinal degeneration, SLS [29]. Nephrocystin-5 contains two IQ calmodulin binding sites; the significance of its interaction with calmodulin is unclear. It colocalises with nephrocystin-1 and nehrocystin-4 in the primary cilium, adherens junctions, and focal adhesions [29] and interacts with nephrocystin-6 [30, 58]. Nephrocystin-5 also complexes with the retinal ciliopathy gene retinitis pigmentosa GTPase regulator (RPGR) [29], explaining the frequent retinal phenotype.

2.6. NPHP6/CEP290 and Nephrocystin-6. Mutations in *NPHP6* cause a full spectrum of extrarenal features with no

apparent genotype-phenotype correlation [36]. It is the commonest genetic cause (21%) of isolated congenital retinal degeneration, Leber's congenital amaurosis (LCA) [59]. It was suggested that oligogenicity and the effect of modifier genes may account for some of the pleiotropy. Oligogenicity has been described in patients with homozygous *NPHP6* mutations and an additional heterozygous mutation in: *NPHP4* resulting in NPHP [36] or SLS [54], *NPHP11* causing BBS or MKS [60], and *AHI1* causing JS [41]. Oligogenicity has also been identified in patients with SLS and JS as a consequence of a homozygous mutation in *NPHP1* and heterozygous mutation in *NPHP6* [41]. NPHP6 interacts with and modulates the transcription factor ATF4, involved in cAMP-dependent renal cyst formation [30]. In addition to nephrocystin-5 [58], another protein interaction partner of nephrocystin-6 is coiled-coil and C2 domain protein (CC2D2A) [61]. In zebrafish models of combined *NPHP6* and *CC2D2A* knockdown, there is synergy of the renal cystic phenotype, suggesting an epistatic, disease-modifying effect [61]. *CC2D2A* mutations cause JS and MKS [62]; however, they have not been identified in patients with isolated NPHP [15, 61].

2.7. NPHP7/GLIS2 and GLIS2. NPHP7 is a rare cause of isolated NPHP [31]; its protein product is a Kruppel-like zinc-finger transcription factor, Gli-similar protein 2, which localises to the primary cilium and nucleus [31]. Interestingly, a *Glis2* knockout mouse model showed an upregulation of genes promoting epithelial-to-mesenchymal transition and histological features of NPHP including fibrosis [31]. This correlation of nephrocystin-7 with GLI transcription factors links the pathogenesis of NPHP to the Hedgehog (Hh) signalling pathway, which is essential for controlling tissue maintenance [63].

2.8. NPHP8/RPGRIP1L and RPGRIP1L. NPHP8 mutations more frequently cause extrarenal manifestations such as cerebello-oculo-renal syndromes, JS [32, 56], and MKS [56] than isolated NPHP. There appears to be some genotype-phenotype correlation with missense mutations causing LCA [64], whilst truncating mutations cause the more severe disorder MKS [56]. RPGRIP1L colocalises with nephrocystin-4 and nephrocystin-6 at basal bodies and centrosomes [56]. RPGRIP1L interacts with nephrocystin-1 and nephrocystin-4 [20].

2.9. NPHP9/NEK8 and NEK8. NPHP9 mutations are a rare cause of both infantile and noninfantile NPHP [33]. Oligogenicity has been identified with a pathogenic homozygous mutation in *NPHP5* and heterozygous *NPHP9* mutation, which may behave as a modifying gene, in an individual with SLS [33]. In some patients with heterozygous *NPHP9* mutations, a second recessive mutation has not been identified. Its protein product, never in mitosis A-related kinase 8 (NEK8), colocalises with various nephrocystins in primary cilia, basal bodies, and centrosomes and appears to be important in regulating the cell cycle [20]. NEK8 has been shown to interact with polycystin-2 (autosomal dominant polycystic kidney disease (ADPKD) protein), to regulate its expression and phosphorylation [65]. NEK8 may thus function in a protein complex with polycystin 1 and 2.

2.10. NPHP10/SDCCAG8 and SDCCAG8. SDCCAG8 was recently identified as *NPHP10* by homozygosity mapping, ciliopathy candidate exome capture, and parallel sequencing [21]. Twelve mutations were identified in ten families with NPHP-related ciliopathies, in particular SLS and BBS. Homozygous *SDCCAG8* mutations account for 3.3% of cases of SLS. Its protein product, serologically defined colon cancer antigen 8 (SDCCAG8), colocalises at centrosomes and cell-cell junctions with nephrocystin-5. SDCCAG8 and nephrocystin-5 colocalise in the transition zone of photoreceptors which is likely of functional significance and correlates with the phenotype of SLS. SDCCAG8 also colocalises with the retinal ciliopathy proteins RPGRIP and RP1. SDCCAG8 interacts directly with the protein oral-facial-digital syndrome 1 (OFD1) [21], although the functional significance of this is currently not clear, it is clearly of interest, as recessive mutations in *OFD1* are an X-linked cause of the NPHP-related ciliopathy JS [66].

2.11. NPHP11/TMEM67/MKS3 and TMEM67. Mutations in *NPHP11* are pleiotropic, having been identified in patients with NPHP and liver fibrosis, extending to patients with related ciliopathies including JS, MKS [34], and COACH syndrome (cerebellar vermis hypoplasia, oligophrenia (developmental delay), ataxia, coloboma, and hypotonia) [67]. Whilst oligogenicitiy has not been described, a patient with JS and an isolated heterozygous *NPHP11* mutation has been identified [68], suggesting that triallelism and the role of *NPHP11* as a modifier gene is possible. Transmembrane protein 67 (TMEM67) or meckelin localises to the membrane of primary cilia and diffusely at basal and basolateral cell surfaces [69]. TMEM67 interacts with several proteins including nesprin 2 [70], MKS1 [69], and TMEM216 [71] which are important in maintaining cellular structure and mitigating centrosome migration, which is essential for ciliogenesis.

2.12. NPHPL1/XPNPEP3 and XPNPEP3. NPHP-like 1 gene (*NPHPL1*) was recently identified in two consanguineous families with NPHP by genome-wide homozygosity mapping [35]. Additional extrarenal manifestations include cardiomyopathy and seizures. This is a novel discovery because it is the first NPHP gene identified whose protein product, X-propyl aminopeptidase 3 (XPNPEP3), does not localise to primary cilia, basal bodies, or centrosomes [35]. Instead, XPNPEP3 localises in mitochondria; however, it has been hypothesised that this enzyme may be able to interfere with cilia function by cleaving certain cilial proteins [17, 35].

2.13. TTC21B and IFT139. Mutations in *TTC21B* have recently been identified in families with isolated NPHP and extrarenal manifestations including the ciliopathy, Jeune

asphyxiating thoracic dystrophy (JATD) [22]. Interestingly, both causal mutations (homozygous or compound heterozygous) and modifier mutations (heterozygous) in *TTC21B* were identified in affected individuals. Oligogenicity was identified between *TTC21B* and several other ciliopathy genes. With regard to NPHP, triallelism was identified in a Turkish family with mutations in both *TTC21B* and *NPHP4*. The protein product of *TTC21B* is a retrograde intraflagellar transport protein IFT139, found in the primary cilium, and is essential for ciliary function.

3. Oligogenicity and Modifier Genes

Oligogenicity has been described above for *NPHP1, NPHP5, NPHP6, NPHP8, NPHP9, NPHP11,* and *TTC21B*. It has been hypothesised that oligogenicity may help to account for the intrafamilial variation in age of onset of ERF and severity of clinical features [38]. Current evidence fails to consistently identify a correlation between genotype and phenotype [17], therefore mutation analysis is required to identify the molecular cause. Making a molecular diagnosis often involves expensive and time-consuming mutation analysis. However, the results may be important when managing patients, to guide appropriate screening for potentially associated complications of the retina, cerebellum, liver and lungs. Understanding the natural history of NPHP and associated complications will hopefully improve following completion of the current clinical trial ongoing in France, which is evaluating the evolution of NPHP and related extrarenal manifestations in children (over 7 years of age) with a confirmed diagnosis of NPHP1–8 (excluding NPHP7) (see http://clinicaltrials.gov/ct2/show/NCT01022957?term=nephronophthisis&rank=1). The results of this study may facilitate understanding in characterising genotype/phenotype associations. Although NPHP-related ciliopathies are heterogenic disorders, the true frequency of oligogenicity remains uncertain. It is however interesting that mutation analysis of 18 NPHP associated ciliopathy disease genes (including 12 *NPHP* genes, *SDCCAG8* was not included) in 120 patients with NPHP and related ciliopathies, using DNA pooling and next generation sequencing, recently failed to identify any evidence of oligogenicity [15]. Remarkably, in 75% of patients in this cohort, no mutations were detected in the known candidate NPHP genes [15].

Since the molecular cause of NPHP remains unidentified in 70–75% of cases, it is anticipated that additional NPHP genes will be discovered. Whilst genes involved in the structure and function of the primary cilium are logical candidates to consider, indeed this approach has resulted in the recent successful identification of *TTC21B* [22], it is interesting that "noncilial" causal genes such as *XPNPEP3* [35] are now being identified. Additionally, genes implicated in maintaining the cell cytoskeleton and cellular junctions are likely involved. Whilst identifying causal genes is fundamental to determine the pathogenesis of NPHP, it is the tip of the iceberg and we need to understand the functional consequences of such mutations within tissues. We will now discuss current hypotheses for the cellular pathophysiology of NPHP.

4. Cystic Kidney Disease as a Ciliopathy

In 2005, realisation that polycystin-1 and polycystin-2 (protein products of ADPKD genes *PC1* and *PC2*) and the discovered nephrocystins (NPHP1–5) were all expressed in the primary cilium, basal body, and centrosomes led to consideration of the term "ciliopathy" as a unifying theory for cystic kidney disease [18]. A few years earlier, the concept of a ciliopathy, a disorder in which abnormal structure or function of cilia/centrosomes is associated with defective proteins encoded by mutated genes, was attributed to the multisystem disorder BBS [72]. In the intervening years, the primary cilium [73] had been extensively studied. Primary cilia are composed of an axoneme containing nine microtubular doublets which extend by a process of intraflagellar transport (IFT) and mediate the trafficking of signals between the extra- and intracellular environments [73]. Cilia are considered to be involved in mechanosensation of urinary flow in the renal tubules [74].

Normally, in healthy kidneys, the renal cortical collecting duct (CCD) concentrates urine by responding to vasopressin, which binds to vasopressin-2 receptors (V_2R). V_2R are coupled to adenylyl cyclase resulting in increased intracellular cyclic AMP (cAMP), leading to phosphorylation of aquaporin-2 water channels (AQP2), which mediate water reabsorption [11]. In NPHP, inability to concentrate urine is the earliest clinical feature and is unresponsive to desmopressin therapy [10]. In animal models of NPHP, vasopressin levels are elevated and thought to contribute to cystogenesis by upregulating cell proliferation [75]. Identification of V_2R in the primary cilium of renal epithelial cells [76] is consistent with the theory of cystic kidney diseases as ciliopathies. This also emphasises the importance of the primary cilium in water reabsorption which has recently been eloquently investigated in patients and renal epithelial cell culture models of the related ciliopathy BBS [77]. Understanding the pathogenesis of this urine concentration defect should help explain the success of vasopressin receptor antagonists in animal models of NPHP, where they induced a reduction in cAMP levels and caused regression of cysts [53].

In NPHP, in response to urinary flow, cilia are considered to alter expression of inversin and potentially influence Wnt signalling pathways and planar cell polarity [17, 78]. Originally, observations in mouse models established the link between primary cilia and cystic kidney disease: the *orpk* mouse model of ARPKD with a *Tg737* mutation (disrupting the protein polaris) has impaired ciliogenesis and renal cysts [79].

The extrarenal manifestations of NPHP including retinal degeneration and SLS, usually associated with *NPHP5* and *NPHP6* mutations, can be explained by ciliary dysfunction. Retinal photoreceptors contain a connecting cilium through which rhodopsin traffics along, mediating photosensation [18]. Both nephrocystin-5 and -6 are expressed in the connecting cilia of retinal photoreceptors and disrupted structure or function of these proteins interferes with rhodopsin transport, leading to retinal degeneration (17, 20).

Through their involvement in various cellular signalling pathways including Hh, calcium, and Wnt, cilia mediate several fundamental processes including cell cycle, proliferation, differentiation, and polarity [73]. Proposed mechanisms for renal cystogenesis include abnormal cell proliferation, fluid secretion, and disorientated cell division leading to cystic expansion rather than longitudinal tubular growth [78]. However, aberrations in the signalling pathways linking cilia to cystogenesis remain incompletely understood, complicated by sometimes contradictory evidence. A recent helpfulreview of mechanisms of NPHP discusses each gene [17]. Here we provide an overview, concentrating on the Wnt signalling pathway.

5. Wnt Signalling and NPHP

Wnt signalling involves several ciliary proteins including PC1, PC2, inversin, nephrocystin-3, jouberin, BBS1, BBS4, BBS6, OFD1, and HNF1β, to regulate cell proliferation and differentiation [80]. It is composed of two pathways: canonical (β-catenin dependent) and noncanonical (or planar cell polarity (PCP)); the functional branch appears to be determined in part by inversin acting as a switch at the base of the primary cilium [49]. Interestingly, both over activation [49, 81] and underactivation [82] of the canonical Wnt pathway have been identified in animal models of NPHP and JS, suggesting that unbalanced canonical Wnt signalling is damaging and mediates cystogenesis [80]. However, this theory has recently been refuted by studies in the *inv* mouse model of NPHP, where no change in canonical Wnt signalling was identified between cystic *inv* mutants and controls [50].

PCP describes the normal intrinsic organisation of cells in a tissue plane perpendicular to their apicobasal polarity [83]. PCP regulates kidney tubule development or recovery from injury by convergent extension and orientated cell division [78, 84], meaning that cells are spatially orientated and divide along an axis to maintain a constant tubule diameter whilst elongating [78, 85]. Several mouse models of cystic kidney disease provide evidence of aberrant PCP signalling [83, 86]. An excellent, comprehensive review of the role of Wnt signalling in cystic kidney disease has been published recently [80].

The other signalling pathway with particular relevance to understanding the pathophysiology of NPHP is the Hh pathway. An association with NPHP was realised when Hh effector proteins, GLI transcription factors, were noted to be related to GLIS2, the protein product of *NPHP7*. Loss of GLIS2 promotes epithelial to mesenchymal translation (EMT), fibrosis and apoptosis, and histological hallmarks of NPHP [31]. Thus although *NPHP7* is a rare cause of NPHP, its discovery has contributed important understanding to the signalling pathways involved.

6. Clinical Management of NPHP

6.1. Diagnosis and Investigations. In order to establish a clinical diagnosis of NPHP, a detailed history, clinical examination, including looking for extrarenal associations (abnormal eye movements, retinopathy, ataxia, polydactyly, and cardiac malformations) is required. A detailed family history must be taken both to facilitate diagnosis and to highlight other individuals who should be invited for review. Appropriate investigations include renal and liver function tests; urine concentration ability, renal and hepatic ultrasound, cerebral imaging if clinically indicated, and referral to an ophthalmologist (Figure 1) [16]. After genetic counselling, blood may be sent for genetic testing (Table 2) to genetic testing organisations (e.g., http://www.ukgtn.nhs.uk/ or http://www.eurogentest.org/) to seek a molecular diagnosis. Regular review is required to appropriately manage CKD/ERF, and individuals with extrarenal manifestations should be referred to appropriate colleagues and are ideally best managed in specialist clinics.

Table 2: NPHP genes available for testing via UK and European gene testing networks.

Gene	Laboratory
NPHP1	NE Thames, London
NPHP1	Glasgow, Scotland
NPHP1	Utrecht, Netherlands
NPHP1	Helsinki and Tampere, Finland
NPHP1	Malaga, Spain
NPHP1	Granada, Spain
NPHP1,2	Gosselies, Belgium
NPHP1,2	Brussels, Belgium
NPHP1–3	Aachen, Germany
NPHP1–4	Paris, France
NPHP1–4	Weißwasser, Germany
NPHP1–4	Tubingen, Germany
NPHP1–4, 7	Rostock, Germany
NPHP1–4, –9, NPHPL1	Barcelona, Spain
NPHP1–4, –9, NPHPL1	Oviedo, Spain
NPHP1–4, –9, NPHPL1	Leuven, Belgium
NPHP1–4, 6–9	Ingelheim, Germany

6.2. Treatment. Presently there is no cure for NPHP and related ciliopathies. Clinicians must focus on optimising the delivery of renal replacement therapy, ideally with renal transplantation where possible. However with a growing understanding of the pathophysiology of NPHP, the future is more hopeful. In recent years various drugs including vasopressin receptor antagonists [53], mTOR inhibitors (mammalian target of rapamycin) [87], triptolide [88] and roscovitine (cyclin-dependent kinase inhibitor) [89] have been shown to be effective in reducing renal cysts in animal models of NPHP and ADPKD. Many of these drugs are currently or have recently been involved in clinical trials in adult patients. Furthermore, large numbers of compounds which could be potential therapies are being screened in zebrafish [90] models of ciliopathies.

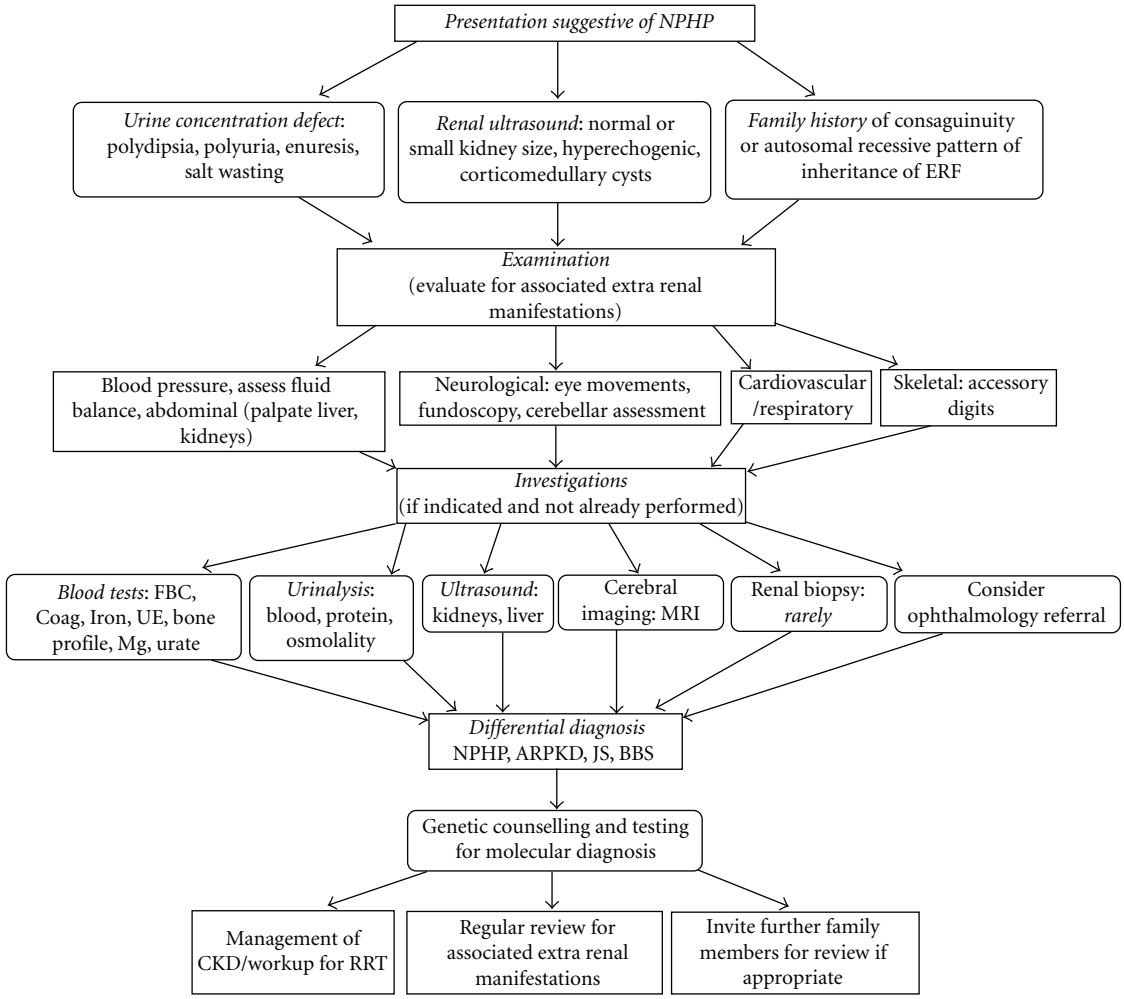

Figure 1: Diagnostic algorithm for suspected cases of NPHP. ARPKD: autosomal recessive polycystic kidney disease; BBS: Bardet-Biedl syndrome; CKD: chronic kidney disease; Coag: coagulation; ERF: established renal failure; FBC: full blood count; JS: Joubert syndrome; LFT: liver function tests; Mg: magnesium; MRI: magnetic resonance imaging; NPHP: nephronophthisis; RRT: renal replacement therapy; UE: urea and electrolytes.

7. Conclusion

Understanding of the molecular genetics of NPHP has advanced considerably over the last few years. We are increasingly aware of the heterogeneity, the pleiotropic nature of mutations, and oligogenicity, all of which contribute to the complexity of NPHP. Identification of further NPHP genes, many of which may be "ciliary," is warranted to provide further clues to its pathogenesis. This will require international multidisciplinary collaboration to sequence patient cohorts with no current molecular diagnosis and to screen candidate genes in animal and cell models. Acknowledgement that the genetic cause is unknown in 70% of NPHP cases, combined with awareness that a mitochondrial gene, *XPNPEP3*, has recently been identified to cause an NPHP-like phenotype, may encourage us to consider other nonciliary candidates, such as genes involved in cell-cell contacts and the cytoskeleton. Ultimately understanding the physiological outcomes at a protein and cellular level should facilitate identifying and developing novel therapeutic targets for affected patients.

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