Review

Therapies for Childhood Polycystic Kidney Disease

Ameya Patil [1], William E Sweeney, Jr [1], Ellis D. Avner [1, 2, *]

1. Medical College of Wisconsin, Department of Pediatrics, Children's Research Institute, Children's Hospital Health System of Wisconsin, Milwaukee, Wisconsin 53226, USA; E-Mails: appatil@mcw.edu, bsweeney@mcw.edu, eavner33@gmail.com
2. Medical College of Wisconsin, Department of Physiology, Children's Research Institute, Children's Hospital Health System of Wisconsin, Milwaukee, Wisconsin 53226, USA

* **Correspondence:** Ellis D. Avner; E-Mail: eavner33@gmail.com

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Abstract

Renal cysts are present in a wide variety of hereditary renal diseases in children. The term polycystic kidney disease (PKD) refers to two specific hereditary diseases, distinguished by the usual age of onset and genetic cause: autosomal recessive polycystic kidney disease/congenital hepatic fibrosis (ARPKD/CHF, MIM *606702) and autosomal dominant polycystic disease (ADPKD-OMIM *601313 and OMIM *173910). ARPKD/CHF is characterized by cystic dilations of the renal collecting ducts and developmental defects of biliary ductal plate remodeling, resulting in varying degrees of congenital hepatic fibrosis. ARPKD/CHF is commonly diagnosed *in utero* or at birth but can remain silent well into adolescence and rarely into adulthood. ADPKD, the most common inherited renal disease is characterized by slow, progressive enlargement of fluid-filled cysts leading to renal failure by the fifth to sixth decade of life in addition to various extrarenal manifestations. ADPKD can manifest in utero, infants, and children and can be a significant cause of morbidity and mortality in this age group. Our understanding of the genetic basis of ARPKD and ADPKD, including mechanisms of transmission and genes involved continues to evolve. Despite

remarkable advances in understanding the basic molecular and cellular pathophysiology, the development of targeted therapies has proven difficult.

The complex structure of the genes and proteins, the tremendous allelic heterogeneity, complex inheritance patterns, and functional interactions of both the genes and proteins products of ARPKD and ADPKD complicate diagnostic and prognostic assessments. None the less, multiple therapeutic interventions are being tested in clinical trials, two clinical trials aimed at pediatric PKD patients are underway and one therapy, tolvaptan, has been approved for adult use in Japan, Europe, Australia, South Korea and recently in the USA. This review will discuss: 1) the clinical manifestations and genetics of childhood PKD; 2) how our understanding of the molecular and cellular pathophysiology of PKD is guiding current therapies; and 3) how recent discoveries may shape future therapies.

Keywords

ARPKD; ADPKD; proliferation; therapy; tyrosine kinase inhibitor; tolvaptan; combination therapy; multi-kinase inhibitors; gene dosage

1. ARPKD/CHF

1.1 Clinical Manifestations

ARPKD/CHF is a congenital dual organ fibrocystic disease and is a significant cause of renal and liver-related morbidity and mortality in children. ARPKD/CHF previously referred to as infantile PKD, is a rare disease commonly diagnosed *in utero* or at birth, and occurs at a frequency of 1:20,000 live births [1]. ARPKD/CHF is characterized by non-obstructive fusiform dilations of the renal collecting ducts and varying degrees of biliary ectasia due to developmental biliary ductal plate malformations leading to congenital hepatic fibrosis [2-5]. ARPKD is highly variable with a wide clinical spectrum of disease that ranges from the severe classical presentation *in utero*, or at birth, of massively enlarged bilaterally cystic kidneys to the presentation in older children, teenagers, or young adults with manifestations of hepatic disease and negligible renal disease [4, 6]. The variability of organ involvement in ARPKD is not well understood, but it is widely acknowledged [7-10].

The renal disease in ARPKD is characterized by nephromegaly, hypertension, and varying degrees of renal insufficiency. The most severely affected children exhibit a characteristic "Potter's" phenotype with enormously enlarged echogenic kidneys, up to 10x the normal size, and pulmonary hypoplasia due to oligohydramnios and a rigid contraction of the diaphragm [10]. Pulmonary hypoplasia, as the result of oligohydramnios, is a serious complication that can lead to respiratory failure that may ultimately be incompatible with survival [10-12]. The perinatal mortality due to pulmonary hypoplasia is estimated to be as high as 30% despite advances in neonatal care [2, 13]. Patients that survive the neonatal period have a one-year survival rate of 85% and a ten-year survival rate of 82% [10, 12, 14]. The majority of patients (70-80%) manifest some degree of impaired renal function just after birth, but function stabilizes as renal development continues and the kidney may get smaller [10, 15, 16]. Most patients have a urinary concentrating

defect with consequent polyuria and polydipsia which can lead to clinically significant electrolyte abnormalities [10, 15, 17-19].

Neonatal survivors and patients diagnosed as older children or adults typically present with signs and symptoms related to CHF [4]. Histological evidence of CHF is invariably present at birth, but the clinical complications of CHF can develop at any time [3, 4, 20]. Portal hypertension (PH), the predominant manifestation of CHF is potentially lethal, and nearly 70% of children with ARPKD will develop PH [4, 6]. PH may lead to hypersplenism with low platelet counts, and varices within the esophagus, stomach, and rectum. Rupture of esophageal or gastric varies can lead to a life-threatening hemorrhage [4, 10].

A subset of affected individuals will develop recurrent or persistent bacterial ascending cholangitis due to dilated bile ducts and stagnant bile flow [4, 10, 21]. Patients may require portosystemic shunting or liver transplantation for complications of portal hypertension [10, 22].

The variability in age and clinical manifestations of the hepatic disorder is related to the relative degree of renal and biliary abnormalities [2, 23]. In neonatal survivors, estimates are that 40% will exhibit severe dual-organ disease, and the remaining 60% is divided equally into three groups: those with severe renal disease/mild hepatobiliary disease; mild renal/severe hepatobiliary kidney disease; mild renal/mild hepatobiliary disease: [4, 22]. Platelet counts are an accurate means of determining the severity of PH in patients with ARPKD [4, 20].

Hypertension is common in both infants and children, and it can be challenging to control [3, 24-26]. Nearly all children with ARPKD will develop hypertension including patients with normal renal function [9, 25, 27]. Combination ACEi/ angiotensin receptor blocker therapy is not recommended because of an increased risk of side effects without a clear-cut benefit in reducing hypertension [28]. The use of ACEi in older adolescents that may become pregnant is contraindicated due to concerns of teratogenicity [29].

Kidney transplantation offers definitive renal replacement therapy in children with ARPKD. Successful kidney transplantation prolongs survival and often accelerates growth and development in young uremic children. Patients suffering from both severe renal and severe hepatic disease may be candidates for combined liver and kidney transplant [22]. A decision tree that guides appropriate choices has been developed and published [22].

Given the multiorgan effects of ARPKD/CHF, a team of pediatric subspecialists including nephrologist, hepatologist, specialized nurses, dietitians, social workers is recommended for optimal comprehensive care for children with ARPKD [9, 10].

1.2 Genetics-ARPKD

The majority of ARPKD/CHF occurs due to mutations in a single gene, the polycystic kidney and hepatic disease 1 (*PKHD1*) gene [30]. As the name of the gene and the disease imply, ARPKD/CHF patients invariably have some degree of CHF along with cystic renal disease [2]. The variability of renal and hepatic disease previously noted does not appear to be related to the type or site of mutations in PKHD1 [5, 31].

ARPKD/CHF was originally thought to be a genetically homogeneous disease caused by mutations in a single gene until the recent discovery of a second locus, the *DZIP1L* gene (Daz interacting protein 1-like (OMIM *617570). Mutations in *DZIP1L* have been identified in seven children from four unrelated consanguineous families [32]. All patients had enlarged

hyperechogenic kidneys, arterial hypertension, and four progressed to end-stage renal disease (ESRD). In animal models, mutations in *DZIP1L* appears to compromise membrane translocation of the ADPKD proteins, polycystin-1 and polycystin-2 (see ADPKD Proteins below). The number of ARPKD/CHF cases caused by mutations in *DZIP1L* is a minute percentage of a rare disease, and nearly all cases are due to *PKHD1*.

PKHD1, the causative gene of ARPKD/CHF, is an exceptionally large gene of 86 exons spanning 470 kb of genomic DNA with 67 exons included in the longest open-reading frame [33]. Alternatively spliced transcripts including some that may be secreted have been identified; however, the function and effect of these isoforms are not known [33, 34]. Attempts to define genotype-phenotype correlations and predict disease outcomes have largely been unsuccessful. To date, only general correlations are possible at best due to extreme allelic heterogeneity [35, 36]. Nearly 750 mutations in *PKHD1* have been identified to date (http://www.humgen.rwth-aachen.de) without apparent grouping at any specific site [37-39]. The majority of families have unique ("private") mutations, and the majority of families are compound heterozygotes [8, 37, 40]. Missense, frameshift and truncating mutations have all been described [41].

Patients with two truncating mutations generally exhibit a very severe phenotype with a high rate of perinatal /neonatal mortality [8, 37, 42]. In contrast, patients harboring at least one missense mutation (amino acid substitutions), have a non-lethal presentation [43, 44]. In an animal model of *Pkhd1,* investigators found 22 novel renal transcripts and observed an assortment of alternative splicing mechanisms including exon skipping, use of alternate acceptor/donor splice sites, and the inclusion of novel exons [34]. These studies suggest that aberrant *PKHD1* splicing may represent an additional pathogenic mechanism in ARPKD.

Finally, several phenocopies exist that can lead to a misdiagnosis of ARPKD. A recent study of 36 unrelated patients originally diagnosed with ARPKD but without an identified mutation in *PKHD1* found that 22% harbored mutations in other genes including PKD1, HNF1b, NPHP1, TMEM67, and PKD1/TSC2) [45].

1.3 ARPKD Proteins

PKHD1 encodes a very large, complex protein of unknown function known as fibrocystin or polyductin (FPC) [33, 40]. The longest open reading frame is comprised of 66 exons resulting in a protein of 4074 amino acids [46]. FPC is predicted to have a single transmembrane spanning domain and multiple copies of an immunoglobulin-like plexin-transcription-factor domain. Alternatively, spliced transcripts including some that may be secreted have been identified; however, the function and significance of these isoforms are unknown [33, 34]. During normal renal development, FPC is strongly expressed in branching ureteric bud. Postnatally it is normally expressed in collecting ducts with reduced amounts expressed in proximal and distal nephron segments [47]. FPC is expressed in multimeric complexes on the apical and basolateral plasma membrane and in the primary cilium/basal bodies. FPC has been found in the same protein complex as the PKD2 protein PC2 [47, 48].

FPC undergoes regulated proteolytic processing similar to NOTCH and releases an intracellular C-terminal fragment which translocates to the nucleus [49].

There is also evidence that FPC undergoes nonsense-mediated alternative splicing. Nonsense-mediated mRNA decay (NMD) is a mRNA quality control mechanism meant to lessen the harmful

effects of truncating mutations. A shortened, truncated protein with some residual function is produced, which may yield a milder phenotype than generally expected [46, 50]. FPC is also normally expressed in the liver, pancreas, and lungs and its precise function in these organs is unknown as well.

Overall, the large size of *PKHD1* and its protein FPC, its complex pattern of splicing, allelic heterogeneity and lack of an accurate paradigm to identify changes as pathogenic, pose substantial challenges to DNA-based diagnostic testing. Despite these challenges, there is a robust demand for early, reliable prenatal diagnosis, for couples considering pre-implantation genetic diagnosis and possible in vitro fertilization. PDG-IVF has been successfully utilized in at-risk families [51]. Given the levels of complexities, genetic counselors should be necessary participants in any discussions and planning based on genetic sequence.

2. ADPKD

2.1 Clinical Manifestations

ADPKD is the most common potentially lethal monogenetic renal disease occurring at a frequency between 1/400 and 1/1000 [52, 53]. ADPKD affects over 600,000 individuals in the USA and greater than 12.4 million people worldwide [54]. ADPKD is generally a late-onset (adult), systemic disease characterized by persistent development and unrelenting growth of bilateral focal renal cysts that eventually compresses normal renal parenchyma. This results in fibrosis and end-stage renal disease (ESRD). Nearly 50% of affected individuals will reach ESRD by the sixth decade of life [52, 55].

ADPKD has extraordinarily high phenotypic variability. Despite the classic presentation as an adult disease, ADPKD can have clinical manifestations including structural kidney disease, in infants, children and young adults [3, 10, 56-58]. As many as 5% of all ADPKD patients present in childhood with a broad phenotypic spectrum, ranging from severe neonatal presentations mimicking ARPKD to renal cysts that remain clinically silent well into adulthood [58-61]. Additionally, as many as 17% of children and adolescents with ADPKD will exhibit unilateral cystic kidneys at initial presentation [10].

ADPKD is a systemic disease with extrarenal manifestations that can include: hepatic cysts; splenic cysts; pancreatic cysts; cardiac abnormalities and vascular abnormalities including intracranial aneurysms. Many of these adult extrarenal manifestations are uncommon in children with ADPKD. The incidence of hepatic cysts increases with age. Early studies of ultrasound screening determined that 10 to 20 percent of ADPKD patients below the age of 30 had detectable hepatic cysts [62, 63]. However, in a study of 230 ADPKD patients between 15 and 46 years old, the use of MRI identified hepatic cysts in 83 percent of patients with ADPKD [64].

Pancreatic and splenic cysts can occur in childhood ADPKD, but the occurrence is rare and generally of no clinical consequence. There has been a single report of a ruptured intracranial aneurysms (ICA) in a patient under 20 years of age, but routine screening is not indicated during childhood [65]. A growing body of evidence suggests that hypertension, cardiovascular abnormalities, and increased kidney volume precede the development of symptoms in affected children [66, 67]. Furthermore, studies have shown that targeting modifiable risk factors early in the disease can significantly slows progression [58, 66-68]. Consequently, clinicians should be aware of these factors and pediatric patients with ADPKD should be monitored closely.

Renal manifestations in children with ADPKD are comparable to renal manifestations in affected adults [58]. In adult ADPKD, creatinine levels rise only in the advance stages of disease after non-cystic parenchyma has been permanently destroyed by expanding renal cysts. Thus, the renal function remains normal well into disease progression in adults. Similarly, renal function in children with ADPKD is generally normal [69].

Renal manifestations in children and adults with ADPKD include hypertension, hypertension induced cardiac disease, hematuria, proteinuria, polyuria, renal stones, urinary tract infections, and acute or chronic flank and abdominal pain [55, 70]. Hematuria can occur with cyst hemorrhage, and overt proteinuria is generally associated with more advanced structural kidney disease [69]. There is little to no correlation between microalbuminuria and either kidney volume or function in children with ADPKD. However, studies link established proteinuria in children with a more aggressive course of the renal disease [69, 71]. Glomerular hyperfiltration is common and indicates an increased risk of progression [72] including a faster decline in eGFR and a higher increased renal enlargement [73].

Hypertension induced cardiovascular disease is a major feature in both adults and children with ADPKD. Up to 30% of children with ADPKD have overt hypertension. Hypertension is particularly common in children with very early onset ADPKD [66, 74, 75]. Hypertension is linked to increased total kidney volume and an accelerated rate of renal function decline and left ventricular hypertrophy (LVH) [76, 77]. Studies imply that a substantial degree of left ventricular hypertrophy (LVH) may be present in children with ADPKD, even those with normal BP [66, 78].

Tight regulation of BP in normotensive children to the 50th percentile was found to halt the progression of LVH and slow the decline in renal function [66, 79-81]. A similar benefit of rigorous BP control (<110/75 mmHg) on kidney volume was confirmed in the HALT-PKD study in adults. ACE inhibition monotherapy in early ADPKD reportedly results in a 14% reduction in total kidney volume growth [68].

A five-year prospective clinical trial designed to evaluate the effectiveness of angiotensin-converting enzyme inhibitor (ACEI) enalapril on BP control provided additional evidence of the critical importance of BP in children with ADPKD. This study found that ACEi treatment in normotensive (BP 75 to 95th percentile) or borderline hypertensive (BP ≤75 percentile) children with ADPKD could prevent or significantly reduce the development of LVH and the decline in renal function [71]. These benefits occurred in the absence of any positive effect on kidney volume [58, 68, 71]. Surprisingly, these secondary benefits of ACEi did not apply to hypertensive ADPKD children [71]. The strong correlation between hypertension, larger kidneys and cardiovascular disease in ADPKD children makes accurate and frequent monitoring of BP essential. [77, 80, 82, 83].

Ambulatory BP monitoring (ABPM) is the most accurate method of detecting hypertension in children [84, 85]. Studies of pediatric ADPKD patients assessed by ABPM have revealed: a) a significant relationship between renal volume, renal length, and number of renal cysts [86]; b) one-third of the hypertensive ADPKD children showed exclusively nocturnal hypertension [86, 87]; and c) nearly 60% of patients who had normal BP by casual measurement were found to have daytime hypertension by ABPM [86]. Similar to the previously noted studies in childhood ARPKD [88], routine ABPM is more sensitive for the diagnosis of hypertension in the pediatric population with ADPKD [75, 86].

Structural kidney disease can be seen in children with ADPKD and in rare instances can be detected *in utero* [3, 56-58]. Structural kidney disease can cause acute or chronic flank pain and may be severe enough to necessitate chronic pain management [58].

In general, the renal manifestations seen in adult ADPKD are also seen in children except for renal failure which rarely occurs in children. It is clear that structural renal damage occurs before most children are diagnosed [66, 77, 79, 89]. The benefits from early and rigorous BP control should prompt clinicians to monitor at-risk children regularly with ABPM to identify hypertension as early as possible. While there are no available data on how long early intervention delays the more severe renal and cardiac manifestations, it is clear that BP control in children with ADPKD is a clinical priority.

In contrast to the renal manifestations seen in ADPKD children and adults, the extrarenal manifestations of ADPKD commonly seen in adults are rarely seen in children.

2.2 Genetics - ADPKD

ADPKD is genetically heterogeneous with two known genes responsible for the vast majority of cases. PKD1 found on chromosome 16 is responsible for 80% of all ADPKD and PKD2, found on chromosome 4 is responsible for approximately 15% of ADPKD cases. Until recently approximately 5% percent of diagnosed ADPKD cases were genetically unresolved (GUR) [52]. Technical advances in whole genome sequencing and next-generation sequencing have spurred reexamination of cohorts of GUR cases. Additional genes are being identified that produce phenotypes that in many respects resemble ADPKD. The two most recent genes are *GANAB* [90], and *DNAJB11* (DnaJ heat shock protein family (Hsp40) member B11) [91].

At least six genes (*ALG8* (OMIM*608103), *SEC61B* (OMIM609214), *LRP5* (OMIM *603506), *SEC63* (*608648), *PRKCSH* (OMIM*177060), and most recently *GANAB* (*104160) [90] have been identified as causative genes for a distinct genetic entity, autosomal dominant polycystic liver disease (ADPLD). All six genes and *DNAJB11* function in the ER as part of a quality control mechanism and mutations in any of these genes lead to reduced levels of PC1.

Genetic testing for the diagnosis of ADPKD is not generally necessary and technical challenges of analyzing PKD1 currently make it expensive. Cases where genetic testing may be helpful include clinical cases where there is no family history of PKD; reproductive counseling, and, for the identification of disease-free living related transplant donors.

Next-generation sequencing technologies offer increasingly cost-efficient diagnostic strategies which will ultimately reduce the cost. Prognostic assessment during the early stages of ADPKD will become increasingly important to identify patients with rapidly progressing disease who may be candidates for new clinical trials.

PKD1 is a large complex gene with 46 exons mapping to chromosome 16 (16p13.3). PKD1's genomic structure has several characteristics that complicate its sequencing and consequently its use for molecular diagnostics:

1. it is a highly GC-rich gene with many CpG dinucleotides;
2. 70% of the PKD1 gene, 5' to exon 1 through exon 33, is duplicated six times proximally on chromosome 16. These duplications encode pseudogenes with high sequence homology (95% identity) to PKD1;

3. PKD1 contains a 2.5 kb polypyrimidine tract in intron 21 (the largest in the human genome) [92, 93];
4. there is extreme allelic heterogeneity in both PKD1 and PKD2, with over 1500 different pathogenic mutations in PKD1, and 250 in PKD2;
5. algorithms for predicting the pathogenicity of DNA changes are lacking.

Genotype-phenotype correlations in ADPKD follow general patterns rather than absolute rules that begin with the well-recognized increased severity of PKD1 compared to PKD2. The average age of ESRD in PKD1 patients is 20 years earlier (53.4 versus 72.7) than PKD2 patients [52]. There is also a clear correlation between the type of mutation and disease severity. Truncating mutations (either frameshift, splicing, or nonsense) have an increased severity while non-truncating PKD1 mutations (missense, in-frame deletion/insertion) generally are associated with milder disease [94].

2.3 ADPKD Proteins

Polycystin 1 (PC1), the protein product of PKD1 is a large integral membrane protein of over 4000 amino acids. PC1 contains 11 transmembrane domains, an extensive extracellular domain with multiple predicted motifs, and a short intracellular C-terminal domain that regulates multiple signaling cascades [95]. PC1 is a putative receptor for an as yet unidentified ligand. The cytoplasmic carboxy-terminal coiled-coil domain of PC1 is known to interact with PC2; this interaction is required for PC1 maturation and trafficking to the cilia where PC1 and PC2 are thought to form a receptor-channel complex [96].

PC1 undergoes a series of post-translational cleavage events that alter the protein and its activity. PC1 undergoes a cleavage event at a juxtamembrane G protein-coupled receptor (GPCR) proteolytic site (GPS) motif generating a large N-terminal fragment (NTF) that remains noncovalently associated with the C-terminal fragment at the plasma membrane [97]. This cleavage seems to be a critical regulatory mechanism of PC1 maturation and trafficking [98]. Also, PC1 undergoes regulated cleavage of the intracellular C-terminal tail (CTT), resulting in the release of cytoplasmic fragments, which can translocate to the nucleus [99] and modulate signaling [100]. The CTT of PC1 has also been shown to translocate to the mitochondria [101]. Many if not most of the functions of PC1 remain unclear.

In contrast to PKD1, the PKD2 gene located on chromosome 4 (4q21) is much smaller, less complex with simpler features and structure [102]. PKD2 has 15 exons, spanning 68 kb of genomic sequence, encoding a 2904 bp open reading frame transcript [102]. Polycystin 2 (PC2), the product of the PKD2 gene, is a member of the transient receptor potential (TRP) family of nonselective cation channels [103]. Polycystin-2 is a calcium-permeable channel with six transmembranes (TM) spanning elements, and both the amino and carboxy termini face the cytoplasmic compartment. Interaction with PC1 is required for full-length PC2 to leave the ER and reach the cilia [96, 104].

3. Pathophysiology of Cyst Formation

The process of cyst formation and progressive growth is a multifaceted, dynamic process with multiple elements all contributing to the process, but never acting autonomously. The *PKD1* and *PKHD1* genes demonstrate a genetic interaction [105] and the proteins FPC and PC2 have been shown to directly interact [104,113,114] [#. PC1, PC2, and PKHD1 are expressed in several cellular

compartments and form multimeric protein complexes that modulate signaling pathways, which control essential cellular functions. This provides a molecular basis for the similar phenotypic expression of childhood ARPKD and ADPKD. For the clinician, it implies that therapies effective in either cystic disease may be effective in the other.

These proteins are localized to apical and basolateral plasma membranes, desmosomes, focal adhesions, basal bodies, and primary cilium. Except for PC2, there is little evidence to indicates the actual function of the PKD proteins PC1 or FPC or how these protein complexes may communicate with each other.

The rapid development of sophisticated techniques in molecular biology and increased specificity in producing targeted gene mutations has led to the development of new orthologous mouse models with conditional and hypomorphic mutations in *Pkd1*, *Pkd2,* and *Pkhd1*[106]. In combination with improved next-generation sequencing techniques, the ability to create specific targeted mutations has allowed animal models with mutations identified in PKD patients to be generated to test a mechanistic hypothesis. Such models have revealed unanticipated results regarding the development and progression of PKD. These new insights will lead to novel methods and novel compounds for therapeutic intervention for PKD.

A remarkable feature of PKD is the variable phenotypic disease expression in both ADPKD and ARPKD. Gaining a better understanding of disease progression and identifying determinants of disease severity will provide multiple benefits. It will identify new therapeutic avenues where adjustments of one or more of determinants of severity can be targeted to reduce disease progression. Further, it will serve to identify candidates most likely to benefit from a proposed clinical trial. Finally, it will identify patients that are unlikely to benefit from a specific therapy thereby preventing unnecessary and possibly harmful side-effects of a clinical trial.

3.1 Molecular Pathophysiology

Factors Affecting Progression or Severity. Variants in PKD alleles, as well as complex transcriptional events, play a key role in modifying progression and severity of childhood PKD. Recent discoveries of diverse transcriptional patterns and allelic variants including: hypomorphic or incompletely penetrant alleles [107, 108]; homozygosity of *PKD1* or *PKD2* [108]; compound heterozygosity [8]; germline mosaicism [109]; digenic inheritance or trans-heterozygosity [110]; co-inheritance of mutations in either *PKD1* or *PKD2* and an additional PKD-causing gene such as *PKHD1* [105], *HNF1B* [14, 111] or the tuberous sclerosis 2 (*TSC2*) gene [112]; all demonstrate that ADPKD and ARPKD have complex inheritance patterns which can contribute to disease severity.

In addition to the molecular transcriptional events listed above, other molecular events that highlight interactions between the PKD genes and proteins have been discovered that influence the severity and rate of cyst formation and total kidney growth as well.

The genetic combination of five murine cystic disease models including orthologous *Pkd1, Pkd2, Pkhd1,* and two orthologous ADPLD genes*, Prkcsh and Sec63* allowed the creation of different combinations of mutant alleles to characterize the functional relationships between these genes [113]. These combinations yielded unanticipated relationships and surprising results. These crosses demonstrated that:
1. PC1 dosage modifies the severity of both ADPKD and ARPKD

2. the threshold level of PC1 necessary for normal tubular morphology varies by nephron segment with collecting ducts being most sensitive;
3. overexpression of *Pkd1* can rescue a mutant *Pkhd1* [113];
4. *Pkhd1* and *Pkd1* interact at the gene level;
5. the overexpression PC1 can cause cysts.

These studies demonstrated that the rate of cyst growth is a regulated trait, which can be adjusted by changes in levels of functional PC1 [114]. These data support a threshold or dosage model of PKD. According to this model, cyst initiation and cystic expansion are governed by Pkd1 gene dosage [113-115]. Every cell or nephron has a threshold level of Pkd1 that is necessary to maintain normal tubular size and maintain cells in a non-proliferative state. When the level of functional PC1 falls below the cystogenic threshold, proliferation and cyst formation follow.

In a similar experiment tissue-specific, conditional and inducible mouse models that deleted structural ciliary genes (*Kif3a* and *Ift20)* and PKD genes (*Pkd1, Pkd2, and Pkhd1*), in a spatiotemporal manner, alone or in combination were examined to determine the functional roles of PKD1 and PKD2 in cilia. The various combinations revealed that:
1. loss of cilia causes less cyst growth than the loss of PC1 or PC2;
2. simultaneous loss of either PC and either the cilia structural proteins Kif3a or Ift20 resulted in greater reduction in cyst formation than that seen when either PC was inactivated; and
3. the length of time that intact cilia existed after the loss of PC's increased disease severity [116, 117]. These data suggest that the existence of a cilia-dependent, cyst-promoting (proliferation promoting) pathway exists which is inhibited by a normal PC1/PC2 complex on the cilia [116-118].

The two-hit theory of cyst development originated from the observation that ADPKD is a molecularly recessive disease and the cystic lesions are focal. All cells in the body have the same mutation, but estimates assert that only 5 to 10% of nephrons become cystic. The two-hit theory states that a second somatic mutation is required for cyst formation to occur. Substantial evidence exists to support this theory [119-121]. However, other factors have been shown to influence disease progression and severity [122]. These include:
1. the developmental timing of *PKD1* inactivation [123-126];
2. reduction in functional PC1 dosage; [107, 108, 113, 114]. The reduction of PKD1 dosage below 50% is sufficient to cause cystic lesions;
3. renal cells cellular and nephron segments show differences in sensitivity to PC1 dosage and have different requirements for the amount of PC1 needed for normal development/function [114];
4. The principal cells of the collecting duct appear to be the most sensitive [108];
5. the effect of one cyst on neighboring cells and nephrons creating a toxic environment or a "snowball effect" leading to cyst development in neighboring nephrons [127];

Therapies targeting genetic mechanisms in ADPKD have inherent limitations. As a result, most experimental therapies at present are aimed at delaying the growth of the cysts and associated interstitial inflammation and fibrosis by targeting cystic tubular epithelial cell proliferation and fluid secretion by the cystic epithelium.

3.2 Targeting the Cystic Phenotype

The specific mechanisms that accelerate cyst formation and progressive enlargement are poorly understood. However; it is clear that essential phenotypic changes are required for a normal absorptive, non-proliferating tubular epithelial cell to become a cystic epithelial cell. These changes include the proliferation of cells which line expanding cysts, fluid secretion into cysts that have lost their connection to the nephron and remodeling of the extracellular matrix surrounding the expanding tubules. In PKD, normal renal epithelial cells change from mature, differentiated, non-proliferative, absorptive cells to partially de-differentiated secretory cystic cells characterized by specific polarity defects and increased rates of proliferation.

Identification of signaling molecules and signaling pathways associated with PKD define the "cystic phenotype." The precise mechanisms by which mutated PKD proteins alter signaling molecules and integrate signaling pathways are not fully understood, and there is little known about the actual function of the proteins PC1 and FPC. Despite this gap in knowledge, key pathogenic features of both ARPKD and ADPKD have been identified and form the basis of current therapeutic interventions. These features include but are not limited to:

1. Altered intracellular cAMP levels coupled with decreased intracellular calcium;
2. Abnormalities in expression, localization, and activity of the epidermal growth factor (EGFR) – a family of receptors and ligands (EGFR axis);
3. Abnormal activity of cSrc, a critical mediator of cross-talk between the EGFR axis and G-protein-cAMP pathways which is required for STAT3 activation by a PC1 tail;
4. Alterations in cell-cell adhesion, and cell-matrix interactions;
5. Specific interstitial-tubular interactions which lead to chronic Inflammation and progressive fibrosis.

Clinical trials to date have been largely based on targeting these aberrant cellular signaling elements which define the cystic phenotype. Figure 1 is a cartoon that includes the major aberrant signaling pathways that comprise the cystic phenotype.
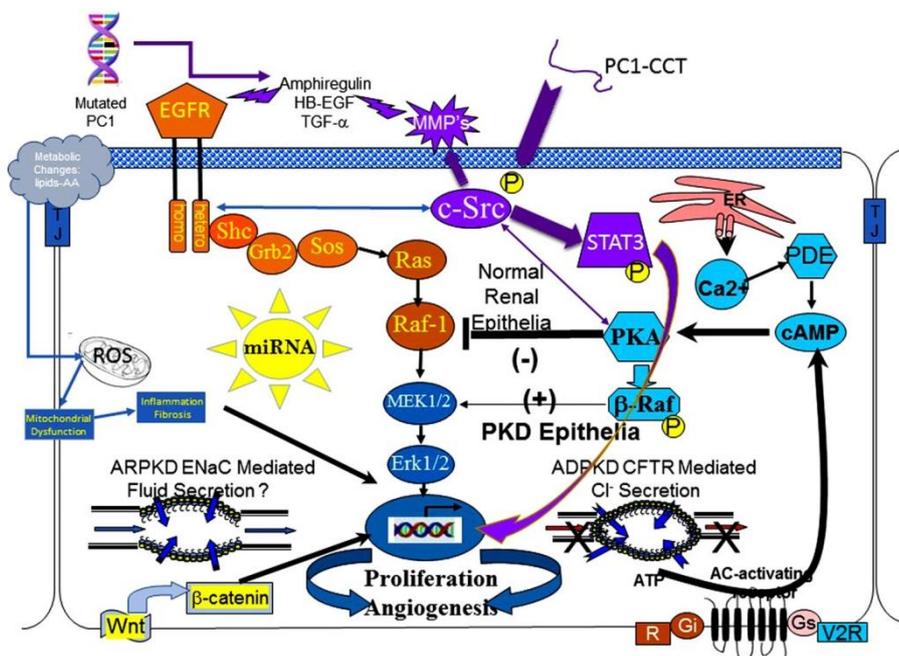
Figure 1 The cystic cellular phenotype. This cartoon is an abridged composite of the abnormal signal transduction pathways reported to be active in PKD. Two main conduits that lead to unchecked proliferation are: 1) the EGFR axis (orange); and 2) a G-protein axis (aqua blue) that leads to increased cAMP and a switch in the phenotypic response of renal epithelia to cAMP. The pathway suggest the following possible scenario: In ARPKD, an apical EGFR becomes activated resulting in reciprocal phosphorylation of the nonreceptor tyrosine kinase cSrc leading to increased cAMP-mediated, and EGFR driven proliferation; and in ADPKD (purple) a mutated PC1 leads to increase amphiregulin, activating EGFR, resulting in increased cSrc phosphorylation; in both ARPKD and ADPKD, cSrc activation alters the cellular response of cAMP resulting in increased proliferation and secretion; in addition, the cytoplasmic tail, PC1-p30, is overexpressed. PC1-p30 interaction with Src causes Src-dependent activation of STAT3 by tyrosine phosphorylation. Increased activity of EGFR and increased cAMP amplify the activation of the Src-STAT3 pathway. Finally, aberrant miRNA expression can impact any pathway and may effect more than one at a time.

cAMP-mediated proliferation and secretion. The cAMP-dependent pathway, or the adenylyl cyclase pathway, is a G-protein-coupled receptor signaling pathway that is abnormally regulated and aberrantly active in PKD. In normal renal epithelia increased intracellular cyclic AMP (cAMP) levels result in reduced epithelial proliferation. However, in cystic renal epithelia, a low intracellular calcium level, (theoretically due to a defective PC1/PC2 calcium channel) and a cSrc dependent phosphorylation of b-Raf, allows the cell to bypass Raf-1 and increase ERK phosphorylation to stimulate cell proliferation in the presence of increased cAMP levels [128, 129]. Increased cAMP in a cell with an active MAPK pathway also changes the cell from an absorptive to a secretory cell which contributes significantly to progressive cyst enlargement in ADPKD [130].

Therapeutically, increased cAMP is reduced by vasopressin R2 receptor antagonists such as tolvaptan (Samsca or Jinarc®) or somatostatin (and its long-acting analogs such as lanreotide that inhibit adenylate cyclase thereby reducing cAMP levels [131, 132].

Tolvaptan has undergone extensive human clinical trials. Tolvaptan slows the increase in total kidney volume and loss of renal function [133-135]. Jinarc® has been approved in Europe, Japan, Canada, Australia, and South Korea for the treatment of adults with ADPKD. Tolvaptan has recently been approved in the USA under the trade name JYNARQUE®. Due to concerns regarding potentially serious adverse liver events, JYNARQUE is only available in the USA through a restricted distribution program called the JYNARQUE Risk Evaluation and Mitigation Strategy which is designed to closely monitor patients for liver or other adverse events. Details can be found at: (www.fda.gov/safety) and (https://www.jynarque.com).

A single report of the use of tolvaptan in severe neonatal ADPKD was found to resolve hyponatremia and stop the growth of the kidneys when administered betweenPN30 to 12 months of age [136]. The medical team instituted a strict monitoring program that included frequent blood pressure, urine, and electrolytes and hepatic function testing [137]. Despite this successful use of tolvaptan in a child, Ttolvaptan is not currently recommended for use in children due to the necessity to closely monitor fluid intake in the pediatric population. Never the less, a Phase III clinical trail of tolvaptan in children between age 4 and 17 years with ADPKD is under way in

Belgium, Italy, Germany and the United Kingdom (NCT02964273). A clinical trial of Tolvaptan in children and adolescents with euvolemic and hypervolemic hyponatremia will provide additional data on the safety, pharmacokinetics and fluid balance issues with tolvaptan use in a pediatric population (Clinical Trials.gov Identifier: NCT02442674).

Palladia Sciences is testing a drug called Lixivaptan that the company believes provides the same benefits of Ttolvaptan in ADPKD without the potential liver issues associated with Tolvaptan use (ELSA study NCT 03487913). In addition to the potential problems on liver function with tolvaptan therapy, the unanticipated finding of increased shedding of HB-EGF in the urine of patients treated with tolvaptan may be problematic especially with lengthy use due to the known role of EGFR in fibrosis [137]. If this data is confirmed, it suggests the addition of a tyrosine kinase inhibitor (TKI) or multi-TKI to the tolvaptan treatment may improve clinical results.

EGFR (ErbB) axis. The epidermal growth factor receptor (EGFR) axis consist of the epidermal growth factor/ErbB family of receptors and ligands. A large body of evidence implicates one or more members of the ErbB receptor family in renal cystic epithelial proliferation, including the epidermal growth factor receptor (EGFR), as well as the related receptors, ErbB2 and ErbB4 [138-140].

In both human ADPKD and ARPKD, and every rodent model of PKD published to date, cystic kidneys display characteristic alterations in EGFR-axis expression. Inhibition or reduction of EGFR activity through genetic manipulation [141] or administration of a tyrosine kinase inhibitor [142, 143], reduces cyst formation and progressive enlargement in murine cystic models.

Recent studies have provided a direct link between PC1 and PC2 proteins and the EGFR axis. These studies have revealed that EGF reduces the threshold of PKD2 activation by releasing it from PIP(2)-mediated inhibition [144] and that mutated PC1 results in increased expression of amphiregulin and increased proliferation [145].

Microarray profiling in normal human and cystic PKD1 kidney cells has demonstrated that the EGF/ErbB family receptor (ErbB4) is a major driver of cyst growth in ADPKD and ErbB4 levels also serve as a potential biomarker for disease progression [146]. This increased expression of ErbB4 *in vivo* in both human ADPKD and *Pkd1* cystic kidneys was associated with a single microRNA (miRNA), mir-193b-3p [146].

cSrc. c-Src is a critical intermediate that integrates signals between two major proliferative pathways in cystic epithelial cells, the EGFR axis, and cyclic AMP pathway [147] (see Figure 1). Also, a proteolytic fragment of the PC1 cytoplasmic tail, PC1-p30, interacts with cSrc, to activate STAT3 stimulating further increases in proliferation [148-150]. Src activation of STAT 3 is amplified by both the increases in activity of the EGFR axis and by increased cAMP levels resulting in a self-perpetuating cycle that accelerates proliferation [150].

These experimental results would predict a pathologic pathway where a mutation in PC1 increases amphiregulin expression, resulting in activation of the EGFR axis (EGFR or ErbB4) and reciprocal activation of Src. In concert with increased cAMP levels and cleavage of the PC1 tail, cSrc assimilates and amplifies input from the EGFR axis, and cAMP signaling pathways to activate STAT3 [150].

In a clinical trial of Bosutinib, a cSrc inhibitor (ClinicalTrials.gov Identifier NCT01233869) was very effective in reducing total kidney volume in ADPKD patients. However, in this limited clinical trial, bosutinib did not affect changes in renal function [151].

mTOR. The mammalian target of rapamycin (mTOR) pathway integrates signals from growth factors (including EGFR), G-protein coupled receptors (which generate cAMP), cellular energy levels, nutrient status and stress conditions to stimulate protein synthesis and cell growth through activation (phosphorylation) of S6K1 and eIF4E [152, 153]. In human ADPKD, ARPKD and a variety of animal models, cyst-lining epithelium reveals an increased activity of mTOR [153-156]. The mTOR inhibitors rapamycin and everolimus have been tested in human clinical trials and were found to be ineffective in slowing total kidney volume or the progressive loss of renal function [157, 158]. It has been hypothesized that effective renal concentration of mTOR inhibitors was not achieved and thereby responsible for the disappointing clinical results. Based on the finding that cystic cells express folate receptors Shillingford et al. conjugated folate to rapamycin. The effectiveness of mTOR inhibition was dramatically improved, and target specificity was increased in a *Pkd1* animal model when rapamycin was conjugated with folate [159]. To date, no clinical trials have been reported utilizing this new approach to mTOR inhibition in PKD.

3.3 Emerging Therapies

Tesevatinib. Tesevatinib (TSV) previously known as KD-019, is a unique multi-kinase inhibitor that targets EGFR, c-Src and KDR. TSV was effective in slowing the proliferation of renal epithelia and cyst growth, slowing progression of fibrosis and slowing the loss of renal function in the BPK and orthologous PCK rat models of ARPKD [160].

TSV is currently being tested in two adult Phase II-III clinical trials in ADPKD [ClinicalTrials.gov Identifier NCT01559363 and NCT03203642. TSV is also the first specific disease-targeted therapy to receive FDA approval for a clinical trial in pediatric ARPKD [ClinicalTrials.gov Identifier NCT03096080]. All three clinical trials are in progress.

20 – HETE. Metabolic changes are known to occur during the development and progression of ARPKD and ADPKD. Bioactive lipids such as 20-HETE, a P450-produced bioactive lipid metabolite of arachidonic acid was shown to correlate with eGFR and TKV in ADPKD patients [161]. Three studies demonstrated the effectiveness of 20 HETE inhibition an animal models of PKD [162-164] indicated that inhibitors of 20-HETE production may have potential use as a therapeutic agent for slowing PKD progression and as a non-invasive biomarker to monitor disease desease progression.

Bardoxolone Methyl or Bard (Reata Pharmaceuticals). Triterpenoids are used for medicinal purposes in many Asian countries because of their anti-inflammatory, antioxidant, antiproliferative, anticancer, and anticarcinogenic properties. Bardoxolone methyl is an experimental and orally-bioavailable semi-synthetic triterpenoid that in pre-clinical studies acts as an activator of the Nrf2 pathway and inhibitor of the transcription factor NF-kB (Reata Pharmaceuticals). Nrf2 regulates the expression of antioxidant proteins that protect against oxidative damage triggered by injury and inflammation, and NF-kb is a pro-inflammatory transcription factor. Inflammation and oxidative damage are both implicated in all forms of CKD including PKD, regardless of initiating cause [165].

Twenty-seven clinical trials have been completed or are in progress utilizing BARD to target cancer and chronic renal diseases. A Phase II multi-center clinical trial (ClinicalTrials.gov Identifier: NCT00664027) of BARD in patients with moderate to severe CKD and type 2 diabetes reported that eGFR increased as much as 10 ml/min/1.73 m² in patients treated with bardoxolone methyl

for 24 months [166]. To date, no therapy targeting ARPKD or ADPKD has shown to improved eGFR. At best, therapies have been shown to reduce the rate of functional decline.

Recruitment is underway for a Phase II Trial of the Safety and Efficacy of Bardoxolone Methyl in Patients With Rare Chronic Kidney Diseases associated with type 1 diabetes (T1D), IgA nephropathy (IgAN), focal segmental glomerulosclerosis (FSGS), and autosomal dominant polycystic kidney disease (ADPKD) (ClinicalTrials.gov Identifier: NCT03366337). The estimated completion date is August 2019. An improved eGFR if found following Bard treatment of PKD would be a remarkable finding.

MicroRNAs. Given the complexity of the ADPKD genes and the multiple interactions discussed above, therapeutic interventions are not likely in the near future with the possible exception of miRNA inhibition. miRNAs are small, non-coding RNAs with a strictly regulated biogenesis. This is combined with an extremely flexible and sophisticated regulatory function of gene expression at the post-transcriptional level. This allows the simultaneous targeting of multiple proteins involved in different crucial biological pathways of specific cell types and tissues. miRNAs are integral to the normal development and physiology of the human kidney. They have also been shown to be central to the pathophysiology of many human disease states including renal diseases. As mentioned above, a recent study in human ADPKD has linked a single miRNA to the progression of human ADPKD [146] and more recently a unique interstitial miRNA signature was found to play a role in the pathogenesis of renal fibrosis in an orthologous animal model of ADPKD [167]. Recent studies have also identified miRNAs that play a role in the pathogenesis of PKD. Deletion or inhibition of the miR-17-92 cluster in four orthologous animal models of PKD, and cell cultures from multiple human cysts result in reduced epithelial proliferation and cyst growth [168-170].

3.4 Non-Specific Therapies Targeting PKD

Hypertension. In ADPKD, hypertension is associated with increased TKV, activation of the renin-angiotensin-aldosterone system (RAS) and progressive loss of renal function. Therefore, the identification and treatment of hypertension is essential in slowing the progression to ESRD in ADPKD.

The NIH sponsored HALT/PKD studies failed to demonstrate any benefit effect of adding an ARB (telmisartan) to an ACEi (lisinopril) on reducing disease progression regardless of level of renal function in adult ADPKD patients. However, in younger patients with moderately preserved renal function, the reduction of blood pressure in children and young adults (age 15 to 49) a lower target level with an ACEi significantly slowed the increase in TKV and decreased urinary albumin excretion and left ventricular mass index but did not slow the decline in renal function. estimated glomerular filtration rate [68, 171].These data support the premise that targeting lower BP goals in ADPKD patients with RAS blockade may have long term beneficial effects in preserving renal function, and may be particularly beneficial to children and young adults.

Statins Therapy. The cholesterol-lowering drugs, 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors, collectively called statins, have anti-proliferative, anti-inflammatory and anti-oxidant effects independent of cholesterol lowering effects [172, 173].

A randomized double-blind placebo controlled pPhase III clinical trial in pediatric and adolescence patients with ADPKD demonstrated that statin therapy significantly slowed increases in TKV and reduced the decline of renal function over a three year period [174]. To date, these

results have not been replicated in adults with ADPKD but a clinical trial still recruiting patients is designed to examine this issue (ClinicalTrials.gov Identifier: NCT03273413).

Dietary Modification.

Water consumption: Suppression of serum AVP by increasing free water intake would be predicted to decrease intracellular cAMP concentrations in collecting duct cells and thereby reduce tubular epithelial cell proliferation and cyst growth. A number of small observational trials have looked at the effect of a short-term increased in water consumption on the progression of PKD. Thus far the data has been intriguing not been persuasive enough to support recommending the use of increased water consumption to slow progression of disease. However, clinical trials are underway to examine the effect of increased water consumption to achieve a urine osmolality < 270 mOsmo/kg or urine specific gravity ≤ 1.010 (ClinicalTrials.gov Identifier: NCT02933268). One such study in the USA is still recruiting patients (ClinicalTrials.gov Identifier: NCT03102632) as is a study in Australia (Trial registration number ANZCTR12614001216606 [173].

Caloric Restriction and Other Dietary Recommendations: A recent study in mouse models of PKD examined the effect of caloric restriction on disease progression [175]. The study revealed a mild to moderate (10%-40%) reduction of food slowed disease progression in a dose-dependent manner. These findings have ignited an interest in examining the effect of caloric restriction on ADPKD progression. A clinical trial to assess the effect of "Daily Caloric Restriction and Intermittent Fasting in Overweight and Obese Adults with ADPKD (ClinicalTrials.gov Identifier: NCT03342742) is underway.

4. Conclusions

The diagnosis of childhood PKD is no longer the terminal diagnosis as it once was. For children with ARPKD advances in neonatal critical care, control of hypertension and hepatic complications, and renal/hepatic transplantation have allowed many to survive into adulthood. Insights into the development and treatment of PH are preventing lethal complications of hepatobiliary disease and provide a better quality of life. Renal transplantation and dual organ transplantation provide an opportunity for these children to live a near normal life [22].

Pre-implantation genetic diagnosis (PGD) and in vitro fertilization (PGD-IVF) holds the possibility of eliminating ARPKD from families who can afford the procedure and who have no religious/ethical contraindications [51]. PGD for ADPKD is becoming more widely available [176] and PGD-IVF for ADPKD is being examined under clinical trial in China (NCT02948179).

As clinical trials to date have shown, targeting a single molecule of an aberrant pathway has not been effective in preventing the loss of renal function as originally hoped. However, even as the targeted therapeutic strategies develop more specificity, they will always need to target (a) cell proliferation; (b) cAMP-mediated proliferation and secretion; and (c) interstitial fibrosis. Proliferation is an essential component that must be targeted and controlled for any therapy to be effective. How much and for how long remains are questions currently under investigation.

In the short term, the most promising therapies will likely target key signaling intermediates that appear to integrate multiple pathways, such as Src, (see Figure 1) [147, 150] and/or utilize multiple compounds to target multiple pathways simultaneously or a single compound that targets multiple pathways. A single compound like TSV that targets multiple pathways [177, 178], offers great promise. Future rational therapies will also require a knowledge of the extent of

disease, as well as the rate of progression, and will likely involve different compounds that will vary at specific stages of disease progression.

There are additional essential factors to consider in developing therapies for childhood PKD. Therapies will become increasingly focused on treating PKD in early childhood where they are likely to provide the maximum long-term benefit. Therapies that target abnormal signaling pathways will need to be carefully tailored, so that pathway activity is reduced to normal basal levels rather than eliminating the signal completely. Promising compounds will be modified to direct the molecule to the site of disease, making such therapies highly specific with low levels of toxicity. This specificity will speed the development of protocols for the ethical treatment of children with PKD so early intervention will provide maximal quality of life. Epigenetic and dietary factors that slow or accelerate the progression of PKD will be discovered, and adherence or avoidance of such factors may slow the rate of progression and may eliminate the need for pharmacological intervention or renal replacement therapy for some.

The importance of gene dosage, modifier genes and somatic mutations in the clinical course of a disease in an individual patient provides a compelling rationale for personalized medicine in PKD. Such therapies will modulate functional gene dosage and base therapy not only on the individual genotypes but will account for the response of the kidney and liver to the therapy itself and any unanticipated response of the kidney or the patient to the therapy.

The progress made in understanding the pathophysiology of PKD has been remarkable and despite the progress, work remains cClinical trials for children with PKD are underway: One for children with ADPKD [Tolvaptan [NCT02964273 Belgium and Italy] and one recently approved for the use of TSV in ARPKD (ClinicalTrials.gov Identifier NCT03096080). A list of current and completed clinical trials can be found at http://clinicaltrials.gov/.

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Author Contributions

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Competing Interests

The authors declare that they have no conflicts of interest concerning research, authorship and publication of this manuscript.

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