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ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent inherited kidney disease. Transepithelial fluid secretion is one of the key factors of cystogenesis in ADPKD. Multiple studies have suggested that fluid secretion across ADPKD cyst-lining cells is driven by the secretion of chloride, essentially mediated by the CFTR channel and stimulated by increased intracellular levels of 3',5'-cyclic adenosine monophosphate. This review focuses on the pathophysiology of fluid secretion in ADPKD based on the pioneering studies of Jared Grantham and colleagues, and on the follow-up investigations from the molecular level to the potential applications in ADPKD patients. Altogether, the studies of fluid and chloride transport in ADPKD paved the way for innovative therapeutic targets to prevent cyst volume expansion and thus, kidney disease progression.

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Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent inherited kidney disease. Transepithelial fluid secretion is one of the key factors of cystogenesis in ADPKD. Multiple studies have suggested that fluid secretion across ADPKD cyst-lining cells is driven by the secretion of chloride, essentially mediated by the CFTR channel and stimulated by increased intracellular levels of 3',5'-cyclic adenosine monophosphate. This review focuses on the pathophysiology of fluid secretion in ADPKD based on the pioneering studies of Jared Grantham and colleagues, and on the follow-up investigations from the molecular level to the potential applications in ADPKD patients. Altogether, the studies of fluid and chloride transport in ADPKD paved the way for innovative therapeutic targets to prevent cyst volume expansion and thus, kidney disease progression.

In 1987, J. Grantham and colleagues reported on a detailed characterization of cysts from 10 sets of kidneys obtained from adult patients with ADPKD. They remarkably observed that the cystic fluid does not originate from the glomerular filtrate but rather from transepithelial fluid secretion [20]. As pointed by Grantham in his 2003 Lillian Jean Kaplan Prize lecture, "respectable mammalian renal tubules are supposed to reabsorb glomerular filtrate [...] and I...
was going to tell that our sacred nephron was converted into the equivalent of a tear duct, or a sweat duct...” [21]. Yet, in original experiments conducted in the early 1970's, Grantham demonstrated that isolated proximal tubules perfused in vitro change from absorbing to secreting fluid when a solute (e.g. para-aminobiphurate, PAH) is added to the external bath and secreted into the lumen by the tubular cells [22]. The existence of such a fluid secretions, regulated by 3’,5’-cyclic adenosine monophosphate (cAMP) is now considered as a key driver for the formation and expansion of kidney cysts in ADPKD [1,21,23–25]. Here, we will briefly review the pathophysiology of fluid secretion in ADPKD based on the classic studies performed by Grantham and colleagues, and the translational insights that led to novel therapeutic pathways in ADPKD.

Electrophysiological properties of kidney cysts

A first step in understanding cyst formation in ADPKD came from the analysis of cyst fluid composition. Assuming that kidney cysts arise from any tubular segment, cyst fluid composition should reflect its tubular origin. Gardner classified ADPKD cysts into two categories according to the Na+ concentration above or below 100 mmol/l [26]. Huseman and Grantham suggested that cysts with a low [Na+] (“gradient cysts”) derive from tight distal nephron segments, whereas cysts exhibiting a high [Na+] (“no gradient cysts”) most probably derive from leaky proximal tubule segments [27]. By measuring the electrical parameters of cyst-line epithelia mounted in Ussing chambers, Per- rone showed that “gradient cysts” exhibit a transepithelial resistance similar to tight epithelia, with a transepithelial potential difference (∼5 mV, lumen negative) inhabitable by apical amiloride [28]. These observations indicate that the current electrical of Na+ re- absorption. Note that a net secretory flux of Na across monolayers of cystic cells was also reported, potentially driven by mispolarized Na+-K+-ATPase or, alternatively, by a primary secretion of Cl− [29].

Transepithelial chloride secretion in ADPKD

The existence of abnormal fluid secretion through the epithelial cells lining the ADPKD cysts came from the observation that a kidney cyst rapidly fills again after drainage [30]. The classical studies of Grantham and colleagues deciphered the in vitro and ex vivo mechanisms involved in ADPKD-associated fluid secretion [23,31]. The transport of Cl− was established as the driving force of fluid secretion in monolayers of ADPKD cyst cells [23,32,33]. Fluid secretion could be stimulated by adenylyl cyclase agonists, as well as by the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) or by 8-Br-cAMP, a form of cAMP insensitive to hydrolysis by phosphodiesterase. Note that forskolin-induced fluid secretion was associated with an increased electronegativity in cyst lumen. Conversely, the addition of bumetanide in the basolateral medium or the Cl− channel inhibitor diphenylamine-2-carboxylate (DPC) on the apical side inhibited fluid secretion in cystic cells [32,33]. The latter observations indicate that a NKCC cotransporter (SLC12A1 or SLC12A2) may play a role in Cl− entrance in cyst-lining cells.

Expression of CFTR and other chloride transporters in ADPKD

The analogy between the properties of the ADPKD cystic epithelium and those of other secretory epithelia suggested that the cystic fibrosis transmembrane regulator (CFTR) channel could be the molecular counterpart of the cAMP-stimulated Cl− secretion in ADPKD cysts (Fig. 1). The expression of CFTR was first evidenced in primary cultures of ADPKD cells and ADPKD kidney extracts, with an immunostaining pattern compatible with localization to the apical membrane of cyst-lining cells [34]. Selective Cl− currents were recorded in cultured ADPKD cyst cells. These currents were stimulated by forskolin or a permeant analogue of cAMP, but were totally inhibited by the CFTR blocker DPC [34]. A significant heterogeneity in CFTR expression was reported in cyst-lining epithelia, possibly caused by cell dedifferentiation [34–36]. Thus, a subset of tubular cells with functional CFTR may be sufficient to drive fluid secretion into the cyst or, alternatively, other types of Cl− transporters might be implicated in fluid secretion [37]. CFTR was also detected at the apical cell membrane of kidney cyst-lining cells in a Pkd1 KO mouse model, consistent with its role in cAMP-dependent fluid secretion [38].

Considering the heterogeneous expression of CFTR in ADPKD cysts, the possibility that alternative pathways participate in CI− and fluid secretion has been explored. In particular, Kunzelmann et al. discovered that the calcium-activated Cl− secretion by anotamin 1 (ANO1, also named TMEM16A), activated through purinergic (P2Y2) receptors, plays a role in epithelial fluid secretion and proliferation, using principal cells from dog (MDCK) and mouse (M1) kidneys and an embryonic kidney cyst model [39,40]. This pathway may be linked to local hypoxia, secondary to cyst growth, and stabilization of the hypoxia-inducible transcription factor-1α (HIF-1α), which regulates cyst growth in vitro via calcium-activated Cl− secretion [41]. The relevance of these findings needs to be substantiated in vivo, as for instance ANO1 appears to be cytoplasmic or even located on the basolateral membrane in human ADPKD cyst-lining cells [42].

The nature of the Cl− transporters involved on the basolateral side of the cells was investigated by Lebeau et al. [43]. They detected NKCC1 in ADPKD cells and kidney extracts, with a basolateral reactivity in about one-third of ADPKD cysts. Staining of serial sections showed that cysts expressing NKCC1 were also positive for CFTR. The fact that most CFTR-positive ADPKD cysts also expressed NKCC1 suggested that transepithelial Cl− secretion in ADPKD involves molecular mechanisms similar to secretory epithelia. This conclusion was supported by the demonstration of NKCC1 in the basolateral membranes of advanced cystic lesions in the Pkd1−/−/− mouse model [44]. Lebeau et al. also showed that the Cl−/HCO3− anion exchanger type I (AE1), which operates in the basolateral membrane of α-type intercalated cells of the collecting ducts, is detected at the basolateral pole of CFTR-positive, NKCC1-negative ADPKD cysts. Accordingly, AE1 might be an alternative basolateral pathway for Cl− conductance in a subgroup of cysts [43].

The sustained activity of NKCC1 in the basolateral membrane of cyst epithelial cells requires basolateral recycling of K+ and Na+, as well as the maintenance of a hyperpolarized membrane potential (Fig. 1). Albaqumi and colleagues demonstrated the role of the Ca2+−activated potassium channel KCa3.1 in mediating the efflux of K+ and maintaining a relatively negative intracellular membrane potential that drives the apical Cl− secretion by CFTR in monolayers of kidney cells derived from patients with ADPKD [45]. Both cAMP and PKA may activate KCa3.1 channels, which appears relevant given the importance of cAMP in the pathogenesis of ADPKD [24].

CFTR structure, function and localization in the kidney

The CFTR protein belongs to the superfamily of the integral membrane ATP-binding cassette (ABC) transporters [46]. These mediators of unidirectional organic solute transport include multidrug resistance proteins, such as MDR and P-glycoprotein, and a number of prokaryotic and eukaryotic small nutrient transporters [47]. CFTR is symmetrically structured in 2 transmembrane domains (TMD1 and TMD2) and 2 nucleotide binding domains (NBD1 and NBD2), separated by a large, polar, regulatory (R) domain. Each membrane-spanning domain is made of 6 α-helices, portions of which form the Cl− pore (Fig. 2). CFTR is regulated by cAMP-dependent phosphorylation of the R domain via the protein kinase A (PKA), followed by ATP-dependent gating events initiated by ATP binding to the cytoplasmic nucleotide-binding domains (NBD1 and NBD2) and resulting in transepithelial Cl− transport [46,48]. CFTR channel gating is strictly coupled to phosphorylation and ATP hydrolysis [49]. Following phosphorylation, the R domain is
disengaged from its inhibitory position; the NBDs form a "head-to-tail" dimer upon binding ATP; and the cytoplasmic pathway is cracked open. Note that local movements of the transmembrane helices may also participate to ion access to the pore even in the NBD-dimerized conformation [49].

The N- and C-termini of CFTR are both oriented to the intracellular compartment. The C-terminus of CFTR harbors a type I PDZ domain-binding motif, which interacts with several PDZ-domain proteins including the Na⁺/K⁺ exchange regulatory factors (NHE-RF1 and NHE-RF2) and the actin-binding protein ezrin [50]. The dynamic regulation of CFTR binding to these scaffolds may participate to its dimeric organization into macromolecular functional units containing regulatory partners and other channels. The insertion of CFTR channels in the plasma membrane also involves a complex of proteins including the PDZ-domain proteins NHERF1 and CAL, as well as the SNARE SYN6 and the Rho GTPase TC10 and several Rab GTPases [51,52]. There, CFTR interacts functionally with other channels, including the outwardly rectifying Cl⁻ channel ENaC, preventing Na⁺ reabsorption from the lumen. The main drugs targeting these transport pathways are indicated in green boxes. For details, see text.

Other abbreviations: CREB, cAMP response element-binding protein; Gs, stimulatory G protein; Gi, inhibitory G protein; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; SST2, somatostatin receptor. (Adapted from Refs. 1, 18, 25).

Fig. 1. Role of chloride transport and cAMP in ADPKD cyst-lining cells.
A cyst-lining tubular cell (from the collecting duct), with right junctions delineating the apical and basolateral poles, is depicted. The complex involving polycystin-1 (PC1) and polycystin-2 (PC2) mediates Ca²⁺ fluxes in response to stimuli sensed by the primary cilium (apical pole). Disruption of the PC1-PC2 complex is involved in the alteration of intracellular Ca²⁺ levels. PC2 is also involved in the regulation of Ca²⁺ stores from the endoplasmic reticulum (ER). The transepithelial secretion of Cl⁻ is mediated by the basolateral Na⁺-K⁺-2Cl⁻ co-transporter NKCC1 and apical Cl⁻ channels including the protein kinase A (PKA)-stimulated CFTR. The Cl⁻/HCO₃⁻ anion exchanger type 1 (AE1) might be an alternative basolateral pathway for Cl⁻ in a subpopulation of cysts. This transepithelial pathway is stimulated by an increased concentration of cAMP, probably reflecting reduced intracellular Ca²⁺ levels (which stimulates of Ca²⁺-inhibitable adenyl cyclase (AC) and/or inhibits the Ca²⁺-dependent phosphodiesterase (PDE)) and stimulation of the vasopressin V2 receptor (V2R) pathway. The activity of NKCC1 requires the recycling of K⁺ via the basolateral K⁺ channel KCa3.1 and that of Na⁺ via the ubiquitous Na⁺-K⁺-ATPase (not shown). The increased cAMP levels stimulate PKA-mediated phosphorylation of various mediators, leading to disruption of flow sensing and tubulogenesis; transepithelial fluid secretion driven by apical CFTR; increased expression of water channels (AQP2); and transcriptional regulation of mediators involved in cell proliferation. The expression of CFTR and the net secretion of Cl⁻ induces a transepithelial movement of Na⁺ (electric coupling) and water (osmotic coupling). Note that CFTR is blocking the apical Na⁺ channel ENaC, preventing Na⁺ reabsorption from the lumen. The main drugs targeting these transport pathways are indicated in green boxes. For details, see text.

Fig. 2. Representative 2-dimensional structure of CFTR.
The CFTR protein contains two symmetric parts made of 1 membrane-spanning domain (including 6 trans-membrane domains (TMD)) and 1 nucleotide-binding domain (NBD), linked by an intracellular, R (regulatory) domain. The phenylalanine most frequently affected in Caucasian patients with cystic fibrosis is located in NBD1 (Phe508). Ivacaftor (IVA, a potentiator of CFTR) binds CFTR at the NDB1/2 interface and the coupling helix of intracellular loops 1 & 4 (L1/L4), whereas Lumacaftor (LUMA, a CFTR corrector) binds NBD1. (Adapted from Refs. 65)
found at the apical surface of both proximal and distal tubules of rat kidney [54]. In mouse kidney, CFTR is mainly present in the apical area of proximal tubule (PT) cells [pars recta, S3 segment], with a subcellular distribution compatible with endosomes [58,59]. In the human kidney, CFTR was detected in the PT, in addition to the thin limbs of Henle's loop, distal tubules and collecting ducts [54-57]. Of note, a functional truncated isoform (TNR-CFTR) made of the TMD1, NBDB1 and R domains has also been reported in rat and human kidney, with a specific ontogeny pattern [55,56,60]. TNR-CFTR may function in intracellular organelles and partially substitute CFTR functions in the kidney medulla [61].

Besides its location in the plasma membrane, CFTR is present in intracellular organelles along the endocytic and the secretory pathways. There, CFTR may act as a pH regulator by importing Cl− in parallel to H+ accumulation and thereby influencing vesicular acidification and, potentially, activity of the endolysosomal and recycling compartments [58,62-64].

Kidney phenotype in cystic fibrosis

Cystic fibrosis (CF; MIM #219700) is the most common lethal autosomal recessive disease in Caucasians, with a prevalence of one in 2500 live births. CF is a multi-systemic disease that basically results from the obstruction of exocrine glands by an excessive production of mucus. The accumulation of thick, poorly hydrated mucus in the airways causes an obstructive lung disease promoting chronic bacterial infection and inflammation [65]. In 1989, Collins, Riordan and colleagues demonstrated that CF was caused by loss-of-function mutations in the CFTR (also named, ABCC7) gene that encodes CFTR [66]. Over 2075 CF-associated mutations have been reported thus far in CFTR (CF Genetic Analysis Consortium, http://www.genet.sickkids.on.ca/cftr/), classified into 5 groups according to their structural or functional consequences on Cl− conductance [65]. The in-frame deletion of 3 bases encoding a phenylalanine residue at position 508 (∆F508) represents the most common mutation in CF population. The ∆F508 mutation affects the correct processing and maturation of CFTR [67]. About 5 to 10% of CFTR mutations are due to premature truncation or non-sense alleles. These are associated with the most severe CF phenotypes [68]. Most other CFTR mutations are unique to a particular family or to only a handful of cases across the world.

The abundant expression of CFTR in the mammalian kidney suggests that CFTR may play a role in (patho)-physiology [69]. Patients with CF are prone to develop episodes of Bartter-like syndrome characterized by dehydration with metabolic alkalosis [70,71]. A decreased capacity to concentrate the urine has also been reported [72]. However, some of these observations may reflect adaptations to excessive losses of NaCl in sweat and feces. The incidence of kidney stones is apparently increased in CF patients, and microscopic nephrolithiasis has been reported [73,74]. Modifications in lithogenic factors and impaired hydration may also contribute to these observations.

CF patients show an enhanced renal clearance of several drugs including aminoglycosides, pointing to a putative defect in receptor-mediated endocytosis in PT cells [75,76]. By analogy to other intracellular Cl− transporters such as the Cl−/H+ exchanger, CIC-5, CFTR may play a role in membrane recycling and/or vesicular pH regulation in kidney cells [57,64]. CFTR-null (Cftr−/−) mice showed a significant increase in the urinary excretion of the low-molecular-weight (LMW) protein Clara Cell protein (CC16, 16 kD) in comparison to controls, reflecting a selective defect in receptor-mediated endocytosis [58]. The Cftr−/− mice also showed a decreased uptake of radiolabeled [35S]β2-microglobulin and a lower renal uptake of aminoglycosides, compared to wild-type littermates, confirming the endocytic defect. The latter was explained by an loss of the apical receptor cubilin in PT cells, due to its increased shedding in the urine, causing a loss of cubilin ligands (e.g. transferrin, CC16) in the urine [58]. An intermediate phenotype was observed in the ∆F508 mice. Taken together, these data suggest that the lack of a functional CFTR in kidney PT cells significantly perturbs receptor-mediated endocytosis, namely via an accelerated shedding of cubilin from the brush border into the urine. Accordingly, a mild but significant albuminuria and LMW proteinuria was reported in a cohort of CF patients, all harboring at least one ∆F508 mutation [58]. These findings may impact on the long-term renal function, since LMW proteinuria can trigger tubule-interstitial injury and chronic kidney disease. Moreover, the increased urinary loss of the cubilin-ligand, transferrin, could participate in the iron deficiency and lower circulating transferrin levels that are commonly reported in CF patients [77].

CFTR may also play a more general role in kidney damage. In a model of unilateral ureteral obstruction (UUO)-induced kidney fibrosis, ∆F508 mice exhibited significantly higher β-catenin activity with aggregated renal fibrogenesis. This could be rescued by overexpression of CFTR, suggesting a potential therapeutic target against fibrosis via CFTR-regulated Wnt/β-catenin signaling [78].

Role of CFTR in ADPKD

The role of CFTR in mediating Cl− fluid secretion in ADPKD was substantiated in vitro by the fact that forskolin-stimulated fluid secretion was dramatically reduced after incubation of monolayers of ADPKD cells with an antisense oligonucleotide against CFTR [35]. Studies using primary cultures of ADPKD vs. control epithelial cells confirmed the role of cAMP in stimulating cell proliferation as well as fluid secretion and cyst enlargement [79]. The use of CFTR blockers (e.g. glibenclamide, NPPB, genistein) reduced cyst growth and cAMP-stimulated Cl− currents in MDCK cyst in collagen gel [80]. Furthermore, the thiazolidinedione inhibitor CFTI3172, which stabilizes the channel in the closed state, inhibits cyst growth in MDCK cells and in metanephric kidney organ culture [81,82]. Two additional CFTR inhibitors (i.e. the tetrazolo-CFTRinh-172 and the phenyl-derived glycine hydrazide Ph-GlyH-101) suppressed cyst growth in MDCK cells without affecting cell proliferation; inhibited cyst number and growth in a cAMP-stimulated embryonic kidney cyst model; and, remarkably, slowed kidney enlargement and cyst growth and preserved renal function in a neonatal, kidney-specific Pkd1 knockout model (Pkd1lox/lox/Ksp-Cre mice) [83].

Jansson et al. observed that physiological concentrations of ouabain in the presence of forskolin or 8-bromo-cAMP significantly enhanced fluid secretion and cyst growth of cultured human ADPKD cell monolayers and microcysts in collagen matrix. Ouabain was also promoting cAMP-dependent cyst enlargement in metanephric organ cultures from Pkd1 compared to wild-type mouse kidneys [84]. In subsequent studies, these investigators demonstrated that apical CFTR activation mediates the cAMP-induced fluid secretion and cyst growth promoted by ouabain [85]. In M1 cells derived from mouse cortical collecting duct, treatment with vasopressin (AVP) increased the levels of CFTR protein and mRNA, which, in turn, increased CFTR-mediated short-circuit currents [86].

From these collective data, the current model of cyst formation in ADPKD starts with the loss of function of the polycystin-1/polycystin-2 complex. When the residual activity of that complex is under a critical threshold, a cascade of changes in the cellular phenotype is triggered. These changes include loss of tubular cell differentiation, abnormal proliferation and changes in the surrounding tissue. Increased levels of cAMP, resulting from the activation of G-protein-coupled receptors including the vasopressin V2 receptor, activate protein kinase A (PKA)-dependent pathways and cAMP-sensitive ion channels, essentially CFTR, that mediate cyst fluid accumulation (Fig. 1). Importantly, CAMP is also stimulating proliferation of cyst-lining cells, via PKA, B-Raf and the MEK-1 and ERK kinase [1,21]. The release of ATP (and adenosine) in the lumen may also stimulate the secretion of Cl−, and thus cyst growth, through other types of Cl− channels [87,88].
Potential therapeutic role of CFTR inhibitors in ADPKD

The data summarized above substantiate a role for CFTR in mediating cyst fluid accumulation in ADPKD. The potential of targeting CFTR to decrease fluid secretion, and thus slow kidney cyst enlargement (e.g. TKV) in ADPKD has long been proposed [34,35,80,89,90]. Genetic evidence apparently concurred, since a milder cystic phenotype was reported in 3 ADPKD patients with concomitant CF, as compared to siblings with ADPKD alone [91,92]. Such protective effect was not confirmed in a detailed analysis of kidney volume and function in 4 ADPKD individuals harboring the ΔF508 mutation at the homozygous or heterozygous state – compared to ADPKD controls. The lack of protective effect in these patients could be due to the persistent expression of mutant CFTR with an apical pattern in a large fraction of ADPKD cysts [93]. To the best of our knowledge, the effect of a genetic deletion of CFTR in an orthologous mouse model of ADPKD has not been reported thus far.

As pointed by Verkman and colleagues, the use of CFTR inhibitors in ADPKD would require a low toxicity profile, due to the nature of disease and presumed long duration of treatment, a good penetration in the cystic kidney, and a minimal accumulation in organs with active CFTR (e.g. lungs, pancreas) [90]. Yang et al. screened small-molecule inhibitors of the thiazolidinedione and glycine hydrizide classes in the MDCK cell model [83]. They identified two lead compounds (tetrozolo-CFTR(inh)-172 and Ph-GlyH-101) which blocked cyst growth without affecting cell proliferation. These compounds also inhibited cystogenesis in an embryonic kidney cyst model. Subcutaneous administration of these two compounds to neonatal, Pkd1 knockout mice for 7 days slowed kidney enlargement and cyst expansion and preserved renal function compared to vehicle-treated mice, at low micromolar EC50 range [83].

The class of (benzo)pyrimido-pyrrolo-quinoxalinedione (PPQ) inhibitors of CFTR was also shown to inhibit cyst growth in an embryonic kidney culture model of PKD, with a particular compound (BPO-27) showing favorable pharmacokinetics and high efficacy (full inhibition of CFTR with IC50 of ~8 nM) [94].

More recently, it was shown that steviol, the major metabolite of the noncaloric sweetener stevioside, was able to inhibit cAMP-activated chloride secretion by targeting CFTR and inhibit MDCK cyst growth, in part by promoting CFTR degradation by the proteasome [95]. Early postnatal administration of steviol in an orthologous mouse model of human ADPKD (Pkd1fl/fl;floxo;Pkd1Cre) for 14 days markedly decreased kidney weight and cystic index and blood urea nitrogen levels values, compared to vehicle administration. Steviol also reduced cell proliferation and CFTR expression (and mTOR/S6K) in kidney cyst-lining epithelial cells. However, these results were only observed with i.p. administration of high doses of steviol (200 mg/kg BW) [96]. The proton pump inhibitor Lansoprazole has also been recently proposed to reduce kidney cysts in vitro (MDCK cells) and in vivo (PCK rat), via activation of the liver X receptor and subsequent down-regulation of CFTR, with an additional anti-proliferative effect [97].

The VX-809 corrector of CFTR (Lumacaftor), which restores the folding and trafficking of ΔF508-CFTR to the plasma membrane and may also interact directly with the CFTR protein Fig. 2, was shown to reduce cyst growth and to improve kidney function in a kidney-specific Pkd1 mouse model and in proximal tubule-derived, cultured Pkd1 knockout cells. VX-809 was shown to reduce proliferation and to decrease both basal and forskolin-stimulated cAMP levels, while reducing the thapsigargin-induced release of Ca2+ from the ER and increasing the level of CFTR expression in the cells [98]. This result, which is counterintuitive when considering the role of CFTR in mediating fluid secretion and cyst growth, is probably due to an effect of the CFTR corrector on cAMP levels, potentially mediated by a specific reduction of adenyl cyclase isoform 3 (AC3), and/or decreasing the Hsp90 levels. Subsequent studies confirmed that VX-809 treatment of Pkd1-null mouse kidneys changed the cyst phenotype from fluid secretion towards fluid reabsorption, which could be due to increased expression of the Na+-H+ exchanger NHE3 (proximal cysts) and Na+-channel ENaC (collecting duct-derived cysts) [38]. The fact that the in vivo effects of VX-809 were observed at doses lower than the doses used for CF pediatric patients and the safety profile of the drug sustain the potential interest of this CFTR corrector in ADPKD. An exploratory, randomized, double-blinded, placebo-controlled multicenter study evaluating the efficacy, safety, tolerability and pharmacokinetics of the orally administered CFTR corrector GLP-2737 in patients with ADPKD has been initiated recently (Galapagos; EuadrCT2019–003521-21).

On the basolateral side of the cyst cells, targeting NKCC1 with loop diuretics could theoretically be envisaged, but the efficacy and safety of such an approach remains very uncertain. In fact, a small-scale, non-randomized study showed that hypertensive ADPKD patients treated with diuretics had a faster loss of renal function compared to those treated with ACE inhibitors, despite similar blood pressure control [99].

The clotrimazole analogue TRAM-34, which specifically inhibits the Ca2+-activated K+ channel KCa3.1, inhibited forskolin-stimulated Cl− secretion across the monolayers (without affecting CFTR or other apical Cl− channels), and inhibited cyst formation and enlargement in collagen matrix. It must be noted that such des-imidazoyl trityl derivatives are already used in phase 3 clinical trials to block KCa3.1 in sickle cell disease without side-effects, opening perspectives for further testing in the ADPKD context [45,100].

Role of cAMP and vasopressin in ADPKD

Increased concentrations of cAMP play a central role in the progression of renal cystic disease in ADPKD (Fig. 1). Stimulation of the V2R by the antidiuretic hormone arginine vasopressin (AVP) is the major source of cAMP production in the collecting ducts [101]. Haploinsufficiency in polyostin-1 in Pkd1ΔF508 mice is associated with excessive AVP signaling, causing increased phosphorylation and recruitment of aquaporin-2 water channels and inappropriate antiuredness [102]. The decreased intracellular Ca2+ concentration secondary to PKD1/2 mutations may downregulate the Ca2+-dependent phosphodiesterase PDE1 and stimulate the Ca2+ inhibitable adenylyl cyclase 6 (AC6), causing an increase in cAMP levels. In turn, increased cAMP stimulates proliferation and growth of ADPKD cells and drives transepithelial chloride and fluid secretion, through activation of protein kinase A (PKA) and pathways including the mitogen-activated protein kinase (MAPK) kinase MEK and the extracellular signal-regulated kinase (ERK), mTOR and Wnt-β-catenin, STAT3 and possibly PAX2 signaling (25).

The crucial involvement of the V2R pathway in ADPKD was demonstrated by the significant effect of V2R antagonists including tolvaptan on kidney cyst development in various rodent models of PKD (1,18). Suppression of endogenous AVP secretion by high water intake was shown to attenuate the progression of kidney cysts in the PCK rat [103]. Furthermore, kidney cyst development was inhibited in PCK rats crossed to Brattleboro rats carrying a spontaneous mutation that renders them deficient in AVP, whereas administration of the V2R agonist 1-deamino-8-d-arginine vasopressin rescued the kidney cyst phenotype [104]. These compelling results motivated the initiation of randomized clinical trials, which demonstrated that the use of tolvaptan effectively slows the rate of decline in kidney function in patients with ADPKD and at risk of rapid disease progression [105,106].

Epilogue: When discussing the forces behind his quest to understand ADPKD, Jared Grantham first mentioned his best childhood friend and his family, affected by the disease. This emotional drive, coupled to a formidable investigative mind, experimental rigor, willingness to translate research insights into clinical gain, and exceptional managerial and personal qualities led to discoveries that transformed the care of ADPKD. The visionary quest of Jared Grantham was rooted in his interest for epithelial transport, paving the way for the first treatment able to slow the progression of ADPKD: blocking the vasopressin-cAMP pathway, thus interfering with cyst fluid
secretion…

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