Senior-Løken Syndrome: A syndromic form of retinal dystrophy associated with nephronophthisis

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Abstract

Senior-Løken syndrome (SLS) is an autosomal recessive disease characterized by development of a retinitis (RP)- or Leber congenital amaurosis (LCA)-like retinal dystrophy and a medullary cystic kidney disease, nephronophthisis. Mutations in several genes (called nephrocystins) have been shown to cause SLS. The proteins encoded by these genes are localized in the connecting cilium of photoreceptor cells and in the primary cilium of kidney cells. Nephrocystins are thought to have a role in regulating transport of proteins bound to the outer segment/primary cilium; however, the precise molecular mechanisms are largely undetermined. This review will survey the biochemistry, cell biology and existing animal models for each of the nephrocystins to understand the photoreceptor biology and the pathogenesis of retinal degeneration.

Keywords: Senior-Løken syndrome; NPHP5; nephronophthisis; retinal degeneration; ciliopathy; NPHP

Abbreviations: NPHP – nephronophthisis; NPHP5 – nephrocystin-5; SLS – Senior-Løken Syndrome; RP – retinitis pigmentosa

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1. Introduction

Inherited retinal diseases are estimated to affect up to 200,000 people in the United States (http://www.mdsupport.org/library/numbers.html). A vast majority of these cases are retinitis pigmentosa (RP), which accounts for 40% or 80,000 people. RP is a disease where the photoreceptors, the light-sensing cells of the eye, die (Hartong et al., 2006). RP clinically presents with nyctalopia (night blindness) and can progress to a reduction of the peripheral visual field (tunnel vision). Central visual acuity and cone function are often preserved until late in the course of the disease. End stage disease will have no light perception at all. Other clinical findings on exam include bone spicule formation (retinal pigmentation), peripheral retinal atrophy, waxy pallor of the optic disk and optic nerve head drusen and vascular attenuation (**Fig. 1**). Diagnosis is done using a detailed clinical and family history, a dilated retinal examination and photography, visual field testing, electroretinography, optical coherence tomography and genetic testing.

RP may associate with development of pathology in other organs. Some examples of syndromic RP include Bardet-Biedl syndrome, Joubert syndrome, Meckel syndrome and Senior- Løken syndrome (**Table 1**). Currently, there is no therapy that stops progression or restores vision in patients with RP, and therefore, prognosis is poor.

Patients with Senior-Løken syndrome develop RP and a kidney pathology called nephronophthisis. Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease and is the most frequent genetic cause of end-stage renal disease

(ESRD) in children and adolescents. A recent review on nephronophthisis can be found in (Wolf and Hildebrandt, 2011) The median age for ESRD is around 13 years old. Children clinically present with polyuria, nocturia or secondary enuresis. Renal ultrasound scans may initially show normally sized kidneys with increased echogenicity, poor corticomedullary differentiation, and corticomedullary cysts and can progress to atrophic kidneys with more prominent cysts. A kidney biopsy may show the triad of corticomedullary cysts, tubular basement membrane disruption and interstitial fibrosis. NPHP is diagnosed using renal biopsy or by mutational analysis. So far, mutations in 11 genes (NPHP1-11) are known to cause nephronophthisis. Most of the mutations found in these genes are also associated with development of retinal degeneration and various pathologies in other organs. The NPHP proteins involved in retinal degeneration are expressed in the photoreceptor connecting cilium, a structural counterpart of the primary cilium found in most cell types.

2. The Photoreceptor Connecting Cilium

Cilia are composed of nine microtubule doublets (called the axoneme) in a cylindrical arrangement that emanate from basal bodies, the microtubule organizing centers of the cell. There are three types of cilia: nodal cilia that are found on cells of the embryonic node and play a role in body patterning, motile cilia that are necessary for locomotion and fluid movement, and primary cilia that serve mainly sensory functions. Motile cilia have an additional microtubule doublet in found in

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the center of the axonemal cylinder giving it a characteristic 9+2 pattern unlike the 9+0 pattern of doublets found in primary cilia. Nodal cilia were initially thought to have 9+0 arrangement but some have recently been found to be 9+2 (Caspary et al., 2007) (Novarino et al., 2011). In addition to the central pair of doublets, motile cilia also have inner and outer dynein arms on the outer doublets that are required for generation of force necessary for motility. The axonemal structure is anchored at the base by the basal body or mother centriole.

The connecting cilium (CC) of vertebrate photoreceptors. 0.5-1.2μm in length and approximately 0.2μm in diameter, connects the biosynthetic inner segment (IS) to the sensory outer segment (OS) (**Fig. 2**). The entire structure consisting of CC and OS is also called the "photosensitive cilium". It is now recognized that mutations in numerous genes, which are expressed in the photoreceptor connecting cilium cause a group of heterogeneous diseases called ciliopathies. Because of the ubiquitous expression of these genes in primary cilia, ciliopathies often affect multiple organs including kidney, retina, brain, or spermatozoa. The most prominent syndromic diseases that affect the retina are Joubert Syndrome, Bardet-Biedl syndrome, and Senior Løken syndrome (**Table 1**).

Senior-Løken syndrome and the mutations in the genes that are associated to the development of this disease will be important to study to understand the basic mechanisms of protein transport through the connecting cilium. Therefore, it is important to understand what the normal function of each of the nephrocystins to understand pathogenesis of retinal degeneration in these patients.

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3. Genes Associated with Development of Senior-Løken Syndrome

By positional cloning, several mutations in *NPHP* genes have been found to cause nephronophthisis (Hildebrandt et al., 2009). The mutations which cause disease are inherited in an autosomal recessive pattern. Transmission can be monogenic (i.e. homozygous and compound heterozygous mutations) or digenic (i.e. double heterozygote mutations). These genes include *NPHP1, NPHP2/Inversin, NPHP3, NPHP4, NPHP5/IQCB1, NPHP6/CEP290, NPHP7/GLIS2, NPHP8/RPGRIP1L, NPHP9/NEK8, NPHP10/SDCAAG8*, *NPHP11/*TMEM67*, NPH12/TTC21B, and NPHP13/WDR19*. The frequency of RP with nephronophthisis (Senior-Løken syndrome) depends on which nephrocystin gene is mutated. The frequency of retinal dystrophy in *NPHP1-4* is less than 35% and reaches 100% in *NPHP5* and *NPHP6* mutations (**Table 2**). It should be noted that mutations in each of the *nephrocystin* genes may give rise to heterogeneous phenotypes and even different syndromes. Abnormalities in multiple organs may develop including the retina, brain, liver and heart (**Table 2**). For example, *NPHP6/CEP290* mutations may be associated with isolated Leber congenital amaurosis (LCA), Senior-Løken syndrome (SLS) or the lethal multi-systemic Meckel Gruber syndrome (MKS) (**Table 1**). The presence of modifier alleles may further contribute to the complexity of the ciliopathies. In contrast, mutations in *NPHP5* are only associated with development of the retinal-renal disease phenotype making this gene the classic Senior-Løken gene (Wolf and Hildebrandt, 2011).

3.1. NPHP1

Nephronophthisis, first described by Fanconi et al. (1951), is caused by mutations in 13 distinct and unrelated genes (NPHP1-13) (**Table 2**) (Wolf and Hildebrandt, 2011). Juvenile nephronophthisis is caused by mutations in *nephrocystin-1* (*NPHP1*), the first of the nephronophthisis genes discovered. The disease has a median age of developing end stage renal disease (ESRD) of 13 years old. *NPHP1* is located on chromosome 2q13 and contains 20 exons. The NPHP1 gene produces a 4.5kb-transcript encoding an 83kDa protein. NPHP1 contains an SH3 domain (Otto et al., 2002), a coiled-coil domain in the N-terminus (Donaldson et al., 2002) and a highly conserved C-terminal domain called a nephrocystin homology domain (NHD) (Donaldson et al., 2002). The major gene defect observed in juvenile nephronophthisis is a large homozygous deletion in the NHD region of *NPHP1* (Otto et al., 2002). Deletion of this region is also associated with development of Cogan syndrome with nephronophthisis (Hildebrandt et al., 1997) (**Table 1**).

In the mouse retina, NPHP1 is expressed in mouse photoreceptors and localizes to the connecting cilium in close proximity to the basal body (Fliegauf et al., 2006). This may explain why a subset of patients with *NPHP1* mutations develops retinal degeneration. Targeted disruption of *NPHP1* in the mouse (deletion of the last C-terminal exon 20) did not produce nephronophthisis, but exhibited rapid retinal degeneration starting at P14-P21 (Jiang et al., 2009) and caused male infertility (Jiang et al., 2008). The $Nphp^{-/-}$ mice failed to develop normal outer segment (OS) but the axoneme of the connecting cilium was still present in the

mutant photoreceptors. At 8 months of age, there is an almost complete absence of the OS and IS (Jiang et al., 2009) (**Table 3**). Rhodopsin, transducin and other phototransduction proteins destined for the OS were predominantly located in the IS at P14, before significant degeneration of photoreceptor cells began (Jiang et al., 2009). However, myosin VIIA, RPGR, KIF3A, centrin-1, and WDR19 localized normally to the connecting cilium.

At P14, there was significant increase of apoptosis in photoreceptor cells which correlates to the increased amount of rhodopsin mislocalized in the IS (Jiang et al., 2009). The retinal phenotype was rescued after crossing the *NPHP1* mutant mice with a transgenic mouse expressing GFP-tagged NPHP1 (Jiang et al., 2009) showing specificity of the effect of a targeted disruption in *NPHP1*. Another important observation in these mice was the mislocalization of specific IFT (intraflagellar transport) particles along the connecting cilium. These include IFT88 but not IFT122 which suggests that NPHP1 may regulate a specific subset of IFT particles and their associated cargos destined to the OS (Jiang et al., 2009) (Donaldson et al., 2002). Importantly, nephrocystin-1 depletion does not completely abolish photoreceptor ciliogenesis and IFT, it only affects the transportation efficiency and sorting mechanism of IFT (Jiang et al., 2009).

3.2. NPHP2/INVS

Genetic linkage to infantile nephronophthisis, which is a form of nephronophthisis that clinically presents before the age of 5 years, has been

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mapped to 9q31 (Haider et al., 1998). The gene *INVS* has a 5.5kb transcript and encodes a 1065 amino acid protein, NPHP2/inversin (Morgan et al., 1998). In human, two transcript variants encoding distinct isoforms (long form, 1065 amino acids, and short form, 895aa) have been identified for this gene. The shorter form lacks an in-frame segment in the coding region. The mouse genome contains only the long form transcript. The protein contains multiple domains and protein-binding motifs including 16 tandem ankyrin repeats in the N-terminus, 2 putative nuclear localization signals (Morgan et al., 1998), 2 IQ-type calmodulin-binding domains (Morgan et al., 2002b), and 2 D-box domains (one is important for binding Apc2, a subunit of the anaphase promoting complex, APC) (Morgan et al., 2002a). The NPHP2 gene is conserved in chimpanzee, dog, cow, mouse, rat, chicken, zebrafish, and *C. elegans*.

The *inv/inv* mouse, which has a deletion of exons 3-11 of *INVS*, resembles the human disease with renal cysts, hepatobiliary duct malformations and other renal histopathological features (Phillips et al., 2004). It also has been shown that recessive mutations in *INVS* cause infantile nephronophthisis with or without presence of *situs inversus* in patients, a classic finding in ciliopathies (Otto et al., 2003). Moreover, disruption of *invs* in zebrafish also causes renal cyst formation (Otto et al., 2003). NPHP2/Inversin is expressed in the retina as shown by fluorescence microscopy using an inversin:GFP transgenic mouse line (Watanabe et al., 2003). However, there is no report of a retinal phenotype in the loss-of-function *invs* mouse and zebrafish models. There has only been one known case of a mutation in *NPHP2* (in exon 13, R907X) causing retinal degeneration (SLS) suggesting that

there may be genetic modifiers in the background of *NPHP2* mutations which lead to development of retinal pathology (O'Toole et al., 2006).

Nphp2/Inversin accumulates at the base of the primary cilium (termed the *Inv* compartment) and acts as an anchor for Nphp3 and NPHP9/Nek8 in this compartment (Sang et al., 2011; Shiba et al., 2010). Nphp2/Inversin has not been found to affect formation or maintenance of the renal primary cilia in the *inv/inv* mouse. This suggests that NPHP2/inversin is not necessary for regulating structure of the primary cilia (Morgan et al., 2002a). Inversin also colocalizes with NPHP1 and β-tubulin in the primary cilium of MDCK cells (Otto et al., 2003) but this interaction has not been validated in photoreceptors.

The ERK pathway, known to regulate cell proliferation, has been thought to play a role in cyst formation. In the *inv/inv* mouse expressing a C-terminal truncated form of *INVS*, the ERK pathway is activated (Watanabe et al., 2003). After administration of a MAP kinase inhibitor there was decreased ERK phosphorylation and inhibition of renal cyst progression (Watanabe et al., 2003). Finally, the canonical Wnt/β-catenin signaling pathway is also activated in renal cells of a patient with NPHP2 suggesting a role of this pathway in cyst formation (Bellavia et al., 2010). However, there is very little data on the role of the ERK and Wnt/β-catenin signaling pathways in photoreceptor cells, cells that are no longer proliferating.

3.3. NPHP3

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NPHP3 is associated with adolescent nephronophthisis that causes ESRD at a median age of 19 years old (Morgan et al., 1998). The NPHP3 gene was mapped on chromosome 3q22 (Omran et al., 2000). The *NPHP3* gene (28 exons) generates a 6.5kb transcript encoding a 1,330-amino acid protein (Olbrich et al., 2003). It is expressed in the retina suggesting that it contributes to development of Senior-Løken syndrome (Olbrich et al., 2003). Linkage on the *NPHP3* loci has been shown in families with Senior-Løken syndrome (Omran et al., 2002).

NPHP3 contains several protein-protein interaction domains including an N-terminal coiled-coil domain, a C-terminal tetrarico peptide repeat domain and a tubulin-tyrosine kinase domain (Olbrich et al., 2003). Furthermore, it is N-terminally mytristoylated and interacts with the acyl-binding proteins UNC119A and UNC199B (Wright et al., 2011). Both UNC119A and UNC119B contain immunoglobulin-like hydrophobic binding pocket to accommodate acyl side chains (Wright et al., 2011; Zhang et al., 2011). Targeting to primary cilia is controlled by N-terminal myristoylation as a NPHP3 (G2A) mutant did not target to cilia (Nakata et al., 2012). Knockdown of UNC119B also prevented cilia targeting, suggesting that both myristoylation of NPHP3 and interaction with the myristoyl-binding protein UNC119B is important for targeting (Nakata et al., 2012; Wright et al., 2011). Furthermore, Wright et al. showed that the GTP-binding protein ARL3 and its GTPase activating protein (GAP), Retinitis Pigmentosa 2 protein (RP2), are involved in release of cargo from UND119B (Wright et al., 2011). Nephrocystin-3 also interacts with NPHP2/inversin, and like inversin, can inhibit canonical Wnt signaling (Bergmann et al., 2008).

The *pcy* mouse, a spontaneous mouse cystic kidney disease model, produces a phenotype that resembles the disease progression of NPHP3 in humans. Synteny has been demonstrated in the human *NPHP3* and mouse *pcy* loci (Omran et al., 2000). However, there is no report of retinal pathology in the *pcy* mouse. The *pcy* mutation is a hypomorphic most likely caused by a missense mutation (Olbrich et al., 2003). Generation of an *NPHP3* loss-of-function mouse is embryonic lethal with additional phenotypes of *situs inversus* and congenital heart defects (Bergmann et al., 2008). In contrast to NPHP1 and NPHP2, loss of function of NPHP3 shows a defect in the primary cilium length suggesting a role in regulating the structure of the primary cilium (Bergmann et al., 2008). Knockdown of nephrocystin-3 in *Xenopus laevis* or zebrafish showed defective convergent extension, which is regulated by non-canonical Wnt pathway activation (planar cell polarity defects) (Bergmann et al., 2008; Zhou et al., 2010).

3.4. NPHP4

NPHP4 is located in chromosome 1p36 and encodes a 1,250-amino acid protein called nephrocystin-4 (Mollet et al., 2002; Schuermann et al., 2002). Development of Senior-Løken syndrome has been shown to associate to this region (Mollet et al., 2002; Otto et al., 2002). NPHP4 has a proline-rich region known to bind SH3 domains and is thought to mediate its binding with NPHP1 (Mollet et al., 2002).

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Nephrocystin-4 interacts with p130Cas and Pyk2, proteins involved in cell-cell adhesion (Mollet et al., 2005). It is also known that NPHP4 negatively regulates Pyk2-dependent tyrosine phosphorylation of NPHP1, which is important for localization of NPHP1 to a trans-Golgi interacting protein (Liebau et al., 2011). NPHP4 localizes to the renal epithelial cell's periphery, primary cilium (where it is colocalized with α-tubulin) and the basal body (Keller et al., 2005; Mollet et al., 2005). NPHP1 and NPHP4 also associate with other tight junction proteins such as PALS1/PATJ and Par6 (Delous et al., 2009). Similar to NPHP2/inversin, NPHP4 is thought to regulate the canonical and noncanonical Wnt-signaling pathways (Burckle et al., 2011).

NPHP4 interacts with the Retinitis GTPase Regulator (RPGR) and RPGR-Interacting Protein 1 (RPGRIP1) and may provide the link to development of retinal degeneration in patients with a mutation in *NPHP4* (Murga-Zamalloa et al., 2009; Roepman et al., 2005). RPGR mutations cause over 70% of X-linked RP cases (Murga-Zamalloa et al., 2009). Mutations in RPGRIP1 are linked to development of Leber congenital amaurosis (LCA) where both rod and cone photoreceptors degenerate early in life (Roepman et al., 2005). RPGRIP1 is localized to the photoreceptor connecting cilia associated with the ciliary axoneme (Hong et al., 2001). Mutations in either *NPHP4* or *RPGRIP1* abolish this interaction (Roepman et al., 2005).

A loss-of-function mouse model of *NPHP4* shows severe retinal degeneration reminiscent of LCA, with mislocalization of rhodopsin and ROM to the IS (Won et al., 2011). However, no renal pathology was observed in this mouse similar to the

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NPHP1 and *NPHP6* mutations in mice (Chang et al., 2006; Jiang et al., 2008; Won et al., 2011).

3.5. NPHP5/IQCB1

Located on human chromosome 3, the *NPHP5* gene encodes a 69kD protein called nephrocystin-5 or NPHP5 (also referred to as IQCB1) (Otto et al., 2005). By sequence analysis, it was found that NPHP5 contains two IQ-calmodulin binding regions (similar to NPHP2/inversin) and several coiled-coiled domains thought to be important for protein-protein interactions (Otto et al., 2005). In contrast to NPHP1-4 where approximately 10-33% of patients develop retinal degeneration, Senior-Løken syndrome is thought to develop in 100% of patients with NPHP5 mutations (Hildebrandt et al., 2009). Moreover, unlike the other nephrocystin genes, which may involve multiple organs, *NPHP5* mutations only associate with the retinal-renal phenotype, making this the Senior-Løken gene (Hildebrandt et al., 2009). *NPHP5* mutations in isolated cases of LCA (without nephronophthisis) have also been found (Estrada-Cuzcano et al., 2011; Stone et al., 2011).

NPHP5 is expressed in the photoreceptor connecting cilia (Otto et al., 2005). It is known to interact with calmodulin, NPHP6 and RPGR, other proteins involved in development of retinal degeneration (Murga-Zamalloa et al., 2010; Schafer et al., 2008). Knockdown of NPHP5 in zebrafish causes mislocalization of opsin suggesting its role in specific transport of proteins to the OS (Zhao and Malicki, 2011).

3.6. NPHP6/Cep290

The *NPHP6/Cep290* gene spans nearly 100 kb and consists of 54 exons, and is located in chromosome 12q21.32 (Sayer et al., 2006). Null mutations in *NPHP6* has been linked in a cohort of patients with nephronophthisis, Joubert syndrome, Meckel syndrome or Senior-Løken syndrome (Baala et al., 2007a; Helou et al., 2007; Sayer et al., 2006) (**Table 1**). Hypomorphic *NPHP6* mutations have been found in isolated LCA patients (den Hollander et al., 2006). The *NPHP6*/*Cep290* gene encodes a 2,472-amino acid protein, which contains several putative coiled-coil domains, an ATP/GTP binding loop (P-loop) and a C-terminal myosin-tail homology domain among others (Chang et al., 2006; Sayer et al., 2006). NPHP6 is localized in the photoreceptor connecting cilium and associates with dynein-dynacting and kinesin-II molecular motor subunits, RPGR, RPGRIP, and NPHP5 (Chang et al., 2006; Moradi et al., 2011; Murga-Zamalloa et al., 2010; Schafer et al., 2008). NPHP6 also directly interacts with the transcription factor ATF4 and mediates activation of ATF4 targets (Sayer et al., 2006).

NPHP6 is important for centrosome and cilia function. Loss-of-function *cep290* mutants in zebrafish exhibited renal, retinal and cerebellar anomalies highly reminiscent of the clinical findings in Joubert syndrome (Sayer et al., 2006). The *rd16* mouse, which has an in-frame deletion of exons 35-39 also develops an early-onset retinal degeneration phenotype associated with kidney and cerebellar abnormalities (Chang et al., 2006). This deletion mostly removes the C-terminal myosin-tail homology domain of NPHP6. Another naturally occurring *Cep290* mutant is the Abyssinian retinal degeneration cat model (*rdAc)* (Narfstrom, 1983).

The retina phenotype of the mutant cat resembles a slowly developing recessive RP, with reduced ERG a-wave amplitudes at 7 months, and complete photoreceptor degeneration at 3-5 years of age. The CEP290 gene defect was recently identified as a SNP of intron 50, generating a new splice site, a 4 bp insertion and a CEP protein that is shortened by 159 amino acids (Menotti-Raymond et al., 2007).

3.7. NPHP7/GLIS2

NPHP7/GLIS2 encodes a 3.8kb transcript containing 6 exons. The protein, a 55kDa Krupel-like transcription factor is important for kidney development (Zhang et al., 2002). Mutations in *NPHP7/GLIS2* cause nephronophthisis in humans (Attanasio et al., 2007) without reports of association of retinal degeneration. The *NPHP7/GLIS2* loss-of-function mouse recapitulates the human disease with atrophic kidneys and presence of fibrosis (Attanasio et al., 2007). It is not known whether NPHP7/GLIS2 is expressed in the mammalian retina and is currently not known whether it has an important role in photoreceptor development and maintenance.

3.8. NPHP8/RPGRIP1L

The *NPHP8/RPGRIP1L* (retinitis GTPase regulator-interacting protein-1 like) is located on chromosome 16q12.2 with 35 exons. It encodes a protein with 1,315 amino acids. NPHP8/RPGRIP1L shares two protein kinase C conserved regions (C2 domains) with RPGRIP, but overall sequence similarity is relatively low (31%) (Arts et al., 2007). The protein further contains five coiled-coil domains in the N-terminus

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and an RPGR-interacting domain (RID) in the C-terminus (Delous et al., 2007). By RT-PCR, RPGRIP1L is expressed in retina, kidney and brain (Arts et al., 2007).

NPHP8/RPGRIP1L is known to interact with NPHP4 and NPHP6/CEP290. When expressed individually in COS cells, NPHP8/RPGRIP1L is soluble, but when co-expressed with NPHP4, it localizes to basal bodies and cilia suggesting that NPHP4 recruits NPHP8/RPGRIP1L to these structures. In photoreceptors, NPHP8/RPGRIP1L localizes to the photoreceptor connecting cilium, basal body, in the ONL and the synaptic region, areas where other NPHP gene products, particularly NPHP4 are located (Arts et al., 2007; Delous et al., 2007).

Loss-of-function mutations in *NPHP8/RPGRIP1L* cause Joubert syndrome (JBTS) or Meckel syndrome (MKS), ataxia and abnormal eye movements (Arts et al., 2007; Delous et al., 2007; Wolf et al., 2007) (**Table 1**) consistent with the notion that it is required for normal brain and kidney development. In *Rpgrip1l* knockout mice (*ftm*, fantom or fused tow mouse), fetuses had head abnormalities and very small or non-existing eyes (Delous et al., 2007). Patients with *NPHP8/RPGRIP1L* null alleles do not develop RP; however, an A229T variant is associated with photoreceptor cell loss (Khanna et al., 2009). This mutation compromises interaction with RPGR and causes degeneration. Absence of the mutation is thought to have a protective effect from RP (Khanna et al., 2009).

3.9. NPHP9/NEK8

Mutations in human *NPHP9/NEK8* are associated with development of nephronophthisis (Otto et al., 2008). It is not clear whether mutations in *NPHP9/Nek8* cause Senior-Løken syndrome. Of the 3 patients reported with mutations in this gene, one patient with a heterozygote mutation in *NPHP9/Nek8* developed RP and was blind at 24 years of age; however, this patient also contained an additional homozygous mutation in *NPHP5* (Otto et al., 2008).

NPHP9/*NEK8* is a serine/threonine protein kinase and a member of the NIMA (never in mitosis-A)-related kinase (Nek) family. *NPHP/NEK8* encodes a 692 amino acid protein, which contains an N-terminal catalytic (kinase) domain, several RCC1-like domain, (RCC1 is a guanine nucleotide exchange factor for Ran GTPase) and a coiled-coil domain in the C-terminus (Holland et al., 2002). Members of this family are thought to regulate coordination of cilium formation and the cell cycle. Like other nephrocystin proteins, *NPHP9/NEK8* are localized to the primary cilium. It is not known whether it is expressed in photoreceptor cells of the mammalian retina.

Mutations in *NPHP9/NEK8* occur in the juvenile cystic kidney (*jck*) mouse, a model of autosomal recessive cystic kidney disease (Atala et al., 1993; Liu et al., 2002). Knockdown studies in zebrafish produce development of pronephric cysts (Liu et al., 2002); however, in both *jck* and zebrafish models, retinal pathology has not been reported. It is more likely that mutations in *NPHP9/NEK8* are not associated with development of retinal degeneration in humans, which correlates well with the vertebrate models.

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3.10. NPHP10/SDCCAG8

Truncating mutations in *NPHP10/SDCCAG8* have been found in several families with nephronophthisis and associated pathologies including retinal degeneration (Otto et al., 2010). Mutations in *NPHP10/SDCCAG8* have also been found in Bardet-Biedl syndrome (BBS) (Schaefer et al., 2011). *NPHP10/SDCCAG8* has 3.3kb transcript encoding an 88kDa protein containing an N-terminal globular domain, a nuclear localization signal and eight putative coiled-coil domains (Otto et al., 2010).

NPHP10/SDCCAG8 is localized to the connecting cilium area in photoreceptor cells particularly in the basal body and transition zone (Otto et al., 2010). It also has been shown that it co-localizes with other proteins associated with development of retinal degeneration including NPHP5, RPGRIP and RP1 (Otto et al., 2010). Knockdown of NPHP10/SDCCAG8 in zebrafish resulted in kidney cysts, hydrocephalus as well as other developmental problems but no retinal phenotype has been reported (Otto et al., 2010).

3.11. NPHP11/MKS3/TMEM67

Hypomorphic mutations in *NPHP11/MKS3/TMEM67* cause nephronophthisis with associated liver fibrosis (Otto et al., 2009) as well as other diseases in the ciliopathy spectrum including Meckel-Gruber syndrome (Smith et al., 2006) and Joubert syndrome (Baala et al., 2007b). Out of the 12 individuals found with a

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mutation in *NPHP11/MKS3/TMEM67*, 3 patients developed retinal degeneration/blindness (Otto et al., 2009).

NPHP11/MKS3/TMEM67 is located in chromosome 8q22 and transcribed into 2 isoforms. The longer isoform 1 (28 exons) contains 995 aa and the shorter isoform 2 (29 exons) contains 914 aa. In the shorter isoform, the three N-terminal exons of isoform 1 are replaced by two alternate exons. The protein encoded by the isoforms, called meckelin, is a transmembrane protein predicted to have an extracellular region with four N-liked glycosylation sites, seven transmembrane regions and a short cytoplasmic tail (Smith et al., 2006).

The *NPHP11/MKS3/TMEM67* locus is syntenic to the *Wpk* (Wistar polycystic kidney) locus in rat, which carries a hypomorphic mutation (P394L) that produces polycystic kidney disease, agenesis of the corpus callosum and hydrocephalus (Gattone et al., 2004; Nauta et al., 2000). Further, the Wpk photoreceptors showed advanced retinal degeneration at 3 weeks of age (Tammachote et al., 2009).

A spontaneous deletion of *NPHP11/MKS3/TMEM67* in mouse (*bpck* mouse – bilateral polycystic kidneys) has also been reported with phenotypes that resemble the human disease (polycystic kidney and hydrocephalus) (Cook et al., 2009). The photoreceptors in *bpck* mice degenerate very early with complete absence of the OS at P24 and reduction of the axonemal tip in the connecting cilia at P14 (Collin et al., 2012). However, at P14, the structure of the CC and the basal body was normal. Rhodopsin and ROM1 were mislocalized at P12, but RPGR, RPGRIP1L and ALMS1 located normally to the CC. At this age, mutant OS were disorganized and arrestin

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and transducin failed to translocate in the photoreceptors when exposed to light (Collin et al., 2012). Cone photoreceptors were also affected in the *bpck* retina. Both ML-opsin and S-opsin were mislocalized and cone outer segments appeared shortened (Collin et al., 2012). These results suggest that *NPHP11*, like other of the nephrocystin proteins in photoreceptors are important in regulation of protein trafficking.

3.12. NPHP12/TTC21B/THM1

NPHP12/TTC21B/THM1 (tetratricopeptide repeat domain 21B) located on chromosome 2q24.3 contains 29 coding exons and encodes, IFT139, a ~150kDa retrograde intraflagellar transport (IFT) protein (Davis et al., 2011). NPHP12/TTCB21 contains eleven tetratricopeptide (TPR) domains and is important for mediating protein-protein interactions and assembly of multimeric complexes. Mutations in *NPHP12/TTC21B/THM1* cause disease phenotypes in the ciliopathy spectrum including isolated nephronophthisis and Jeune syndrome (asphyxiating thoracic dystrophy) (Davis et al., 2011) (**Table 1**). Mutations in this gene also contribute as a modifier allele with other disease-causing genes (Davis et al., 2011).

In IMCD3 cells, NPHP12/TTCB21B colocalizes with Acetyl-tubulin in cilia, and was shown to be present throughout the axoneme, reminiscent of IFT88 localization (Tran et al., 2008). Live cell imaging in NPHP12/TTCB21B-deficient IMCD3 cells revealed IFT88-EYFP at the distal ends of cilia, consistent with impairment of retrograde IFT (Tran et al., 2008).

The *alien* mouse (*aln*), an *NPHP12/TTC21B* null mutant generated by ENU mutagenesis, is embryonic lethal at E18.5. *Aln* shows skeletal abnormalities, neural tube defects as well as delayed development of the eye and brain (Tran et al., 2008), similar to the hallmark features in individuals with Jeune syndrome. In rodents, specific knockdown of *NPHP12/TTC21B* show abnormal photoreceptor structure (Davis, Zhang et al. 2011).

3.13. NPHP13/WDR19

NPHP13/WDR19 belongs to the family of WD-repeat proteins. The WDR domain consists of a conserved 40 amino acids domain flanked by glycine-histidine and tryptophan-aspartic acid dipeptides (WD) (Lin et al., 2003). WD-repeat containing proteins are thought to be important in vesicle formation and vesicular trafficking (Lin et al., 2003). The *NPHP13/WDR19* gene contains 36 exons located on chromosome 4p15-4p11. It encodes, IFT144, a protein containing six WD repeats, a clathrin heavy-chain repeat and three transmembrane domains (Lin et al., 2003). IFT144 is a member of the intraflagellar transport (IFT) complex A (Bredrup et al., 2011). Proteins in the IFT-A complex regulate retrograde ciliary transport in contrast to the IFT-B complex, which regulate anterograde transport.

Compound heterozygous mutations in *NPHP13/WDR19* have been shown to associate with development of Sensenbrenner syndrome characterized by renal insufficiency leading to chronic renal failure (nephronophtisis-like), RP and various skeletal anomalies including polydactyly, short fingers, and other bone

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abnormalities (Bredrup et al., 2011). Compound heterozygous and homozygous missese mutations in *NPHP13/WDR19* have also been identified in isolated nephronophthisis and in Jeune syndrome, a syndrome with similar clinical findings as that of Sensenbrenner syndrome (Bredrup et al., 2011).

NPHP13/WDR19 localizes to the primary cilium of fibroblast cells and when absent, negatively affects cila formation (Bredrup et al., 2011). In disease-causing mutations, which still express the protein, IFT-B particles accumulate in the tip of cilia highlighting the importance of NPHP13/WDR19 in retrograde trafficking.

Of the six patients with *NPHP13/WDR19* mutations associated with disease development (Sensenbrenner, isolated nephronophthisis and Jeune syndrome), three develop retinal abnormalities (50%) with two being diagnosed with RP (Bredrup et al., 2011). It is not known whether NPHP13/WDR19 localizes to the photoreceptor connecting cilium; however, presumably, it has an important role in retrograde protein trafficking in photoreceptor cells. It will be important to elucidate proteins which NPHP13/WDR19 bind and transport from the photoreceptor OS to IS. Also, animal models will be important in elucidating the molecular mechanism of NPHP13/WDR19.

4. Conclusions and Perspectives

Mutations in 13 'nephronophthisis' genes have now been reported and there will be many more discovered in the next five years. Mutations in these genes cause heterogeneous phenotypes affecting several organs. The emergence of a 'unifying'

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theory of ciliopathies greatly improves how we think mutations in a single gene can cause such widespread effects on different organs; however, fundamental questions remain which include why mutations in each of genes cause such discrete phenotypes that only affect certain organs and why apparently similar mutations cause a heterogeneous phenotype. The answer to the second question is most likely the presence of modifier alleles; however, to answer the first question, a more detailed understanding of the molecular mechanism of these proteins is needed.

The occurrence of RP with nephronophthisis or Senior-Løken syndrome (SLS) is one of the more common co-segregation pattern of pathologies observed in mutations of NPHP genes. Many but not all of the nephrocytin genes cause SLS; it is striking that all mutations found in *NPHP5* cause SLS without much heterogeneous variability as it is not associated with development of pathologies in other organs. Therefore, to answer the first question posed above, specifically, why mutations in *NPHP5* cause only a kidney and retina phenotype relies on establishing robust *in vitro* and *in vivo* model systems to understand the molecular mechanism that NPHP5 controls. Nevertheless, it is important to study all nephrocystin genes associated with development of retinal degeneration to understand whether there is a 'unifying' mechanism which they control – particularly, trafficking through the connecting cilium.

Outside of regulation of trafficking of proteins in photoreceptor cells, another research area that is emerging focuses on signaling pathways which nephrocystin regulate. It is tempting to speculate that some of the signaling pathways important for kidney development and development of kidney pathology may be important for

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photoreceptor maintenance and survival. For example, misregulation of calcium signaling is known to be important for kidney cyst formation; however, not much is known in how any of the nephrocystin genes regulate local calcium homeostasis in the connecting cilium/basal body region.

Finally, we recommend a closer association of researchers in the visual sciences and ophthalmology and nephrology. Collaboration in both areas is critical to understand the etiology of Senior-Løken syndrome to advance early diagnosis of patients and devise treatment strategies to delay or inhibit disease progression.

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Fig. 1. Fundus photo of a patient diagnosed with Senior-Løken syndrome. Shown are classic findings in RP: bone-spicule shaped deposits, attenuation of blood vessels with macular sparing.

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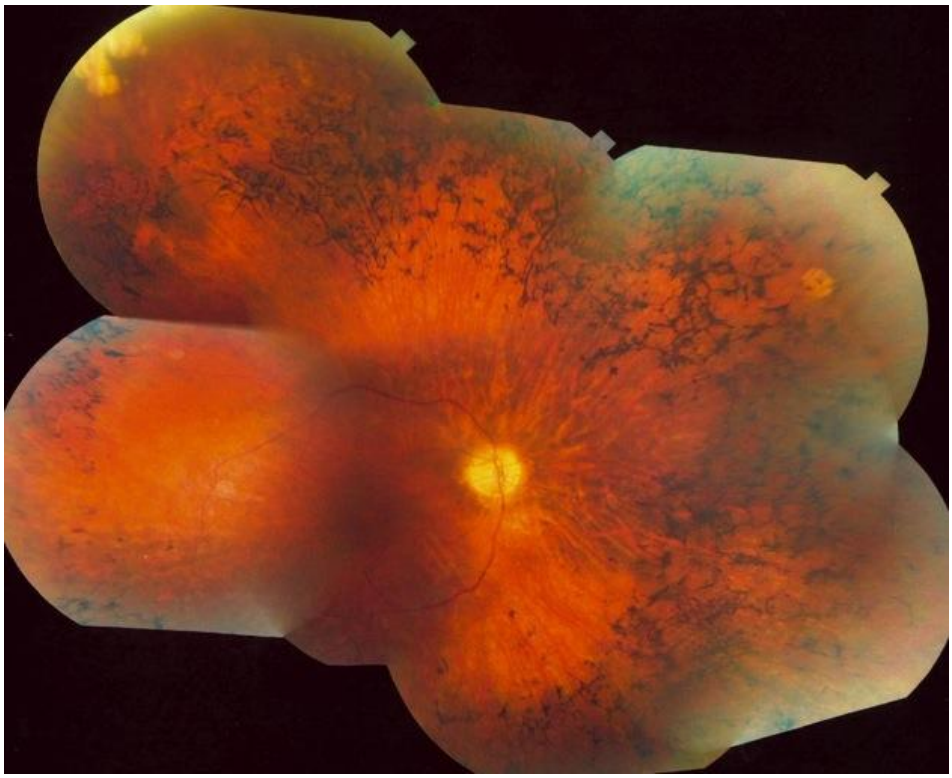

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Fig. 2. Cartoon of the connecting cilium (CC), the inner segment (IS), and the proximal part of the outer segment (OS) of a photoreceptor. Shown in the left is a selection of ciliopathy genes involved in retinal diseases (in parenthesis). In red are the NPHP genes, which causes Senior-Løken syndrome (SLS). Also shown is the localization of the proteins in the photoreceptor. Most of the nephrocystin proteins are localized in the basal body and connecting cilium. RP – retinitis pigmentosa; MD – macular degeneration; LCA – Leber congenital amaurosis; SLS - Senior-Løken syndrome; BBS – Bardet-Biedl Syndrome; JS – Joubert syndrome

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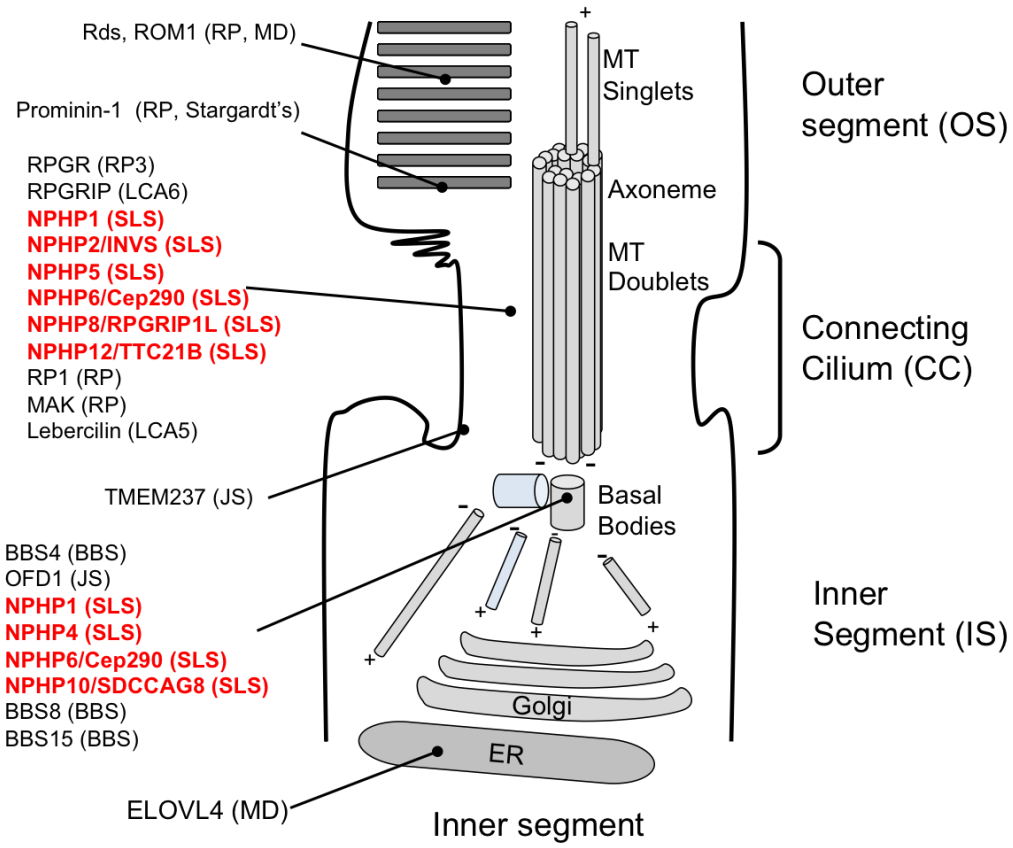

Table 1. Syndromes associated with mutations from different NPHP genes. *

denotes that gene may be a genetic modifier for the disease

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Syndrome	NPHP Gene Mutations	Other pathologies besides RP
Bardet Biedl Syndrome	6, 11*	obesity, mental retardation, polydactyly,hypogenitalism, renal abnormalities
Senior-Løken syndrome	1, 5, 6, 10	nephronophthisis
Cogan syndrome	4	corneal inflammation, hearing loss, dizziness
COACH syndrome	8, 11	mental retardation, ataxia due to cerebellar hypoplasia, and hepatic fibrosis, coloboma, renal cysts
Joubert syndrome	1, 6, 8, 11	cerebellar ataxia, oculomotor apraxia, hypotonia, neonatal breathing abnormalities, psychomotor delay, vermis hypoplasia, renal cysts
Meckel syndrome	3, 6, 8, 11	encephalocele, hepatic ductal dysplasia and cysts, and polydactyly
Jeune syndrome	12, 13	thoracic hypoplasia, brachydactyly, chronic nephritis
Renal-Hepatic-Pancreatic hyperplasia (RHD)	3	pancreatic fibrosis, renal dysplasia, hepatic dysgenesis
Sensenbrenner syndrome	13	microcephaly, narrow thorax, heart defects, liver fibrosis, hypoplasia of the corpus callosum,

Table 2. Retinal degeneration (RD) in NPHP mutations and mouse models and their retina-renal phenotype. NPHP = nephronophthisis; SLS = Senior-Løken syndrome; JS, Joubert syndrome; LCA= Leber congenital amaurosis; MKS = Meckel syndrome; BBS = Bardet-Biedl syndrome.

* aln mouse is lethal at E18.5 so retinal and renal phenotype cannot be determined; there is a knockdown model in the rat that has retinal degeneration

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Gene	Locus	RD	Mouse model	Retinal Phenotype	Renal Phenotype	Associated Human Diseases
NPHP1	2q13	6%	Nphp1$^{-/-}$	RD	No	NPHP, SLS
NPHP2/INVS	9q31	10%	*inv/inv*	?	Yes	NPHP, SLS
NPHP3	3q22	13%	*pcy*	?	Yes	NPHP, SLS
			Nphp3$^{-/-}$	ND (embryonic lethal)	ND (embryonic lethal)	
NPHP4	1p36	33%	Nphp4$^{-/-}$	RD	Yes	NPHP, SLS
NPHP5/IQCB1	3q21.1	100%	No	?	?	SLS, LCA
NPHP6/CEP290	12q21.32	100%	*rd16*	RD	No	NPHP, JS, MKS, SLS, LCA
NPHP7/GLIS2	16p13.3	0%	Nphp7$^{-/-}$	No	Yes	NPHP
NPHP8/ RPGRIP1L	16q12.2	100%	*ftm*	No	Yes	NPHP, JS, MKS, SLS
NPHP9/NEK8	17q11.1	0	*jck*	No	Yes	NPHP
NPHP10/ SDCAAG8	1q43	0	No	?	?	NPHP, SLS, BBS
NPHP11/MKS3/ TMEM67	8q21.1	0	*Bpck* *wpk* (rat)	RD RD	Yes Yes	NPHP (+liver fibrosis), MKS, JS

NPHP12/ TTC21B	2q24.3	0	*aln*	ND (embryonic lethal)*	ND (embryonic lethal)*	NPHP, Jeune
NPHP13/ WDR19	4p14-p11	50%	No	?	?	Sensenbrenner, Jeune

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