Metabolism and mitochondria in polycystic kidney disease research and therapy

*Valeria Padovano[1], Christine Podrini[2], Alessandra Boletta[2] and Michael J. Caplan[1]**

Abstract | Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common, potentially lethal, monogenic diseases and is caused predominantly by mutations in polycystic kidney disease 1 (*PKD1*) and *PKD2*, which encode polycystin 1 (PC1) and PC2, respectively. Over the decades-long course of the disease, patients develop large fluid-filled renal cysts that impair kidney function, leading to end-stage renal disease in ~50% of patients. Despite the identification of numerous dysregulated pathways in ADPKD, the molecular mechanisms underlying the renal dysfunction from mutations in *PKD* genes and the physiological functions of the polycystin proteins are still unclear. Alterations in cell metabolism have emerged in the past decade as a hallmark of ADPKD. ADPKD cells shift their mode of energy production from oxidative phosphorylation to alternative pathways, such as glycolysis. In addition, the polycystins seem to play regulatory roles in modulating mechanisms and machinery related to energy production and utilization, including AMPK, PPARα, PGC1α, calcium signalling at mitochondria-associated membranes, mTORC1, cAMP and CFTR-mediated ion transport as well as the expression of crucial components of the mitochondrial energy production apparatus. In this Review, we explore these metabolic changes and discuss in detail the relationship between energy metabolism and ADPKD pathogenesis and identify potential therapeutic targets.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common, life-threatening monogenetic diseases, affecting ~1 in 1,000 individuals worldwide[1], although regional epidemiological analyses have reported a lower incidence of ADPKD (2.4–4.7 in 10,000 individuals)[2,3]. ADPKD is characterized by the development of large fluid-filled renal cysts that compress and destroy surrounding healthy tissue, dramatically enlarging the kidneys and progressively reducing their function, leading to end-stage renal disease in ~50% of patients by the fifth to sixth decade of life[4]. Although renal cysts are the most prominent phenotype, ADPKD is a systemic disorder with various cystic and non-cystic extrarenal manifestations, including liver and pancreatic cysts, cerebral aneurysms and cardiovascular abnormalities[5]. Mutations in two genes, *PKD1* and *PKD2*, which encode polycystin 1 (PC1) and PC2, respectively, are the most common cause of ADPKD. Mutations in *PKD1* account for ~85% of ADPKD cases and result in markedly more rapid disease progression[6] than mutations in *PKD2*, which account for most of the remaining ADPKD cases. Interestingly, mutations in these genes have not been detected in ~9% of affected families[7,8], which might be explained by atypical mutations in *PKD1* or *PKD2* or by mutations in one or more unidentified polycystic kidney disease (PKD) loci. For example, mutations in *GANAB* (which encodes neutral α-glucosidase AB) have been identified in patients with a mild form of ADPKD who lack mutations in *PKD1* or *PKD2* (REF.[9]). Furthermore, the high degree of phenotypic variability among family members carrying the same mutation in one of the *PKD* genes and the focal nature of renal cysts are both consistent with the possibility that a two-hit process initiates cystogenesis. In this two-hit model, somatic mutations in the wild-type *PKD1* or *PKD2* allele occur randomly throughout the lifespan of a patient who has a germline ADPKD mutation and are required to trigger ADPKD[10]. Another possible explanation for the phenotypic heterogeneity observed in patients with ADPKD is the dosage of functional polycystin proteins. Several reports have found that homozygous *PKD1* or *PKD2* hypomorphism and incompletely penetrant alleles result in variable disease severity[11–14].

PC1 is a large 4,302-residue transmembrane protein comprising a substantial 3,000-residue amino-terminal extracellular domain, 11 transmembrane domains and a short intracellular carboxy-terminal tail[15,16]. PC2 is a

[1]*Department of Cellular and Molecular Physiology, Yale School of Medicine, New Haven, CT, USA.*

[2]*Division of Genetics and Cell Biology, Dibit, San Raffaele Scientific Institute, Milan, Italy.*

**e-mail: michael.caplan@yale.edu*

https://doi.org/10.1038/s41581-018-0051-1

Key points

- Metabolic reprogramming has emerged as an important aspect of the pathogenesis of autosomal dominant polycystic kidney disease (ADPKD).
- Increased glycolysis, defective fatty acid β-oxidation and altered mitochondrial function have been observed both in vitro and in vivo in animal models of ADPKD and in tissues from patients with ADPKD.
- Polycystin proteins can directly regulate mitochondrial function; for example, the polycystin 1 (PC1)–PC2 complex at mitochondria-associated membranes can directly regulate oxidative phosphorylation by mediating mitochondrial calcium uptake.
- Polycystin proteins can indirectly affect mitochondrial function through regulation of calcium signalling, reduction of cAMP levels, inhibition of miR-17, maintenance of mitochondrial DNA (mtDNA) copy number and modulation of mitochondrial morphology.
- The energy sensor AMP-activated protein kinase (AMPK) regulates at least two key processes that are altered in ADPKD, mechanistic target of rapamycin complex 1 (mTORC1) signalling and the activity of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel.
- Targeting the metabolic alterations in ADPKD ameliorates cyst progression in rodent and non-rodent models of ADPKD, and thus, these alterations might be novel therapeutic targets.

968-residue transmembrane protein comprising a short N-terminal intracellular domain, six transmembrane domains[17,18] and a short intracellular C-terminal tail. PC2 is a calcium-permeable non-selective cation channel of the transient receptor potential (TRP) channel family[19], which modulates calcium release from the endoplasmic reticulum (ER)[20]. PC1 and PC2 interact through their C-terminal regions and extracellular loops, forming a heterodimeric complex that is thought to have a role in regulating intracellular calcium release[21–23]. PC1–PC2 complexes are detected in the ER membrane, the plasma membrane, exosomes and primary cilia[24–27], where they have been suggested to mediate calcium influx in response to fluid flow[28]. Although the function of polycystin proteins in cilia and their involvement in cilium-associated calcium fluxes are intensely debated[29], it is generally accepted that the cilium is central in the pathogenesis of ADPKD. In fact, ADPKD was one of the first described ciliopathies[30]. Although the functions of the PC1–PC2 complex and its role in the pathogenesis of ADPKD are unclear, polycystin proteins have been shown to modulate numerous signalling pathways (FIG. 1) that mostly fall into six functional categories: G protein signalling, mechanistic target of rapamycin complex 1 (mTORC1) signalling, cAMP signalling, calcium signalling, growth factor signalling, cell cycle regulation and WNT signalling (the evidence implicating these pathways in ADPKD has been reviewed extensively elsewhere[31] and is not discussed in detail here). However, in the past few years, this list of ADPKD-related pathways has grown after studies found that metabolic alterations occur in ADPKD and might be involved in disease pathogenesis.

Although the polycystin proteins were discovered more than two decades ago, the mechanisms underlying the formation of renal cysts when *PKD1* or *PKD2* are deleted or mutated have not been established. The molecular connections between polycystins and the signalling pathways that are dysregulated in ADPKD are largely unknown, and the hierarchical relationship between these signalling pathways, whether polycystin proteins directly affect these pathways and the contribution of each pathway to cyst initiation and progression are the subject of active research that has yet to produce consensus. However, despite this complexity, broad agreement exists about several processes that contribute to cyst expansion, including the proliferation of cyst-lining epithelial cells and the active transepithelial transport of fluid into the cyst lumen[32]. Although it has not been established whether the newly identified metabolic alterations that occur in ADPKD directly cause this proliferation and fluid secretion, cellular metabolic pathways clearly have a central role in determining the capacity of a cell to produce the energy required for proliferation and active transport. In this Review, we discuss the evidence for metabolic changes in ADPKD and the mechanisms that might underlie them. We examine the relationship between energy metabolism and the pathogenesis of ADPKD and evaluate the potential utility of targeting the metabolic alterations in ADPKD for the development of novel therapeutic approaches.

Metabolic pathways altered in ADPKD

Studies have shown that metabolic reprogramming might be a key feature of ADPKD and offer a novel therapeutic target in patients with ADPKD[33,34]. Here, we describe the most prominent metabolic alterations that have been reported to date.

Glucose metabolism. The first evidence of a metabolic alteration in ADPKD came from in vitro studies. Mouse embryonic fibroblasts (MEFs) lacking *Pkd1* were found to have increased glucose uptake and used glycolysis as their main source of energy, even in normoxic conditions[33]. Importantly, this effect mostly depends on the upregulation of mTORC1 signalling and results in the inhibition of AMP-activated protein kinase (AMPK; also known as PRKAA2), which led to the hypothesis that, in ADPKD, cells preferentially use aerobic glycolysis for energy production[33,35], the so-called Warburg effect that was first described in tumour cells. Consistent with this hypothesis, glucose starvation in *Pkd1*$^{-/-}$ MEFs resulted in decreased proliferation, increased apoptosis and defective autophagy[33]. Increased glycolysis has been detected in proliferating cells, including cancer cells, and is used to generate ATP and promote the efficient conversion of glucose into macromolecules that are needed to construct new cellular components[36]. Subsequent studies of cells lacking *PKD1* reported contradictory results; some studies confirmed that glycolysis was upregulated and that the transcription of the key glycolytic genes hexokinase 1 (*HK1*) and *HK2* was increased in human ADPKD primary cells in vitro[37,38], whereas others failed to detect substantially enhanced glycolysis[34,39]. The reason for this discrepancy has not been definitively established, but the different methods of primary renal cell isolation and immortalization to create cell lines might be one explanation. An alternative possibility is that slightly different culture conditions in different studies might result in substantial differences in the baseline metabolic characteristics of cells, which has been documented in other studies[40,41].

Warburg effect
The use of glycolysis as the major cellular energy source without using oxygen, even when it is available, resulting in lactate production. First reported in cancer cells but also observed in other hyperproliferative cell types.

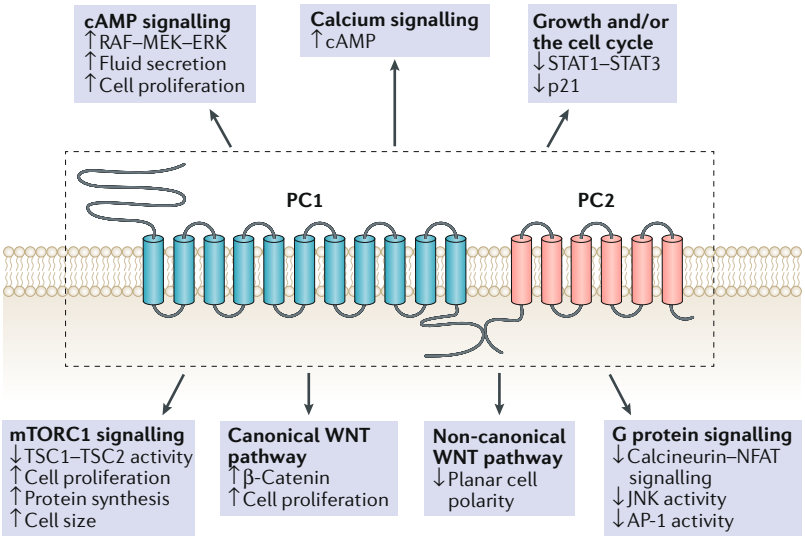

Fig. 1 | **Signalling pathways that are regulated by polycystin proteins.** The polycystin proteins have been implicated in the regulation of several signalling pathways that can lead to cyst formation and expansion in autosomal dominant polycystic kidney disease (ADPKD) when their function is reduced. The pathways regulated by polycystin 1 (PC1) and PC2 include G protein-mediated signalling, mechanistic target of rapamycin complex 1 (mTORC1) signalling, cAMP signalling, calcium signalling, growth factor signalling, cell cycle regulation, and both canonical and non-canonical WNT signalling pathways. Arrows indicate the effects on the pathways of the loss or mutation of the *PKD* genes that encode the polycystin proteins. AP-1, activator protein 1; ERK, extracellular signal-regulated kinase; JNK, JUN N-terminal kinase; MEK, MAPK/ERK kinase; NFAT, nuclear factor of activated T cells; STAT, signal transducer and activator of transcription; TSC1, tuberous sclerosis complex 1.

However, of note, for both studies that did not detect a substantial increase in glycolysis in cells lacking *PKD1*, the results are consistent, in principle, with increased glycolysis. In one study, Menezes et al. reported reduced oxidative phosphorylation (OXPHOS) in *Pkd1*$^{-/-}$ cells that had fatty acids as their primary energy source, suggesting that fatty acid β-oxidation (FAO) is reduced[34], which is typically accompanied by a compensatory increase in glycolysis. In the other study, Warner et al. found that the key glycolytic enzyme hexokinase 2 (HK2) is transcriptionally upregulated in cells lacking *Pkd1* (REF.[39]), which is also predicted to result in increased glycolysis. Additional well-controlled studies using comparable conditions and validated tools need to be carried out to reconcile these discordant findings. However, despite these discordant results, the in vitro data show clearly that metabolic reprogramming, including enhanced glycolysis, occurs in PKD.

In addition to the in vitro studies, investigators found that the expression of key glycolytic genes is upregulated in cystic epithelia from patients with ADPKD and in MEFs and cystic kidneys of *Pkd1*$^{-/-}$ mice[33]. Furthermore, investigators followed the fate of labelled glucose (^{13}C-glucose) in orthologous murine models of ADPKD, and injection of ^{13}C-glucose resulted in increased glucose uptake by polycystic kidneys and a concomitant increase in the level of ^{13}C-lactate, providing in vivo evidence of the Warburg effect in animal models of ADPKD[33,42]. Of note, mTORC1 upregulation correlates with these metabolic alterations in vivo. Similarly, in two mouse models of PKD, activation of AMPK using metformin ameliorated cyst progression[43]. As AMPK is involved in many cellular functions, including sensing the energy status of the cell, this observation provides indirect evidence that metabolic rates and the AMP:ATP ratio are altered in ADPKD. Indeed, in preclinical studies, targeting glycolysis is an effective therapy for ADPKD[33,42]. Additional studies in the Han:SPRD rat and in an orthologous minipig model of ADPKD further confirmed the increased renal glycolysis[37,44]. Furthermore, in heterozygous Han:SPRD rats, the expression of genes encoding key glycolytic enzymes was upregulated, and administration of 2-deoxyglucose effectively slowed disease progression[37].

Lipid metabolism and mitochondrial energy production. A renal transcriptomic analysis and urine metabolomic analysis in a mouse model of early-onset ADPKD (generated by inducible *Pkd1* deletion) identified metabolic pathways that are potentially linked to cyst formation. Among these pathways, the investigators have found high levels of acetylcarnitine in the urine of ADPKD mice, suggesting defective metabolism of fatty acids in mitochondria. Furthermore, the transcription factor hepatocyte nuclear factor 4α (HNF4α; encoded by *Hnf4a*) was identified as a transcriptional network node[45]. Inactivation of HNF4α in *Pkd1*$^{-/-}$ mice exacerbates the PKD phenotype, suggesting that upregulation of HNF4α is protective in ADPKD[45]. Indeed, HNF4α is a central regulator of several metabolic pathways in cells and has been implicated in the regulation of fatty acid metabolism in the liver. Mutations in *HNF4A* are also associated with a form of metabolic syndrome termed maturity onset diabetes of the young, type 1 (MODY1)[46].

Additional important studies have implicated reduced FAO and OXPHOS[34,47] as being involved in ADPKD. Transcriptional profiling and metabolomics analysis of orthologous models of slowly progressive ADPKD found substantial alterations in lipid metabolism[34]. The impairment of FAO in mice with renal-tubule-specific *Pkd1* mutation seems to occur via signalling involving the microRNA (miRNA) miR-17 and the transcription factor peroxisome proliferator-activated receptor-α (PPARα)[47], as inhibition of miR-17 ameliorated the impairment of FAO and reduced cyst growth in short-term and long-term PKD mouse models[47]. Of note, the regulatory network comprising PPARα and its target genes is among the most downregulated networks in both mouse and human renal cysts[47,48]. Importantly, mutations in genes encoding components of the OXPHOS and FAO pathways, as well as in PPARα target genes, result in clinical disorders that sometimes include cystic kidneys[49]; for example, cystic kidneys occur in patients with mutations in mitochondrial carnitine *O*-palmitoyltransferase 2 (CPT2)[49], an enzyme that transports fatty acids into mitochondria, or in patients with Zellweger syndrome[50,51], a disorder caused by defective peroxisomes. Similarly, renal cysts occur in patients with glutaric acidaemia type II[50], which is caused by mutations in genes encoding electron transfer flavoprotein subunit-α (ETFα), ETFβ or ETF dehydrogenase (ETFDH), which are components

Oxidative phosphorylation (OXPHOS). The production of ATP from ADP using the electrochemical gradient created through the activity of the electron transport chain of the inner membrane of mitochondrion.

Fatty acid β-oxidation (FAO). The production of energy by the catabolism of fatty acids.

Han:SPRD rat
A non-orthologous rat model of chronic, progressive renal cystic disease that is caused by a missense mutation in ankyrin repeat and sterile α-motif domain-containing 6 (*Anks6*; also known as *Pkdr1*).

Network node
A central hub where different gene expression networks interconnect.

Maturity onset diabetes of the young, type 1 (MODY1). An autosomal dominant condition, usually with an early adult onset and characterized by features of metabolic syndrome.

of an OXPHOS enzyme, ETF complex. Consistent with these metabolic changes in PKD, reducing lipid intake can slow cyst progression, both in orthologous PKD models[34,39] and in the non-orthologous Han:SPRD rat[51]. Finally, as discussed in detail below, polycystins seem to affect the function and morphology of mitochondria[52,53], and, thus, mitochondrial dysfunction might initiate the metabolic reprogramming in PKD.

Of note, in the study that reported defective FAO in an orthologous mouse model of ADPKD, a prominent glycolytic defect was not detected in the cystic kidneys[34]. A major obstacle preventing direct comparison of the results discussed above is their use of different animal models of ADPKD, including orthologous models, such as mice with kidney-specific inactivation of *Pkd1* (REFS[33,47]) or with inducible ubiquitous *Pkd1* inactivation[34], as well as non-orthologous models, such as Han:SPRD$^{Cy/+}$ rats[37,51]. In future studies, researchers should agree on the use of standardized cell lines and animal models of ADPKD. Taken together, these studies suggest that metabolic changes are important in the pathogenesis and/or progression of PKD and therefore are potential therapeutic targets.

Mechanisms of ADPKD metabolic changes

A key question from the studies discussed above is the underlying cause or causes of the metabolic switch to glycolysis that occurs in ADPKD. The compromised mitochondrial function in *Pkd1*$^{-/-}$ proximal tubule cells[52] could explain, at least in part, the metabolic alterations in ADPKD. Indeed, the oxygen consumption rate in *Pkd1*$^{-/-}$ cells is lower than in *Pkd1*$^{flox/-}$ cells that have one wild-type *Pkd1* allele and thus express PC1 (REF.[52]). Importantly, both the localization and activity of PC1 are regulated by oxygen levels — hydroxylation of proline residues in the PC1 cytosolic domain by the cellular oxygen sensor proxyl hydroxylase domain-containing protein 3 (PHD3; also known as EGLN3)[52] links PC1 to cellular oxygen-sensing pathways. In addition, PC1 localizes to areas of the ER that are in close contact with mitochondria[52], the so-called mitochondria-associated membranes (MAMs), which mediate the transport of calcium from the ER lumen into mitochondria. As this calcium influx positively regulates components of the OXPHOS machinery in mitochondria, OXPHOS is probably also affected by cellular oxygen levels through PHD3-mediated regulation of PC1 function. Thus, PC1 might regulate mitochondrial functions in response to oxygen availability, although it is likely that the function of the polycystins at MAMs might be regulated by other pathways. Clearly, further studies are needed to improve our understanding of the regulation of the polycystins and their channel activity at MAMs and other cellular locations. The altered calcium transport into mitochondria resulting from loss of PC1 could, in principle, explain the glycolytic switch in PKD. Of note, a cleaved carboxy-terminal fragment of PC1 accumulates in mitochondria and affects their morphology[53], suggesting that PC1 directly modulates mitochondrial function[53].

The reduced oxygen consumption rate in cells lacking polycystins and in animal models of ADPKD might be due to mechanisms other than the polycystin-mediated calcium influx into mitochondria[34,47,54]. For example, the expression of miR-17 is substantially increased in human and mouse ADPKD cells both in vivo and in vitro. miR-17 represses the expression of a master regulator of mitochondrial biogenesis, PPARα[47,55], and in this way, modulates mitochondrial function. Importantly, increasing miR-17 levels by treatment with miR-17 mimics reduces the oxygen consumption rate, which is restored by re-expression of *Ppara*[47]. Furthermore, deletion of the miR-17~92 cluster in two different mouse models of ADPKD resulted in the upregulation of genes involved in OXPHOS and FAO, further supporting a role for PPARα in the regulation of FAO[56]. Interestingly, treatment with an anti-miR-17 oligonucleotide or the PPARα agonist fenofibrate enhances FAO and reduces the kidney weight:body weight ratio, cystic index and proliferation in vivo[57], suggesting that PPARα-mediated metabolic reprogramming plays a crucial role in PKD progression.

Mitochondrial abnormalities are present in *Pkd1*$^{-/-}$ mouse renal epithelial cells, in renal tissue from patients with ADPKD[53] and in both mouse and rat models of ADPKD[54]. Mitochondria are highly dynamic organelles, and their morphology and function are tightly linked. Mitochondrial fragmentation results in decreased mitochondrial respiration and increased production of reactive oxygen species (ROS)[58]. Furthermore, a role for mitochondria in metabolic reprogramming in tumour cells has been suggested, as reduced mitochondrial DNA (mtDNA) copy number correlates with the expression of mitochondrial metabolic genes (and thus mitochondrial activity) in some types of cancer[59]. Interestingly, the mitochondria in cyst-lining cells from Ksp-Cre *Pkd1*$^{flox/flox}$ mice and Han:SPRD$^{Cy/+}$ rats are more fragmented and have increased ROS production and reduced mtDNA copy number compared with their wild-type counterparts[54]. In addition, decreased calcium signalling and increased cAMP levels in these cells resulted in reduced expression of PPARγ co-activator 1α (PGC1α), which is a master regulator of mitochondrial biogenesis. These findings led researchers to propose that, analogous to cancer cells[60], mitochondrial abnormalities and enhanced ROS production in PKD activate extracellular signal-regulated kinase 1 (ERK1)–ERK2 signalling, which leads to increased cell proliferation, suggesting that targeting the mitochondrial defects in PKD slows cyst progression by arresting abnormal cell proliferation (REF.[54]). Interestingly, ERK1–ERK2 activity was also shown to be central in the metabolic shift in the kidneys of *Pkd1*$^{-/-}$ mice and patients with ADPKD, leading these researchers to propose that ERK1–ERK2 reduces AMPK activity by phosphorylating liver kinase B1 (LKB1; also known as STK11) (reducing its ability to activate AMPK) and by increasing mTORC1 signalling, glycolysis and ATP production[33]. Another study, however, has reported that although activation of the mitogen-activated protein kinase (MAPK)–ERK pathway is observed in vivo in an orthologous mouse model of ADPKD, it may not represent the basis for cyst expansion, as treatment with an ERK inhibitor does not slow disease progression[61]. Taken together, these studies suggest that the mitochondrial defects in ADPKD are

upstream and causative of the increased glycolysis and cell proliferation. The cause or causes of the mitochondrial defects in ADPKD, such as the decreased calcium signalling, increased cAMP levels and reduced PGC1α levels induced by *PKD1* or *PKD2* mutations, are not known. Therefore, it is possible that the mitochondrial defects in PKD (caused by reduced function of the polycystin complex, defective FAO and/or other unknown mechanisms) result in reduced OXPHOS and increased ROS production, which in turn activate ERK1–ERK2 signalling, increase glycolysis, inhibit AMPK activity and increase cell proliferation. However, polycystin dysfunction clearly does not result in only metabolic defects, and other pathways are most likely involved in the pathogenesis of PKD. Nonetheless, the metabolic perturbations in ADPKD are a new intriguing area of research and a potential therapeutic target. In healthy conditions, polycystins can regulate mitochondrial function by various molecular mechanisms (FIG. 2).

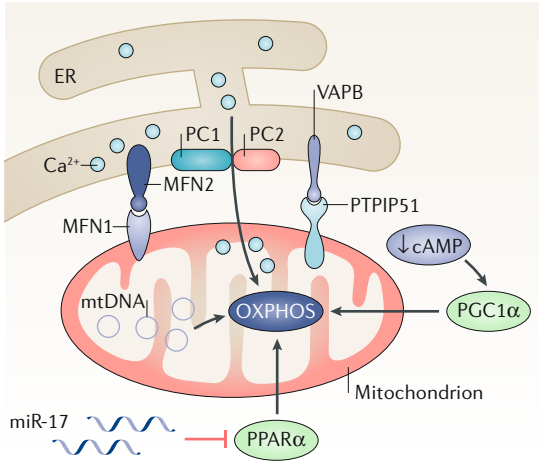

Fig. 2 | **Polycystin proteins positively regulate mitochondrial function by several mechanisms.** The polycystin complex is a heterodimer of polycystin 1 (PC1) and PC2, which is localized in the endoplasmic reticulum (ER) in membranes that are in close apposition to mitochondria, the mitochondria-associated membranes (MAMs). Through this localization and its channel activity, the PC1–PC2 complex directly modulates mitochondrial function by mediating calcium release from the ER and uptake by mitochondria. Mitochondrial function can also be indirectly regulated by the polycystins through their inhibition of miR-17 expression, which results in increased peroxisome proliferator-activated receptor-α (PPARα) levels. Increased PPARα activity stimulates the expression of genes that are involved in fatty acid β-oxidation (FAO), thus increasing substrate availability for mitochondrial energy production. Furthermore, polycystins are implicated in the maintenance of mitochondrial DNA (mtDNA) copy number and prevention of mitochondrial fragmentation, and their activity results in increased Ca^{2+} signalling and decreased cAMP levels, thus increasing expression of PPARγ co-activator 1α (PGC1α). OXPHOS, oxidative phosphorylation; MFN, mitofusin; PTPIP51, protein tyrosine phosphatase interacting protein 51; VAPB, vesicle-associated membrane protein-associated protein B/C.

AMPK regulation of metabolism in ADPKD

As the primary sensor of cellular energy stores, AMPK ensures that metabolic energy production and utilization are coordinated[62,63]. When cellular ATP levels fall, AMP levels increase owing to the activity of adenylate kinase, which generates ATP from ADP, producing AMP in the process[64]. AMPK is a heterotrimeric complex comprising a catalytic α-subunit and a regulatory β-subunit and γ-subunit[65]. The binding of AMP to the γ-subunit induces a conformational change in the α-subunit that exposes Thr172 for phosphorylation by regulatory kinases, including LKB1 and calcium/calmodulin-dependent protein kinase kinases (CaMKKs)[63]. Thus, reduced cellular energy levels lead to the activation of AMPK, which in turn activates energy-generating pathways and inhibits energy-consuming pathways (reviewed in REFS[66,67]); for example, AMPK inhibits the energy-consuming process of fatty acid synthesis by phosphorylating, and thereby inhibiting, acetyl-CoA carboxylase (ACC), and, conversely, AMPK phosphorylation of TBC1 domain family member 1 (TBC1D1) and TBC1D4 stimulates the translocation of glucose transporter type 4 (GLUT4; also known as SLC2A4) to the plasma membrane in skeletal muscle cells, thus increasing their capacity to import glucose and to fuel energy production.

At least two key pathways that are regulated directly by AMPK are linked to ADPKD pathogenesis: mTORC1 signalling and the activity of the channel cystic fibrosis transmembrane conductance regulator (CFTR).

Regulation of mTORC1 signalling by AMPK. mTORC1 integrates information from nutrient sensing pathways[68], and its activation leads to increased protein synthesis and cell growth[69,70]. The catalytic subunit of mTORC1, mTOR kinase, is activated by the small GTP-binding protein RAS homologue enriched in brain (RHEB) in its GTP-bound form[71]. The tuberous sclerosis complex (TSC), which comprises tuberous sclerosis 1 (TSC1; also known as hamartin); TSC2 (also known as tuberin) and TBC1D7, is a RHEB GTPase-activating protein (GAP) that stimulates the intrinsic GTPase activity of RHEB, resulting in GTP hydrolysis and formation of inactive GDP-bound RHEB[71]. AMPK stimulates the GAP activity of the TSC by phosphorylating TSC2, thus inhibiting mTOR kinase activity and consequently reducing the rates of protein synthesis and cell growth[72]. Elevated mTOR activity has been documented in many, but not all, animal models of ADPKD[73] and is thought to be a major driver of the cell proliferation that contributes to cyst progression[74–83] (reviewed in REF.[77]).

Regulation of CFTR by AMPK. CFTR is an ATP-gated chloride channel that is thought to be principally responsible for the secretion of cyst fluid and thereby contributes to cyst dilation[84,85]. AMPK phosphorylation of CFTR reduces the single channel open probability of CFTR and thus its capacity to mediate transepithelial ion transport[86–88]. As both protein synthesis and electrolyte transport are energy-consuming processes, it is perhaps not surprising that activation of AMPK results in inhibition of both processes.

Regulation of cAMP levels by AMPK. AMPK might also modulate a third pathway that has been clearly linked to the pathogenesis of PKD: cAMP signalling. The levels of cAMP are elevated in renal epithelial cells from patients with ADPKD and might contribute directly to cystogenesis[89]. In hepatocytes, AMPK phosphorylates and activates cAMP-specific 3′,5′-cyclic phosphodiesterase 4B (PDE4B), resulting in increased breakdown of cAMP and decreased cellular cAMP levels[90]. Thus, AMPK activity might protect against the elevated cAMP levels that occur in ADPKD cells, although this requires confirmation in renal cells.

AMPK activity is markedly reduced in *Pkd1*$^{-/-}$ mice and minipigs[33,44], which is consistent with AMPK inhibiting cystogenesis pathways. The increased glycolytic production of ATP in *Pkd1*$^{-/-}$ cells might directly suppress AMPK activity[33]. Alternatively, increased ERK1–ERK2 activation caused by elevated cAMP levels could lead to a reduction in AMPK activity[91,92]. In either case, it is tempting to speculate that, in normal conditions, AMPK acts as a brake on mTORC1 signalling and CFTR channel activity. The metabolic alterations in *PKD1*$^{-/-}$ cells are predicted to reduce AMPK activity, resulting in an upregulation of mTORC1 signalling, CFTR channel activity and perhaps cAMP levels, as well as their cystogenic effects. According to this hypothesis, the metabolic changes in cells that lose polycystin expression might be a primary cause of the PKD phenotype rather than a secondary consequence of the morphological and physiological changes that accompany cystic formation.

Interestingly, in this context, loss of LKB1, which is one of the upstream kinases that activate AMPK, substantially exacerbates the cystic phenotype that is associated with loss of the AMPK target and mTOR suppressor protein TSC1 (REF.[93]). Furthermore, the *Lkb1*$^{-/-}$*Tsc1*$^{-/-}$ mice provide further confirmation for the mechanism that has been proposed to account for the metabolic dysregulation in PKD, which suggests that this dysregulation is dependent on alterations in the parallel branches of the mTOR and AMPK pathways acting synergistically with one another[33]. The survival and correct development of the kidneys in these animals is highly dependent on the availability of glutamine as an energy source. Interestingly, restricting the availability of glutamine slows cyst formation in embryonic explants from *Lkb1*$^{-/-}$*Tsc1*$^{-/-}$ mice and from orthologous *Pkd1*-knockout models of renal cystic disease. In addition, treating mice with the glutaminase inhibitor BPTES slows cyst growth in vivo, both in *Pkd1*-knockout mice and in *Lkb1*$^{-/-}$*Tsc1*$^{-/-}$ mice[93].

Metabolic targets for ADPKD therapy

If the metabolic perturbations in ADPKD cells and animal models drive disease pathogenesis rather than simply being a secondary consequence of cyst formation, then targeting these perturbations should slow disease progression. Furthermore, even if the metabolic perturbations themselves are not the primary drivers of cyst formation, the energy production required for the cellular proliferation and ion secretion that mediate cyst dilation means that metabolic pathways are still potential targets for therapeutic intervention. These ideas have motivated the design of several promising interventions that might slow PKD progression (FIG. 3). The simplest method targets the alterations in energy generation in cyst epithelial cells by reducing the systemic availability of nutrients.

Targeting nutrient availability. Glucose uptake into cells requires transport proteins that bind to glucose and facilitate its transport across the plasma membrane. A common method of inhibiting glycolysis involves the use of 2-deoxyglucose, a form of glucose that is transported into cells but that cannot undergo glycolysis and is therefore a competitive inhibitor of glucose utilization in glycolytic pathways[94]. Cyst epithelial cells seem to avidly consume glucose and are highly dependent upon its availability to sustain their growth[33] — thus, they are predicted to be particularly sensitive to even small reductions in glucose levels. Consistent with this hypothesis, growing *PKD1*$^{-/-}$ cells without glucose substantially reduced their growth rate[33]. Furthermore, 2-deoxyglucose treatment slowed the progression of cystic disease in an orthologous mouse model of ADKPD[37,42]. The reported dependence of ADPKD cells on lipid metabolism as an energy source has been similarly tested in animal models. A low-fat diet modestly but significantly reduced cyst burden in an orthologous mouse model of ADPKD[34], and a low-calorie diet dramatically slowed disease development in a mouse model of slowly progressing ADPKD[39,95]. As 2-deoxyglucose has been tested previously in clinical trials as a cancer therapy[96], it should be simple to test its therapeutic use in clinical trials of patients with ADPKD. Similarly, clinical trials of low-fat or low-calorie diets should be straightforward to implement. Promising results in these trials would provide strong support for metabolism as an appropriate target for ADPKD therapies that can be accessed through low-tech and low-cost approaches.

Thiazolidinediones. The thiazolidinediones (also known as glitazones) are PPARγ agonists that have been used extensively to treat metabolic syndrome and type 2 diabetes mellitus. Thiazolidinediones increase sensitivity to insulin and enhance insulin-induced hypoglycaemia, in part through their effects on adipose tissue[97]. Thiazolidinediones inhibit cell proliferation and CFTR channel activity (thus potentially reducing cyst fluid secretion)[98], which has instigated preclinical studies to assess their efficacy as a PKD therapy — thiazolidinediones reduce both cyst growth and the proliferation of cyst-lining cells in several animal models of ADPKD[99–104]. Thiazolidinediones also reduce the circulating levels of both glucose and fatty acids[105], and thus, their beneficial effects in ADPKD might derive, at least in part, from this reduction in cellular energy sources. Consequently, the ameliorative effects of thiazolidinediones, 2-deoxyglucose and calorie restriction on the cystic phenotype might have a common underlying metabolic mechanism.

Targeting mitochondrial dysfunction in ADPKD. The mitochondrial phenotypes in ADPKD have also been explored as potential therapeutic targets. The expression of the oncoprotein MYC is elevated in ADPKD, which leads to increased expression of the miR-17~92

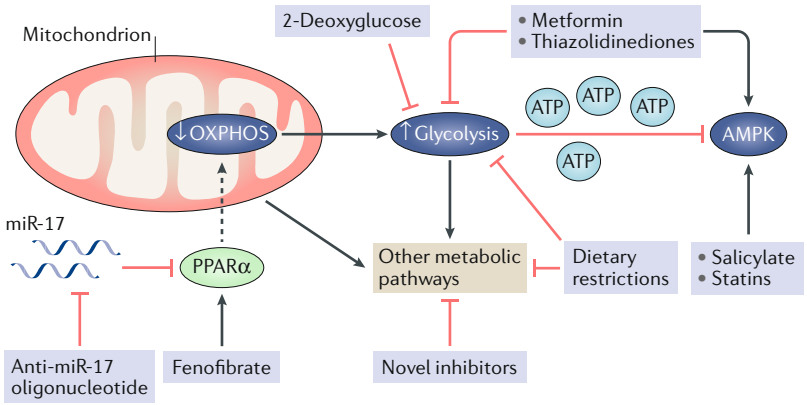

Fig. 3 | **Therapeutic targets in metabolic pathways that are affected in ADPKD.** Reduced oxidative phosphorylation (OXPHOS) in mitochondria stimulates glycolysis and results in increased ATP production and reduced AMP-activated protein kinase (AMPK) activity. These aspects of the metabolic reprogramming in autosomal dominant polycystic kidney disease (ADPKD) can be targeted for therapeutic intervention. The OXPHOS defects in ADPKD can be rescued by modulation of a key regulator of mitochondrial metabolism, peroxisome proliferator-activated receptor-α (PPARα), through, for example, downregulation of the PPARα inhibitor miR-17 (using an anti-miR-17 oligonucleotide) or using the PPARα agonist fenofibrate, which ameliorates the ADPKD cystic phenotype. The increased glycolysis in ADPKD can be targeted using the glycolysis inhibitor 2-deoxyglucose or dietary restriction (such as a low-calorie or low-fat diet), and similar reductions in energy production through glycolysis can also be obtained by treatment with metformin and thiazolidinediones. AMPK is another therapeutic target that can be modulated by the AMPK activators metformin and salicylates and perhaps also by thiazolidinediones and statins. Furthermore, alterations in glycolysis and OXPHOS might result in the deregulation of other metabolic pathways that have not yet been linked to ADPKD, thus increasing the number of possible therapeutic targets.

miRNA cluster[47]. As discussed above, miR-17 reduces the functional capacity of mitochondria through its inhibition of PPARα and OXPHOS, and blocking miR-17 function reduces cyst progression[47]. Thus, activation of PPARα is predicted to overcome at least some of the reduction in mitochondrial function and, by correcting this PKD-associated metabolic defect, might alter the disease course in patients with ADPKD. Treatment with the PPARα agonist fenofibrate increased mitochondrial mass and function and also slowed cyst growth and the proliferation of cyst epithelial cells in a mouse model of ADPKD[57].

Targeting AMPK activity with AMP analogues. The inhibition of several cystogenic pathways by AMPK (see above) has prompted efforts to explore its potential as a therapeutic target. Activation of AMPK can be achieved by several pharmacological approaches, including through its normal physiological regulation by AMP (for example, elevating cytosolic levels of AMP or providing AMP analogues). Small-molecule analogues of AMP, including 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), are highly effective AMPK activators in vitro and produce AMPK-associated physiological effects, such as hypoglycaemia, when administered in vivo in animal models (reviewed in REFS[106,107]). AICAR has been shown to induce hypoglycaemia in clinical trials of patients with type 2 diabetes, although it is not clear whether this effect was entirely through the effects of AICAR on AMPK[108–110]. As AICAR is not orally bioavailable and must be administered by injection, it is less than ideal as a long-term therapy for PKD.

Targeting AMPK activity with metformin. Metformin is a biguanide drug that is widely used to treat type 2 diabetes and polycystic ovary syndrome. Almost a century after the discovery of metformin and more than 50 years after its first clinical use, its mechanisms of action remain the subject of active debate. Metformin inhibits complex I of the mitochondrial electron transport chain, which in turn reduces mitochondrial ATP synthesis. This decrease in ATP levels has been suggested to result in elevated AMP levels, leading to activation of AMPK[111], although metformin seems to also activate AMPK independently of changes in AMP levels[112]. It is not clear whether the hypoglycaemic and anti-gluconeogenic effects of metformin and its effects on AMPK activity are related[113,114], but it is clear that metformin activates AMPK in vitro and in vivo[106,115] and thus could be used for the treatment of PKD. Importantly, metformin suppresses cystogenic pathways in vitro and slows the progression of cystic disease in orthologous mouse models of ADPKD[43], and it suppresses cyst formation in human and mouse renal cells[116] and in *PKD2*$^{-/-}$ zebrafish[117].

Of note, although metformin activates AMPK, it is not clear whether AMPK activity is the sole or even the most important target of metformin. In the liver, adenylyl cyclase activity is inhibited by the elevated AMP levels that result from metformin inhibition of mitochondrial complex I, resulting in increased cAMP degradation and reduced cAMP levels[118]. Furthermore, the anti-gluconeogenic effects of metformin, which are sufficient to reduce fasting blood glucose levels in patients with insulin-resistant type 2 diabetes[119], might reduce the quantity of glucose available to cyst epithelial cells and thus slow cyst progression through the same mechanisms as 2-deoxyglucose. Thus, metformin might slow cyst progression both directly by its activation of AMPK and indirectly through its effects on other pathways. Despite this uncertainty regarding its mechanism of action, its well-established safety profile makes metformin an attractive candidate for clinical trials of PKD treatment. The Metformin as a Novel Therapy for Autosomal Dominant Polycystic Kidney Disease (TAME) trial is currently recruiting patients with ADPKD to assess the safety of metformin and will monitor total kidney volume, renal function and pain, and quality of life of these patients before, during and after a 24-month course of metformin treatment[120].

Targeting AMPK with other agonists. A number of other agents that activate AMPK directly or indirectly have been shown to slow ADPKD progression, both in preclinical models and in clinical trials. In addition to activating AMPK through their effects on PPARγ, thiazolidinediones might, like metformin, inhibit mitochondrial complex I and thus elevate cytosolic levels of AMP[121–123]. Thus, some of the beneficial effects of thiazolidinediones in preclinical studies of ADPKD might be attributable to their effects on AMPK via AMP levels. Similarly, statins are hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors that have shown

promise in ameliorating cystic phenotypes in animal models of ADPKD as well as in clinical trials in paediatric patients with early-onset ADPKD[124–129]. Although HMG-CoA reductase and cholesterol synthesis are their primary targets, statins also seem to activate AMPK through mechanisms that are yet to be elucidated[130–132], and at least some of their anti-cystic effects might be mediated through their activation of AMPK. Finally, salicylates, such as aspirin, potently activate AMPK by directly binding to it independently of AMP levels[133,134]. Interestingly, salicylates might slow cyst progression in a rat model of PKD[135]. In conclusion, it seems that numerous compounds with well-understood pharmacological properties activate AMPK and show at least some promise as potential treatments for ADPKD. In future studies, it will be interesting to determine whether the PKD-suppressive effects of these drugs are due entirely or in part to their activation of AMPK and whether combinations of these drugs produce synergistic effects on AMPK activation and cyst suppression.

Conclusions

Although the biological roles of the polycystin proteins have not been unambiguously established, it is clear that a multitude of cellular and metabolic processes are altered in the absence of polycystins. Which of these perturbations drive cyst formation and how the polycystin proteins function in such a diverse assemblage of interwoven pathways are unclear. However, a consensus is building that cells lacking polycystins exhibit substantial metabolic disruptions, including alterations in both glucose and fatty acid metabolism. Growing evidence suggests that the biogenesis of mitochondria and/or regulation of their function are, at least in part, the proximate cause of these metabolic disruptions in PKD. The altered metabolic state in PKD cells might lead to reduced activity of the energy-sensing kinase AMPK, which in healthy individuals inhibits a number of cellular pathways that are thought to contribute to cyst growth. Although the precise origin of these metabolic alterations is the subject of active research, these alterations have already emerged as appealing targets for novel PKD therapies. Simple treatment strategies, such as calorie reduction, exploit the altered energy requirements of PKD cells to dramatically slow cyst progression. Furthermore, a number of agents that have shown efficacy in preclinical models and in human trials might exert their therapeutic benefits, at least in part, by altering the availability of different energy sources or by modulating the activity of the energy sensor AMPK. Metabolism is clearly an exciting new focus in ADPKD research and, as our understanding improves, it promises to be a fertile source of novel, readily testable therapeutic strategies.

Published online 17 August 2018

1. Harris, P. C. & Torres, V. E. Genetic mechanisms and signaling pathways in autosomal dominant polycystic kidney disease. *J. Clin. Invest.* **124**, 2315–2324 (2014).
2. Solazzo, A. et al. The prevalence of autosomal dominant polycystic kidney disease (ADPKD): a meta-analysis of European literature and prevalence evaluation in the Italian province of Modena suggest that ADPKD is a rare and underdiagnosed condition. *PLoS ONE* **13**, e0190430 (2018).
3. Willey, C. J. et al. Prevalence of autosomal dominant polycystic kidney disease in the European Union. *Nephrol. Dial Transplant* **32**, 1356–1363 (2017).
4. Halvorson, C. R., Bremmer, M. S. & Jacobs, S. C. Polycystic kidney disease: inheritance, pathophysiology, prognosis, and treatment. *Int. J. Nephrol. Renovasc Dis.* **3**, 69–83 (2010).
5. Gabow, P. A. Autosomal dominant polycystic kidney disease. *N. Engl. J. Med.* **329**, 332–342 (1993).
6. Cornec-Le Gall, E. et al. Type of PKD1 mutation influences renal outcome in ADPKD. *J. Am. Soc. Nephrol.* **24**, 1006–1013 (2013).
7. Rossetti, S. et al. Comprehensive molecular diagnostics in autosomal dominant polycystic kidney disease. *J. Am. Soc. Nephrol.* **18**, 2143–2160 (2007).
8. Audrezet, M. P. et al. Autosomal dominant polycystic kidney disease: comprehensive mutation analysis of PKD1 and PKD2 in 700 unrelated patients. *Hum. Mutat.* **33**, 1239–1250 (2012).
9. Porath, B. et al. Mutations in GANAB, encoding the glucosidase iiα subunit, cause autosomal-dominant polycystic kidney and liver disease. *Am. J. Hum. Genet.* **98**, 1193–1207 (2016).
10. Qian, F., Watnick, T. J., Onuchic, L. F. & Germino, G. G. The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type I. *Cell* **87**, 979–987 (1996).
11. Hopp, K. et al. Functional polycystin-1 dosage governs autosomal dominant polycystic kidney disease severity. *J. Clin. Invest.* **122**, 4257–4273 (2012).
12. Leeuwen, I. S. L.-v. et al. Lowering of Pkd1 expression is sufficient to cause polycystic kidney disease. *Hum. Mol. Genet.* **13**, 3069–3077 (2004).
13. Rossetti, S. et al. Incompletely penetrant PKD1 alleles suggest a role for gene dosage in cyst initiation in polycystic kidney disease. *Kidney Int.* **75**, 848–855 (2009).
14. Losekoot, M. et al. Neonatal onset autosomal dominant polycystic kidney disease (ADPKD) in a patient homozygous for a *PKD2* missense mutation due to uniparental disomy. *J. Med. Genet.* **49**, 37 (2012).
15. van Adelsberg, J. S. & Frank, D. The PKD1 gene produces a developmentally regulated protein in mesenchyme and vasculature. *Nat. Med.* **1**, 359 (1995).
16. Nims, N., Vassmer, D. & Maser, R. L. Transmembrane domain analysis of polycystin-1, the product of the polycystic kidney disease-1 (PKD1) gene: evidence for 11 membrane-spanning domains. *Biochemistry* **42**, 13035–13048 (2003).
17. Cai, Y. et al. Identification and characterization of polycystin-2, the PKD2 gene product. *J. Biol. Chem.* **274**, 28557–28565 (1999).
18. Mochizuki, T. et al. *PKD2*, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* **272**, 1339–1342 (1996).
19. Gonzalez-Perrett, S. et al. Polycystin-2, the protein mutated in autosomal dominant polycystic kidney disease (ADPKD), is a Ca^{2+}-permeable nonselective cation channel. *Proc. Natl Acad. Sci. USA* **98**, 1182–1187 (2001).
20. Koulen, P. et al. Polycystin-2 is an intracellular calcium release channel. *Nat. Cell Biol.* **4**, 191–197 (2002).
21. Qian, F. et al. PKD1 interacts with PKD2 through a probable coiled-coil domain. *Nat. Genet.* **16**, 179 (1997).
22. Tsiokas, L., Kim, E., Arnould, T., Sukhatme, V. P. & Walz, G. Homo- and heterodimeric interactions between the gene products of PKD1 and PKD2. *Proc. Natl Acad. Sci.* **94**, 6965–6970 (1997).
23. Salehi-Najafabadi, Z. et al. Extracellular loops are essential for the assembly and function of polycystin receptor-ion channel complexes. *J. Biol. Chem.* **292**, 4210–4221 (2017).
24. Yoder, B. K., Hou, X. & Guay-Woodford, L. M. The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *J. Am. Soc. Nephrol.* **13**, 2508–2516 (2002).
25. Hogan, M. C. et al. Characterization of PKD protein-positive exosome-like vesicles. *J. Am. Soc. Nephrol.* **20**, 278–288 (2009).
26. Scheffers, M. S. et al. Distinct subcellular expression of endogenous polycystin-2 in the plasma membrane and Golgi apparatus of MDCK cells. *Hum. Mol. Genet.* **11**, 59–67 (2002).
27. Ibraghimov-Beskrovnaya, O. et al. Polycystin: in vitro synthesis, in vivo tissue expression, and subcellular localization identifies a large membrane-associated protein. *Proc. Natl Acad. Sci.* **94**, 6397–6402 (1997).
28. Nauli, S. M. et al. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat. Genet.* **33**, 129 (2003).
29. Delling, M., DeCaen, P. G., Doerner, J. F., Febvay, S. & Clapham, D. E. Primary cilia are specialized calcium signalling organelles. *Nature* **504**, 311 (2013).
30. Hildebrandt, F., Benzing, T. & Katsanis, N. Ciliopathies. *N. Engl. J. Med.* **364**, 1533–1543 (2011).
31. Saigusa, T. & Bell, P. D. Molecular pathways and therapies in autosomal-dominant polycystic kidney disease. *Physiology* **30**, 195–207 (2015).
32. Takiar, V. & Caplan, M. J. Polycystic kidney disease: pathogenesis and potential therapies. *Biochim. Biophys. Acta* **1812**, 1337–1343 (2011).
33. Rowe, I. et al. Defective glucose metabolism in polycystic kidney disease identifies a new therapeutic strategy. *Nat. Med.* **19**, 488–493 (2013).
This study provides early evidence of metabolic reprogramming in ADPKD.
34. Menezes, L. F., Lin, C., Zhou, F. & Germino, G. G. Fatty acid oxidation is impaired in an orthologous mouse model of autosomal dominant polycystic kidney disease. *EBioMedicine* **5**, 183–192 (2016).
35. Priolo, C. & Henske, E. P. Metabolic reprogramming in polycystic kidney disease. *Nat. Med.* **19**, 407 (2013).
36. Vander Heiden, M. G., Cantley, L. C. & Thompson, C. B. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* **324**, 1029–1033 (2009).
37. Riwanto, M. et al. Inhibition of aerobic glycolysis attenuates disease progression in polycystic kidney disease. *PLoS ONE* **11**, e0146654 (2016).
38. Chen, L. et al. Macrophage migration inhibitory factor promotes cyst growth in polycystic kidney disease. *J. Clin. Invest.* **125**, 2399–2412 (2015).
39. Warner, G. et al. Food restriction ameliorates the development of polycystic kidney disease. *J. Am. Soc. Nephrol.* **27**, 1437–1447 (2016).
This study shows that the metabolic alterations in ADPKD can be targeted by dietary changes, resulting in reduced cyst progression.

40. Muir, A. et al. Environmental cystine drives glutamine anaplerosis and sensitizes cancer cells to glutaminase inhibition. *eLife* **6**, e27713 (2017).
41. Cantor, J. R. et al. Physiologic medium rewires cellular metabolism and reveals uric acid as an endogenous inhibitor of UMP synthase. *Cell* **169**, 258–272.e17 (2017).
42. Chiaravalli, M. et al. 2-deoxy-d-glucose ameliorates PKD progression. *J. Am. Soc. Nephrol.* **27**, 1958–1969 (2016).
43. Takiar, V. et al. Activating AMP-activated protein kinase (AMPK) slows renal cystogenesis. *Proc. Natl Acad. Sci. USA* **108**, 2462–2467 (2011).
This study provides evidence that the energy sensor AMPK might be a target of drugs that were developed to slow cyst progression.
44. Lian, X. et al. The changes in glucose metabolism and cell proliferation in the kidneys of polycystic kidney disease mini-pig models. *Biochem. Biophys. Res. Commun.* **488**, 374–381 (2017).
45. Menezes, L. F. et al. Network analysis of a Pkd1-mouse model of autosomal dominant polycystic kidney disease identifies HNF4α as a disease modifier. *PLoS Genet.* **8**, e1003053 (2012).
46. Yamagata, K. et al. Mutations in the hepatocyte nuclear factor-4α gene in maturity-onset diabetes of the young (MODY1). *Nature* **384**, 458 (1996).
47. Hajarnis, S. et al. microRNA-17 family promotes polycystic kidney disease progression through modulation of mitochondrial metabolism. *Nat. Commun.* **8**, 14395 (2017).
This study demonstrates that elevated expression of miR-17 might account for some of the mitochondrial alterations in ADPKD and that miR-17 might be a therapeutic target.
48. Song, X. et al. Systems biology of autosomal dominant polycystic kidney disease (ADPKD): computational identification of gene expression pathways and integrated regulatory networks. *Hum. Mol. Genet.* **18**, 2328–2343 (2009).
49. Hackl, A. et al. Disorders of fatty acid oxidation and autosomal recessive polycystic kidney disease-different clinical entities and comparable perinatal renal abnormalities. *Pediatr. Nephrol.* **32**, 791–800 (2017).
50. Whitfield, J. et al. Fetal polycystic kidney disease associated with glutaric aciduria type ii: an inborn error of energy metabolism. *Amer J. Perinatol* **13**, 131–134 (1996).
51. Jayapalan, S., Saboorian, M. H., Edmunds, J. W. & Aukema, H. M. High dietary fat intake increases renal cyst disease progression in Han:SPRD-cy rats. *J. Nutr.* **130**, 2356–2360 (2000).
52. Padovano, V. et al. The polycystins are modulated by cellular oxygen-sensing pathways and regulate mitochondrial function. *Mol. Biol. Cell* **28**, 261–269 (2017).
This study provides evidence that reduced calcium uptake by mitochondria is a potential cause of mitochondrial dysfunction in ADPKD cells.
53. Lin, C. C. et al. A cleavage product of Polycystin-1 is a mitochondrial matrix protein that affects mitochondria morphology and function when heterologously expressed. *Sci. Rep.* **8**, 2743 (2018).
54. Ishimoto, Y. et al. Mitochondrial abnormality facilitates cyst formation in autosomal dominant polycystic kidney disease. *Mol. Cell. Biol.* **37**, e00337-17 (2017).
This research shows that both mitochondrial function and morphology are altered in ADPKD.
55. Fan, W. & Evans, R. PPARs and ERRs: molecular mediators of mitochondrial metabolism. *Curr. Opin. Cell Biol.* **33**, 49–54 (2015).
56. Desvergne, B.a. & Wahli, W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev.* **20**, 649–688 (1999).
57. Lakhia, R. et al. PPARα agonist fenofibrate enhances fatty acid β-oxidation and attenuates polycystic kidney and liver disease in mice. *Am. J. Physiol. Renal Physiol.* **314**, F122–F131 (2018).
58. Picard, M., Shirihai, O. S., Gentil, B. J. & Burelle, Y. Mitochondrial morphology transitions and functions: implications for retrograde signaling? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **304**, R393–R406 (2013).
59. Reznik, E. et al. Mitochondrial DNA copy number variation across human cancers. *eLife* **5**, e10769 (2016).
60. Weinberg, F. et al. Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. *Proc. Natl Acad. Sci.* **107**, 8788–8793 (2010).
61. Shibazaki, S. et al. Cyst formation and activation of the extracellular regulated kinase pathway after kidney specific inactivation of Pkd1. *Hum. Mol. Genet.* **17**, 1505–1516 (2008).
62. Hardie, D. G. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat. Rev. Mol. Cell Biol.* (2007).
63. Towler, M. C. & Hardie, D. G. AMP-activated protein kinase in metabolic control and insulin signaling. *Circ. Res.* **100**, 328–341 (2007).
64. Atkinson, D. E. & Walton, G. M. Adenosine triphosphate conservation in metabolic regulation. Rat liver citrate cleavage enzyme. *J. Biol. Chem.* **242**, 3239–3241 (1967).
65. Davies, S. P. et al. Purification of the AMP-activated protein kinase on ATP-γ-sepharose and analysis of its subunit structure. *Eur. J. Biochem.* **223**, 351–357 (1994).
66. Hardie, D. G. & Sakamoto, K. AMPK: a key sensor of fuel and energy status in skeletal muscle. *Physiology* **21**, 48–60 (2006).
67. Hardie, D. G. & Pan, D. A. Regulation of fatty acid synthesis and oxidation by the AMP-activated protein kinase. *Biochem. Soc. Trans.* **30**, 1064–1070 (2002).
68. Sarbassov, D. D., Ali, S. M. & Sabatini, D. M. Growing roles for the mTOR pathway. *Curr. Opin. Cell Biol.* **17**, 596–603 (2005).
69. Mamane, Y., Petroulakis, E., LeBacquer, O. & Sonenberg, N. mTOR, translation initiation and cancer. *Oncogene* **25**, 6416–6422 (2006).
70. Dowling, R. J. et al. mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. *Science* **328**, 1172–1176 (2010).
71. Astrinidis, A. & Henske, E. P. Tuberous sclerosis complex: linking growth and energy signaling pathways with human disease. *Oncogene* **24**, 7475–7481 (2005).
72. Inoki, K., Zhu, T. & Guan, K. L. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **115**, 577–590 (2003).
73. Ma, M., Tian, X., Igarashi, P., Pazour, G. J. & Somlo, S. Loss of cilia suppresses cyst growth in genetic models of autosomal dominant polycystic kidney disease. *Nat. Genet.* **45**, 1004–1012 (2013).
74. Shillingford, J. M. et al. The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. *Proc. Natl Acad. Sci. USA* **103**, 5466–5471 (2006).
75. Becker, J. U. et al. The mTOR pathway is activated in human autosomal-recessive polycystic kidney disease. *Kidney Blood Press Res.* **33**, 129–138 (2010).
76. Belibi, F., Ravichandran, K., Zafar, I., He, Z. & Edelstein, C. L. mTORC1/2 and rapamycin in female Han:SPRD rats with polycystic kidney disease. *Am. J. Physiol. Renal Physiol.* **300**, F236–244 (2011).
77. Distefano, G. et al. Polycystin-1 regulates extracellular signal-regulated kinase-dependent phosphorylation of tuberin to control cell size through mTOR and its downstream effectors S6K and 4EBP1. *Mol. Cell. Biol.* **29**, 2359–2371 (2009).
78. Fischer, D. C. et al. Activation of the AKT/mTOR pathway in autosomal recessive polycystic kidney disease (ARPKD). *Nephrol. Dial Transplant* **24**, 1819–1827 (2009).
79. Ibraghimov-Beskrovnaya, O. & Natoli, T. A. mTOR signaling in polycystic kidney disease. *Trends Mol. Med.* **17**, 625–633 (2011).
80. Wahl, P. R. et al. Inhibition of mTOR with sirolimus slows disease progression in Han:SPRD rats with autosomal dominant polycystic kidney disease (ADPKD). *Nephrol. Dial Transplant* **21**, 598–604 (2006).
81. Wu, M. et al. Everolimus retards cyst growth and preserves kidney function in a rodent model for polycystic kidney disease. *Kidney Blood Press Res.* **30**, 253–259 (2007).
82. Zafar, I., Belibi, F. A., He, Z. & Edelstein, C. L. Long-term rapamycin therapy in the Han:SPRD rat model of polycystic kidney disease (PKD). *Nephrol. Dial Transplant* **24**, 2349–2353 (2009).
83. Zafar, I., Ravichandran, K., Belibi, F. A., Doctor, R. B. & Edelstein, C. L. Sirolimus attenuates disease progression in an orthologous mouse model of human autosomal dominant polycystic kidney disease. *Kidney Int.* **78**, 754–761 (2010).
84. Davidow, C. J., Maser, R. L., Rome, L. A., Calvet, J. P. & Grantham, J. J. The cystic fibrosis transmembrane conductance regulator mediates transepithelial fluid secretion by human autosomal dominant polycystic kidney disease epithelium in vitro. *Kidney Int.* **50**, 208–218 (1996).
85. Magenheimer, B. S. et al. Early embryonic renal tubules of wild-type and polycystic kidney disease kidneys respond to cAMP stimulation with cystic fibrosis transmembrane conductance regulator/Na(+),K(+),2Cl(-) Co-transporter-dependent cystic dilation. *J. Am. Soc. Nephrol.* **17**, 3424–3437 (2006).
86. Hallows, K. R., Kobinger, G. P., Wilson, J. M., Witters, L. A. & Foskett, J. K. Physiological modulation of CFTR activity by AMP-activated protein kinase in polarized T84 cells. *Am. J. Physiol. Cell Physiol.* **284**, C1297–C1308 (2003).
87. Hallows, K. R., McCane, J. E., Kemp, B. E., Witters, L. A. & Foskett, J. K. Regulation of channel gating by AMP-activated protein kinase modulates cystic fibrosis transmembrane conductance regulator activity in lung submucosal cells. *J. Biol. Chem.* **278**, 998–1004 (2003).
88. Hallows, K. R., Raghuram, V., Kemp, B. E., Witters, L. A. & Foskett, J. K. Inhibition of cystic fibrosis transmembrane conductance regulator by novel interaction with the metabolic sensor AMP-activated protein kinase. *J. Clin. Invest.* **105**, 1711–1721 (2000).
89. Belibi, F. A. et al. Cyclic AMP promotes growth and secretion in human polycystic kidney epithelial cells. *Kidney Int.* **66**, 964–973 (2004).
90. Johanns, M. et al. AMPK antagonizes hepatic glucagon-stimulated cyclic AMP signalling via phosphorylation-induced activation of cyclic nucleotide phosphodiesterase 4B. *Nat. Commun.* **7**, 10856 (2016).
91. López-Cotarelo, P. et al. A novel MEK-ERK-AMPK signaling axis controls chemokine receptor CCR7-dependent survival in human mature dendritic cells. *J. Biol. Chem.* **290**, 827–840 (2015).
92. Damm, E., Buech, T. R. H., Gudermann, T. & Breit, A. Melanocortin-induced PKA activation inhibits AMPK activity via ERK-1/2 and LKB-1 in hypothalamic GT1-7 cells. *Mol. Endocrinol.* **26**, 643–654 (2012).
93. Flowers, E. M. et al. Lkb1 deficiency confers glutamine dependency in polycystic kidney disease. *Nat. Commun.* **9**, 814 (2018).
94. Wick, A. N., Drury, D. R., Nakada, H. I. & Wolfe, J. B. Localization of the primary metabolic block produced by 2-deoxyglucose. *J. Biol. Chem.* **224**, 963–969 (1957).
95. Kipp, K. R., Rezaei, M., Lin, L., Dewey, E. C. & Weimbs, T. A mild reduction of food intake slows disease progression in an orthologous mouse model of polycystic kidney disease. *Am. J. Physiol. Renal Physiol.* **310**, F726–F731 (2016).
96. Pelicano, H., Martin, D. S., Xu, R. H. & Huang, P. Glycolysis inhibition for anticancer treatment. *Oncogene* **25**, 4633–4646 (2006).
97. Mudaliar, S. & Henry, R. R. New oral therapies for type 2 diabetes mellitus: the glitazones or insulin sensitizers. *Annu. Rev. Med.* **52**, 239–257 (2001).
98. Nofziger, C. et al. PPARγ agonists inhibit vasopressin-mediated anion transport in the MDCK-C7 cell line. *Am. J. Physiol. Renal Physiol.* **297**, F55–F62 (2009).
99. Dai, B. et al. Rosiglitazone attenuates development of polycystic kidney disease and prolongs survival in Han:SPRD rats. *Clin. Sci.* **119**, 323–333 (2010).
100. Muto, S. et al. Pioglitazone improves the phenotype and molecular defects of a targeted Pkd1 mutant. *Hum. Mol. Genet.* **11**, 1731–1742 (2002).
101. Raphael, K. L. et al. Effect of pioglitazone on survival and renal function in a mouse model of polycystic kidney disease. *Am. J. Nephrol.* **30**, 468–473 (2009).
102. Yoshihara, D. et al. PPAR-γ agonist ameliorates kidney and liver disease in an orthologous rat model of human autosomal recessive polycystic kidney disease. *Am. J. Physiol. Renal Physiol.* **300**, F465–F474 (2011).
103. Blazer-Yost, B. L. et al. Pioglitazone attenuates cystic burden in the PCK rodent model of polycystic kidney disease. *PPAR Res.* **2010**, 274376 (2010).
104. Flaig, S. M., Gattone, V. H. & Blazer-Yost, B. L. Inhibition of cyst growth in PCK and Wpk rat models of polycystic kidney disease with low doses of peroxisome proliferator-activated receptor γ agonists. *J. Transl Int. Med.* **4**, 118–126 (2016).
105. Nolan, J. J., Ludvik, B., Beerdsen, P., Joyce, M. & Olefsky, J. Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. *N. Engl. J. Med.* **331**, 1188–1193 (1994).
106. Hardie, D. G., Ross, F. A. & Hawley, S. A. AMP-activated protein kinase: a target for drugs both ancient and modern. *Chem. Biol.* **19**, 1222–1236 (2012).

107. Kim, J., Yang, G., Kim, Y., Kim, J. & Ha, J. AMPK activators: mechanisms of action and physiological activities. *Exp. Mol. Med.* **48**, e224 (2016).
108. Babraj, J. A. et al. Blunting of AICAR-induced human skeletal muscle glucose uptake in type 2 diabetes is dependent on age rather than diabetic status. *Am. J. Physiol. Endocrinol. Metab.* **296**, E1042–1048 (2009).
109. Boon, H. et al. Intravenous AICAR administration reduces hepatic glucose output and inhibits whole body lipolysis in type 2 diabetic patients. *Diabetologia* **51**, 1893–1900 (2008).
110. Cuthbertson, D. J. et al. 5-Aminoimidazole-4-carboxamide 1-β-D-ribofuranoside acutely stimulates skeletal muscle 2-deoxyglucose uptake in healthy men. *Diabetes* **56**, 2078–2084 (2007).
111. Rena, G., Hardie, D. G. & Pearson, E. R. The mechanisms of action of metformin. *Diabetologia* **60**, 1577–1585 (2017).
112. Hawley, S. A., Gadalla, A. E., Olsen, G. S. & Hardie, D. G. The antidiabetic drug metformin activates the AMP-activated protein kinase cascade via an adenine nucleotide-independent mechanism. *Diabetes* **51**, 2420–2425 (2002).
113. Foretz, M. et al. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *J. Clin. Invest.* **120**, 2355–2369 (2010).
114. Shaw, R. J. et al. The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science* **310**, 1642–1646 (2005).
115. Seo-Mayer, P. W. et al. Preactivation of AMPK by metformin may ameliorate the epithelial cell damage caused by renal ischemia. *Am. J. Physiol. Renal Physiol.* **301**, F1346–F1357 (2011).
116. Mekahli, D. et al. Polycystin-1 but not polycystin-2 deficiency causes upregulation of the mTOR pathway and can be synergistically targeted with rapamycin and metformin. *Pflugers Arch.* **466**, 1591–1604 (2014).
117. Chang, M. Y. et al. Metformin inhibits cyst formation in a zebrafish model of polycystin-2 deficiency. *Sci. Rep.* **7**, 7161 (2017).
118. Miller, R. A. et al. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. *Nature* **494**, 256–260 (2013).
119. Cusi, K., Consoli, A. & DeFronzo, R. A. Metabolic effects of metformin on glucose and lactate metabolism in noninsulin-dependent diabetes mellitus. *J. Clin. Endocrinol. Metab.* **81**, 4059–4067 (1996).
120. Seliger, S. L. et al. A randomized clinical trial of metformin to treat autosomal dominant polycystic kidney disease. *Am. J. Neph.* **47**, 352–360 (2018).
121. Fryer, L. G., Parbu-Patel, A. & Carling, D. The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. *J. Biol. Chem.* **277**, 25226–25232 (2002).
122. LeBrasseur, N. K. et al. Thiazolidinediones can rapidly activate AMP-activated protein kinase in mammalian tissues. *Am. J. Physiol. Endocrinol. Metab.* **291**, E175–E181 (2006).
123. Brunmair, B. et al. Thiazolidinediones, like metformin, inhibit respiratory complex I: a common mechanism contributing to their antidiabetic actions? *Diabetes* **53**, 1052–1059 (2004).
124. Fassett, R. G., Coombes, J. S., Packham, D., Fairley, K. F. & Kincaid-Smith, P. Effect of pravastatin on kidney function and urinary protein excretion in autosomal dominant polycystic kidney disease. *Scand. J. Urol. Nephrol.* **44**, 56–61 (2010).
125. Gile, R. D. et al. Effect of lovastatin on the development of polycystic kidney disease in the Han:SPRD rat. *Am. J. Kidney Dis.* **26**, 501–507 (1995).
126. Klawitter, J. et al. Effects of lovastatin treatment on the metabolic distributions in the Han:SPRD rat model of polycystic kidney disease. *BMC Nephrol.* **14**, 165 (2013).
127. Namli, S. et al. Improvement of endothelial dysfunction with simvastatin in patients with autosomal dominant polycystic kidney disease. *Ren. Fail.* **29**, 55–59 (2007).
128. van Dijk, M. A., Kamper, A. M., van Veen, S., Souverijn, J. H. & Blauw, G. J. Effect of simvastatin on renal function in autosomal dominant polycystic kidney disease. *Nephrol. Dial Transplant* **16**, 2152–2157 (2001).
129. Zafar, I. et al. Effect of statin and angiotensin-converting enzyme inhibition on structural and hemodynamic alterations in autosomal dominant polycystic kidney disease model. *Am. J. Physiol. Renal Physiol.* **293**, F854–F859 (2007).
130. Babcook, M. A. et al. Synergistic simvastatin and metformin combination chemotherapy for osseous metastatic castration-resistant prostate cancer. *Mol. Cancer Ther.* **13**, 2288–2302 (2014).
131. Sun, W. et al. Statins activate AMP-activated protein kinase in vitro and in vivo. *Circulation* **114**, 2655–2662 (2006).
132. Ma, L., Niknejad, N., Gorn-Hondermann, I., Dayekh, K. & Dimitroulakos, J. Lovastatin induces multiple stress pathways including LKB1/AMPK activation that regulate its cytotoxic effects in squamous cell carcinoma cells. *PLoS ONE* **7**, e46055 (2012).
133. Hawley, S. A. et al. The ancient drug salicylate directly activates AMP-activated protein kinase. *Science* **336**, 918–922 (2012).
134. Yue, W., Yang, C. S., DiPaola, R. S. & Tan, X. L. Repurposing of metformin and aspirin by targeting AMPK-mTOR and inflammation for pancreatic cancer prevention and treatment. *Cancer Prev. Res. (Phila)* **7**, 388–397 (2014).
135. Ibrahim, N. H. et al. Cyclooxygenase product inhibition with acetylsalicylic acid slows disease progression in the Han:SPRD-Cy rat model of polycystic kidney disease. *Prostaglandins Other Lipid Mediat.* **116–117**, 19–25 (2015).

Acknowledgements
The authors thank their colleagues in their laboratories for very helpful and thought-provoking discussions. Relevant work from the authors' laboratories was supported by the US National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) grant R01DK072614 (M.J.C.), US Department of Defense (DoD) grant W81XWH-15-1-0419 (M.J.C.), the Italian Ministry of Health grant RF11-12 (A.B.), the PKD Foundation grant 187G14a/b (A.B.) and the Italian Association for PKD (AIRP) (A.B.).

Author contributions
All authors contributed to researching data for the article, discussion of its content, writing the article and reviewing and editing the manuscript before submission.

Competing interests
The authors declare no competing interests.

Publisher's note

Reviewer information
Nature Reviews Nephrology thanks J. Calvet, V. Torres and the other anonymous reviewer(s) for their contribution to the peer review of this work.