Pediatr Nephrol (2007) 22:765–769
DOI 10.1007/s00467-007-0434-1

EDITORIAL COMMENTARY

Nephrocystin and ciliary defects not only in the kidney?

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Received: 28 October 2006 / Revised: 28 December 2006 / Accepted: 2 January 2007 / Published online: 20 February 2007

Abstract Cystoproteins have been recognized to play a major role in the development of cystic kidney diseases (CKDs) via interaction with the cilia/centrosome complex. We highlight our present knowledge on nephrocystin as the defective protein in nephronophthisis type I. Nephrocystin has been localized to the ciliary transition zone not only of renal tubule cells but also of respiratory and retinal cilia. Thus, multi-system involvement as in Senior–Løken-syndrome (retinal degeneration plus nephronophthisis) can be explained by a functional ciliary defect in various tissues. In addition, we illustrate that ciliated respiratory cells have a high potential for diagnostics in CKDs and will further aid understanding of the underlying molecular mechanisms.

Keywords Cilia · Transition zone · Nephronophthisis · Cystoproteins · Cystic kidney disease · Ciliogenesis · Senior–Løken-syndrome

From cysts to cilia: the discovery of cystoproteins

Cystic kidney diseases (CKDs) are among the most frequent causes of chronic renal failure in childhood, adolescence and adulthood. Thus, great effort has been put into the successful characterization of their molecular bases during the past two decades [1, 2]. Positional cloning was primarily used to identify affected genes. After confirmation of mutant alleles in family studies, the encoded proteins have been characterized in a number of CKD types, e.g. in autosomal dominant polycystic kidney disease and autosomal recessive polycystic kidney disease (ADPKD/ARPKD), in six different types of the nephronophthisis (NPHP) complex, and in more then ten genes causing the multi-system disease Bardet–Biedl syndrome [2, 3].

Most of the affected proteins were subsequently shown to localize to structures associated with primary renal monocilia—an almost "forgotten organelle"—near their basal bodies, the centrosomes, or the axonemes [4]. This led to the hypothesis that, in CKDs, "all roads lead to the cilium" [5], and research in ciliary function has gained widespread interest among nephrologists [1].

The term "cystoproteins" was introduced to describe those functionally related proteins involved in the pathogenesis of CKDs. Among those cystoproteins, we focus attention on nephrocystin, which is either absent or mutated in nephronophthisis type 1 (NPHP1) [6, 7]. NPHP1 (OMIM #256100) frequently causes end-stage renal failure (ESRF) in children and adolescents (estimated prevalence of all forms of nephronophthisis, 1 in 50.000 [1]; estimated percentage with NPHP1, 62% [8]). Patients typically present with polyuria and renal failure, sometimes associated with manifestations of extra-renal diseases including retinitis pigmentosa or Leber congenital amaurosis (both referred to as Senior–Løken-syndrome), Cogan's oculomotor apraxia, cerebellar vermis aplasia (Joubert syndrome), liver fibrosis, cone-shaped epiphyses and, rarely, situs inversus. Renal histology reveals cysts predominantly at the corticomedullary border in normal sized or small kidneys with tubular atrophy and interstitial fibrosis. These changes are typical but not pathognomonic [9]. The various manifestations of extra-renal disease indicate that the protein encoded by *NPHP1* exhibits distinct roles in diverse tissue types.

In most NPHP1 patients, a large homozygous deletion involving the *NPHP1* locus on chromosome 2q12–q13 has

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been described [10]. However, point mutations are also found, e.g. a stop codon at arginine 586 is the prevalent *NPHP1* mutation in Italian NPHP1 patients [11], while some patients carry point mutations in combination with a heterozygous deletion.

NPHP1 encodes a 733 amino acid protein called nephrocystin 1, which has been shown to interact with a number of proteins involved in focal adhesion complexes and at sites of cell-to-cell contact in renal epithelial cells [2]. In addition, nephrocystin interacts with other nephronophthisis proteins (nephrocystin-2, -3 and -4), which suggests that these proteins assemble into a multimeric protein complex. Renal monocilia have been supposed to act as sensors of renal tubular fluid flow, thereby initiating an intracellular Ca^{2+}-dependent signalling cascade that is thought to be essential for the structural integrity of the tubular epithelium [12]. Loss of the sensory activity has been hypothesized to lead subsequently to cyst formation, and, therefore, common molecular mechanisms have been postulated to be involved in the different types of CKDs. Cilia appear to control cell division, allow—as a cellular positioning device—orientation of tubular epithelial cells along the nephron axis, and help to establish subcellular asymmetry and polarization during cell differentiation [13]. Since many of these cystoproteins are closely linked to ciliary function, CKD is now regarded as a defect of the renal monocilia, whereas extrarenal disease manifestations are likely to be explained by associated dysfunctions of other cilia types (e.g. photoreceptor-connecting cilia in Senior–Løken-syndrome).

From cilia to cysts: a link to multi-system involvement

The new concept of ciliary involvement in multi-systemic renal diseases led to increased studies of cilia. These organelles are evolutionary conserved structures and are either motile or immotile. Each cilium extends from a specialized centriole, called a basal body. Located between the basal body apparatus and the ciliary axoneme is the so-called transition zone, where the centriolar triplet microtubular structure converts to the axonemal doublet microtubular structure.

The basic axonemal structure of most motile cilia types (i.e. respiratory epithelial cells) consists of nine peripheral doublet microtubules surrounding two central single microtubules (9+2 structure). Cilia with absent central microtubules (9+0)—also referred to as primary cilia—are of special interest in renal physiology. These cilia belong to a class of organelles that usually emerge as a single cilium from the apical cell surface (monocilium).

While a mechanical function of motile cilia and flagella seems obvious, i.e. in respiratory cells or spermatozoa, the role of immotile primary monocilia is currently at the focus of extensive research. New evidence suggests that they function as sensory organelles to detect environmental cues of a mechanical and/or chemical nature and, also, osmotic, photonic, hormonal or olfactory signals [14].

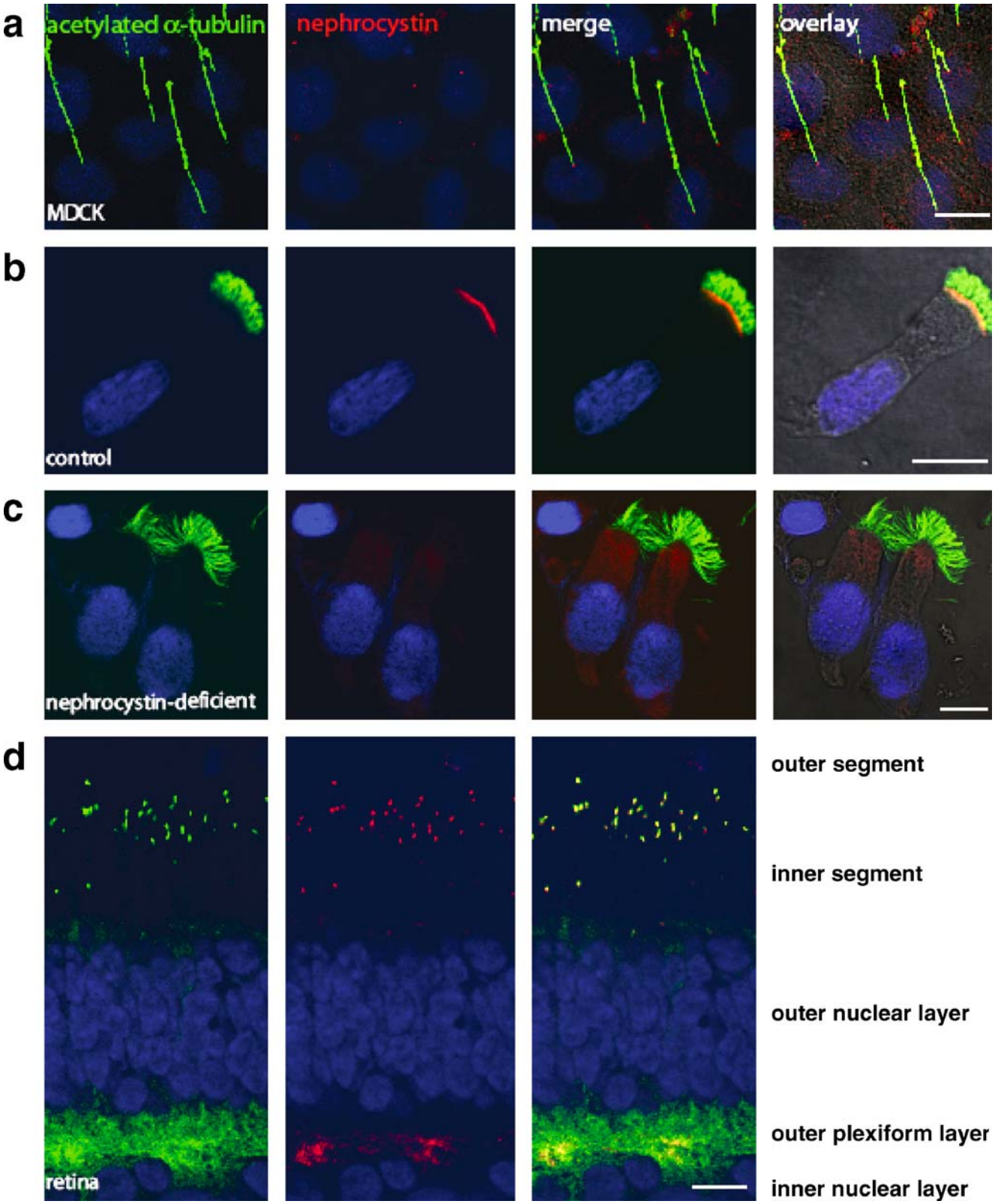
Cilia are of great importance for planar cell polarity. This concept refers to the required cellular asymmetry of, e.g., renal tubular cells, with specialized cellular components such as luminal and basolateral membranes [13]. In the case of disturbed cell polarity, misorientated cell division occurs, and specialized structures such as the nephron may be disrupted by non-directional cell division and cyst formation [15, 16].

Since the discovery of the *NPHP1* gene in 1997 much progress has been achieved concerning the possible function of its gene product nephrocystin [6, 7]. Initial studies suggested a primary role for nephrocystin in the signalling processes of focal adhesions, as reviewed by Hildebrandt and Otto [17]. However, in later work nephrocystin was found to be also localized to the renal monocilium [18]. Recent data demonstrate that nephrocystin is targeted to the ciliary base by a PACS-1 mediated process involving phosphorylation by casein kinase 2 [19]. Using specific monoclonal and polyclonal antibodies directed against nephrocystin, we found that protein localization is not restricted to renal monocilia [19]. It can also be detected in a variety of different tissues and cilia types, including the cilia of respiratory cells and photoreceptors. Photoreceptors are specialized neuronal cells, where the outer segment filled with pigments develops from a modified monocilium. In the mature photoreceptor a connecting cilium remains, bridging the outer and inner segments [8]. Importantly, in all cilia types so far analysed, nephrocystin specifically localizes to the ciliary transition zone or its homologous structure such as the connecting cilium (Fig. 1). This observation that proteins that have

Fig. 1 Nephrocystin localizes to the transition zone of renal ▶ monocilia, human respiratory epithelial cilia and to photoreceptor-connecting cilia, demonstrated by high-resolution confocal immunofluorescence imaging using antibodies against the cilia-specific protein acetylated tubulin (*green*) and rabbit-anti-nephrocystin antibodies (*red*). Nuclei were stained with Hoechst 33342 (*blue*). Merged and overlay images are shown in the *right-hand panels*. The *scale bars* represent 10 μm. **a** In confluent, polarized Madin Darby canine kidney (MDCK) cells (a renal epithelial cell line) specific nephrocystin staining is observed at the base (transition zone) of each monocilium. **b** In ciliated human respiratory epithelial cells, obtained by non-invasive transnasal brush biopsy, specific nephrocystin staining is also only observed at the ciliary bases, within the transition zone. **c** In ciliated respiratory epithelial cells derived from nephronophthisis patients with *NPHP1* deletions, no specific nephrocystin staining is observed. Nephrocystin deficiency does not impair the polarization of respiratory epithelial cells or cilia formation. **d** In the retina (from pig, cryosection) nephrocystin co-localizes with acetylated tubulin, which marks the connecting cilium bridging the inner and outer photoreceptor segments

previously been implicated in the sensory function of renal monocilia are also localized to other cilia types indicates that nephrocystin displays functional properties in motile and immotile cilia.

Detailed analysis using high-resolution immunofluorescence microscopy showed that nephrocystin does not exhibit a staining pattern similar to that of intraflagellar transport proteins, which are involved in intraciliary protein



transport. Based on this observation it is unlikely that nephrocystin is a component of the intraflagellar transport machinery. Interestingly, we could not identify specific localization of nephrocystin at focal adhesions. However, this does not rule out that nephrocystin molecules that are not detectable by immunofluorescence microscopy play a role at that site. In addition, we could demonstrate that nephrocystin-deficient respiratory cells do not have an altered process of cilia generation. Thus, nephrocystin is probably not mandatory for the process of ciliogenesis, which is also supported by data derived from *C. elegans* [20].

Because we found strong and specific localization of nephrocystin within the transition zone, we speculate that nephrocystin is a component of the ciliary (flagellar) pore complex at the ciliary base, a supramolecular structure that regulates access of proteins to the ciliary compartment [21]. Integrating previous and novel findings, one can speculate that nephrocystin does not have a primary function at focal adhesions but, possibly, is involved in cilia-mediated signalling processes that also affect focal adhesions. In addition, our novel data indicate that ciliary dysfunction in several distinct cell types might contribute to the multi-organ involvement in diseases like nephronophthisis, where extra-renal manifestations such as retinal degeneration can occur.

Analysis of respiratory cells: future potential for diagnosis of CKDs

Based on the observation that respiratory cells can be used to analyse function and sub-localization of nephrocystin, we studied whether this knowledge can be exploited to confirm diagnosis of NPHP1. This approach is similar to that in Alport's syndrome, where confocal microscopy of skin biopsies can contribute to the diagnosis in many cases [22]. Indeed, we found that nephrocystin deficiency can be demonstrated in respiratory cells derived from patients with homozygous *NPHP1* deletions (Fig. 1) [8]. This novel approach may also offer a great diagnostic potential for other CKD types. In addition, in vitro ciliogenesis of respiratory cellular cultures introduces new options for the study of the functional properties of cystoproteins during cilia formation and cell differentiation [8].

With increasing knowledge of disease mechanisms involved in CKDs, novel therapeutic options now arise. Recently, progression of cystic kidney disease has been significantly slowed down in an animal model for adolescent nephronophthisis by drugs interfering with intracellular cAMP-signalling. Vasopressin V2 receptor antagonists led to reduced levels of cAMP and inhibition of cyst development [23]. Other substances, such as rapamycin and long-acting somatostatin, have also shown promising effects on slowing down cyst formation. These concepts of addressing high levels of cAMP to counteract the altered pathways of reduced intracellular calcium in CKDs are now entering clinical trials [24]. In addition, very recently, Bukanov and co-workers have been able to show that, by inhibition of the cyclin-dependent kinase, which is involved in ciliary mediated cell cycle regulation, an effective and long-lasting arrest of murine polycystic kidney disease can be achieved [25].

In summary, by the identification of cystoproteins and their ciliary function in CKDs, new insights into the pathology of cyst formation have evolved, offering fascinating diagnostic and therapeutic options.

References

1. Guay-Woodford LM (2006) Renal cystic diseases: diverse phenotypes converge on the cilium/centrosome complex. Pediatr Nephrol 21:1369–1376
2. Hildebrandt F, Otto E (2005) Cilia and centrosomes: a unifying pathogenic concept for cystic kidney disease? Nat Rev Genet 6:928–940
3. Badano JL, Teslovich TM, Katsanis N (2005) The centrosome in human genetic disease. Nat Rev Genet 6:194–205
4. Davenport JR, Yoder BK (2005) An incredible decade for the primary cilium: a look at a once-forgotten organelle. Am J Physiol Renal Physiol 289:F1159–F1169
5. Zhang Q, Taulman PD, Yoder BK (2004) Cystic kidney diseases: all roads lead to the cilium. Physiology (Bethesda) 19:225–230
6. Hildebrandt F, Otto E, Rensing C, Nothwand HG, Vollmer M, Adolphs J, Hanusch H, Brandis M (1997) A novel gene encoding an SH3 domain protein is mutated in nephronophthisis type 1. Nat Genet 17:149–153
7. Saunier S, Calado J, Heilig R, Silbermann F, Benessy F, Morin G, Konrad M, Broyer M, Gubler MC, Weissenbach J, Antignac C (1997) A novel gene that encodes a protein with a putative src homology 3 domain is a candidate gene for familial juvenile nephronophthisis. Hum Mol Genet 6:2317–2323
8. Fliegauf M, Horvath J, von Schnakenburg C, Olbrich H, Muller D, Thumfart J, Schermer B, Pazour GJ, Neumann HP, Zentgraf H, Benzing T, Omran H (2006) Nephrocystin specifically localizes to the transition zone of renal and respiratory cilia and photoreceptor connecting cilia. J Am Soc Nephrol 17:2424–2433
9. Hildebrandt F, Omran H (2001) New insights: nephronophthisis-medullary cystic kidney disease. Pediatr Nephrol 16:168–176
10. Saunier S, Calado J, Benessy F, Silbermann F, Heilig R, Weissenbach J, Antignac C (2000) Characterization of the NPHP1 locus: mutational mechanism involved in deletions in familial juvenile nephronophthisis. Am J Hum Genet 66:778–789
11. Caridi G, Dagnino M, Trivelli A, Emma F, Perfumo F, Ghiggeri GM (2006) Stop codon at arginine 586 is the prevalent nephronophthisis type 1 mutation in Italy. Nephrol Dial Transplant 21:2301–2303
12. Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AE, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J (2003) Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nat Genet 33:129–137
13. Benzing T, Walz G (2006) Cilium-generated signaling: a cellular GPS? Curr Opin Nephrol Hypertens 15:245–249
14. Praetorius HA, Spring KR (2005) A physiological view of the primary cilium. Annu Rev Physiol 67:515–529

15. Simons M, Walz G (2006) Polycystic kidney disease: cell division without a c(l)ue? Kidney Int 70:854–864
16. Ross AJ, May-Simera H, Eichers ER, Kai M, Hill J, Jagger DJ, Leitch CC, Chapple JP, Munro PM, Fisher S, Tan PL, Phillips HM, Leroux MR, Henderson DJ, Murdoch JN, Copp AJ, Eliot MM, Lupski JR, Kemp DT, Dollfus H, Tada M, Katsanis N, Forge A, Beales PL (2005) Disruption of Bardet–Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. Nat Genet 37:1135–1140
17. Hildebrandt F, Otto E (2000) Molecular genetics of nephronophthisis and medullary cystic kidney disease. J Am Soc Nephrol 11: 1753–1761
18. Otto EA, Schermer B, Obara T, O'Toole JF, Hiller KS, Mueller AM, Ruf RG, Hoefele J, Beekmann F, Landau D, Foreman JW, Goodship JA, Strachan T, Kispert A, Wolf MT, Gagnadoux MF, Nivet H, Antignac C, Walz G, Drummond IA, Benzing T, Hildebrandt F (2003) Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left–right axis determination. Nat Genet 34:413–420
19. Schermer B, Hopker K, Omran H, Ghenoiu C, Fliegauf M, Fekete A, Horvath J, Kottgen M, Hackl M, Zschiedrich S, Huber TB, Kramer-Zucker A, Zentgraf H, Blaukat A, Walz G, Benzing T (2005) Phosphorylation by casein kinase 2 induces PACS-1 binding of nephrocystin and targeting to cilia. EMBO J 24:4415–4424
20. Winkelbauer ME, Schafer JC, Haycraft CJ, Swoboda P, Yoder BK (2005) The C. elegans homologs of nephrocystin-1 and nephrocystin-4 are cilia transition zone proteins involved in chemosensory perception. J Cell Sci 118:5575–5587
21. Rosenbaum JL, Witman GB (2002) Intraflagellar transport. Nat Rev Mol Cell Biol 3:813–825
22. Muda AO, Massella L, Giannakakis K, Renieri A, Rizzoni G, Faraggiana T (2003) Confocal microscopy of the skin in the diagnosis of X-linked Alport syndrome. J Invest Dermatol 121:208–211
23. Gattone VH, Wang X, Harris PC, Torres VE (2003) Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist. Nat Med 9:1323–1326
24. Harris PC, Torres VE (2006) Understanding pathogenic mechanisms in polycystic kidney disease provides clues for therapy. Curr Opin Nephrol Hypertens 15:456–463
25. Bukanov NO, Smith LA, Klinger KW, Ledbetter SR, Ibraghimov-Beskrovnaya O (2006) Long-lasting arrest of murine polycystic kidney disease with CDK inhibitor roscovitine. Nature 444: 949–952