Reconsidering Genetic Testing for Neonatal Polycystic Kidney Disease

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Received 16 March 2020; revised 30 April 2020; accepted 4 May 2020; published online 23 May 2020

Kidney Int Rep (2020) **5,** 1316–1319; https://doi.org/10.1016/j.ekir.2020.05.008

Polycystic kidney disease (PKD) is a condition typified by numerous renal cysts and enlarged kidneys. Types of PKD are generally distinguished by the genetic mode of inheritance, either autosomal dominant (ADPKD) or autosomal recessive (ARPKD). In addition, ADPKD and ARPKD are characterized by differences in clinical and pathological presentations. Whereas ADPKD presents with bilateral renal enlargement and macrocysts, extrarenal cysts (hepatic), intracranial aneurysms, mitral valve prolapse, and a biphasic pattern of progression to end-stage renal disease (ESRD) in later decades of life, ARPKD is characterized by early and rapid enlargement of the kidneys, childhood progression to ESRD, and frequent liver involvement leading to congenital hepatic fibrosis.

Because of these differences, family history and clinical presentation are the primary factors used to direct the genetic testing ordered to establish a molecular diagnosis. When a family history includes suspected ADPKD affecting individuals in 1 or more generations, sequencing and/or deletion/duplication analysis of *PKD1* and *PKD2* is considered. When a family history includes a child with either prenatal or early pediatric onset of PKD, sequencing and/or deletion/duplication analysis of *PKHD1* is pursued. Although pathogenic variants in *PKD1* and *PKD2* account for nearly all PKD cases that appear to be AD, pathogenic variants detected in *PKHD1* account for only ~75% of PKD cases that appear to be AR.[1] Historically, it has been unclear whether these ostensibly AR cases of PKD without detected *PKHD1* pathogenic variants represent atypical early manifestations of ADPKD or whether there are yet-unknown genes responsible for an early-onset AR presentation of PKD. Although this landscape is rapidly changing with increasing use of massively parallel sequencing in the form of either gene panels or exome sequencing, particularly in a research setting, clinical practice has been slow to change—although many providers now offer gene panels for evaluation, particularly in the neonatal setting.

Research to further understand this phenomenon has yielded important information about the mechanism of disease in PKD. Historically, a "2-hit" hypothesis was supported based on the combination of germline and somatic mutations seen in cysts and the severe, lethal phenotype of homozygous knockout *Pkd1* mice.[2–4] However, further research in mice with reduced *Pkd1* expression has established a dose-dependent model of disease, suggesting that modifier alleles may contribute to the severity and onset of cyst development.[5,6] Multiple studies of families with ADPKD have implicated variants in *PKD1*, *PKD2*, *PKHD1*, or *HNF1B* inherited in *trans* with known pathogenic *PKD1* variants as a cause of earlier-onset, more severe cystic kidney disease.[7–9,S1]

In addition to identifying hypomorphic alleles that can account for some of the variable expressivity seen in ADPKD families, it has been proposed that the inheritance of 2 such hypomorphic alleles in *trans* may also cause disease in humans.[S2] Here we report a family with 2 siblings diagnosed with PKD *in utero*, both found to have biallelic inherited mutations in *PKD1*.

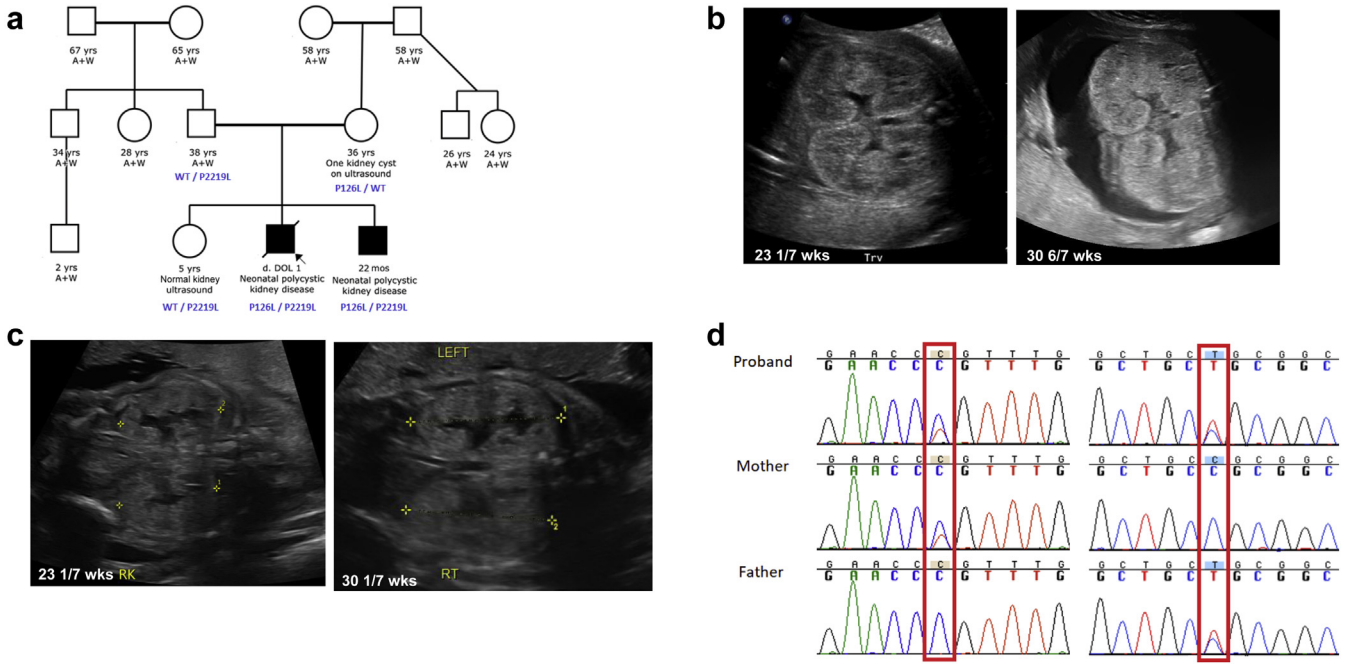

Figure 1. Biallelic mutations in *PKD1* identified in a family with 2 children with neonatal polycystic kidney disease (PKD). (a) A 3-generation family history was negative for cystic kidney disease, including extrarenal manifestations such as intracranial aneurysm, early-onset hypertension, liver disease, and cysts in other organs. Genotype is indicated where available (WT = wild type). Unaffected individuals who have had renal ultrasound are indicated. (b) Fetal renal ultrasounds for the proband. Ultrasound at 23 1/7 weeks showed that fetal kidneys were markedly enlarged and echogenic, raising concern for neonatal PKD (right kidney 6.6 cm, left kidney 6.4 cm). Ultrasound at 30 6/7 weeks showed that fetal kidneys were markedly enlarged and more echogenic. Fetal hydrops was present as well as anhydramnios, prompting delivery. (c) Fetal renal ultrasounds for second affected child. Ultrasound at 23 1/7 weeks gestation showed that fetal kidneys were mildly enlarged and echogenic (right kidney 3.4 cm, left kidney 3.3 cm). Ultrasound at 30 1/7 weeks gestation showed that the fetal kidneys remained enlarged and echogenic (right kidney 5.5 cm, left kidney 5.7 cm). Amniotic fluid was normal, and there were no signs of fetal hydrops. (d) Whole-exome sequencing identified a maternal missense mutation (c.377C>T, p.Pro126Leu) and a paternal missense mutation (c.6656C>T, p.Pro2219Leu) in *PKD1*. Sanger sequencing confirmed that both affected children were compound heterozygous for the mutations.

CASE PRESENTATION

The proband was a male fetus found at 20 3/7 weeks gestational age (GA) to have bilateral enlarged, cystic kidneys during an otherwise unremarkable pregnancy with no family history of renal cysts or other features of PKD (Figure 1a). The infant was delivered at 30 6/7 weeks GA due to the development of fetal hydrops in the setting of oligohydramnios and unfortunately died in the delivery room (Figure 1b). *Post mortem* sequencing and deletion/duplication analysis of *PKHD1* was negative. The parents of the proband had a subsequent pregnancy with a male fetus affected with echogenic kidneys at 18 weeks GA, and enlarged, cystic kidneys at 23 1/7 weeks GA. The infant was delivered at 37 weeks GA and was postnatally confirmed to have enlarged echogenic kidneys with innumerable cysts (Figure 1c). He is currently alive at 22 months, and his kidneys remain enlarged with microcysts, but they have not grown rapidly.

RESULTS

Exome sequencing was performed on DNA samples from the proband and parents (see Supplementary Methods). Biallelic variants in *PKD1* were identified in the proband, and Sanger sequencing using standard techniques identified both variants in the other affected male child (RefSeq: NM_001009944): a maternally inherited missense variant (c.377C>T, p.Pro126Leu) and a paternally inherited missense variant (c.6656C>T, p.Pro2219Leu) (Figure 1d). Both variants are absent from ExAC and gnomAD databases, predicted to be deleterious by *in silico* models (maternal variant: CADD score 25, paternal variant: CADD score 29, both variants assessed as "probably damaging" by Polyphen, "damaging" by SIFT, "disease causing" by MutationTaster), and the amino acid residues are highly conserved in vertebrates. Neither variant has been previously reported in the literature. Interestingly, *PKD1* occurs in a segmentally duplicated portion of the genome. Thus, detecting variants using exome sequencing is complicated by the presence of multiple homologous pseudogenes, which limits the sensitivity of this modality, although the specificity remains high[S3]; our variants appeared to be of high quality and were Sanger confirmed in the similarly affected sibling. Although both variants are formally classified as variants of uncertain significance, we propose them as

disease causing for our family, as we are proposing a novel mechanism and mode of inheritance (as opposed to the typical single loss-of-function variants seen in ADPKD).

We further evaluated the exome data for rare, predicted damaging variants in known ARPKD genes such as *PKD2, PKHD1,* and *HNF1B* and did not find any candidate variants.

DISCUSSION

We present a case of early-onset PKD attributed to biallelic variants in *PKD1*. After the proband's variants were identified, we believed this combination of variants to be lethal; however, the live-born second child with the same variants exhibits an attenuated phenotype with an unclear prognosis. At this time, the second affected child is thriving with normal renal function and normal blood pressure despite enlarged cystic kidneys that appear consistent with the phenotype of ARPKD.

Although ARPKD is classically associated with biallelic variants in *PKHD1*, hypomorphic variants in multiple genes in *trans* with pathogenic variants in *PKD1*/*PKD2* have been found to cause a more severe cystic kidney disease presentation in ADPKD families.[7,8] One such hypomorphic variant in *PKD1*, p.R3277C, has been found recurrently to account for early onset of disease in ADPKD individuals with cyst development in the pediatric period.[7,S2] Although this recurrent hypomorphic variant has not been reported in a human in a homozygous state, it has been proposed that the inheritance of 2 such hypomorphic alleles in *trans* may also cause disease in humans. In a couple with 2 children diagnosed with PKD *in utero* with no *PKHD1* variants identified, analysis of *PKD1* and *PKD2* revealed that each healthy parent carried a known or suspected hypomorphic *PKD1* variant, and each affected child had inherited these mutations in *trans*.[S2] Our case is the second family documented with PKD *in utero* caused by biallelic *PKD1* hypomorphic variants, although phenotypic variability is seen within this family, as described above.

Given the current evidence, we propose that compound heterozygous or homozygous hypomorphic variants in *PKD1* may represent a proportion of early-onset PKD cases that are not accounted for by either biallelic variants in *PKHD1* or null mutations in *PKD1*/*PKD2* modified by a hypomorphic allele in another PKD-related gene. Because of the demonstrated variability in genes that can contribute to early-onset PKD, broadening the genetic testing approach taken toward a molecular diagnosis could benefit families affected by early-onset PKD. In a recent study of 36 individuals with a clinical diagnosis of ARPKD, 8 (22%) did not carry *PKHD1* variants and instead carried variants in other kidney disease–associated genes.[S4] Although recommendations have been made against single-gene analysis of *PKHD1* as a first-line diagnostic approach in light of the contribution of additional genes to phenocopy disorders,[S5] this was a standard practice for many years and continues to be practiced in some clinical settings.

In order to move toward more comprehensive genetic testing for early-onset PKD, the historical model delineating early- and later-onset PKD by gene(s) needs to be reconsidered. The current model of defining ADPKD by 1 pathogenic variant in *PKD1* or *PKD2* and ARPKD by 2 pathogenic variants in *PKHD1* no longer encompasses what is known about the dose-dependent mechanism responsible for disease development and the multitude of genes involved. A model of describing early-onset PKD as 2 variants in PKD-related genes (*PKD1, PKD2, PKHD1, HNF1B)* and later-onset PKD as 1 variant in a PKD-related gene would more accurately represent our knowledge of the underlying causes of PKD.

DISCLOSURE

TEM is an employee of Quest Diagnostics. All the other authors declared no competing interests.

ACKNOWLEDGMENTS

MHW received support from T32 GM007748. Sequencing and analysis were provided by the Broad Institute of MIT and Harvard Center for Mendelian Genomics (Broad CMG) and was funded by the National Human Genome Research Institute, the National Eye Institute, and the National Heart, Lung, and Blood Institute grant UM1HG008900 and in part by National Human Genome Research Institute grant R01 HG009141. The authors wish to thank the family for their participation in the research.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

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Treatment of Nephrogenic Diabetes Insipidus Patients With cGMP-Stimulating Drugs Does Not Mitigate Polyuria or Increase Urinary Concentrating Ability

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Received 31 March 2020; revised 28 April 2020; accepted 4 May 2020; published online 23 May 2020

Kidney Int Rep (2020) **5,** 1319–1325; https://doi.org/10.1016/j.ekir.2020.05.015

Nephrogenic diabetes insipidus (NDI) is usually caused by a lack of responsiveness of the collecting ducts to the arginine vasopressin (AVP). It is characterized by excessive urinary output caused by impaired urinary concentration ability. In severe cases, adult patients excrete more than 10 L per day, making them at constant risk for severe dehydration. Water homeostasis critically depends on replenishing water loss, and this compensatory fluid intake, combined with the continuous large diuresis, significantly impairs activities of daily living.[1] No causal treatment for the disease exists, and current approaches for symptomatic treatment aim to ameliorate symptoms with adequate water supply, nonspecific pharmacological therapies and dietary restrictions, as reviewed elsewhere.[2] The diagnosis of congenital NDI is often reached in infancy; clinical findings include polyuria, polydipsia, hypernatremia, plasma hyperosmolality, and hypoosmolar urine.[3] In NDI, there is a normal increase in vasopressin/copeptin plasma concentrations during water deprivation but no sensitivity to exogenous or endogenous AVP.[3] Nephrogenic diabetes insipidus results from impaired vasopressin receptor signaling at target cells or defect aquaporins, and may be acquired (secondary NDI) or congenital (primary NDI).[4] Primary NDI can be caused by mutations in genes encoding aquaporin 2 (AQP2) (autosomal recessive/dominant, 10% of primary NDI cases) or the arginine vasopressin 2 (AVP-V2) receptor (X-linked, 90% of primary NDI cases).[1] Activation of the AVP-V2 receptor promotes water reabsorption through increases in intracellular cyclic adenosine monophosphate (cAMP) level and protein kinase A activity (Figure 1a). No treatment has yet efficiently bypassed an inactive AVP-V2 receptor to raise cAMP in principal cells. *In vitro* studies found a cAMP-independent effect of nitric oxide, L-arginine, and atrial natriuretic peptide to increase the translocation of AQP2 from the cytoplasm to the apical membrane through an increase in cyclic guanosine monophosphate (cGMP).[5] Similarly,