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The Oak Ridge Polycystic Kidney Mouse: Modeling Ciliopathies of Mice and Men

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Abstract

The Oak Ridge Polycystic Kidney (ORPK) mouse was described nearly 14 years ago as a model for human recessive Polycystic Kidney Disease. The ORPK mouse arose through integration of a transgene into an intron of the *ift88* gene resulting in a hypomorphic allele (*Ift88*^{Tg737Rpw}). The *Ift88*^{Tg737Rpw} mutation impairs intraflagellar transport (IFT), a process required for assembly of motile and immotile cilia. Historically, the primary immotile cilium was thought to have minimal importance for human health; however, a rapidly expanding number of human disorders have now been attributed to ciliary defects. Importantly, many of these phenotypes are present and can be analyzed using the ORPK mouse. In this review, we highlight the research conducted using the OPRK mouse and the phenotypes shared with human cilia disorders. Further, we describe an additional follicular dysplasia phenotype in the ORPK mouse, which alongside the ectodermal dysplasias seen in human Ellis-van Creveld and Sensenbrenner's syndromes, suggests an unappreciated role for primary cilia in the skin and hair follicle.

Keywords

Cilia; ciliopathies; hair follicle; skin; IFT88

Introduction

The Oak Ridge Polycystic Kidney (ORPK) mouse has a long history as a model for cystic kidney disease and later cilia dysfunction. The ORPK mouse was first described in 1994 and arose as part of a large-scale transgene-induced insertional mutagenesis project at the Oak Ridge National Laboratory. In the case of the *orpk* allele (currently designated as *Ift88*^{Tg737Rpw}) the transgene integrated into an intron near the 3′ end of the gene partially disrupting the expression and function of the intraflagellar transport 88 (IFT88, Tg737, polaris) protein, which is required for the formation of motile and immotile cilia. In contrast to the *Ift88* null mutations (*Ift88*^{tm1Rpw}, *Ift88*$^{tm1.1Bky}$, and *Ift88*^{fxo}), which are embryonic lethal around the beginning of organogenesis, the hypomorphic nature of the IFT88 allele in the ORPK mouse allows these homozygous mutant mice to survive into young adulthood. As such, the ORPK mouse has been a good model to analyze the role of primary cilia in a variety of tissues and to

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evaluate the molecular, cellular, and physiological connections between ciliary dysfunction and disease pathogenesis seen in human ciliopathies.

The gross phenotype of the ORPK mouse was originally described with a triad of scruffy fur, severe growth retardation, and preaxial polydactyly on all limbs (Moyer et al., 1994). The ORPK mouse is best known for its cystic renal phenotype, which resembles that of human autosomal recessive polycystic kidney disease (ARPKD). It was also the first mammalian model to establish a connection between cystic kidney disease and ciliary dysfunction (Pazour et al., 2000; Taulman et al., 2001; Pazour et al., 2002b). In addition to the cystic kidney disease, histological analyses of the ORPK mice revealed hepatic and pancreatic ductal abnormalities and cysts, retinal degeneration, skeletal defects, cerebellar hypoplasia, and hydrocephalus (Moyer et al., 1994; Pazour et al., 2002a; Cano et al., 2004; Banizs et al., 2005; Zhang et al., 2005; Chizhikov et al., 2007; Haycraft et al., 2007). In light of the importance of the ORPK model in understanding the ciliopathies, we present here a brief review of the ORPK mouse with additional findings regarding the development of the scruffy fur phenotype that implicate primary cilia in hair follicle and skin homeostasis.

The ORPK mouse and the link to cilia

The connection between Tg737/OSM-5/IFT88 and cilia resulted from a convergence of research from several laboratories using a variety of model organisms including mouse, *Caenorhabditis. elegans (C. elegans)*, and *Chlamydomonas reinhardtii* (*C. reinhardtii*) (Murcia et al., 2000; Pazour et al., 2000; Haycraft et al., 2001; Qin et al., 2001; Taulman et al., 2001). These studies demonstrated that the *Ift88* gene was highly conserved and that it was required for ciliogenesis. The IFT88 protein is needed for the bidirectional movement of proteins between the tip and base of the cilium, a process called intraflagellar transport (IFT), which was initially described in *C. reinhardtii* flagella (Kozminski et al., 1993). The ORPK mouse has a hypomorphic allele of IFT88 ($Ift88^{Tg737Rpw}$), leading to cilia which are stunted and malformed, but not completely abolished.

The multiple facets of the ORPK phenotype and its human analogs

Cilia can be either motile or immotile (primary cilia) and are present on nearly every cell in the mammalian body (see http://www.bowserlab.org/primarycilia/cilialist.html for a list of ciliated cells). The importance of motile cilia in processes such as mucus clearance and cerebrospinal fluid movement are well known. In contrast, the primary cilium was thought to be of minimal importance for mammalian development and health. In part, data from the ORPK mouse with its wide spectrum of phenotypes affecting numerous tissues has changed this perception (Figure 1). Although not mutually exclusive, for the purpose of this communication, the pathogenesis of the phenotypes in the ORPK mouse can be grouped according to several factors based on: (1) defects in cilia mediated sensory function (ciliary dysesthesia), (2) defects in ciliary structure maintenance, (3) defects in ciliary motility (ciliary dyskinesia), and (4) defects in ciliary mediated signal processing (e.g. the hedgehog pathway). These phenotypes in the ORPK mouse are comparable to several of the ciliopathies observed in humans caused by mutations in cilia proteins that impair cilia function (Table 1).

There is remarkable overlap and diversity in the expressivity of phenotypes associated with ciliary defects in humans. Several factors could contribute to this phenomenon. In part this may reflect the localization of the affected proteins at the basal body, cilia, or subdomain within the cilium in the different human ciliopathies. Data indicate there is also allelic variation among the human ciliopathies. This is evident in the case of Meckel syndrome, Nephronophthisis, and Joubert syndrome. Despite having multiple distinct phenotypes, mutations in shared genes have been identified as the underlying cause of several forms of JBS, MKS, and NPH with the phenotypic outcomes depending on the nature of the mutation (Baala et al., 2007a; Baala et

al., 2007b) *NPHP1/JBTS4* (Parisi et al., 2004), *MKS4/NPHP6/JBTS5* (Sayer et al., 2006; Baala et al., 2007a), and *MKS5/NPHP8/JBTS7* (Arts et al., 2007; Delous et al., 2007). Additionally, in some ciliopathies such as Bardet Biedl syndrome or Nephronophthisis there is oligogenic or triallelic inheritance whereby the resulting phenotype is modified or arises through a combination of mutations in two or more of the BBS or NPH genes (Katsanis, 2004; Badano et al., 2006a; Hoefele et al., 2007). Modifier gene effects are also evident in the case of the ORPK mouse (Moyer et al., 1994). ORPK mutants on the FVB/N background (ORPK-FVB/N) normally die prior to weaning with hydrocephalus and severe renal, hepatic and pancreatic phenotypes (Figure 1). In contrast, on the C3HeB/FeJLe background (ORPK-C3H) the ORPK mice can survive for longer than a year and have a much milder slower progressing renal and hepatic phenotype with a significant increase in fibrosis. In contrast to the ORPK-FVB/N mouse, the pancreas in the ORPK-C3H mouse is characterized by a greatly enlarged cyst that can encompass much of the abdominal cavity (Figure 1C, insert) (Sommardahl et al., 2001). Thus, genetic analyses using the ORPK mouse inbred on different backgrounds will provide important insights into potential modifier genes associated with ciliary dysfunction as reported by Sommardahl et al. Based on the modifier effects seen in BBS and NPH, it is feasible that this may yield additional loci involved in the human ciliopathies.

Defects in cilia mediated sensory function

The ORPK mouse has a number of phenotypes associated with primary ciliary dysfunction on epithelial cells. Most notably, this includes cysts in the renal tubules and ducts of the pancreas and liver and associated pancreatic and biliary ductal hyperplasia and interstitial fibrosis (Figure 1). In most cases, the pathogenesis of these abnormalities secondary to cilia dysfunction in the ORPK mouse remain poorly understood. In all three of these tissues, the cilium extends into the tubule or duct lumen where data suggest they function as mechanosensors that detect fluid movement (Praetorius and Spring, 2001;Praetorius and Spring, 2003). In the kidney, deflection of the cilium initiates a calcium signaling pathway which is dependant on the ciliary proteins polycystin 1 (PC1) and polycystin 2 (PC2) (Nauli et al., 2003). Mutations in either PC1 or PC2 cause cyst formation in mice and humans (Mochizuki et al., 1996;Wu et al., 1998). Defects in this flow response were demonstrated in perfused tubules isolated from the ORPK mutant mice (Liu et al., 2005b).

The impaired cilia function in ORPK animals also results in changes in membrane channel organization and activity. In ORPK renal epithelium there are altered Na+/H+ exchanger (NHE) and Epithelial Sodium Channel (ENAC) activities, deregulated apical calcium entry abnormal ciliary distribution of the cation channel polycystin 2 (Pazour et al., 2002b; Olteanu et al., 2006; Siroky et al., 2006). In addition, the choroid plexus of ORPK mice has altered chloride and sodium transport, defects in intracellular pH regulation, and a marked increase in intracellular cAMP levels (Banizs et al., 2005; Banizs et al., 2007). Although the pathogenesis of these defects remains incompletely understood, it is thought they are due to defects in IFT mediated channel/receptor transport into or along the cilium and/or transmission of signals from the cilia back into the cytosol.

An important aspect of cilia research is how loss of this organelle leads to cyst expansion. Data obtained recently from two independent rodent models have shown that there are defects in the orientation of cell divisions in cystic kidney disease which are thought to cause an expansion of the tubule diameter (cyst formation) rather than tubule elongation (Fischer et al., 2006). In contrast to the ORPK mouse, the genes mutated in these two models (renal deficient HNF1β in mice and *pck* mutant rat) do not disrupt the cilia assembly process but do affect cilia signaling activities. These data raise the possibility that the cilium or signals from the cilium have a role in establishing the direction of mitotic spindles. This could occur if cilia regulate Wnt signaling and influence the planar cell polarity pathway or function to position the basal body/centrioles,

which are located at the base of the cilium. Furthermore, this could involve the mechanosensory function of the cilium which would provide spatial cues regarding the axis of the tubule by sensing the movement of fluid through the tubule lumen. In the case of the ORPK mouse, the cilium is stunted and would have defects in establishing this spatial information.

An alternate hypothesis is that ciliogenic proteins such as IFT88 have a direct role in regulating the cell cycle. This was demonstrated in cell culture using siRNA to knockdown IFT88 expression, which promoted cell-cycle progression to S and G2/M phases (Robert et al., 2007). Further this study demonstrated a direct interaction between IFT88 and CHE-1, a protein that is known to inhibit retinoblastoma's (Rb) growth suppressing function. Intriguingly, proliferative defects due to loss of IFT88 *in vivo* have given disparate results. For example, analysis of proliferation in the ducts of the pancreas showed that ORPK mutants have a relative increase in proliferation rates compared with other pancreatic cell types, such as the acini, which do not have a cilium or express IFT88 (Cano et al., 2004; Zhang et al., 2005). In contrast, in other tissues, such as the cerebellum, the phenotype is characterized by a failure of progenitor cell expansion (Chizhikov et al., 2007). Thus, roles for IFT proteins in cell-cycle regulation must be tissue or cell type specific and must be further analyzed.

Whether the renal, pancreatic, and hepatic phenotypes in ORPK mutants are due to loss of a mechanosensory role for cilia, changes in cell cycle regulation, or altered transport and localization of channels/transporters in the cilium remains uncertain. However, recent data have raised questions regarding the fluid-flow mechanosensory based model of cyst formation. This comes from several studies indicating that the severity of the cystic phenotypes in *Pkd1* (*Pkd1*$^{tm2.1Ggg}$), *Kif3a* (*Kif3*^{atm2Gsn}, IFT kinesin) and *Ift88* (*Ift88*$^{tm1.1Bky}$) conditional mutant mice is dependent on the time at which cilia function is disrupted. In these studies, cysts were found to develop rapidly if the genes were disrupted prior to postnatal day 13 (P13) but very slowly if disrupted only a few days later (Davenport et al., 2007; Piontek et al., 2007). Furthermore, analysis in the *Pkd1* conditional mutants revealed no significant difference in proliferation between cystic and non-cystic age matched controls. These findings do not fit well with the model that loss of cilia mediated mechanosensation in itself is sufficient for cyst development or that cysts develop solely from an increase in cell proliferation due to loss of cell-cycle control. However, the data can be incorporated into the existing theories if cysts result from a defect in cilia mediated mechanosensation and also require that this occurs in a proliferative environment where defects in mitotic spindle orientation would become evident. Proliferation in the mouse kidney remains high until around P14, drop significantly at P16, and then become relatively quiescent in the adult kidney (Piontek et al., 2007). This corresponds closely with when the rates of cyst formation in the inducible cilia mutants change from a mechanism of rapid to slow progression.

Defects in specialized cilia maintenance

The ORPK mouse has additional phenotypes resulting from loss of maintenance of cilia structures for sensory reception. The cilia on the retinal rods and cones in the eye are a highly modified form of the primary cilium that contains proteins required for photo-sensation. In the ORPK mouse, the outer segments of the photoreceptors are lost and the cells eventually apoptose, resulting in age-related retinal degeneration (Figure 1) (Pazour et al., 2002a). These studies indicate that transport of components into the rod and cone outer segments relies on IFT and that this is essential for their maintenance.

Retinitis pigmentosa and retinal dystrophy are also present in many of the human ciliopathies or retinal ciliopathies including McKusick-Kaufman syndrome, Senior-Loken syndrome, Usher syndrome, Alstrom syndrome, Meckel syndrome, Joubert syndrome, and Jeune syndrome as well as in some forms of nephronophthisis (Adato et al., 2005; Adams et al.,

2007; Overlack et al., 2007; Roepman and Wolfrum, 2007). In general, the genes disrupted in these syndromes do not encode IFT proteins, such as IFT88 in the ORPK mice, but may have a role in regulating IFT movement or cargo entry into the cilium or IFT particle assembly. The one exception to this has been the recent identification of a hypomorphic mutation in IFT80 in a form of Jeune asphyxiating thoracic dystrophy (Beales et al., 2007).

In addition to retinal degeneration, there are several other sensory deficits associated with human ciliopathies. They include neurosensory hearing loss, impaired nociception, and anosmia (Tosi et al., 2003; Kulaga et al., 2004; Liu et al., 2007; Tan et al., 2007). The presence of these phenotypes in the ORPK mouse has not yet been evaluated.

Ciliary motility defects

IFT88 is required for formation of both immotile and motile cilia. In contrast to the ubiquitous presence of primary cilia throughout the mammalian body, motile (9+2) cilia are relatively restricted and are found on epithelia such as the trachea, efferent duct of the testes, on the oviduct of the female reproductive tract, and ependymal cells lining the ventricles of the brain. In addition to its role in elucidating the functional importance of primary cilia, the ORPK mouse has also contributed to data regarding abnormalities associated with defects in motile cilia. Both female and male ORPK mice are sterile when analyzed on the FVB/N or C3H inbred backgrounds ((Moyer et al., 1994; Pazour, 2004)Yoder unpublished). In human ciliopathies sterility is evident in males with Primary Ciliary Dyskinesia (PCD) and is associated with immotile sperm flagella or loss of normal ciliary beating on epithelium of the efferent duct. Beating of cilia on the efferent duct in the testes is thought to propel sperm, which at this point have not become motile, in the direction of the epididymis. In human females, fertility is somewhat reduced in PCD and Kartagener's syndrome (PCD with *situs inversus*). This is thought to be due to impaired movement of cilia which function in transport of the egg through the oviduct. Although the cause of the sterility in the ORPK mutants has not been fully evaluated, it is likely due to similar mechanisms seen in human PCD.

Another example of a phenotype associated with defects in motile cilia in ORPK mutants that is shared with human ciliopathies is hydrocephalus. The ependyma of the brain ventricles are heavily ciliated and have a highly coordinated beat that directs the flow of cerebrospinal fluid (CSF). In ORPK mice the morphology and coordinated beating of the ependymal cilia is impaired which disturbs normal fluid movement (Banizs et al., 2005). Additionally, small tufts of motile as well as primary cilia are present on epithelial cells of the choroid plexus which produce CSF. In the ORPK mouse, loss of cilia function in the choroid plexus is associated with abnormal regulation of intracellular pH, increased intracellular cAMP, and altered ion transport that contributes to the excess CSF production exacerbating the hydrocephalus phenotype (Banizs et al., 2005; Banizs et al., 2007). In humans, hydrocephalus has been associated with some cases of PCD, though the phenotype's frequency in patients with PCD is a subject of debate (Afzelius, 2004). Mutations in three dynein chain genes DNAI1, DNAH11, and DNAH5 have been identified in human PCD (Pedersen and Mygind, 1980; McComb et al., 1986; Ibanez-Tallon et al., 2002; Wessels et al., 2003; Schwabe et al., 2007). As in the ORPK mouse, these mutations can affect the ultrastructure of the cilium on ependymal cells and cause abnormalities in ciliary beating impairing the ability to direct fluid movement. Immotile cilia also cause an increase in respiratory tract infections in humans with PCD and other ciliopathies; however, this has not been evaluated in the ORPK mouse.

Another function of motile cilia that has been assessed using the ORPK mouse was the role of ependymal cilia in directing neuroblast migration. Analysis suggests that the flow of CSF in the ventricles of the adult brain in mice direct the migration of new-born neurons forming in the stem cell region of the subventricular zone (SVZ) towards their destination in the olfactory

bulb. Sawamoto et al. demonstrated that the neuroblasts fail to align and migrate properly in ORPK mutants and they attribute this to loss of coordinated ciliary beat on the ependymal cells that disrupts CSF movement. Reciprocal neuroblast transplant data indicate the defect is not intrinsic to migratory cells but rather is dependant on the CSF environment and CSF flow. This appears to involve establishing a gradient of a chemorepellent produced by the choroid plexus which is dispersed by CSF flow generated by ependymal cilia (Sawamoto et al., 2006). Whether similar defects in neuroblast migration are present in human ciliopathies that contribute to phenotypes such as mental retardation or sensory deficits (e.g. nociception or anosmia) is not known.

Cilia mediated signal reception and processing

Another exciting class of phenotypes seen in the ORPK mouse or cell lines derived from these mutants involves defects associated with cilia mediated regulation of signaling pathways such as hedgehog (Hh) and platelet derived growth (PDGFR-α/PDGF-A).

Regulation of the Hh pathway has been reviewed recently and will not be described in detail here (Oro, 2007; Riobo and Manning, 2007). Briefly, Hh proteins (Shh, Ihh, and Dhh) are secreted factors that have a complex regulatory network. Hh regulates the function of both transcriptional activators and repressors of the pathway, in part through post translational modifications and proteolytic cleavage steps. Intriguingly, a large number of the Hh signaling components have now been localized to the cilium and recent data indicate the Hh pathway may be initiated by direct binding of Hh with its receptor patched-1 in the cilia membrane. Data from several independent *Ift88* null mutant mice have revealed that cells lacking cilia are unable to respond to Hh ligands (Haycraft et al., 2005; Liu et al., 2005a). Normally in the presence of an Hh ligand, Gli1 and Gli2 function as transcriptional activators while processing of Gli3 to its repressor form is blocked. In contrast, when an Hh ligand is absent Gli1 and Gli2 transcriptional activity is blocked and Gli3 becomes proteolytically cleaved to its repressor form inhibiting the pathway. However, in cells lacking cilia both the repressor and activator functions of the Gli transcription factors appear to be impaired causing the pathway to become deregulated. The resulting phenotype is determined by whether the Gli3 repressor or Gli1 and Gli2 activators are the major factors governing development of a specific tissue.

The ORPK mouse has numerous abnormalities expected for defects in the Hh signaling pathway. Here again the hypomorphic nature of the allele in the ORPK mouse allowed analyses at later developmental and postnatal stages than in mice in which cilia function is completely disrupted. ORPK mouse phenotypes associated with Hh signaling defects include cerebellar hypoplasia with ataxia, preaxial polydactyly, stunted long bone growth, supernumerary teeth, and defects in skin and hair follicle development (Figure 1 and see below) (Zhang et al., 2003;Chizhikov et al., 2007;McGlashan et al., 2007). In the cerebellum, cilia were found to play an important role in Shh mediated regulation of precursor cell proliferation (Chizhikov et al., 2007). This phenotype is also evident in human patients with Joubert syndrome, in which several of the affected proteins localize to the cilium axoneme (Sayer et al., 2006;Arts et al., 2007;Hildebrandt and Zhou, 2007). The sonic hedgehog (Shh) pathway is also important for patterning of the distal portion of the limb with defects resulting in the formation of either excess (loss of Gli3 repressor activity) or too few (loss of Shh) digits (Litingtung et al., 2002). Together Shh and the level of Gli3 repressor function to pattern the AP axis of the autopod and constrain polydactylous potential. Another phenotype in ORPK mice associated with abnormal regulation of the Shh pathway is preaxial polydactyly (Figure 1), which is due in part to defects in the formation of the Hh signaling pathway repressor Gli3. This is supported by genetic studies conducted between the ORPK mouse and the $Gli3^{XT\text{-}J}$ mutant and from the analysis of conditional IFT88 null ($Ift88^{tm1.1Bky}$) mutants in which cilia are lost in the limb bud mesenchyme using the Prx1-Cre deletor (Zhang et al., 2003;Haycraft et al., 2007).

Interestingly, limb bud patterning abnormalities are observed in many of the human ciliopathies including Bardet-Biedl syndrome, Ellis-van Creveld syndrome, Joubert syndrome, Orofaciodigital syndrome type I, and Meckel syndrome (Badano et al., 2006b). Indian hedgehog (Ihh) also has a role in the developing limb in regulating endochondrial bone formation (Long et al., 2004;Lai and Mitchell, 2005). In ORPK mice, dysfunction of the primary cilium results in stunted linear growth, altered chondrocyte differentiation, and delayed chondrocyte hypertrophy within the growth plate (McGlashan et al., 2007). Similar bone phenotypes are seen in Ellis-van Creveld syndrome, a rare genetic disorder characterized by short-limb dwarfism, polydactyly, and malformation of the bones of the wrist. One gene (*evc*) responsible for this disorder has recently been cloned and found to encode a protein that localizes to the base of cilia on chondrocytes. In mice, mutations disrupting the *evc* gene result in loss of Ihh reception in the growth plate (Ruiz-Perez et al., 2000;Ruiz-Perez et al., 2007). Similarly, disruption of *ift88* or *kif3a* using the Col2a-Cre (Tg(Col2a1-cre)1Bhr) was found to cause premature loss of the growth plate resulting from reduced proliferation and accelerated hypertrophic differentiation of the chondrocytes. Interestingly this also altered columnar orientation of the chondrocytes which may indicate a role for cilia in regulation of planar cell polarity (Song et al., 2007).

Cilia also have been implicated in PDGF signaling, in particular in the PDGF-AA/PDGFR-αα pathway (Schneider et al., 2005). PDGFR-α and PDGF-A are regulators of cell migration, proliferation, survival, tissue remodeling, and deposition of extracellular matrix both during development and in adults (Hoch and Soriano, 2003). Mutations disrupting the PDGF-A/PDGFR-α pathway have a wide range of phenotypic consequences and many are embryonic lethal (Hoch and Soriano, 2003). PDGFR-α is located in the cilia membrane, and ligand-dependent activation of PDGFR-α results in activation of Mek1/2 within the cilium and at the basal body (Schneider et al., 2005). Although none of the phenotypes in the ORPK mouse has yet been linked to defects in PDGFR-α signaling, fibroblasts isolated from ORPK mice are unable to respond to PDGF-A and fail to chemotax properly towards PDGF-A (personal communication by Søren Christensen and Biology of the Cilia and Flagella FASEB Conferences, Saxtons River 2007 ((Sloboda and Rosenbaum, 2007)meeting review) .

Finally, primary cilia also appear to have a role in Wnt signaling by regulating the switch between canonical and the non-canonical or planar cell polarity pathway (Simons et al., 2005; Benzing and Walz, 2006). Knockdown of BBS4 or Kif3a expression in cells prevents noncanonical Wnt signals (i.e. Wnt5a) from repressing the canonical Wnt (i.e. Wnt3a) pathway (Gerdes et al., 2007; Corbit et al., 2008). BBS4 is a basal body/cilia localized protein which is disrupted in Bardet-Biedl syndrome patients while Kif3a is the IFT kinesin required for cilia assembly. Thus defects in the cilium lead to elevated β-catenin levels causing an increase in signaling through the canonical pathway. Interestingly, abnormalities in the Wnt and PCP pathways are further supported from the phenotype in the inner ear when IFT88 is disrupted. The data from this analysis by Jones et al. show that proper positioning of ciliary basal bodies and the formation of polarized cellular structures, such as the stereocilia, require ciliary proteins and, surprisingly, the core PCP proteins were found to partition normally along the polarization axis of the cell (Jones et al., 2007). Thus, IFT88 has a distinct function in basal body positioning and morphological polarization during PCP regulation. Despite the lack of effect on the distribution of PCP proteins, a role for IFT88 in PCP was confirmed through its genetic interaction with the known core PCP gene Vangl2 (Jones et al., 2007). IFT88 may be required for a permissive response of core PCP proteins to Wnt signals mediated through the cilium or IFT88/cilia could function independent of the PCP proteins in a parallel pathway regulating polarizing signals that subsequently interact with the core PCP proteins to establish the alignment of stereocilia in hair cells of the organ of Corti. It will be interesting to determine if there are similar causes seen with the misaligned cell division in the cystic kidney phenotype as well as in the altered rotation of chondrocyte seen in the limbs of IFT88 mutant mice. In

contrast to the conditional IFT88 mutant, only minor defects in the inner ear were observed in the ORPK mutants. Although to our knowledge direct abnormalities in PCP pathways have not yet been reported in human ciliopathies, similar phenotypes have been described in the inner ear of mouse models of BBS and vestibular or sensorineural hearing defects are seen in ciliopathies such as Alstroms syndrome and mildly in BBS. (Ross et al., 2005; Badano et al., 2006b; Marshall et al., 2007).

Obesity is associated with a number of the human ciliopathies such as BBS and Alstrom syndrome as well as in the corresponding mouse models (Mykytyn et al., 2004; Nishimura et al., 2004; Collin et al., 2005; Arsov et al., 2006). This phenotype is not present in the OPRK mice, which is likely due to an early death and health issues from phenotypes observed in multiple other tissues. Using the conditional mutation approach, cilia located on proopiomelanocortin (POMC) neurons in the hypothalamus were identified as being critical for normal satiety responses. Current data suggest that cilia may function as part of the leptin-melanocortin signaling axis to regulate feeding behavior (Davenport et al., 2007).

Mental retardation is another feature seen in a large number of the human ciliopathies. In ORPK mice, neuronal cilia are largely disrupted; however, possible cognitive deficits in these mice have not been assessed, in part due to the hydrocephalus (Fuchs and Schwark, 2004; Banizs et al., 2007). In light of the data indicating cilia are functionally important for POMC neuron regulation of satiety responses; it is suggestive that this organelle is involved in reception of signaling peptides that influence neuronal activity. This is further supported by the presence of somatostatin (SSTR3) and serotonin ($5HT_6$) receptors in the cilium of neurons (Fuchs and Schwark, 2004). Overall, the roles of cilia in neuronal patterning and in regulation of neuronal activity remain enigmatic and promise an exciting area of research in the future.

Future Directions for the ORPK model: from “Scruffy” to Sensenbrenner’s syndrome

In addition to the relatively well characterized phenotypes described above, the ORPK mouse has abnormalities in multiple other tissues that have yet to be analyzed. One of these phenotypes is the scruffy appearance that was noted in the initial manuscript describing the ORPK mouse (Moyer et al., 1994). Skin and hair abnormalities in ORPK mice are present at birth prior to onset of severe disease phenotypes in other tissues, thus, it is unlikely to be caused by other factors associated with general health of the mutants, though health status may contribute to the expressivity. It is highly penetrant on the FVB/N background, but has variable expressivity, as seen in several other phenotypes in the ORPK mutants (Moyer et al., 1994; Sommardahl et al., 2001), with a few animals being mildly affected and others having flaky skin and sparse hair (Figure 2).

We have determined that primary cilia are present on most cells in the developing and cycling hair and skin with the exception of more differentiated cells in the stratum corneum and granular cell layers of the epidermis and the differentiating hair shaft (Figure 2 D-G, Lehman et al. manuscript in preparation). Similar to that seen in other tissues of the ORPK mouse, the cilia on cells in the hair follicle and skin are stunted. Histologically, the skin of ORPK mice is characterized by mild orthokeratotic hyperkeratosis and follicular dysplasia, with delayed follicular development, accumulation of keratinaceous debris in mildly cystic follicles, and sparse granulomatous or pyogranulomatous folliculitis (Figure 3). This phenotype is evident by P17, and keratin filled cystic follicles typically are still visible in older (P25) mice (Figure 3 F). By P25, a delayed hair growth cycle also is evident in ORPK mice, in which the follicles remain in the first telogen (Figure 3 C), whereas follicles of wild type mice have progressed to anagen (Figure 3 F).

One possible explanation for the hair phenotype is that the early hair follicle delay may precurse later abnormal development that leads to hair shaft structural defects or twisted keratin fibers. These twisted fibers lead to intrafollicular keratin accumulation when the keratinocytes that form the hair shaft do not differentiate or proliferate properly, leading to follicular cysts, some of which rupture. Consistent with this hypothesis is that PDGF-$A^{-/-}$ transplanted skin develops abnormal hair shafts and keratinized cystic structures with defects in proliferation (Karlsson et al., 1999). Also, transgenic over-expression of Wnt3a in mice using the Keratin 14 promoter, which results in overactivation of β-catenin in the epidermis, also have accumulation of keratinaceous debris in the follicles and formation of follicular cysts (Millar et al., 1999). During catagen, when the hair follicle degenerates, these defects may lead to leakage of keratin, provoking a granulomatous reaction. The timing of this phenotype also suggests the possibility that the ability of the follicles to undergo the first anagen is impaired.

It is important to note that the normal transitions from anagen to catagen and telogen to anagen involve the PDGFR-α pathway, and that proper proliferation of keratinocytes during anagen requires both the PDGFR-α and Shh pathways (St-Jacques et al., 1998; Karlsson et al., 1999; Mill et al., 2003; Mill et al., 2005; Tomita et al., 2006). As discussed above, cilia are required for the normal activity of these pathways (Haycraft et al., 2005; Huangfu and Anderson, 2005; Liu et al., 2005a; Schneider et al., 2005; Rohatgi et al., 2007). Indeed, RT-PCR analysis of skin from P14 ORPK mice further supports this hypothesis. Although Shh expression levels are normal in the skin of ORPK mice as standardized to 18S RNA, activation of the pathway is impaired as determined by expression of downstream targets *Ptch1* and *Gli1* (Figure 3 G). These data indicate that the cilia defects in ORPK mice likely contribute to improper cycling or structural defects in the follicles. The precise molecular causes for the follicular dysplasia remain uncharacterized, but inasmuch as the ORPK mouse mutation is an *Ift88* hypomorph, it is likely that the Wnt, Shh, and PDGFRα pathways contribute to the phenotype.

These new findings strengthen the argument for clinical awareness of potential ectodermal dysplasia phenotypes in human ciliopathies. Potential examples of human ciliopathies with ectodermal dysplasia phenotypes are Ellis-van-Creveld syndrome (EVC) (Ruiz-Perez et al., 2007) and Sensbrenner's syndrome or Cranioectodermal dysplasia (CED). A gene responsible for one form of EVC has recently been identified and found to encode a cilia protein (Ruiz-Perez et al., 2007), while the gene for CED has not been identified. However, the spectrum of phenotypes seen in CED patients suggests it will be an additional member of the ciliopathies. They includes tubulo-interstitial renal disease, congenital hepatic fibrosis, skeletal defects, hypoplasia of the corpus callosum, and retinitis pigmentosa (Tsimaratos et al., 1998; Costet et al., 2000; Zaffanello et al., 2006). CED also has an extensive ectodermal dysplasia features, including a sparse hair phenotype.

In summary, the ORPK mouse has served as a model for analyzing defects in a variety of human organ systems caused by ciliary dysfunction. In this communication, we extend this analysis to the "scruffy fur" phenotype and illustrate the continuing applicability of this model for human disease in the rapidly expanding world of the ciliopathies.

Perspectives

Nearly fourteen years ago, the ORPK mouse was described as an interesting and novel cystic kidney disease mutant by Dr. Woychik's laboratory and the primary cilium was considered to be a vestigial organelle with minimal to no importance in human health. Today, our perspective regarding the primary cilium has changed remarkably, in part through the analyses conducted in the ORPK mouse. Defects in ciliary function are now recognized as the cause of one of the most common genetic diseases (ADPKD) and contribute to the most common form of combined blindness/deafness (Usher syndrome). It is now appreciated that cilia function is

required for normal development and viability and is an essential component of important signaling pathways such as hedgehog, PDGFR-α, Wnt, and for regulating neuronal signals involved in satiety responses. Although research on cilia has advanced at an amazing pace over the past decade, there is still a great deal that needs to be learned. The current goals are to uncover how cilia regulate or influence cell behavior and how dysfunction of this organelle alters tissue and cellular physiology. Understanding these key features will initiate pharmacological and molecular approaches to attenuate disease progression associated with human ciliopathies. Ciliopathy models, such as the ORPK mouse, will continue to be instrumental in realizing these goals, testing therapeutic approaches, and to maintain this rapid progress in cilia related research.

Methods

Confocal immunofluorescence (IF) microscopy was performed using 0.5um optical sectioning of 40um sections from dorsal skin using standard IF protocols and anti-Polaris/IFT88 (B1700) diluted 1:750 and nuclei were stained with Hoechst 33258 (Sigma) diluted 1:1,000 in PBS. Stacks were rendered using Voxx2 imaging software (Indiana Center for Biological Microscopy, Indiana University). For histological analysis sections were fixed in buffered formalin and stained with hematoxylin and eosin. The *Ift88*^{Tg737Rpw} homozygous (ORPK) mice used in this study were described previously (Moyer et al., 1994). The QRT-PCR experiments were performed with a SmartCycler instrument (Cepheid) using TaqMan Assays-on-Demand (Applied Biotechnologies) primers and probes. RNA was isolated from the skin of 14-day-old mutant and littermate control mice. Expression levels of each gene were normalized to the housekeeping gene 18S rRNA for mutant and wildtype mice. Then relative fold differences were determined using the $2^{-\Delta\Delta CT}$ method by comparing the normalized gene expression level for each gene between the mutant and the control. All mice were maintained as heterozygous crosses on an FVB/N background in accordance with the IACUC regulations at the University of Alabama at Birmingham.

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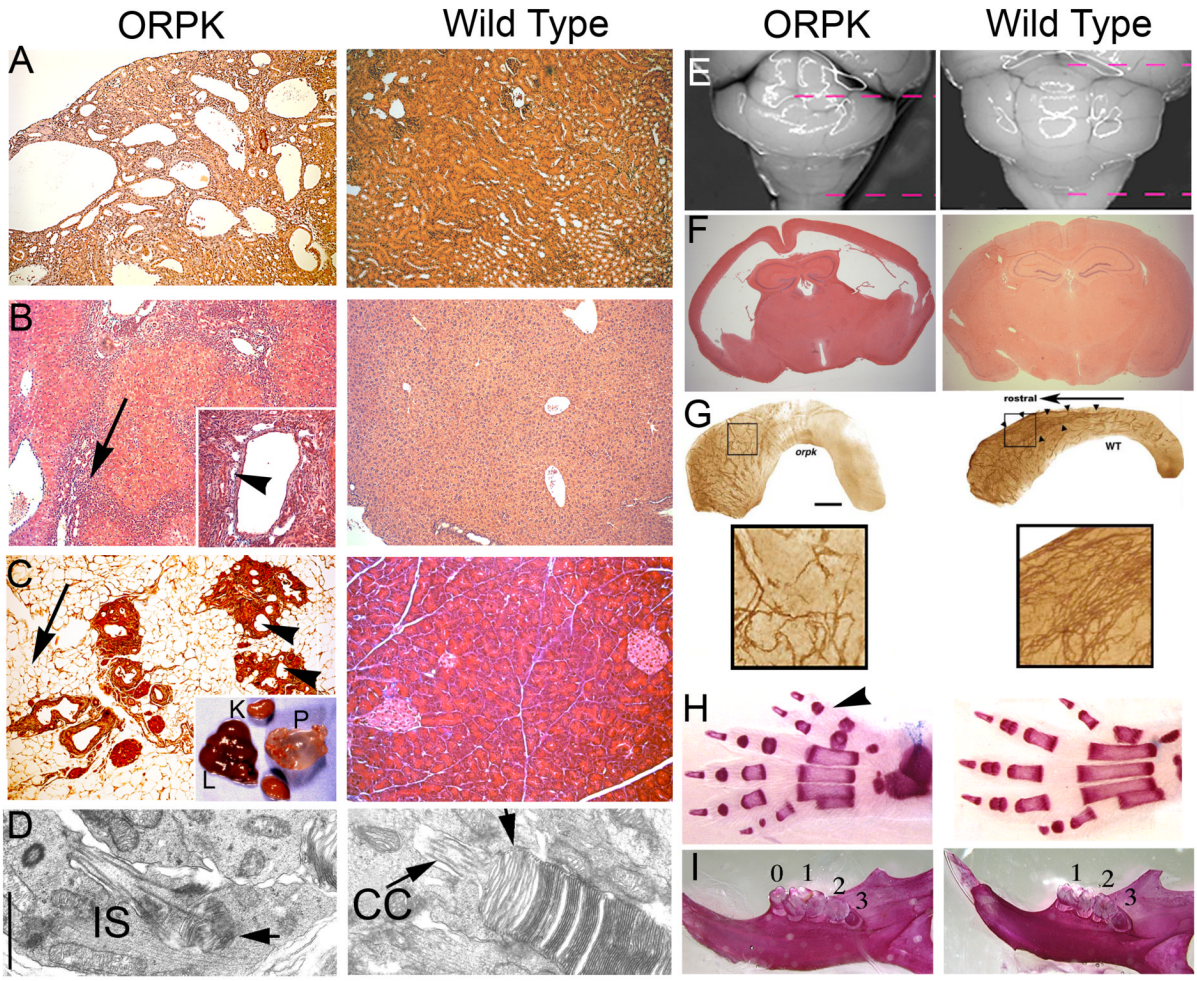

Figure 1. Summary of phenotypes characterized in the ORPK mouse

The ORPK (FVB/N) mouse (left panels) has phenotypes in numerous tissues compared to wild type controls (right panels). (A) Cystic lesions are seen in the kidney. (B) The liver is characterized by biliary (arrow) and bile duct (arrow head) hyperplasia. Inset is a higher magnification view of a central vein showing the multiple dysplastic bile ductules. (C) In the pancreas, ducts become dilated (arrowhead) and acini atrophy (arrow). Insert shows the typical pancreatic phenotype seen in ORPK-C3H mutants (P is pancreas, L is liver, and K is kidney). (D) In contrast to wild type mice, the ORPK mutant has disrupted discs and outer segments that are misshapen and filled with amorphous material that extends into the inner segment of the rod and cone photoreceptors (data is from 10 day old mice, IS=inner segment, and CC=connecting cilium). The images were adapted with permission from Pazour et al. (Pazour et al., 2002a). Reproduced from *The Journal of Cell Biology, 2002, 157:103-113*. Copyright 2002 The Rockefeller University Press. (E) The ORPK mutants are ataxic due to hypoplasia of the cerebellum which is associated with loss of Shh signaling. Red bars indicate the length of the wild-type and ORPK cerebellum along the anteroposterior axis. Images adapted from Chizhikov et al. (Chizhikov et al., 2007). Copyright 2007 by the Society for Neuroscience. (F) ORPK mutants develop hydrocephalus. (G) ORPK mutants have defects in neuroblast migration. Shown are neuroblasts in whole mounts of the lateral ventricle stained with an antibody against Poly-Sialated Neural Cell Adhesion Molecule (PSA-NCAM). In wild type (WT) controls there are well-organized chains of neuroblasts migrating toward the olfactory

bulb. In ORPK mutants, chains of neuroblasts form but the direction of migration is disorganized. Higher magnification views of the boxed region are shown in the inserts. Images were adapted from (Sawamoto et al., 2006). Reprinted with permission from AAAS. (H and I) ORPK mutants also exhibit numerous skeletal defects including preaxial polydactyly (H, arrow head) and formation of an extra molar tooth (I, labeled 0). Images adapted from Zhang et al. (Zhang et al., 2003) Copyright 2003 Wiley-Liss, Inc.

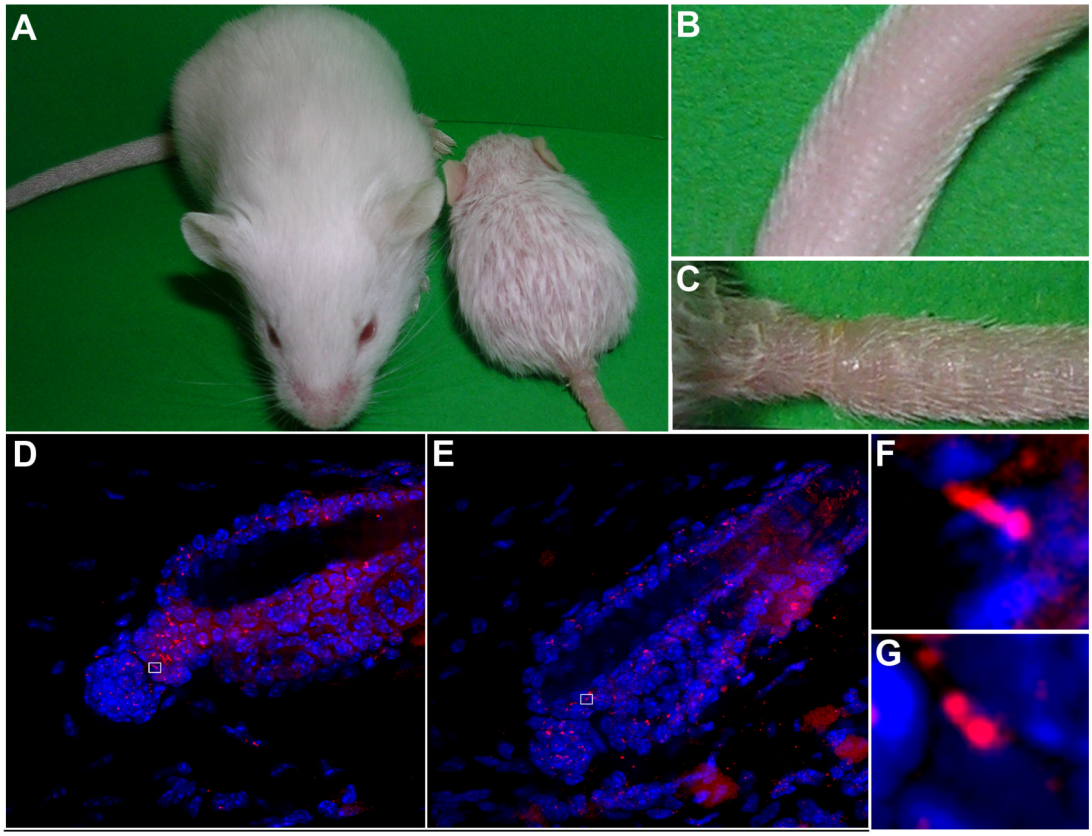

Figure 2. Gross epidermal and cilia phenotypes in the ORPK mouse

(A) Dry and flaky skin with sparse fur is noticeable in the ORPK mutant (right, C) by P16 compared to its wild type littermate control (left, B). (D-E) Primary cilia are found in the follicles of both adult (D) wild type and (E) ORPK mutant hair follicles by 3-D immunofluorescence confocal imaging for IFT88 (Red) and DAPI nuclear stain (Blue). (F-G) Higher magnification images of the cilia found in the hair follicle from the boxed regions in wild type (F) and ORPK (G) mutants. As seen in other tissues the primary cilia are stunted in ORPK mice compared to wild type controls.

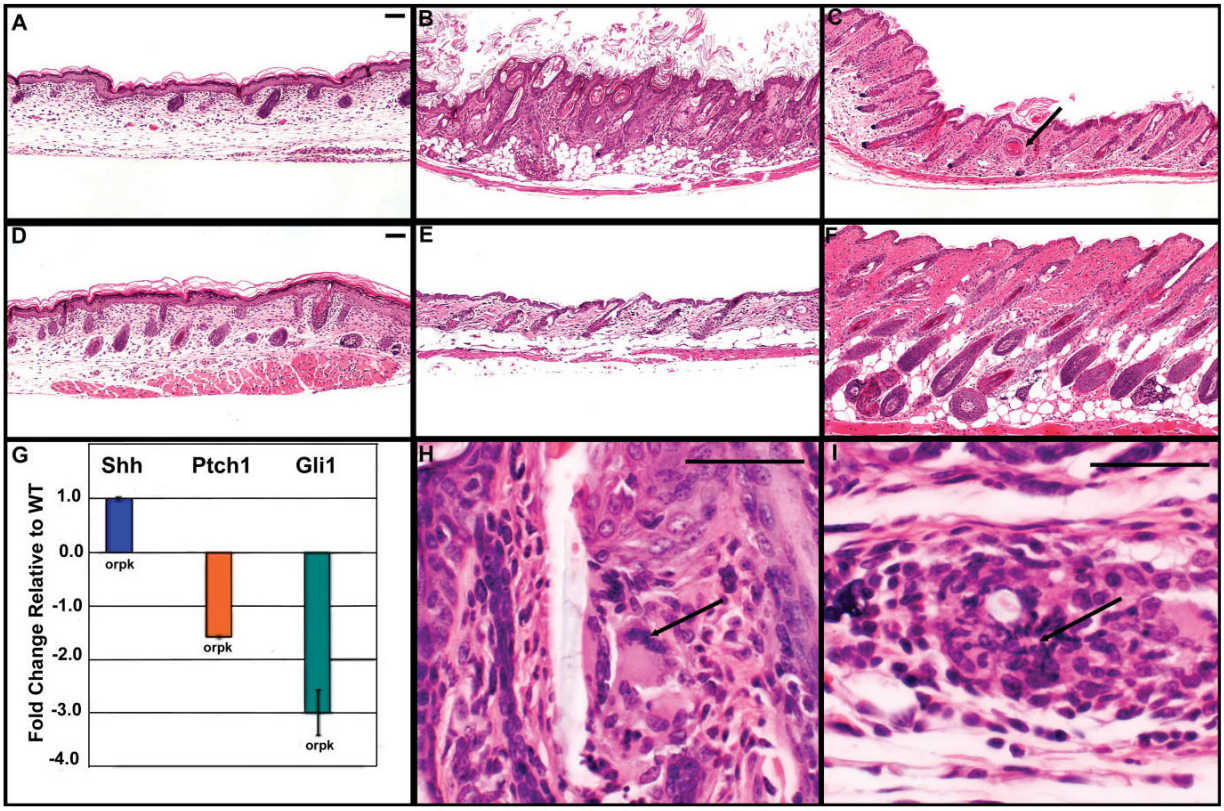

Figure 3. Skin and follicular abnormalities in the ORPK mouse

At P1 (A) ORPK mice have mild growth delay with reduced adnexa in early follicular development compared to (D) wild type littermates. (E) P18 wild type littermates have normal follicles in telogen, whereas the skin of ORPK mice (B) is characterized by orthokeratotic hyperkeratosis, follicular accumulation of keratinaceous debris, and follicles in catagen. (H) Some follicles rupture, leading to a giant cell inflammatory response (arrow) and foreign body granulomas (trichogranulomas) (I, arrow). At P25, wild type follicles (F) are in anagen, whereas the follicles of ORPK mice (C) remain in first telogen. (G) Relative quantification of Shh pathway expression as determined by quantitative RT-PCR standardized to 18S RNA. Relative fold change was calculated by the $2^{-\Delta\Delta CT}$ method by comparing the normalized gene expression level for each gene between the mutant and the control (±SD). *Shh* expression is identical between P14 ORPK skin and littermate controls, but downstream components of the pathway *Ptch1* and *Gli1* are downregulated. Scale bars= 50 microns.

Table 1

Phenotypes and Associated Clinical Features of the ORPK Mouse and Selected Human Ciliopathies

Ciliopathies	**ORPK (*Ift88 Tg^{737Rpw}*)**	**ARPKD**	**ADPKD**	**SLSN**	**MKS**	**JBTS**	**BBS**	**NPH**	**OFD1**	**CED^{+}**	**EVC**	**USH$^{\wedge}$**	**PCD**	**ALS**
OMIM*		**263200**	**173900**	**266900**	**249000**	**213300**	**209900**	**256100**	**311200**	**218330**	**225500**	**276900**	**242650**	**203800**
Retinal degeneration (**M**)	X			X	X	X	X	X		X		X		X
Renal cystic disease(**S**)	X	X	X	X	X	X	X	X	X	X				X
Polydactyly (**P**)	X				X	X	X		X		X			
Situs inversus/isomerism (**D,S**)	-			X	X	X	X	X (nph-2)			X	X (USH1+KS)	X (KS)	
Obesity (?)	-						X							X
Mental retardation (?)	ND			X	X	X	X	X	X			X		
Nociception defects (**S,M**)	ND						X							
Cerebellar vermis hypoplasia/aplasia (**P**)	X			X	X	X	X	X			X			
Hypoplasia of the corpus callosum (?)	ND				X	X	X		X	X				
Hydrocephalus (**D,S**)	X			X	X	X	X	X			X		X	
Respiratory Tract Infections (**D**)	ND									X		X (USH1)	X	X
Hearing defects (**M,S**)	ND			X			X(mild)					X		X
Hepatic Fibrosis (**S**)	X	X	X	X	X	X	X	X		X				X
Ectodermal Dysplasia (**P**)	X									X	X			
Skeletal Defects (**P**)	X			X					X	X	X			X

Legend: ARPKD=Autosomal recessive polycystic kidney disease, ADPKD=Autosomal dominant polycystic kidney disease, SLSN=Senior Loken, MKS = Meckel, JBTS=Joubert syndrome, BBS=Bardiet-biedl syndrome, NPH= Nephronophthisis, OFD1=Orofaciodigital syndrome 1, CED=Cranioectodermal dysplasia, EVC=Ellis-van creveld syndrome, USH=Usher syndrome, PCD= Primary cilia dyskinesia, KS=Kartagener's syndrome. Contributing Ciliary defects: (**D**)yskinesia, (**S**)ensation, Signal (**P**)rocessing, Ciliary (**M**)aintenance, and (?) unknown. (ND)=Not determined.

$^{+}$Cranio-ectodermal dysplasia (CED) is a candidate ciliopathy.

$^{\wedge}$Usher syndrome is a retinal ciliopathy.

*Note: Many of the ciliopathies have significant genetic and allelic heterogeneity and resulting variation in phenotype.