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Urinary Concentration Defects and Mechanisms Underlying Nephronophthisis

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Key Words

Cystic kidney · Urinary concentration defect · Aquaporin · Nephronophthisis · Cilia · Adherens junction · Vasopressin receptor · Collecting duct

Abstract

The cystic kidney disease nephronophthisis (NPHP) is the commonest genetic cause of end-stage renal failure in young people and children. Histologically the disease is characterized by interstitial fibrosis, tubular atrophy with corticomedullary cyst development and disruption of the tubular basement membrane. Affected children present with polydipsia and polyuria, secondary to a urinary concentration defect, before these structural changes develop. Recently, molecular genetic advances have identified several genes mutated in NPHP, providing novel insights into its pathophysiology for the first time in decades. Here we review the normal physiological mechanisms of urinary concentration and explain, in the context of recent discoveries, the possible mechanisms underlying urinary concentration defects in patients with NPHP. The pattern of a ciliary and adherens junction subcellular localization of nephrocystin proteins is discussed. Recent animal models of cystic kidney disease and treatment with vasopressin V2 receptor antagonists are reviewed and a hypothesis regarding urinary concentration defects in NPHP is proposed. Understanding the cellular mechanisms underlying NPHP and other cystic kidney diseases will provide the rationale for therapeutic interventions in this disease. Early urinary concentration defects provide both a clue to clinical diagnosis of NPHP and potential therapeutic targets for pharmacological treatment of this condition.

Introduction

Nephronophthisis (NPHP) is the commonest genetic cause of end-stage renal failure in young people and children [1]. NPHP literally means loss or wasting away of nephrons. It is an autosomal recessive condition and affected children have progressive renal failure leading to end-stage renal failure. Presenting symptoms include polydipsia, polyuria, growth failure and anaemia. Histologically, NPHP is characterized by the triad of interstitial fibrosis, tubular atrophy with cyst development and disruption of the tubular basement membrane [2]. Interestingly, like many other renal cystic diseases, these patients develop polyuria secondary to a urinary concentration defect, even before these structural changes develop [3]. The purpose of this paper is to review the normal physiological mechanisms of urine concentration and to explain the possible mechanisms underlying urinary concentration defects in patients with NPHP.

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Normal Physiology

In healthy kidneys, concentration of urine occurs through the ability of the renal tubules to reabsorb filtered free water. In this way the normal kidney is able to produce urine with an osmolality of 1,200 mosm/kg, approximately five times the normal plasma osmolality. The kidneys receive about 20% of the cardiac output thereby producing 180 litres/day of glomerular filtrate. Only a fraction of this is excreted as final urine, typically 1% (1.8 litres/day), requiring the remaining 99% to be reabsorbed.

About 60–70% of the water in the glomerular filtrate is absorbed by the highly permeable proximal tubule, facilitated by its microvillous surface and the aquaporin (AQP)-1 water channels. The remaining 30–40% of glomerular filtrate enters the loop of Henle where further reabsorption of water occurs without solute, leading to concentration of urine. The descending thin limb of the loop of Henle is highly permeable to water and about 15% of the filtrate is reabsorbed, via AQP1 water channels in long-loop nephrons and by undetermined mechanisms in short-loop nephrons [4]. In contrast, the thin ascending loop and the thick ascending loop of Henle are impermeable to water, with an absence of AQP channels.

Passive reabsorption of sodium chloride occurs in the thin ascending loop down its concentration gradient, from the tubular lumen, into the tubular cells. In the thick ascending loop, electrogenic reabsorption of sodium and potassium and chloride absorption occur facilitated by Na^+-K^+-$2Cl^-$ transporter, the luminal (apical) membrane of thick ascending loop, with recycling of potassium back to the tubular lumen through the renal outer medullary K^+ channels. Tubular potassium recycling via renal outer medullary K^+ channels facilitates electrogenic transport of ions such as calcium and magnesium via paracellular routes in the thick ascending loop. Hence, the remaining filtrate exiting the thick ascending loop and entering the distal tubule is hypotonic compared to plasma.

The distal tubule is relatively impermeable to passive transcellular or paracellular movement of both water and sodium, due to its thick tight junctions. Remaining sodium chloride and calcium ions are actively reabsorbed in the distal tubule by the Na^+-Cl^- cotransporter and a voltage-dependent calcium channel, respectively. The dilute filtrate then enters the connecting segment and the cortical collecting duct. Here, the water within the filtrate can either be absorbed or excreted, but this is entirely dependent on the available levels of antidiuretic hormone (ADH). The cortical collecting duct has low water permeability in its basal state, but in the presence of ADH it can absorb water in the filtrate, allowing urine osmolality to range from 60 to 1,200 mosm/kg, depending upon whole-body water composition and requirements. ADH binds to a V_2 receptor (a G protein-linked receptor) on the basolateral surface of the principal cells of the collecting duct. This activates adenylyl cyclase to increase the intracellular cAMP, causing stimulation of the intracellular protein kinases, which in turn phosphorylates key residues in the carboxyl-terminal domain of AQP2 and allows fusion of cytoplasmic vesicles containing water channel proteins (AQP2) to the luminal membrane. Once inserted, AQP channels allow flow of water across a concentration gradient from the hypotonic nephron lumen to the hypertonic interstitium, thus concentrating the urine. Basolateral AQP3 and AQP4 channels transfer water within the collecting duct cells to the renal interstitium and subsequently into the circulation. In the absence of ADH, the AQP2 water channels are retrieved by an endocytic process – or indeed released into the urine in exosome structures [5] – and water permeability returns to its original low basal rate. Movement of AQP2-containing vesicles requires a dynamic actin cytoskeleton [6], which can reorganize and allow AQP2-containing vesicles to traffic to the plasma membrane.

One of the major enabling factors in the concentration of urine is the countercurrent mechanism [7]; this is enabled by the complex anatomical arrangements between the loops of Henle, the medullary interstitium with its associated blood vessels and the collecting ducts. The countercurrent mechanism generates a hypertonic medullary interstitium, which enables the kidney to produce concentrated urine. To maintain the hypertonic medullary interstitium, water is absorbed in the ADH-sensitive distal and cortical collecting duct nephron segments. In the presence of ADH, both the outer and inner medullary collecting ducts increase their permeability to water; in the absence of ADH, the urine cannot be concentrated, causing polyuria. The outer medulla is impermeable to urea. In contrast, the inner medulla is highly permeable to urea (via urea transporters UT-A1 and UT-A3), with permeability increasing by fourfold in the presence of ADH. This allows urea to accumulate in the medullary interstitium, making it hypertonic and thereby allowing water reabsorption, leading to the production of a concentrated urine.

Urinary Concentration Defects

ADH is a nonapeptide, synthesized in the supra-optic and paraventricular nuclei of the hypothalamus and stored and released from the posterior pituitary. When there is impaired production or release of ADH, it results in a poorly concentrated, low-osmolar urine (<300 mosm/kg), a condition known as central (or cranial) diabetes insipidus. Nephrogenic diabetes insipidus is due to absent or reduced response of ADH on the principal cells of the collecting duct and may be an inherited disorder or an acquired defect. Acquired defects are often secondary to lithium toxicity, hypercalcaemia, hypokalaemic, chronic renal failure, bilateral ureteral obstruction and tubulo-interstitial disease. Inherited forms are secondary to defects in the vasopressin-2 receptor (V2R) or mutations in the *AQP2* gene (autosomal dominant and occasionally recessive), resulting in defective trafficking of AQP2 to the apical membrane [8, 9]. Acquired forms cause a polyuria syndrome by downregulating AQP2 channels in the inner medullary collecting duct [10–13].

High-osmolar urine is produced classically by syndrome of inappropriate secretion of ADH. This condition may be caused by increased levels of ADH secondary to drugs, systemic causes and ectopic secretion of ADH. Increased levels of ADH result in hyponatraemia, hypo-osmolar serum (<260 mosm/kg), natriuresis (urinary sodium >40 mM) and inappropriately concentrated urine (>500 mosm/kg). These biochemical manifestations, when seen with undetectable ADH levels, can be secondary to gain-of-function mutations of the V2R (nephrogenic syndrome of inappropriate antidiuresis) [14].

Causes of Polyuria in Cystic Kidney Disease

Inherited renal cystic diseases are associated with a loss of ability to concentrate urine. This has been demonstrated most repeatedly in autosomal dominant polycystic kidney disease (ADPKD) [15–17], but is also a reported feature of NPHP [3, 18], medullary cystic kidney disease [19] and Bardet-Biedl syndrome [20]. Typically a patient with NPHP will develop symptoms of polydipsia and polyuria at the age of 4–6 years, related to the underlying defect in urinary concentration ability, and a failure to concentrate urine following a water deprivation test is a characteristic finding in NPHP [21]. In a historical review, Gardner [3] points to the fact that a defect in maximal urinary concentrating ability is the best single sign of early NPHP. A concentrating defect may be present in children who are well and who have preserved renal function [22]. Indeed, 'life-long' polyuria and polydipsia was noted in 2 teenage siblings [23]. Detailed biochemical analysis revealed that both siblings, who had developed moderate renal impairment, failed to achieve a urinary osmolality greater than that of plasma, despite administration of vasopressin (Pitressin) [23]. Giselson et al. [24] describe biochemical findings in 2 siblings with NPHP before the onset of renal impairment. The elder sibling was admitted to hospital at the age of 2 years and 3 months, and investigations revealed, despite a normal creatinine clearance, that the urine specific gravity was low (maximum 1.013 g/ml). This patient went on to develop severe progressive renal failure leading to his death at the age of 17 years. The younger sibling had normal renal function at the age of 15 years (creatinine clearance 114 litres/24 h/1.73 m^2), but a maximal urine specific gravity of 1.020 g/ml. However, in this case, polyuria was not pronounced (just 2 litres/day), but of note, urine acidification defects were seen simultaneous to urinary dilution defects in this patient. Additional reports of juvenile NPHP have confirmed this inability to concentrate urine [25]. Bodaghi et al. [18] describe 3 cases of juvenile NPHP, in whom established renal failure was evident before 1 year of age. The authors admit that symptoms of excessive thirst and polyuria were present earlier and could have led to a more timely diagnosis. Unfortunately, these reports represent a rather historical perspective and data correlating urine concentration defect with a confirmed genetic diagnosis of NPHP have not been published. A similar finding of reduced urine concentration capacity has been demonstrated in the *pcy* mouse model of adolescent NPHP (NPHP type 3) [26]. Dissection of the underlying mechanisms of loss of urinary concentration may lead to insights into cystic kidney disease pathophysiology.

Vasopressin as a Driver of Cystic Disease

In the collecting duct, driven by vasopressin, intracellular cAMP is a prerequisite for insertion of AQP2 channels to the apical plasma membrane to absorb luminal water. This acute response is modulated by long-term changes in AQP2 expression, with cAMP-responsive element in the *AQP2* gene controlling AQP2 protein expression. Low AQP2 protein levels have been associated with severe polyuria in mice with high levels of cAMP phosphodiesterase activity and low cellular cAMP levels, con-

firming that AQP2 protein expression is mediated by cAMP [27].

Recently, rodent models of cystic kidney disease have been used to demonstrate that V2R antagonists such as OPC-31260 may successfully suppress cystogenesis [26, 28, 29]. This raises the question whether vasopressin is driving cyst progression in polycystic kidney diseases. For example, the *Pck* rat model of autosomal recessive polycystic kidney disease (ARPKD) demonstrates very early upregulation of V2R and AQP2 mRNAs. Treatment with OPC-31260 led to a reduction in kidney size, renal cyst volume and renal cAMP concentration [26]. Similarly, the *pcy* mouse model of NPHP also demonstrates upregulation in mRNA for the V2R and AQP2. Treatment with OPC-31260 was effective at limiting cystogenesis and cyst enlargement [26]. In normal renal epithelia, polycystin-2, regulated by polycystin-1, acts as a gateway for intracellular entry of Ca^{2+}, with both proteins colocalizing to the renal cilium which acts as a mechanosensor [30]. Disruption of this pathway will result in reduced entry of Ca^{2+} and this in turn stimulates adenylyl cyclase which inhibits cAMP phosphodiesterase and increases intracellular cAMP levels. Such alterations of intracellular calcium regulation may switch the phenotype of principal cells so that cAMP drives (via B-Raf proto-oncogene serine/threonine protein kinase and Elk-related tyrosine kinase) cell proliferation (rather than inhibiting proliferation). In this situation, vasopressin stimulation would also promote this proliferation [31, 32]. Proof of principle for vasopressin driving this mechanism has been recently obtained by suppression of vasopressin by drinking water, which was sufficient to prevent cyst formation in PCK rats [33]. More recently, PCK rats were crossed with Brattlebro rats (deficient in arginine vasopressin; AVP) and the resulting PCK AVP–/– animals had lower renal cAMP levels and reduced cystogenesis [34]. Vasopressin, therefore, is key to the progression of cystic kidney disease and by inhibiting its effect on the collecting duct (by physiological and pharmacological methods) ameliorates cyst progression (in rodents at least).

Interestingly, vasopressin also has a role in actin depolymerization [35] which allows reorganization of the terminal web and vesicle fusion. In the CPK mouse model of ARPKD, AQP2 protein was present throughout the cell rather than in the apical compartment, suggestive of misregulation in sorting and insertion of AQP2 [36, 37]. The V2R antagonist OPC-31260 was able to ameliorate the cystic disease but did not concentrate the urine in these mice. On treatment with OPC-31260 there was an increase in V2R mRNA expression with a decreased expression of AQP2, associated with a halt in the progression of the cystic disease, but no change in the hypotonicity of the urine [36].

Differences between ADPKD and ARPKD and NPHP

Important anatomical differences between the type of cyst formation in ADPKD and ARPKD which include NPHP are worth noting. In ADPKD cysts are detached and spheroid and require fluid secretion for expansion. Cysts may form along the entire length of the nephrons, and are not restricted to the collecting duct [38]. ADPKD cysts are characterized by abnormal cell proliferation rates, defects in membrane protein polarity, disordered cell-matrix interactions and increased ion and fluid secretion [38]. In contrast, in ARPKD and NPHP, the tubules are elongated and ectatic with diverticula, with distal segments predominately affected. Here, tubular anion secretion may play less of a role in cystogenesis. Is NPHP a true cystic kidney disease? NPHP is characterized by formation of corticomedullary cysts, thickening and attenuation of the tubular basement membranes, tubular atrophy and interstitial fibrosis [39]. Typically electron microscopy of the tubular basement membrane in NPHP reveals thickening, tortuosity and lamination of the atrophic tubular basement membrane [40], sometimes with complete disappearance [41]. Accompanying the tubular basement membrane changes is moderate to massive interstitial fibrosis. Before underlying molecular genetic mutations had been discovered, a primary abnormality of the tubular basement membrane was postulated [42]. Descriptions of kidney cysts in patients affected with NPHP state that cysts and diverticula involve predominantly the loops of Henle and distal tubules [43]. In addition, confocal microscopic studies of the *inv* mouse, a model of infantile NPHP (NPHP type 2), where cystic changes are seen in utero, revealed tubular dilatations in collecting ducts, proximal tubules, thick ascending loop of Henle as well as Bowman's capsule [44].

Renal Cilium and Cystic Kidney Disease

Genetic identification of now eight genes implicated in NPHP *(NPHP1–6, AHI1, GLIS2)* [45–48] has led us away from the tubular basement membrane and the emphasis has been placed on the renal primary cilia. The primary cilium is a cell surface projection which acts as an 'an-

tenna'. This specialized organelle extends from the basal body and consists of an axoneme comprising nine microtubular doublets and no central pair of microtubules (termed 9 + 0). Assembly of the axoneme occurs via a process called intraflagellar transport where proteins are moved up and down the cilium [45]. No protein synthesis occurs within the cilium. Recent data has suggested that the renal cilium is central to the pathogenesis of cystic kidney disease, with a common pathway converging at the cilium/basal body complex. It seems likely that cilial expression is likely to be confirmed for all nephrocystin proteins. Thus the cilium acts as a subcellular domain at which nephrocystins form complexes with themselves and other related proteins, in order to facilitate signalling cascades. Primary cilia may be able to sense tubular luminal flow (of urine) and regulate calcium entry (mediated by polycystin-2 channels) [30].

Deflection of the cilia results in a polycystin-1- and polycystin-2-dependent increase in intracellular calcium [30]. A reduction in urine flow or perceived reduction, as might occur in a cyst or dilated tubule, would reduce this sensory signal, leading to downstream effects which may include unregulated cell proliferation and cyst expansion. Nephrocystins may be part of such signalling complexes.

The 'Nephrocystin' Protein Family

To date, the nephrocystin proteins form a diverse group of multidomain proteins, with numerous protein-protein interactions and postulated intracellular roles. To demonstrate their many known properties, the known nephrocystins are detailed below. Unlike polycystins, none of the nephrocystin proteins (to date) possess transmembrane domains, thereby excluding channel activity. However, like polycystins, nephrocystin proteins may have multiple cellular locations, with the possibility for a predefined role at each location of the cell, and different roles in different cell or tissue types. In the mouse kidney, expression of nephrocystin-1, nephrocystin-3 and nephrocystin-4 is seen in renal tubules at the corticomedullary junction, the site at which cyst formation tends to occur [49].

NPHP1 encodes the protein nephrocystin-1, a primary cilia protein [50] which possesses a coiled-coil domain and an Src homology domain-3 (SH3). Nephrocystin-1 has been localized to the renal cilium [50] and to epithelial cell adherens junctions, where it colocalizes with p130(Cas) and tensin [51, 52]. Nephrocystin-1 also physically interacts with other nephrocystins (types 2–4, jouberin) [53–57] and there is evidence that this complex of proteins may function at the cilium, cell-cell adherens junctions and at focal adhesions [50–52, 58].

Inversin (alias nephrocystin-2) is a protein containing 16 tandem ankyrin repeat domains, a nuclear localization signal (bipartite), D-boxes and two IQ calmodulin-binding domains [59, 60]. Like nephrocystin-1, inversin has multiple localizations, with a dynamic distribution during cell cycle [61] and renal cilia expression [50, 61, 62], where it is associated with tubulin [63].

Intriguingly, studies concerning inversin have opened up a further role of nephrocystin proteins, that of Wnt signalling, which comprises several distinct pathways. These may be grouped into canonical (β-catenin-dependent) and non-canonical pathways. Landmark studies by Simons et al. [64] identified a role for inversin as a switch between canonical and non-canonical Wnt signalling pathways where inversin targets cytoplasmic dishevelled for degradation, inhibiting the canonical Wnt signalling (β-catenin) pathway. A further role for inversin was also discovered whereby in development it is required for normal convergent extension movements as part of the non-canonical Wnt signalling pathway [64]. This switching may be secondary to mechanosensing of the cilium, as inversin protein expression in ciliated tubular epithelium increased with flow stimulation. Thus, inversin defects may also change planar cell polarity signalling, resulting in misorientation of the mitotic spindle during cell division, leading to a circumferential rather than linear tubular growth [65]. Thus nephrocystin-2 plays a role in the developing nephron, but may also act in the ongoing maintenance of the tubular architecture during periods of regeneration following injury or ischaemia.

Nephrocystin-3 is another multidomain protein, with a single coiled-coil domain, a tubulin tyrosine ligase (TTL) domain and a tetratricopeptide repeat domain. A STAND (signal transduction ATPase with numerous domains) domain is found within the TTL domain and may have a role in apoptotic pathways [49]. Tissue expression of the murine nephrocystin-3 is widespread, with localization including the renal tubules, liver, biliary tract and retina, accounting for the extended phenotype of patients with NPHP type 3, which includes liver fibrosis [53].

Nephrocystin-4 (alias nephroretinin) is a highly conserved protein but lacks any known protein domains, except for a proline-rich region within its centre. Its N terminus interacts with the C terminus of nephrocystin-1 [55], and there is evidence for this complex acting at cell-cell junctions and complexing with actin cytoskeleton-

organizing proteins, including p130(Cas) and Pyk2 [54]. Nephrocystin-4 is an interaction partner of RPGRIP1L (alias nephrocystin-8) [66] and localizes to the primary cilium [54].

The nephrocystin-5 protein contains two IQ calmodulin-binding sites which surround a coiled-coil domain. Similar to inversin, nephrocystin-5 interacts directly with calmodulin, with which it colocalizes to the primary cilium, and forms a complex with retinitis pigmentosa GTPase regulator [67].

NPHP6 encodes a large centrosomal protein, CEP290 (alias nephrocystin-6), which has 13 coiled-coil domains and numerous other domains including RepA/Rep+ protein KID domains, a P loop (ATP/GTP-binding site motif A) and tropomyosin homology domains. Nephrocystin-6 directly interacts with the cAMP-related transcription factor, CREB2 [47].

AHI1 encodes the protein jouberin and mutations may cause isolated Joubert syndrome [68, 69] as well as the cerebello-oculo-renal syndrome, which includes NPHP as a renal phenotype [48]. Jouberin possesses a coiled-coil domain, six WD40 domains and an SH3 domain and interacts directly with nephrocystin-1 [57]. Using a novel anti-jouberin antibody we have recently demonstrated that jouberin is expressed in human collecting ducts and colocalizes with AQP2 [70]. This is the first example of a 'nephrocystin' protein localization to a specific nephron segment and allows some speculation regarding its functional role (see hypothesis below).

GLIS2 (alias *NPHP7*) encodes a Krüppel-like zinc finger transcription factor. Mutations in *GLIS2* were found in 3 individuals with NPHP who developed end-stage renal failure by 8 years of age [46]. Its expression in MDCK-II cells is along the ciliary axoneme, in a punctuate pattern, typical of other nephrocystins. The zinc finger domains may play a role in this localization, given that cilial localizing transcription factors Gli2 and Gli3 have a similar zinc finger motif [46]. A mouse model that allowed staining of GLIS2 mutant kidneys revealed a redistribution of E-cadherin from the basolateral membrane to a cytoplasmic location in some of the tubules, suggesting disruption of epithelial cell polarity in these cells [46].

NPHP8 (alias RPGRIP1L) encodes a basal body protein named RPGRIP1L and is the latest member of the nephrocystin protein family. It possesses coiled-coil domains, two protein kinase C-conserved region 2 (C2) domains and two leucine zipper motifs [66].

Polyuria in NPHP – a Hypothesis

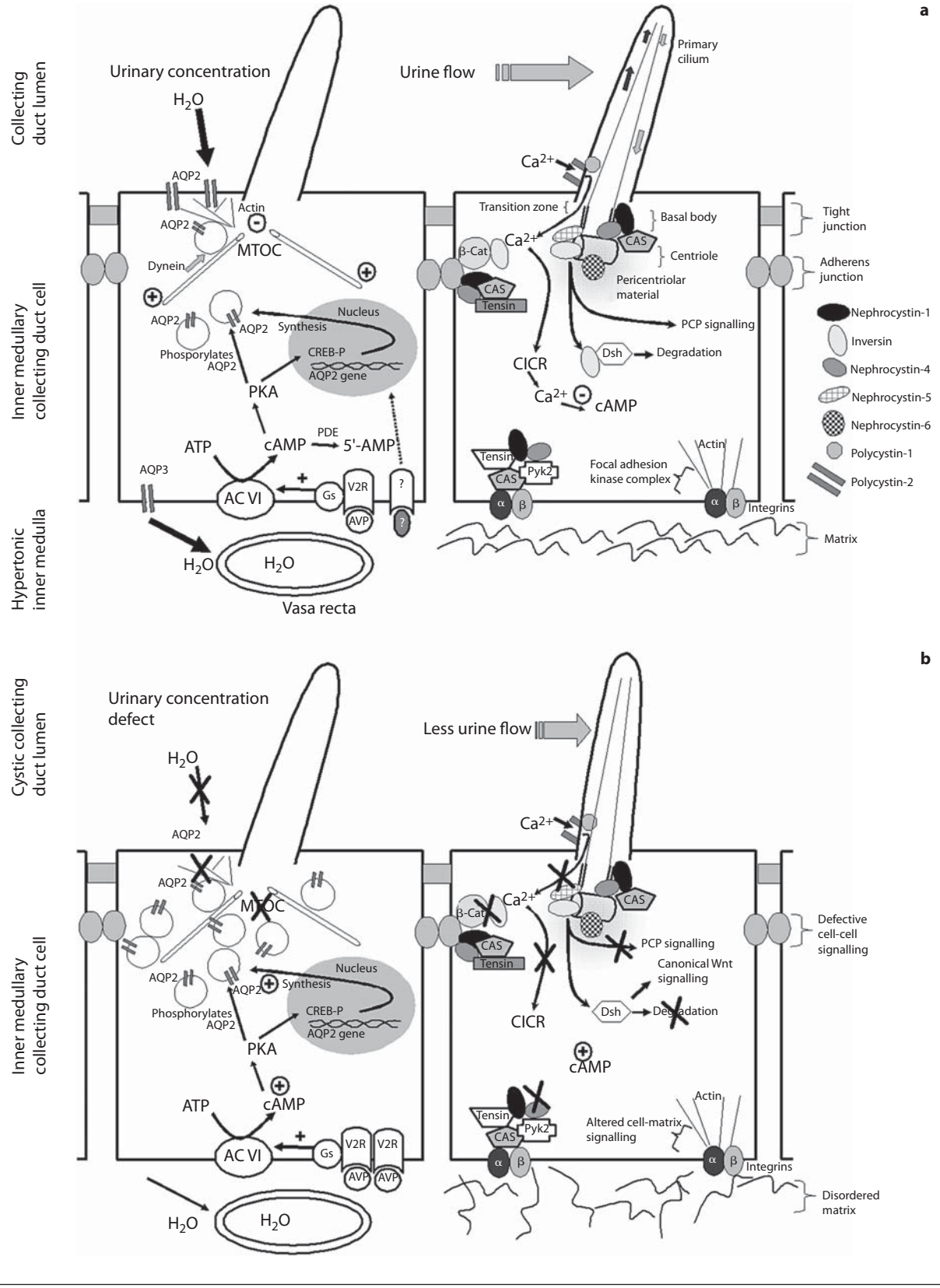
To summarize the normal renal physiology, concentration of urine requires three essential factors working in concert: (1) medullary collecting ducts must be located within a hypertonic interstitium; (2) collecting duct cells must express functional V2R, and (3) there must be the presence of appropriate water channels (AQP2 at the luminal membrane and AQP3 and AQP4 at the basolateral membrane) within the collecting duct to allow transcellular movement of water [71]. Thus, in the normal situation, an intact collecting duct epithelium is able to respond appropriately to vasopressin, via its receptor, and co-ordinate insertion of apical and basolateral AQP channels. This process may be regulated acutely via cAMP mechanisms and chronically via gene transcription regulation (fig. 1a). An intact vasa recta is able to remove extracellular water to prevent dilution of the medullary interstitium. Primary cilia may be intimately involved in these processes, by detecting urine flow, allowing calcium entry and thus regulating adenylyl cyclase VI activity (fig. 1a).

What are the potential mechanisms of loss of urinary concentration in NPHP? If we accept that urinary concentration ability is disrupted early on in the disease, we must consider: (1) an abnormality in epithelial polarity; (2) a disruption of transcellular transport of water (and solutes), and (3) a defect in extracellular matrix.

Epithelial cell polarity is required for normal renal development, for the maintenance of adherens junctions and cell-cell signalling. A key feature of nephrocystin proteins is their strong expression at cell-cell contacts and direct interactions with components of the adherens junction (fig. 1b).

We hypothesize that defects at cell-cell junctions secondary to mutant nephrocystins would compromise both epithelial integrity (in growth, maintenance and repair) and cell-cell signalling. Defective adherens junctions within the collecting duct would reduce the osmotic gradient for effective water reabsorption leading to polyuria.

A defect in transcellular water transport may be secondary to abnormal AQP2 protein regulation in collecting duct cells. For example, a loss of microtubular organization and actin web defects, secondary to abnormal nephrocystin protein complexes, might lead to defective AQP protein insertion or regulation. Loss of transcellular water transport would lead to a compensatory overexpression of vasopressin receptors, and a dysregulation of cAMP, as seen in animal models of cystic kidney disease (fig. 1b).



Abnormal nephrocystin expression within the focal adhesion kinase complexes at the basolateral membranes would lead to altered cell-matrix signalling. This would promote interstitial fibrosis, leading to renal scarring and a 'disappearance of nephrons'. This would impact on the overall concentrating ability of the kidney, in terms of both nephron number and the ability of the countercurrent mechanism to be effective (fig. 1b). Abrupt transition in the tubular basement membrane on electron microscopy is characteristic of NPHP and can occur with or without subsequent cyst formation [72]. These tubular basement membrane changes could contribute to the resistance to ADH seen in NPHP patients, causing low-osmolar urine and the increase in urinary sodium loss [23].

Given the cilial localization of nephrocystin proteins, mutated nephrocystin may lead to abnormal downstream cilial signalling, promoting a phenotypical change in the cell response to calcium, disrupting planar cell polarity signalling and promoting persistent canonical Wnt signalling. Increased proliferation and apoptosis would lead to disruption in renal tubular architecture and promote cystogenesis. Tubular dilatation would perpetuate abnormal flow, reduced ciliary bending and abnormal ciliary signalling (fig. 1b).

Fig. 1. Mechanisms of action of nephrocystin proteins in health and disease. **a** Model of urine concentration and nephrocystin function in the collecting duct. Left: The urine concentration is dependent upon an intact collecting duct and a hypertonic medullary interstitium, where permeability to water is regulated by the vasopressin V2 receptor (V2R) and the water channel aquaporin-2 (AQP2) in principal cells. Following stimulation of the V2R by vasopressin on the basolateral membrane, the GTP-binding protein G_s activates adenylyl cyclase VI (AC VI) and increases the production of cAMP from ATP. cAMP binds to the regulatory subunit of protein kinase A (PKA), which activates the catalytic subunit of PKA. PKA is then able to phosphorylate AQP2 in intracellular vesicles. cAMP also controls in part the long-term regulation of AQP2 by phosphorylating transcription factors such as CREB-P (cAMP-responsive element-binding protein), which increases AQP2 gene transcription. Synthesis of new AQP2 enters the cytoplasmic pool of vesicular AQP2. Additional receptors (?) may regulate AQP2, independent of vasopressin. At the apical membrane, phosphorylated AQP2 translocates from the storage vesicles to be inserted and allows water movement into the cell. Microtubules, essential for this translocation, are polarized and arise from the microtubular organizing centre (MTOC). Here the minus end is anchored, and the plus ends radiate out into the cell cytoplasm. The motor protein dynein together with dynactin allow the movement of AQP2-bearing vesicles along the microtubules. Actin provides a network to anchor vesicles and reorganization of the apical actin network, with actin depolymerization, allows AQP2 translocation into the plasma membrane, facilitated by docking and fusion proteins (not shown). In parallel, AQP3 protein synthesis takes place which is inserted at the basolateral membrane and allows water exit from the cell into the hypertonic medullary interstitium where it is removed by the vasa recta. Right: Primary cilia of the renal epithelium are mechanosensors which sense urinary flow. Deflection of the cilium leads to calcium influx, mediated by the transmembrane proteins polycystin-1 and polycystin-2, which causes a calcium-induced calcium release (CICR) from the endoplasmic reticulum and leads to downstream signalling events including inhibition of adenylyl cyclase VI and stimulating cAMP-dependent phosphodiesterases, both of which reduce cAMP levels. Flow also upregulates inversin, which targets cytoplasmic dishevelled (Dsh) for degradation. Unlike the polycystins, nephrocystin proteins are cytosolic. Nephrocystins localize to the cilia/basal body complex, the adherens junction (cell-cell junctions) and the focal adhesion complexes. Inversin co-precipitates with N-cadherin and β-catenin (β-Cat). Nephrocystin-1 and nephrocystin-4 interact with p130Cas (CAS), Pyk2 and the actin-binding molecules tensin and filamin. Nephrocystins and inversin also associate with microtubule components, α- and β-tubulins. Nephrocystin-1, inversin, nephrocystin-4, nephrocystin-5 and nephrocytsin-6 all localize to the base of the cilium (basal body) and in often a punctate pattern along the ciliary axoneme. In mitotic cells, inversin, nephrocystin-4 and nephrocystin-6 have been localized to the microtubule-organizing centre and to the mitotic spindle. Inversin protein also promotes planar cell polarity (PCP) signalling during development and may have a continued role in maintenance and remodeling of the renal tubule morphology during adult life. Nephrocystin-6 interacts directly with and modulates the activity of cAMP-binding protein CREB2, implicating a role for nephrocystin-6 in transcriptional control, cell proliferation and differentiation. **b** Model of loss of urinary concentration and disease mechanisms in NPHP. Left: Loss in the ability to concentrate urine causes overexpression of V2R and AQP2 levels. This loss of concentration ability may be secondary to a disrupted MTOC and disordered actin web at the cell apex leading to defective AQP2 insertion into the plasma membrane, reducing effective water reabsorption. Increased cAMP is a driving factor for increased AQP2 production. Right: Defects in cell-cell complexes would contribute to a defect in medullary interstitial concentration, reducing the osmotic gradient for water resorption. At the cilium, defects in nephrocystins may contribute to altered calcium signalling, leading to a stimulation of adenylyl cyclase VI and inhibition of phosphodiesterase, leading to increased intracellular cAMP, driving cell proliferation/apoptosis. Nephrocystin protein defects could also contribute to cell-matrix abnormalities and lead to interstitial fibrosis. Specific to inversin, in reduced flow, such as in a dilated (cystic) tubule, inversin levels would be reduced, allowing dishevelled (Dsh) to act along in the β-catenin-dependent (canonical) Wnt pathway, leading to cell proliferation. Finally, planar cell polarity (PCP) signalling may also be regulated by inversin and defects may cause misorientation of mitotic spindles, which in combination with deregulated cell proliferation leads to cyst formation.

A collecting duct localization of nephrocystin proteins, as we have demonstrated with jouberin [70], needs to be confirmed, but provides a logical argument for a protein complex involving nephrocystins to be involved in the regulation of water transport. If nephrocystin proteins colocalize in part with AQP2, we speculate that in NPHP defects in nephrocystin proteins would contribute to the inability to concentrate urine, secondary to misregulation of AQP2 protein in collecting duct cells. A specific collecting duct localization of nephrocystins may also explain the milder and more distal cystic phenotype seen in NPHP kidneys. These speculations become relevant, giving the growing evidence for the use of pharmacological agents to treat cystic kidney disease. Clinical trials to date have focused on treating ADPKD, with a rationale for the use of V2R antagonists based on basic science observations in many rodent models, which included animals with ARPKD and NPHP [26, 28, 29, 34]. It seems appropriate that if the mechanism of disease underlying NPHP includes cAMP dysregulation, which promotes cystogenesis, then agents which reverse this affect may be a useful therapeutic. The paradox is that V2R antagonists are aquaretic agents, promoting tubular water loss in the context of poor urinary concentration ability; but as we have outlined, there may be a scientific rationale to use these agents in patients with NPHP. Outcomes of clinical trials in ADPKD are eagerly awaited, but opportunities to test these agents in ARPKD should not be ignored, given the often much faster rate of progression of renal impairment. These studies may even provide more definitive evidence of therapeutic benefit, with a much shorter time needed to treat before a clear effect might be shown.

Conclusion

The kidney is a highly specialized organ which allows continuous filtration via glomeruli and reabsorption of filtrate via tubules in order to produce urine which eliminates toxins and maintains salt and water balance. Such fine regulation relies upon many specific cell types along the length of the nephron. Collecting duct segments are responsible for final regulation of the urine concentration. Cystic kidney diseases, which include NPHP, manifest themselves by early-onset urinary concentration defects. Understanding the normal physiological mechanisms responsible for urinary concentration and the known localizations and interactions of nephrocystin proteins allow us to hypothesize roles of nephrocystin proteins in the renal tubular epithelial cells and understand their pathophysiology.

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