results of prior studies by this group, particularly those in KCP<sup>−/−</sup> mice; in the absence of congenital renal abnormalities, such null mice display increased susceptibilities to renal fibrosis.\(^1\)\(^1\) In summary, KCP is unique because it enhances BMP-7 activity (while other known extracellular modulators of BMP-7 activity serve as inhibitors) and because it also affects TGF-β1 activity.

The unique function of KCP as extracellular enhancer of BMP-7 activity is of particular interest in the context of existing challenges to translate the antifibrotic activity of BMP-7 into clinical use. Because production of recombinant BMP-7 in quantities needed for clinical use has been difficult, alternate strategies have been sought to exploit the undisputed antifibrotic activity of BMP-7 for clinical application. One successful approach has been development of synthetic peptides, which mimic antifibrotic activity of BMP-7 in the kidney while circumventing production challenges encountered with recombinant BMP-7;\(^1\)\(^2\) one such peptide (AA123) is being tested in a clinical trial.\(^1\)\(^2\)

An alternate approach is to boost the renoprotective activity of endogenous BMP-7 in the kidney through stimulation of BMP-7 expression in the kidney while circumventing production challenges encountered with recombinant BMP-7;\(^1\)\(^2\) the unique function of KCP as extracellular enhancer of BMP-7 activity serves as inhibitors) and because it also affects TGF-β1 activity (while other known extracellular modulators of BMP-7 activity serve as inhibitors). An alternate approach is to boost the renoprotective activity of endogenous BMP-7 in the kidney through stimulation of BMP-7 expression in the kidney while circumventing production challenges encountered with recombinant BMP-7;\(^1\)\(^2\) one such peptide (AA123) is being tested in a clinical trial.\(^1\)\(^2\)

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**DISCLOSURES**

None.

**REFERENCES**


See related article, “Kielin/Chordin-Like Protein Attenuates both Acute and Chronic Renal Injury,” on pages 897–905.

**Working Out Nephronophthisis Genetics One Family at a Time**

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Congenital anomalies and cystic and hereditary nephropathies are among the most frequent causes of ESRD in childhood, adolescence, and early adulthood. Among these causes, nephronophthisis (NPHP) and related ciliopathies (NPHP-RCs) are a heterogeneous group of degenerative recessive diseases.

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that affect the kidney and multiple other organs, manifesting as renal cysts, retinal degeneration, polydactyly, mental retardation, and obesity.\(^1,2\) The renal histopathologic findings include tubular basement membrane disruption, tubulointerstitial nephropathy, and dilation of tubules with cyst formation mainly at the corticomedullary border.\(^3\) Renal ultrasonography shows normal or slightly decreased in size kidneys for age with increased echogenicity with loss of corticomedullary differentiation.\(^3\) However, renal cysts are not typically identified on initial ultrasound examination, and early clinical findings are nonspecific (polyuria and hyposthenuria). NPHP-RCs have a wide clinical spectrum with varying patterns of onset and must be considered among the differential diagnosis of any cause of childhood renal failure of unknown origin. Hence, these disorders can clinically overlap with renal hypodysplasia, medullary cystic kidney disease complex, early-onset autosomal dominant polycystic kidney disease (PKD), autosomal recessive PKD, or acquired tubulointerstitial injury, posing a major diagnostic challenge to clinicians. Obtaining a precise diagnosis would help physicians in predicting outcome and potential extrarenal complications and inform parental counseling.

NPHP-RCs are largely inherited as autosomal recessive diseases. After Hildebrandt et al.\(^3\) identified the first gene, NPHP1, in 1997, they went on to identify many of the 21 other genes now implicated in the development of NPHP-RCs.\(^4–8\) Amazingly, these known genes still only account for less than 50% of NPHP-RC cases, indicating remarkable genetic heterogeneity.\(^2\) To make matters more complicated, the phenotypes may be modified by heterozygous mutations in other genes in the pathway (triallelic inheritance model). For example, Katsanis et al.\(^9\) screened a cohort of 163 Bardet–Biedl syndrome (BBS) families for mutations in both BBS2 and BBS6 and report the presence of three mutant alleles in affected individuals in four pedigrees. Hoefele et al.\(^8\) also described oligogenic inheritance in NPHP, detecting two mutations in one of the NPHP genes combined with a third mutation in another NPHP gene in six different families. These data suggest, in some instances, that a potential third mutation might modify the age of onset and/or severity of the clinical phenotype.

To date, studies have implicated dysfunction of the microtubule-based primary cilium in the pathogenesis of NPHP-RC pathogenesis.\(^2\) This highly conserved sensory organelle, present in virtually every mammalian cell, is involved in the chemo-, photo-, and mechanosensations that allow a cell or an organism to interact with and respond efficiently to its environment. However, despite the convergence of genetic data in NPHP-RCs, the exact mechanisms linking dysfunction of components of the cilium and centrosomes to kidney injury remain unclear. The recent identification of pathogenic mutations in XPNPEP3, ZNF423, and CEP164 genes suggests that NPHP genes may not be exclusively ciliary and may also involve mitochondrial proteins or components of the DNA damage response pathway.\(^4,7\) These more recent findings broaden the spectrum of pathways yielding NPHP-like phenotypes and open new avenues of research for understanding kidney injury.

In this issue of JASN, Hurd et al.\(^10\) report identification of a mutation in the renal Mg\(^{2+}\) transporter gene, SLC4A1A, in a consanguineous family manifesting a renal disorder that phenocopies NPHP-RC clinically, ultrasonographically, and histologically. The presence of bronchiectasis in two affected siblings with tubulointerstitial kidney disease as well as a cousin without nephropathy strongly suggested the diagnosis of a type of ciliopathy affecting both respiratory and renal epithelia. This finding has precedent in autosomal dominant PKD, where systematic studies by computed tomography reveal increased prevalence of bronchiectasis.\(^11\) Using a well validated genetic approach, Hurd et al.\(^10\) performed homozygosity mapping and whole-exome sequencing, detecting a homozygous splice acceptor site mutation (c.698G>T) in SLC4A1A, which results in the skipping of exon 6 and an in-frame deletion of a transmembrane helix. The SLC4A1A mutation was not detected in relatives with bronchiectasis without nephropathy, suggesting that another locus is responsible for the pulmonary phenotype in this family. Hurd et al.\(^10\) also performed detailed follow-up studies, showing that protein expression was almost completely lost in the kidneys from affected individuals. Although the mutation did not affect protein trafficking, the mutant protein had significantly impaired Mg\(^{2+}\) transport in cell lines, indicating a null or severely hypomorphic allele.

In the last decade, the zebrafish has emerged as an excellent vertebrate model system to study cilia biology and the earliest cellular defects occurring during renal cyst formation. The pronephros of zebrafish is a simple organ consisting of two nephrons, and genes that cause cystic kidney diseases in humans cause pronephric dilation in zebrafish. Thus, in another functional study, Hurd et al.\(^10\) performed morpholino-mediated knockdown of SLC4A1A in zebrafish. This knockdown resulted in ventral body curvature, hydrocephalus, and tubular dilation, the same phenotypes produced by knockdown of other NPHP-RC genes.

Human SLC4A1A maps to chromosome 1q31–32 and is known to be directly and specifically involved in Mg\(^{2+}\) transport.\(^12,13\) It is the first member of the Solute Carrier family 41, which has a recognized homology to the prokaryotic MgtE Mg\(^{2+}\) transporter family. It is ubiquitously expressed, with the highest expression levels in the human heart, testis, and kidney.\(^12\) Kolisek et al.\(^14\) recently found that the expression of human SLC4A1A in HEK293 cells leads to Mg\(^{2+}\) efflux, which was strictly dependent on extracellular Na\(^+\) and blocked by nonspecific Na\(^+\)/Mg\(^{2+}\) exchanger inhibitors, such as imipramine and quinidine. On the basis of these findings, Kolisek et al.\(^14\) proposed that SLC4A1A functions as an Na\(^+\)/Mg\(^{2+}\) exchanger. Hurd et al.\(^10\) performed immunohistochemical analysis of SLC4A1A on normal human kidney sections and revealed expression primarily in the distal convoluted tubules, thick ascending limbs of Henle, and the tubules adjacent to
the macula densa, where location is entirely consistent with the region of cystogenesis observed in NPHP-RCs. Moreover, Hurd et al.10 showed that SLC4A1 colocalizes with Claudin-16, a major paracellular protein mediating Mg2+ and Ca2+ transport.

In patients with SLC4A1 mutations, the diagnosis of NPHP was proposed based on the renal biopsy findings showing periglomerular fibrosis, tubular ectasia, tubular basement membrane disruption, and tubulointerstitial infiltrations, but the patients did not exhibit Mg2+ and Ca2+ defects. It is still unclear why dysfunction of intracellular Mg2+ homeostasis leads to an NPNHP-RC phenotype. Moreover, there is precedent for mutations in Mg2+ transporters or channels manifesting as tubulointerstitial injury with and without extrarenal defects in mammals. For example, recessive mutations in Claudin-16 (CLDN16) and CLDN19 cause familial hypercalciuric hypomagnesemia with nephrocalcinosis.15 Mutations in CLDN19 additionally cause macular coloboma or pigmentary retinitis, similar to some NPNH-RC cases.15 However, in Japanese black cattle, recessive mutations of the CLDN16 gene only cause renal tubular dysplasia without hypomagnesemia and nephrocalcinosis,16 suggesting that phenotypic manifestations of Mg2+ transport defect may be species-dependent.

Based on the study by Hurd et al.,10 SLC4A1 mutations represent an extremely rare cause of NPHP-RC, because screening of 1000 NPHP-RC patients did not reveal additional patients with SLC4A1 defects. These studies, thus, set the paradigm for identifying mutations underlying heterogeneous disorders, such as NPHP-RC, where a standard clinical workup cannot provide a definitive diagnosis and virtually every individual may have a unique mutation in a different gene. Moreover, the study by Hurd et al.10 is extremely valuable, because it offers a new link between Mg2+ homeostasis and tubular defects manifesting as NPHP-like disease. SLC4A1 is the first transporter to be identified as causing an NPHP-RC phenotype, and the study by Hurd et al.10, thus, extends the spectrum of both genes and phenotypes associated with NPHP. Now, we need to determine if there is a biologic interaction between Mg2+ homeostasis and ciliary function or if we are dealing with phenotypic overlap because of limitations of clinical diagnosis. As ever larger cohorts of patients with nephropathy are sequenced, we will likely encounter additional families with SLC4A1 mutations.

The present findings will inform future sequencing studies, indicating that variants in genes involved in divalent ion homeostasis should be scrutinized in nephropathy phenotypes. Finally, this study illustrates the future of clinical medicine, where genetic tools will enable physicians to obtain a precise molecular diagnosis and individualize care one family or one person at a time.

DISCLOSURES

None.
The Mutation, a Key Determinant of Phenotype in ADPKD

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Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common monogenic diseases (frequency 1:500–1000) and a classic dominant disease with a penetrance of practically 100%, if defined as the development of multiple bilateral cysts in a family member that inherits a defined mutation. However, the severity of disease, even in mutation characterized cases, varies greatly. At one extreme are those that live into old age and develop just a few cysts with no renal insufficiency, whereas at the other end of the spectrum are neonicotides that die shortly after birth with hugely enlarged cystic kidneys, reflecting the phenotype commonly seen in autosomal recessive polycystic kidney disease (ARPKD).

The typical phenotype is of progressive cyst development and expansion leading to ESRD in late middle age. However, even in this textbook view of ADPKD, the age at first presentation of symptoms and their manifestations as well as the age at ESRD varies greatly. This is not only true for renal disease; the occurrence and severity of cystic and noncystic extrarenal manifestations that characterize ADPKD are also highly variable. Identification of the disease-causing genes, PKD1 (16p13.3) and PKD2 (4p21), in the 1990s was the first step toward understanding the etiology. However, the location of PKD1 in a complex duplicated region with six pseudogenes elsewhere on chromosome 16 has hindered the screening of large disease populations, at least until recently.

In populations identified in the setting of the renal clinic, PKD1 accounts for the majority of resolved cases (approximately 85%), with PKD2 responsible for the remainder of approximately 15%. However, even in comprehensive studies, approximately 9% of cases remained genetically unresolved. Soon after genetic heterogeneity was identified, divergent renal disease severity was noted between the two loci (average age at ESRD of approximately 54 years for PKD1 versus approximately 74 years for PKD2). As described in the ADPKD Mutation Database, mutation screening shows a high level of allelic heterogeneity with 929 PKD1 mutations accounting for 1266 pedigrees and 167 PKD2 mutations in 322 pedigrees; the most frequent mutation, PKD1: c.5014_5015delAG, accounting for just 2.2% of PKD1 families. In typical populations, mutations predicted to truncate the protein (frameshifting, nonsense, and splicing) account for approximately 70% of PKD1 and approximately 82% of PKD2 families, with nontruncating (missense and in-frame changes) accounts for the remainder.

Up until recently there was rather limited evidence of allelic effects in PKD1 or PKD2, with just a relatively weak influence of mutation position in PKD1 on the severity of renal disease and the occurrence of intracranial aneurysms. However, in the past couple of years, studies of families with unusual presentations of ADPKD have identified key roles for incompletely penetrant (hypomorphic) alleles. For instance, the PKD1 allele p.R2220W has been studied in detail and found in homozygosity to be associated with adult onset ADPKD and in heterozygosity to result in the development of just a few cysts. This variant was also inherited in trans with a truncating PKD1 mutation in an ADPKD family, resulting in early onset ADPKD. In a second family with early onset presentation in siblings and a negative family history, which was mistakenly diagnosed with ARPKD, p.R3277C was found in trans with a second PKD1 variant, p.R2220W, in the severe cases. Generation of a mouse knock-in model of p.R3277C proved its hypomorphic nature, resulting in slowly progressive disease as a homozygote (homozygous mice for a fully inactivating mutation.