New Ways of Finding New Genes for Old Diseases

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In this issue of *JASN*, Besse *et al.*[1] report the identification of loss of function variants in *ALG9* as a novel cause of renal and hepatic cysts, ranging from few to a polycystic-like phenotype, in a cohort of genetically uncharacterized patients with autosomal dominant polycystic kidney disease (ADPKD) and autosomal dominant polycystic liver disease (ADPLD) genes. *ALG9* encodes α-1,2-mannosyltransferase and was a strong candidate gene for these diseases, because other defects in the endoplasmic reticulum (ER) protein biogenesis pathway result in overlapping phenotypes.[2] Biallelic mutations in *ALG9* are associated with congenital disorder of glycosylation type 1L, one of a group of diseases also associated with polycystic kidney disease (PKD).[3] Its role in cystogenesis is confirmed using functional assays of polycystin-1 (PC1), the *PKD1* gene product, and protein maturation and uniquely, by using a "genotype"-first approach of identifying loss of function *ALG9* mutations in individuals with renal cysts from a large population-based cohort with linked exome and electronic health care record data. In addition to adding to our knowledge of the genetic architecture and molecular pathology of renal cystic disease, this study elegantly demonstrates how such a "genotype"-first approach can be used to unambiguously assign and refine a gene-disease association when the initial observation is made in a singleton or few individuals.

The majority of individuals with ADPKD harbor a mutation in either *PKD1* or *PKD2*.[4] These genes encode a large ion channel complex localized to the primary cilium of uncertain function. Studies on the basis of linkage analysis or direct sequencing in well characterized cohorts suggest that approximately 80% will have an identifiable pathogenic or likely pathogenic variant in *PKD1* and that approximately 15% will have an identifiable pathogenic or likely pathogenic variant in *PKD2*, with the remainder having no identifiable mutation in either gene.[5] In parallel with advances in and access to clinical and research molecular testing, renal imaging has defined the natural history of ADPKD through measurement of renal size, renal volume, and cyst burden. Mutations in *PKD1* are associated with larger absolute kidney size compared with mutations in *PKD2* and predict a faster rate of decline in renal function.[6] This is reflected in ESKD occurring some 20 years earlier in individuals with a *PKD1* mutation compared with *PKD2*.[5] Loss of function mutations in *PKD1* also predict a more rapid decline in renal function compared with missense mutations.[5] However, there is considerable clinical variability in disease progression between and within families that is not accounted for simply by these genic and allelic effects.

Mutations in *PKD1* and *PKD2* are also associated with polycystic liver disease. In contrast, ADPLD is associated with minimal renal cystic disease and greater genetic heterogeneity. Mutations in *PRKCSH*, *SEC63*, *LRP5*, *ALG8*, *SEC61B*, and *PKHD1* have been identified in ADPLD, revealing that disruption of the ER protein biogenesis pathway is a principle mechanism in hepatic cystogenesis.[2]

Imaging in ADPKD has also defined a group of patients with atypical renal cystic disease in whom the mutation detection rate is less than that in those with typical disease.[7] In atypical disease (unilateral, asymmetric, segmental, or lopsided cystic disease or unilateral or bilateral reduction in renal size), the mutation detection rate may be up to 45%, suggesting that other genes are responsible for multiple renal cysts.[7] This is well documented for rare conditions, such as von Hippel Lindau disease, tuberous sclerosis, *COL4A1*-related disease, orofacial digital syndrome type 1, *HNF1B*-associated renal disease, and autosomal dominant tubulointerstitial kidney disease, where other clinical features are typically present to suggest the diagnosis. However, clinical presentations, including multiple renal cysts in normal-sized kidneys with or without liver cysts, especially at the extremes of age with no clear family history can present a diagnostic dilemma with implications for monitoring, treatment, family screening, and reproductive counseling.

More recently, additional genes have been identified in individuals with renal and/or hepatic cysts. These include *GANAB* and *DNAJB11*.[8,9] Both studies used genetically unresolved individuals with ADPKD and ADPLD and either whole-exome or targeted next generation sequencing to identify pathogenic mutations in multiple families, but these still comprised a small proportion of those in the discovery cohorts. *GANAB* encodes glucosidase II subunit-α, a functional partner of glucosidase II subunit-β (the *PRKCSH* gene product), and was shown to regulate PC1 maturation. *DNAJB11* encodes an ER protein involved in protein folding and assembly and was also shown to regulate PC1 maturation *in vitro*.

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This further confirms that disruption of the ER protein biogenesis pathway is a common mechanism in renal as well as hepatic cystogenesis.

Besse *et al.*[1] identified two patients with loss of function mutations in *ALG9* from a discovery cohort of 122 patients with genetically unresolved ADPKD or ADPLD using *in vitro* assays to confirm disruption of PC1 processing. To obtain further support for *ALG9* as a cause of ADPKD or ADPLD, individuals with a predicted loss of function *ALG9* mutation, no other known ADPKD or ADPLD gene mutation, preserved native kidney function, and accessible abdominal imaging were identified from the MyCode Community Health Initiative.[10] This is a large unselected population–based cohort of >190,000 individuals with combined longitudinal electronic health record and whole-exome sequencing data. From this study, an additional 21 patients were identified from >90,000 exomes, 14 of which had at least one imaging study. Compared with controls, the majority of patients had four or more renal cysts over the age of 50 years. There was extreme clinical variability as has been seen previously in ADPLD and in the rare causes of ADPKD with a low risk of developing ESKD. Therefore, *ALG9* can be added to *GANAB* and *DNAJB11* as a cause of renal and hepatic cystic disease, often with atypical and mild features. Although accounting for a very small proportion of individuals suspected of having ADPKD or ADPLD, testing these genes in parallel with *PKD1* and *PKD2* will provide an opportunity for those who present with atypical disease to be given more precise information about clinical and family risk.

The use of large integrated datasets of genomic and health information has proved extremely valuable in supporting genome-wide and phenome-wide association studies, with the resultant benefit in understanding disease mechanisms, refining disease classification, and identifying drug targets to improve health outcomes. This has been particularly important for identifying genetic determinants of kidney function and CKD.[11] In addition to MyCode, many other similar studies have been established, including electronic medical records and genomics, the Million Veteran Program, the National Institutes of Health All of Us Research Program, and the United Kingdom Biobank. The ability to test rare and private mutations in addition to common variation for association with the numerous and specific phenotypes, including those derived from imaging studies, contained within an individual's electronic health record is now possible with the rapid reduction in the cost of whole-exome and whole-genome sequencing, and it has the promise of fundamentally altering the way that medicine is delivered.

As this study reveals, these integrated datasets can also be simply and elegantly used for single-gene rare variant–single-phenotype association studies to rapidly accelerate gene discovery as well as genome-wide and phenome-wide association studies. However, there are some limitations to the former. There may be a lack of family history information, and patients may not be recallable for additional clinical and research studies. Therefore, we currently do not know the penetrance and variability of heterozygous *ALG9* mutations in additional family members or why the patients identified initially underwent abdominal imaging, for example.

However, the identification of ALG9 as a cause of renal and hepatic cysts adds to our knowledge of the mechanisms underlying cystogenesis and provides opportunities to extend genetic testing to individuals with atypical disease in whom current testing is negative. It also identifies *ALG9* as a potential modifier of disease expression due to *PKD1* or *PKD2* mutations. Further studies are required to address this directly.

The ability to confirm gene disease associations and refine genotype-phenotype correlations using resources, such as MyCode, will significantly enhance the delivery of precision medicine in the general nephrology clinic.

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See related article, "*ALG9* Mutation Carriers Develop Kidney and Liver Cysts," on pages 2091–2102.

Another Tool in the Fight Against Phosphate Toxicity: Where Will It Fit and What Does It Tell Us about Phosphate Homeostasis?

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Efforts to control phosphate retention/hyperphosphatemia in CKD have focused on limiting gastrointestinal phosphate absorption through dietary and pharmacologic means. Suffice it to say that these measures have proven to be woefully inadequate for individuals with advanced kidney failure. Thomas *et al.*[1] have presented evidence that blocking renal phosphate reabsorption through an orally administered agent (PF-06869206; designated Npt2a-I) that selectively inhibits the type IIa sodium phosphate cotransporter (Npt2a) effectively lowers serum phosphate in a mouse model in the setting of normal kidney function and CKD (5/6 nephrectomy model). These findings raise hopes that we now have a more effective and palatable therapy for this universal complication of CKD, a complication implicated in the development of accelerated cardiovascular disease and disabling bone disease in this patient population. This agent may also be useful in expanding our understanding of phosphate homeostasis in particular and mineral metabolism generally.

Reabsorption of filtered phosphate is accomplished through the coordinated actions of Npt2a, Npt2c, and Pit2 (a type III sodium phosphate cotransporter) located on the apical membrane of renal proximal tubule cells, which transport phosphate from glomerular ultrafiltrate into the cells, and an as yet uncharacterized protein or proteins located on the basolateral membrane, which transport reabsorbed phosphate into the blood compartment. In mouse, Npt2a accounts for 70%–80% of the reabsorption of filtered phosphate. Predictably, Thomas *et al.*[1] show that Npt2a-I dramatically increased urine phosphate excretion and decreased serum phosphate in the C57BL/6J mouse model after 3 hours accompanied by an equally predictable increase in the apical membrane expression of Npt2a. The increase in apical membrane expression of Npt2a was likely due to both stimulation of the forward trafficking of Npt2a molecules already present in the cell and inhibition of endocytosis of Npt2a already present in the apical membrane, because total cellular Npt2a expression was not different at 3 hours than at baseline. The parathyroid hormone (PTH) level after Npt2a-I administration rapidly decreased, correlating with the decline in serum phosphate, and this was followed by a "rebound" overshoot in both serum phosphate and PTH levels at the 24-hour mark when Npt2a-I would be absent. At 24 hours, the Npt2a expression was actually lower in the inhibitor-treated kidneys than the vehicle-treated kidneys, likely a result of the rebound increase in serum PTH levels. The PTH response to serum phosphate level in the absence of any change in serum calcium reinforces the growing recognition that, even in the absence of CKD, serum phosphate is a potent and independent regulator of PTH. Likewise, the rapidity of the changes in apical membrane expression of Npt2a in response to serum phosphate and PTH reinforces the role of this transporter as an immediate responder. In contrast, there was no change in fibroblast growth factor 23 (FGF23) at the 3-hour time point but an increase at the 24-hour timepoint after Npt2a-I administration at the time when both phosphate and PTH were higher than baseline. This finding is consistent with human studies showing no change in FGF23 level 3 hours after a dietary phosphate load[2] and suggests a more chronic role for FGF23 in regulation of phosphate homeostasis.

There was an additive effect of PTH and Npt2a-I on urine phosphate excretion and serum phosphate. The authors suggest that the decrease in Npt2a transporter surface expression with PTH facilitated the ability of Npt2a-I to inhibit the remaining transporters. An alternative explanation is that there is a pool of Npt2a not regulated by PTH but inhibitable by the pharmacologic agent. This conclusion is supported by the observation that the proximal tubule handling of phosphate with advancing CKD does not correlate completely with GFR and rising PTH or FGF23 levels. Studies in a model of endogenous hyperparathyroidism demonstrate persistent presence of Npt2a even with significantly elevated PTH levels.[3]

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