Renal channelopathies: Disease mutations in plasma membrane and organellar ion channels and transporters

Marc Rogers, PhD
featured by Nanion Technologies

After academic research as a neuroscientist and 20 years of commercial ion channel drug discovery, Marc is now a freelance blogger, advisor and consultant for clients worldwide, where he shares his expertise and enthusiasm for all aspects of ion channel screening. He is particularly interested in automated patch clamp, and exploiting the potential of human iPS stem cell assays to facilitate the successful translation of new drugs into the clinic.

The trigger for writing this blog was a comment left on one of my recent ion channel drug discovery blogs on LinkedIn that included news of the clinical progress of Vertex's APOL1 blocker Inaxaplin for chronic kidney disease, with the reader not aware that renal ion channels are the subject of drug discovery work. Maybe I'm an outlier but during my interesting journey through this realm I have been involved with several different renal ion channel programs and am aware of several targets so figured it was a good time to bring this information together to highlight work over last 30+ years on these ion channels and their role in different kidney diseases. This issue will include all the good stuff like classical biophysics and pharmacology, high resolution structure-function and virtual screening studies, gene therapy programs, and highlight the use of automated patch clamp (APC) and organellar recordings in these drug discovery efforts.

Ion channels in kidney disease

Several ion channels are associated with kidney disease, including plasma membrane ClC-K and organellar ClC-5 anion channel transporters (Bartter and Dent disease), SLC transporters, TRPC and TRPP ionotropic receptors, and ROMK and APOL1 transmembrane proteins (Figure 1). Genetic variants (e.g. GWAS, SNPs) and channelopathy mutations in several of these ion channel families have been found in the general population and rare disease patients, and mouse genetic models recapitulate many features of chronic kidney disease, validating the pursuit of these ion channels as drug discovery targets in kidney disease. Sodium chloride is taken up apically by the combined activity of NKCC2 (Na^+-K^--2Cl- cotransporters) and ROMK potassium channels, while chloride ions exit from the cell through basolateral ClC-K chloride channels. Both ClC-Ka and ClC-Kb pore forming subunits are associated with accessory Barttin proteins, which facilitate trafficking, stability and function of these ion channel proteins on the cell surface.[1,2] Missing from this image are the organellar ClC-5 anion co-transporters that regulate endocytosis, and TRPC ionotropic channels and APOL1 channels in glomerular podocytes.

There are several major types of human kidney disease, distinguished by their mechanism or cell type/function, as well as several rare genetic syndromes:

- Steroid Resistant Nephrotic Syndrome (SRNS)
- Focal Segmental Glomera Sclerosis (FSGS)
- Membrane Glomerulonephritis
- IgA nephropathy (IgAN)
- Bartter Syndrome (diverse channelopathies)
- Dent's Disease (ClC-5 channelopathies)

In most cases these syndromes are progressive and lead to chronic kidney disease (CKD) and often result in end stage renal failure requiring regular dialysis and organ transplantation. IgA nephropathy is being addressed by a variety of immunotherapies, while FSGS has been tackled preclinically in several TRPC drug discovery projects. Drug discovery interest in other renal channels are validated by human disease mutations (channelopathies) such as those in ROMK K^+ channels and TRPP ionotropic receptors, APOL1 channels, and in ClC-K and ClC-5 anion channels and transporters or via defects in the barttin ClC-K channel accessory subunit. Not all renal diseases involve ion channels, but the deterioration in renal function (ion homeostasis, hormone regulation, drug clearance) may be ameliorated by modulating the activity of key kidney ion channels.

Bartter Syndromes (Barttin Disease)

There are five types of Bartter Syndrome[4] which affect salt reabsorption and electrolyte balance (K^+, Cl^-, Mg^{2+}) to impact fluid volume and blood pressure regulation across several human organ systems, with most linked to defects in ion channels and transporters in the kidney (review [5]):

- Type I results from mutations in the Na-K chloride cotransporter gene NKCC2

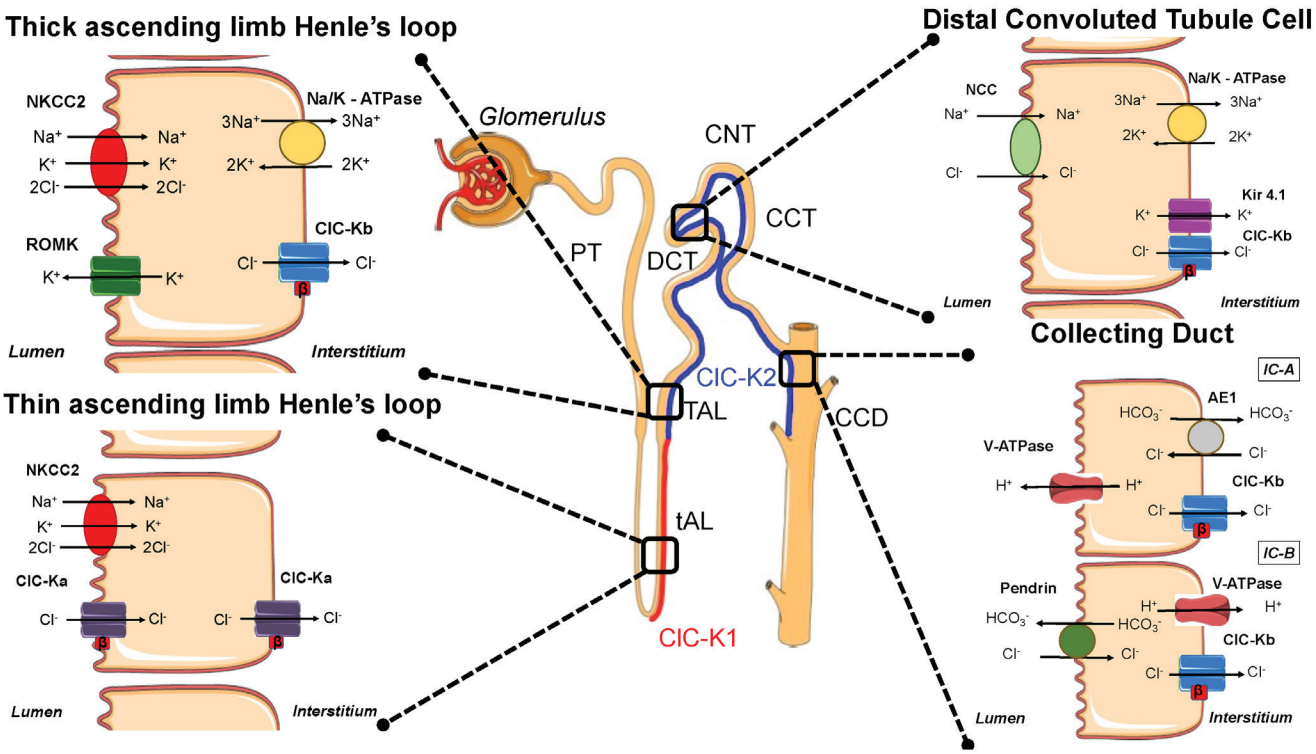


Figure 1 ***Location of plasma membrane ion channels and transporters in kidney cellular compartments.*** *This review also includes APOL1 and TRPC2 channels in glomerular podocytes, TRPP ionotropic receptors, and organellar CLC5 channel transporters. Image from[3] used under CC4.0 license.*

- Type II results from mutations in the KCNJ1 gene encoding tubule ROMK ion channels
- Type III results from mutations in the CLCNKB gene encoding the basolateral ClC-Kb chloride channel
- Type IV results from genetic mutations in the ClC-K channel accessory protein barttin
- Type V results from mutations in an extracellular Ca^{2+} sensor receptor, but also linked to alterations in ClC-Ka and ClC-Kb chloride channels

Bartter Syndrome II : ROMK $K^{\pm}$ channel mutations

The renal outer medullary K^+ channel (ROMK, Kir1.1) is encoded by the KCNJ1 gene which produces two transmembrane domain inwardly-rectifying channel subunits, with several different splicing isoforms differentially localised along the kidney nephron (Figure 2; review[6]). ROMK channels mediate K^+ efflux across the apical membrane of proximal tubule epithelial cells which is coupled to the activity of the NKCC2 transporter to regulate NaCl transport and Ca^{2+} reabsorption in the kidney, and control K^+ excretion in the distal nephron (alongside BK channels). The latter activity is closely regulated, involving hormonal and kaliuretic factors and phosphorylation-dependent trafficking of ROMK channel proteins from endosomes to the plasma membrane and kinase-sensitive internalisation. ROMK channel biophysics, pharmacology and role in renal ion homeostasis have been extensively studied over past decades as it was one of the first mammalian ion channel (and 1st of 16 inward rectifiers) genes cloned in the 1990s. Shortly after loss-of-function mutations in ROMK channels were identified in Bartter Disease patients which gave greater understanding of ROMK structure-function and pathophysiology mechanisms. Indeed, heterozygous carriers of ROMK mutations (e.g. in the general population and Bartter Syndrome family members) exhibit lower risk of hypertension, and ROMK inhibitors phenocopy Bartter Syndrome when dosed in animals[7], demonstrating the key role of ROMK channels in regulating kidney function and validating them as drug discovery targets.

Over 50 ROMK channel mutations are linked to Bartter Syndrome (OMIM # 241200) which are mostly loss-of-function missense, nonsense or frameshift changes. These either produce truncated non-functional proteins (N-terminus variants), protein processing defects that lead to ER retention, misfolding and enhanced degradation resulting in few channels at the cell surface (Ig domain b-sheets), or channels with reduced activity via changes in gating, selectivity or PIP2 modulation.[9,10,11] A recent computational screen of UK biobank and ClinVar genetic databases revealed novel ROMK mutations linked to Bartter Syndrome that similarly reduce surface expression by altering protein trafficking and degradation or reduce channel function.[12]

ROMK was the subject of hypertension drug discovery efforts in the 2010s as Kir1.1 inhibitors held promise as novel diuretics. US academics at Vanderbilt University used thallium flux and Nanion APC platforms to screen their extensive compound library to discover the selective Kir1.1 inhibitor VU591 that binds in the inner pore of Kir1.1,12 and have gone on to use this workflow to discover modulators of other Kir channels for diverse therapeutic indications. In parallel, Merck conducted an extensive drug discovery program using high throughput flux and APC assays to discover several series of selective ROMK inhibitors, revealing a serendipitous story of uncovering a potent impurity in a

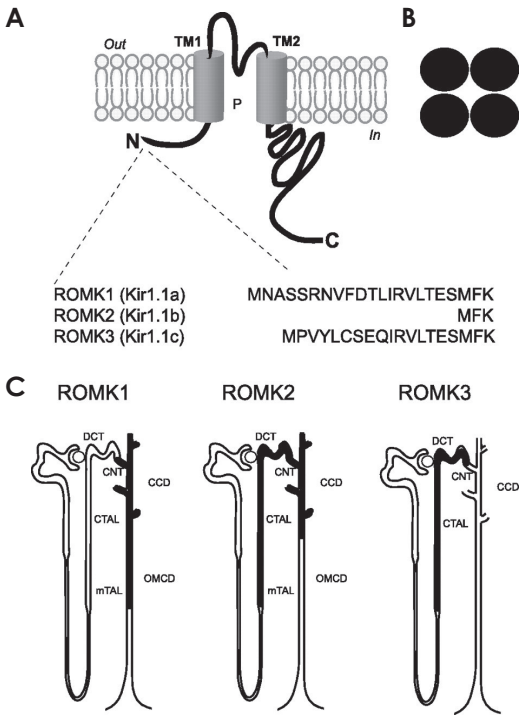


Figure 2 ***ROMK Kir1.1 subunit structure, tetramerization and isoform location in renal tubules.*** **A , B** *ROMK channels share the canonical inwardly rectifying K□ channel architecture with two transmembrane domains, a conserved selectivity filter, and cytoplasmic N- and C-termini, assembling as tetramers.* **C** *ROMK1–3 (Kir1.1a–c) isoforms differ in their N-terminal regions and show distinct expression patterns along the nephron. Figure adapted from[6] and used under CC4.0 open access license.*

HTS compound and subsequent SAR optimisation of hERG selectivity and PK properties in several scaffolds to result in 2 clinical candidates.[13,14] MK-7145 produced diuresis, natriuresis and hypotensive effects in preclinical models with less K^+ excretion compared to existing diuretics and entered clinical trials but suffered from a short plasma half-life, so the back-up MK-8153 was developed.[15,16]

Bartter syndrome type III : ClC-K channelopathies

ClC-Ka and ClC-Kb are members of the ClC family of plasma membrane and organellar chloride channels and transporters (Table 1), and these two related transmembrane channels are key players in maintaining ion homeostasis in the kidney.[17] ClC-Ka is highly expressed in the thin ascending limb of Henle's loop while ClC-Kb is found in both the thick ascending limb of Henle's loop and the distal convoluted tubule. These channels facilitate the reabsorption of chloride ions from the glomerular filtrate back into the bloodstream, and thus play an important role in maintaining the body's fluid and electrolyte balance. Additionally, these channels are expressed in the inner ear, where they are vital for normal hearing and balance.

The ClC family of channels and transporters have an unusual double-barrelled stoichiometry as each dimer subunit has two ion transport pathways, yielding complex gating mechanisms and sub-conductance states. Human ClC-Ka and Kb anion currents are weakly time- and voltage-dependent with little inactivation when expressed in mammalian cells (unlike other human ClC and rat ClC-K ion channels or in Xenopus oocyte recordings), and so studies of high resolution crystal structures have focused on gating and permeation mechanisms, renal disease mutations and drug binding residues.[18,19,20]

Over 50 mutations in the CLCNKB gene encoding ClC-Kb channels have been found in Bartter Syndrome III kidney disease patients[19,21] which present with highly variable phenotypes and disease severity (OMIM #602023). Profiling of these mutations has been slow work as it has relied on manual patch clamp recordings, and human (but not rat) ClC-K channels are usually inactive after expression in heterologous systems in the absence of barttin so this channel activation function is essential,[22] with strong co-localisation of these integral membrane proteins seen in native renal and auditory cells as well as in heterologous cells. Channelopathy profiling reveals a predominance

"Decades of genetic, electrophysiological, and clinical evidence show that mutations in renal ion channels and transporters are central drivers of kidney disease, making targets such as ClC-K/ClC-5, ROMK, TRPC/TRPP, and APOL1 a compelling and still underexploited space for renal drug discovery."

Marc Rogers, Founder and Director, Albion Drug Discovery Services Ltd

	β-subunit	expression	function	mouse model	human disease
plasma membrane					
CIC-1		skeletal muscle	stabilization of membrane potential	myotonia congenita (adr mouse)	recessive & dominant myotonia
CIC-2	± glialCAM	wide	transepithelial transport extracell. ion homeostasis regulation excitabiliity	degener. retina & testes leukodystrophy	leukodystrophy (loss of function) aldosteronism (gain of function)
CIC-Ka	/barttin	kidney, inner ear	transepithelial transport	diabetes insipidus	?
CIC-Kb	/barttin	kidney, inner ear	transepithelial transport	renal salt loss	Bartter III (renal salt loss)
vesicles (endo/lyso)					
CIC-3		wide (brain, kidney, liver...)	acidification & ion homeostasis of late endosomes (and synaptic vesicles?)	degeneration of CNS & retina	?
CIC-4		wide (brain, kidney, muscle...)	ion homeostasis of endosomes	no obvious phenotype	mental retardation epilepsy
CIC-5		kidney (also: intestine...)	acidification & ion homeostasis of endosomes	impaired renal endocytosis	Dent´s disease (proteinuria and kidney stones)
CIC-6		neuronal	ion homeostasis of late endosomes	lysosomal storage in neurons	?
CIC-7	/Ostm1	wide	lysosomal ion homeostasis & acidification osteoclast resorption lacuna	recessive osteopetrosis with CNS & retina degeneration, dominant ostepetrosis	recessive osteopetrosis assoc. with CNS & retina degeneration, or dominant ostepetrosis

Bartter IV (incl. deafness)

Table 1 *ClC family of anion plasma membrane channels and organellar transporters. Image from[20] used under CC4.0 open access license.*

of loss-of-function variants (Figure 3), with the most severe affecting barttin binding sites, dimer subunit interface regions or the selectivity filter, and those that retain variable amounts of anion channel activity (27-100% in 20 profiled mutations) or Ca^{2+} sensitivity presenting with less severe but variable symptoms (disease onset age, plasma ion homeostasis, calciuria). Thus, the genotype-phenotype spectrum for ClC-Kb channelopathies is a product of severe chaperone and trafficking defects linked to barttin interactions and more variable effects on channel function for missense variants able to reach the cell surface. There is also a gain-of-function polymorphism T481S linked to hypertension (and hearing acuity) in multiple global population studies which can promote ClC-Kb activity in the absence of barttin.[23]

Bartter Syndrome type IV : barttin mutations

This is an autosomal recessive inherited rare disease (1/1,000,000) caused by mutations in the barttin gene (BSND) which cause impaired Na^+ and Cl^- reabsorption in the kidney and leads to severe renal salt and fluid excretion into the urine and results in excess urine production (polyuria), dehydration, constipation and stunted neonatal growth; defects on barttin protein in the inner ear are linked to congenital sensorineural hearing loss. Many of these mutations impair the interaction of barttin

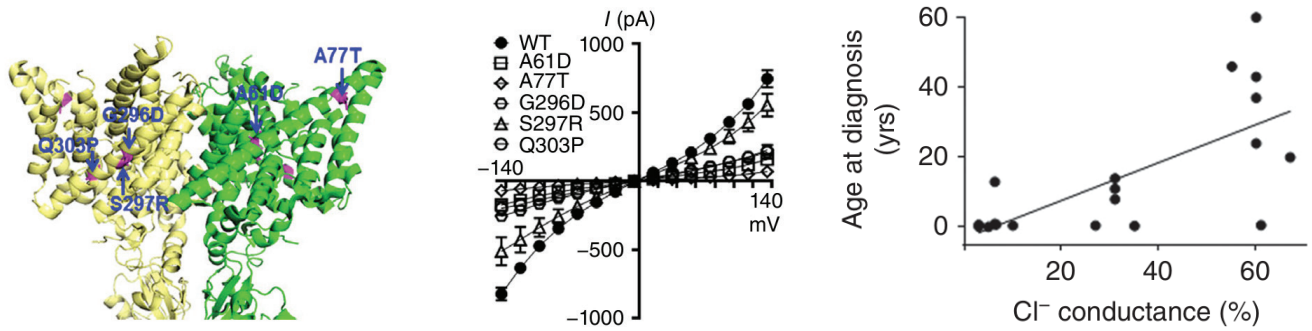


Figure 3 ***Bartter Syndrome III loss-of-function mutations in ClC-Kb anion channel barttin-interaction sites.*** *Images from reference [21] obtained from a PMC open access pdf.*

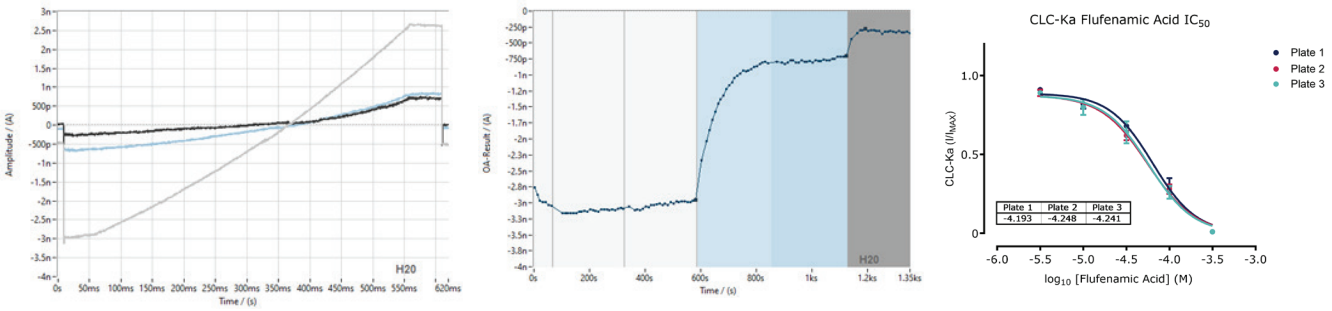


Figure 4 ***ClC-Ka channel recordings on SyncroPatch 384 showing modulation by fenamates.*** *Representative current trace, time course and concentration response curve showing the effect of inhibitor against CLC-Ka and reproducibility across 3 plates. An example of superimposed CLC-Ka current traces under control conditions (grey line), in the presence of 100 µM Flufenamic acid (blue line) and a saturating concentration of 300 µM Flufenamic acid (black line). Data source: https://www.sbdrugdiscovery.com/project/clc-k-electrophysiology/*

accessory subunits with ClC-K channels, and can variably affect channel trafficking and/or function depending on their location in transmembrane or cytosolic domains, respectively, leading to either complete or partial loss of ion transport in the kidney.

Several studies have identified the genotype-phenotype relationship of barttin mutations and linked them to disease severity.[23] For example, missense mutations near the N-terminus were most severe with complete loss of ClC-K channel function despite their insertion into the membrane suggesting loss of accessory subunit 'activation', while the Q32X nonsense mutation was equally severe but via prevention of both barttin and ClC-K membrane trafficking, and the milder G47R mutation decreased ClC-K channel activity through reduced barttin binding and was associated with delayed adult-onset Bartter Syndrome.[24] In contrast, the E88X nonsense mutation disrupted polarised apical-basolateral trafficking and ion transport in and out of the kidney, while the I12T variant reduced trafficking but retained ClC-K channel activation and was not linked to renal disease (but was associated with deafness).

Given that hypertension is far more widespread than patients with rare Bartter Syndromes, the focus of most drug discovery studies has been to discover and optimise ClC-K channel inhibitors to pharmacologically phenocopy Bartter Syndrome,[3,25] but there are a few examples of selective activators or potentiators (but no 'correctors' to continue the analogy to CFTR modulators for cystic fibrosis) which could reverse the effect of loss-of-function mutations in ClC-Kb channel and barttin genes in kidney disease patients. The amino acid divergence between ClC-K isoforms produces some selective pharmacology, especially for ClC-Ka where DIDS, BIM1, valsartan, CPP and Flufenamic acid are antagonists, but Niflumic acid is a mM activator of both ClC-Ka and Kb and Flufenamic acid (200 mM) activates ClC-Kb currents.[26] However this effect is only seen in Xenopus oocytes but not in mammalian cell expression systems,[27] where ClC-K channel open probabilities are much higher. CLC-K channel blockers appear to interact with an extracellular site within the outer pore, while fenamate potentiation involves residues in the extracellular I-J loop that also interact with the barttin accessory subunit[28] which may be accessible in oocytes that don't need barttin to express ClC-K channels but occluded in HEK cells which obligately require barttin association that may interfere with the fenamate potentiation binding site. Interestingly, a structure-based virtual screening and drug repurposing study to find ClC-Kb inhibitors also identified several activators from a list of 39 virtual hits,[19] including approved drugs Carprofen (a veterinary NSAID with different structure to fenamates) and Chlorphenesin carbamate (a central-acting skeletal muscle relaxant). More efficient and high throughput screening for ClC-K channel activators is now possible thanks to stable mammalian cell lines and optimisation of APC assays (Figure 4), which will hopefully deliver useful reagents to treat Bartter Syndrome patients.

ClC-5 mutations in Dent's disease

ClC-5 is another member of the diverse ClC anion channel family and functions as an organellar antiporter of anions and protons (Table 1). It was identified by cloning the locus affected in patients with Dent's disease (OMIM # 300009), another rare kidney indication associated with proteinuria and electrolyte imbalance.[20] ClC-5 is localised to recycling, sorting and early endosomes (alongside H^+-ATPase) where it helps acidify endosomal pH and regulate fluid-phase endocytosis and receptor-mediated internalisation plasma membrane proteins in the proximal tubule. Like other organellar ion channels such as TMEM175 and TRPML1, heterologous over-expression drives enough functional ClC-5 protein to the cell surface for electrophysiology recordings of outwardly rectifying Cl^- currents. Much like ClC-K channels, the ClC-5 protein assembles as a dimer with each subunit having twin permeation pathways, with H^+ transport out of the endosomes gated by conserved glutamate residues (which are absent from ClC channels). Dent's disease is X-linked with 60% of patients having mutations in the CLCN5 gene, with another 15% showing variants in the OCRL1 phospholipid enzyme.[29] ClC-5 mutations are

usually severe or total loss such that males lose all ClC-5 channel activity and females are heterozygous, and mouse knockout models phenocopy Dent's disease in terms of kidney dysfunction (electrolyte imbalance and proteinuria, reduced endocytosis). Dent's disease ClC-5 mutations are divided into 3 classes:

- Class 1 mutations impair protein processing and folding such that ClC-5 channels are trapped in the endoplasmic reticulum and targeted for proteosome degradation
- Class 2 mutations exhibit a delay in protein processing and reduced ClC-5 stability, manifest as drastically reduced cell surface expression and channel activity
- Class 3 mutations do not affect protein trafficking but directly alter channel activity

Nearly 200 CLCN5 gene variants have been identified in Dent's patients, and as with ClC-K channels in Bartter Syndrome the ClC-5 mutations (deletions, nonsense, missense, promoter and frameshift mutations, splicing site edits) are largely loss-of-function and so few have been functionally assessed by over-expression to the cell surface for electrophysiological recordings.[30] Notably, most ClC-5 mutations are found at the dimer interface[31] and physiological studies indicate that endosomal acidification is variably affected by ClC-5 mutations, and a study in immortalised patient-derived proximal-tubular epithelial cell lines found that endocytosis was severely impaired.[32]

I'm not aware of any commercial ClC-5 drug discovery programs, but academic groups have recently published on their work. A UK group tested chaperone-like molecules for potentiator or corrector effects and found that the FDA-approved drug 4-phenylbutyrate could restore mutant ClC-5 protein trafficking and channel activity for non-Class 1 variants.[33] A US group is using mouse knockout models of Dent's disease to develop gene therapies, with mixed results; non-selective lentivirus delivery of the hCLCN5 gene produces transient disease reversal in adult mice but targeted expression to proximal tubule cells using selective promoters was ineffective, while gene delivery under EF1 promoter control to the kidneys of newborn animals produced sustained transgene expression and reduced proteinuria.[34]

More extensive screening for ClC-K and ClC-5 potentiators and correctors has to overcome the difficulty in studying organellar ion channels and transporters, especially in a physiological context. Nanion Technologies has been developing several electrophysiology platforms capable of such recordings, including use of the SURFE^{2}R device to study ClC transporters,[35] and optimisation of specialised APC chips to enable high throughput screening of lysosomal channels such as TMEM175 in native organelles (Nanion App Note). This suggests there are good prospects for mechanistic and drug discovery studies of ClC channels and transporters in the near future, alongside physiological studies of organelle pH, autophagy and endocytosis as translational and target engagement readouts.

SLC transporters

Another class of kidney transporter subject to a gene therapy program are solute carrier proteins (SLCs), which are the target of an siRNA oligonucleotide program recently revealed by Judo Bio in the US. The company launched in 2024 with $100 million funding to develop a novel class of 'ligand-siRNA conjugates' that utilise the Megalin (LRP2) apical cell-surface endocytic receptor to facilitate receptor-mediated uptake of tagged oligonucleotides into kidney proximal tubule epithelial cells to silence genes linked to renal disease.[36] Megalin is highly expressed on proximal tubule epithelial cells and exhibits rapid internalization, slow degradation and high recycling capacity which make it an ideal entry point and cell tropism substrate for intracellular delivery of kidney-specific siRNAs, and there are a variety of megalin receptor ligands that can selectively boost gene delivery to kidney cell lines and *in vivo* compared to naked siRNA (Figure 5). Interestingly, megalin is co-localised with ClC-5 transporter channel proteins in proximal tubule cells and both are reduced in Dent's disease models.[20] It is not publicly disclosed which SLCs or specific kidney diseases Judo Bio are targeting, although human genetics reveal polymorphisms in SLC22A2 (an organic cation transporter), SLC22A12 (URAT1) and SLC6A13 (GABA transporter) linked to CKD susceptibility and reduced drug efficacy, and SLCs are key to removing uremic toxins and metabolites in patients with reduced kidney function.[37,38]

Also worth mentioning are UK company Purespring Therapeutics who are developing podocyte-specific AAV viral capsid vectors and promoters for renal disease gene therapy. They have a complement-dependent signalling modulator for IgAN in Ph I/II trials, and are also developing genetic treatments targeting podocin gene NPHS2 for FSGS and other nephrotic syndromes, which have been shown to reduce proteinuria in preclinical mouse models.[40]

Maze Therapeutics are another company working on kidney solute transporters, publicly disclosing their preclinical and clinical work on small molecule inhibitors of the Na^+-dependent neutral amino acid transporter SLC6A19 (B0AT1) in recent years, culminating in a successful Ph I study of clinical candidate MZE782 in 2025 according to company press releases and meeting abstracts. This renal drug is being lined up for Ph II efficacy studies in 2026 to target patients with phenylketonuria and CKD.

TRPC channels

Several TRPC ionotropic receptors were the target of renal drug discovery efforts at pharma and biotech companies in the last decade, but interest seems to have faded as many

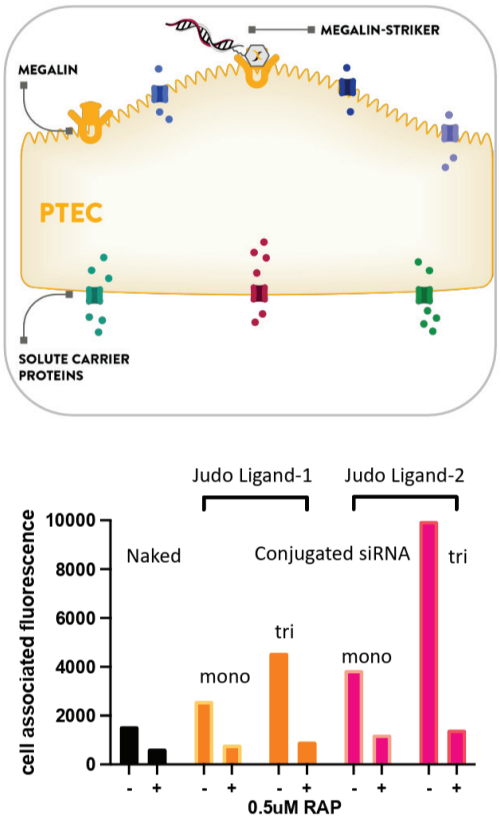


Figure 5 *Megalin endocytic receptor facilitates SLC siRNA-ligand delivery to kidney proximal tubule cells.* *OK cells were treated with 3μM Cy5-labeled unconjugated siRNA or siRNAs conjugated to selected ligands of megalin +/- 0.5μM RAP for 1h. Figure addapted from Judo Bio poster published on company website.* [39]

ligands lacked potency and sub-type selectivity, and there was conflicting data on the relative roles of TRPC channel isoforms in different models of renal disease. A particular focus, based on TRPC receptor location and function as Ca^{2+} permeable channels, is on podocytes which form foot processes that line the slit diaphragm of the glomerulus that is key to regulating kidney filtration and urinary production. Ca^{2+} influx initiates cytosolic signalling cascades including Calcineurin phosphatase activation and cytoskeleton remodelling which ultimately induces podocyte damage and leaky glomerular filtration leading to proteinuria, FSGS and CKD, and inhibition of TRPC channels can protect against kidney patho-physiology. While most TRPC isoforms are expressed in kidney tubules and glomeruli, the strongest target validation is for TRPC3, TRPC5 and TRPC6 channels (reviews[41-45]), although things are complicated by formation of heteromers with other TRPC subunits. TRPC3 subunits are expressed in glomerular podocytes and mesangial cells, but may only be functional if heteromerised with TRPC6 subunits. For example, combined global knockout of TRPC3, TRPC6 and TRPC7 protected against proteinuria and renal histopathology in STZ-induced diabetic mice. TRPC5 knockout mice are protected from chemical-induced proteinuria and small molecule TRPC4/C5 inhibitors have similar effects,[46] while TRPC5 channels in rodent and human iPSC podocytes are stimulated by GPCR-dependent signalling. There is more extensive validation for TRPC6 in renal disease, such as injury-induced and transgenic gene upregulation producing fibrosis and proteinuria in mice that is reduced after TRPC6 knockout and pharmacological inhibition, and increased TRPC6 gene expression in patients with FSGS. TRPC6-mediated Ca^{2+} influx is also increased in diabetic nephropathy by Angiotensin II and reactive oxygen species. Most compelling is human genetic data accumulated over the last 20 years in FSGS patients of a growing number of TRPC6 gain-of-function variants which increase Ca^{2+} influx and channel activity in HEK cells and podocytes, with the magnitude of these TRPC6 channelopathies correlating with disease onset and severity (e.g. proteinuria, age of renal failure). There is also indirect genetic validation for TRPC6 in chronic kidney disease, as TRPC6 expression and activity is inhibited by a-Actinin, nephrin and podocin which are highly expressed in glomerular podocytes and linked to various human kidney proteinuric diseases (Figure 6). Inactivating mutations in these genes upregulate TRPC6 levels; for example, the severity of podocin down-regulation correlates with kidney proteinuria.

Several tool compounds have been discovered which are useful for TRPC target validation and assay development, but their weak potency and lack of selectivity, significant off-target activities and poor drug-like properties have undermined their potential for drug development (Reviews[49-52]).

- AC1903 was discovered by US researchers as a TRPC5 inhibitor (4 mM IC_{50} on SyncroPatch) that reduces proteinuria in kidney disease models,[53] but others have shown it to be a non-selective 2-20 mM blocker of most TRPC isoforms[54]
- Clemizole was identified by German academics from the LOPAC library as a selective 1 mM blocker of TRPC5 channels with 5-10 fold selectivity over other TRPC isoforms[55]
- ML-204 is a 3 mM blocker of TRPC4 channels found in a screen of the Johns Hopkins compound library, and while sparing TRPC6 it also inhibits TRPC5 channels
- Pico145 is a potent <nM but non-selective blocker of TRPC1/4/5 channels, first exemplified in a 2014 patent from Hydra Biosciences (HC-608) and profiled by the Beech laboratory in the UK56

Recent TRPC channel cryo-EM structures have revealed the binding sites of several compounds,[57-59] but most TRPC drug discovery predates structure-based methods and relied on library screening and natural product analogues.

GSK worked on a number of TRP receptor targets

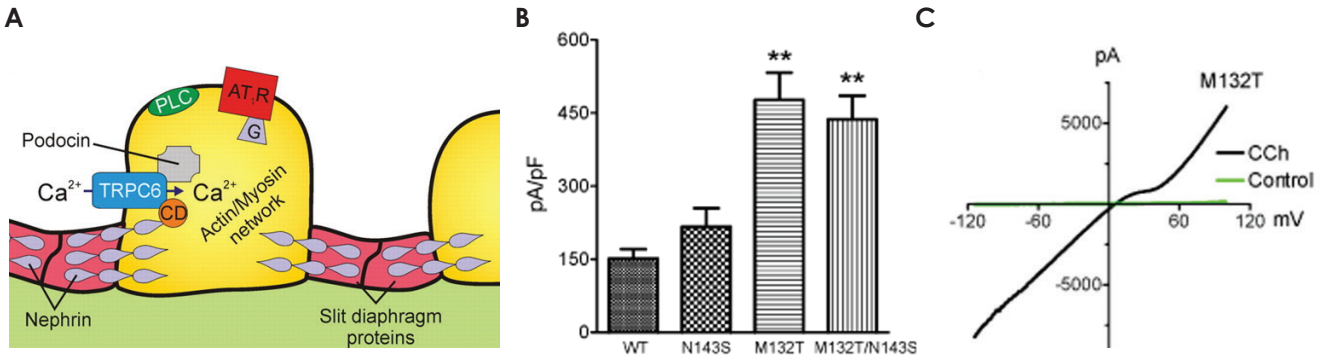


Figure 6 ***TRPC6 gain-of-function variants affect Ca^{2+} signalling and glomerular podocyte integrity.*** **A** *Schematic of the glomerular filtration barrier showing podocyte foot processes connected by the slit diaphragm, overlying the glomerular basement membrane and fenestrated capillary endothelium; TRPC6 is localized at the slit diaphragm in association with podocin, CD2AP, and nephrin.* **B** *Mean outward current amplitudes of wild-type (WT) TRPC6 and mutant channels measured at +100 mV, showing significantly larger currents for the M132T and M132T/N143S variants compared with WT.* **C** *Representative whole-cell currents from the M132T variant elicited using a voltage-ramp protocol (−120 to +100 mV) in the presence of 100 μM carbachol. Figures adapted: A from reference[47]; B, C from reference[48]. Images used under CC4.0 license.*

over the years, and disclosed potent TRPC3 and C6 agonist (GSK1702934A) and antagonist tool compounds (GSK417651A, GSK2293017A) discovered from a HTS that were active in cardiovascular assays but unsuitable for chronic dosing.[60] MPC data showed potent but non-selective effects (EC_{50} 100-400 nM, IC_{50} 10-40 nM). The company went onto develop more potent and selective TRPC3 and C6 blockers (GSK2833503A, GSK2332255B, GSK417654A) which are commercially available so no longer in development; BTDM is an analogue used to reveal a binding site in the TRPC6 channel.[52]

Novartis published on development of medium and high throughput APC assays for TRPC3, C6 and C7 channels expressed in CHO cells expressing M3 mAChRs to allow for carbachol Gq activation,[61] but they did not reveal which therapeutic indication(s) this was for although respiratory diseases are likely given their other clinical work. Nanion APC Patchliner and SyncroPatch platforms have also been used to study TRPC5 channels and develop analogues of AC1903.[63,64]

SAR7334 is a moderately selective and potent (8 nM) TRPC6 inhibitor developed by Sanofi-Aventis with 20-30 fold weaker antagonism of TRPC3 and C7 channels,65 and appears to have been developed to treat pulmonary and renal fibrotic disease. A Ca^{2+} imaging HTS of a pharmacophore library of 1,200 SKF96365 analogues identified more potent and selective compounds which also inhibited TRPC6 patch clamp channel activity with similar potency. Significantly, SAR7334 reduced apoptosis of renal proximal tubular cells *in vitro* and protected against kidney glomerular permeability and volume increases *in vivo*,[50] but rapid clearance precluded clinical development.

Goldfinch Bio was founded in 2016 and raised over $200 million over three financing rounds to support their preclinical and early clinical work on TRPC5 ionotropic receptors for CKD and FSGS. Small molecule inhibitors were identified in a 400K compound HTS screen in a FLIPR Ca^{2+} imaging assay and SAR of the pyridazinone series conducted using a plate-based membrane potential dye assay and APC, taking the 5 mM TRPC5 hit (TRPC6 IC_{50} > 30 mM) to the optimised lead candidate GFB-8438 with patch clamp potency of 200-300 nM against TRPC5 and good selectivity over TRPx and cardiac ion channels, but equipotency against TRPC4.[66] GFB-8438 protects against chemical-induced podocyte damage and kidney proteinuria. The clinical candidate GFB-887 inhibits TRPC5 channels with an IC_{50} of 37 nM and is nearly as efficacious as the tool compound ML-204 but 300 times more potent.[67] The company developed a groundbreaking preclinical PK-PD study using human iPSC-derived kidney organoids transplanted into mice to demonstrate oral drug exposure and target engagement, with GFB-887 producing equivalent protection against protamine sulfate-induced podocyte damage as the clinical comparator cyclosporine A, a calcineurin inhibitor. GFB-887 demonstrated mid-stage clinical efficacy and tolerability, and Goldfinch inked a major collaboration with Gilead in 2019 for diabetic kidney disease, but the company ran out of money in 2023 and their preclinical and clinical renal programs were purchased by Karuna Therapeutics to re-purpose for CNS neurological disorders.

Hydra Biosciences and Boehringer Ingelheim collaborated on a program to discover TRPC4 and C5 receptor antagonists using a Ca^{2+} imaging HTS and filed several patents in the mid-2010s, publishing preclinical data on HC-608 and HC-070 which were potent but non-selective inhibitors (IC_{50} of 0.5 nM by patch clamp) that also blocked heteromers containing TRPC1 subunit. However, this program appears focused on drugs for CNS indications rather than renal disease.[68] However, Boehringer Ingelheim also published on BI 749327 which is a 13 nM TRPC6 inhibitor with

40-80 fold selectivity over TRPC3 and C7 channels, little TRPC5 activity and good oral bioavailability discovered from a membrane potential assay HTS, which was used for preclinical studies of cardiac and renal disease.[69] The compound suppressed NFAT activation (a driver of fibrosis) triggered by activation of wildtype and FSGS mutant TRPC6 channels in HEK cells, and reduced kidney fibrosis pathology and transcriptome markers after oral administration.

Interest in pursuing TRPC5 and/or TRPC6 channels as renal disease targets never seemed to catch fire, for various reasons. Industry prefers a clear target I.D. so working on one of two closely related proteins raises doubts, not helped by the lack of potent and isoform-selective modulators until recent years (e.g. activators for assay specificity and inhibitors for target engagement and validation). There has also been conflicting animal model data from TRPC5 and TRPC6 knockout mice, but potential impact of species differences on TRPC translation remains unclear. The human genetic validation of TRPC6 in renal disease via podocin and FSGS gain-of-function mutations is compelling, but this metric only became a key driver of target selection in the last 5 years and TRPC6 mutation data is now more heterogeneous. We know that drug discovery targets with human genetics are more likely to be clinically successful, so it remains to be seen if TRPC6 drug discovery projects will start again and how they might compare to clinical progress of APOL1 programs (see below).

- AC1903 is non-selective mM TRPC6 blocker specifically tested as a renal disease drug in animal models of against FSGS, but lacks target specificity and potency
- TRPC5-selective compounds from Goldfinch Bio were specifically developed to treat renal disease and showed efficacy against podocyte damage and kidney proteinuria preclinically (GFB-8438) and clinically (GFB-887)
- The potent (13 nM) and selective TRPC6 inhibitor BI 749327 disclosed in 2019 was able to reduce signalling by FSGS TRPC6 gain-of-function mutations and reduce renal fibrosis *in vivo*
- Sanofi's SAR7334 is a moderately selective 10 nM TRPC6 inhibitor disclosed in 2015, and reduced renal proximal tubular cell apoptosis and protected against kidney glomerular permeability and volume increases *in vivo*
- SAR7334 analogue DS88790512 is an 11 nM orally bioavailable TRPC6 inhibitor disclosed by Daiichi-Sankyo[70] albeit with unknown selectivity or therapeutic applications

Most exciting is a recent structure-function paper from Amgen that revealed AM-1473 (another SAR7334 analogue) as the most potent TRPC6 inhibitor to date (220 pM IC_{50}) with 40 fold selectivity over TRPC3 channels. The company obtained high resolution 3.1Å cryo-EM structures of TRPC6 with agonist (AM-0883) and antagonist (AM-1473) bound, which coupled with analysis of FSGS-related mutations suggested that channel activation by small molecules and gain-of-function variants involves inter-subunit interactions. In contrast, other FSGS mutations cluster in cytosolic regions of the N- and C-terminus and seem to perturb this intra-subunit interaction and destabilise the closed state, which increases TRPC6 channel activity without changes in cell surface expression.[57] AM-1473 binds to a pocket between the S1-S4 VSD and pore helix not occupied by non-selective GSK antagonists; the agonist AM-0883 was found in a HTS. This recent progress in TRPC6 structure-function and pharmacology may facilitate future work to develop small molecule blockers, and perhaps also gene therapies, to treat patients with TRPC6 channelopathies and FSGS.

TRPP1/2 (PKD, PC) channels in polycystic kidney disease

Perhaps less well known are the TRPP1 and TRPP2 (Transient Receptor Potential Polycystin) ionotropic receptors encoded by the PKD1 and PKD2 genes, also known as polycystin proteins (PC1 and PC2) as mutations in this gene family are linked to autosomal dominant polycystic kidney disease (ADPKD) where large cysts form on the kidneys that inevitably lead to CKD and kidney failure (reviews[71,72]). 80% of ADPKD patients have a mutation in PKD1 and an average age of 55 for end-stage kidney failure, whereas 20% of mutations are in PKD2 which lead to later onset kidney

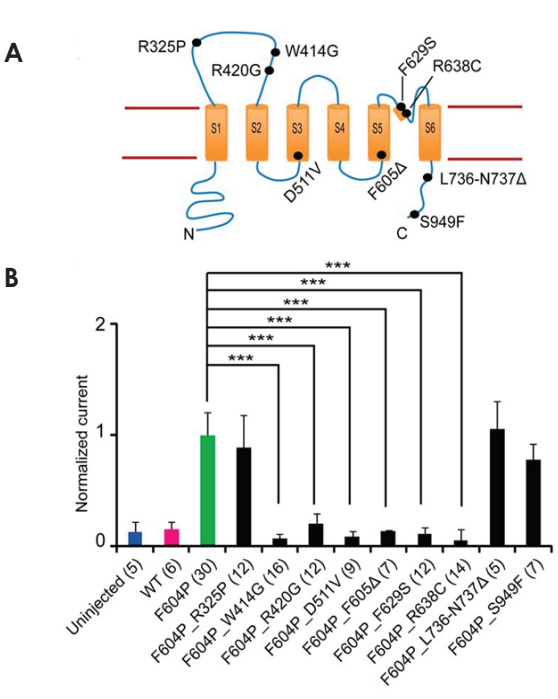


Figure 7 ***Effects of TRPP2 ADPKD mutations on current amplitude.*** **A** *Schematic topology of TRPP2 highlighting the locations of nine disease-associated point mutations.* **B** *Normalized currents of TRPP2_F604P and mutant channels showing the impact of pathogenic variants on channel function. Figure adapted from reference [79] under open access publication rights.*

failure; there are an estimated 160,000 patients in the US alone and 12 million worldwide, making ADPKD one of the most common monogenic human diseases. TRPP1 is a large transmembrane glycoprotein with extensive extracellular domains that are thought to underlie roles in cell adhesion, cellular sensing and organ development, and varied cytoplasmic domains for protein-protein interactions and G-protein signalling. TRPP1 acts as a regulatory subunit and promotes TRPP2 channel translocation from the ER to the plasma membrane, forming a 1:3 subunit tetraheteromer with TRPP2 as revealed by cryo-EM structure.[73] Like other TRPx receptors, TRPP2 channels are Ca^{2+} permeable but weakly voltage-dependent, and also exhibit prominent mechanosensation gating important for renal function.[74,75] Interestingly, TRPP1/2 proteins are notably expressed on the primary cilia of renal tubule cells where they contribute to mechanosensitive Ca^{2+} signalling, and a recent paper explored the novel proteosome of this intriguing structure, including data on proteins expressed in cilia of a kidney proximal tubule cell line.[76]

Over 250 pathogenic loss-of-function mutations in TRPP2 have been identified in kidney disease patients (pkd.mayo.edu, OMIM 173910), most of which are truncating (missense, nonsense, frameshift) and prevent protein expression and membrane insertion, along with splicing site mutations and ~30 single and double point deletion and substitution variants that can affect protein trafficking or ion channel function (Figure 7). ADPKD-related mutations occur throughout the protein and affect the function of each major domain, such as the N-terminus (protein localisation, trafficking to the cilia and subunit oligomerisation), external S1-S2 polycystin and glycosylation linker, S3-S4 voltage sensor domain and S5-S6 pore (gating, ionic selectivity), and various elements of the large C-terminus important for Ca^{2+} modulation (EF hand) and TRPP1/2 heteromerization (coiled-coil domain), suggesting that disruption of any of these domains can lead to catastrophic loss of function and severe kidney disease.[72] The R742X mutation lacking the putative EF hand cytoplasmic domain can be expressed in oocytes and rat sympathetic neurons, exhibiting similar biophysics but higher single channel Po and no Ca^{2+} activation.[77,78] In contrast, ADPKD mutations spread across extracellular and transmembrane domains of the TRPP2 channel protein (e.g. W414G, R420G, D511V, F605D, F629S, R638C) act as dominant negative suppressors of ionic currents (but not surface expression) when co-expressed with an artificial gain-of-function mutant F604P in Xenopus oocytes.[79] ADPKD is a heterogeneous genetic disease as each mutation is only found in <2% of patients, making for a diverse and complicated genotypic landscape (but largely consistent disease phenotype).

The only FDA-approved drug for ADPKD is the Vasopressin V2 receptor inhibitor tolvaptan, but this has a black box warning for potentially fatal liver failure so other treatments targeting the TRPP protein complex are now being developed. Importantly, mouse genetic models show that knockout of PKD1 and/or PKD2 produces severe kidney disease and cyst formation, and conditional re-expression in adult animals can rapidly reverse disease symptoms and pathology.[80] Macroscopic TRPP2 channel activity is hard to detect in whole-cell recordings, as it likely functions primarily as an ER Ca^{2+} release channel and no known agonist or opener exists apart from mechanical activation. Couple this to the fact that ADPKD mutations are loss-of-function and this makes drug discovery efforts to find potentiators and correctors very challenging. The F604P gain-of-function mutant may be a useful reagent for patch clamp screening as long as it retains the ability for additional augmentation, and it may also be a potential gene therapy to correct the TRPP2 loss-of-function phenotype in ADPKD.

Regulus have been pursuing a different gene therapy approach, developing an antisense oligonucleotide (ASO) to suppress the microRNA miR-17, which is upregulated in ADPKD and acts to repress expression of PKD1/2 genes and PC1/2 proteins which then leads to kidney cyst formation and proteinuria.[81] Preclinical data showed that several clinical candidates (RGLS4326 and RGLS8429 which became the successful clinical candidate Farabursen) rapidly and selectively accumulate in the kidney after s.c. administration and enter renal tubule cells and bind to miR-17, interrupting mRNA signalling and reducing cyst growth in several genetic mouse models (Figure 8). RGLS4326 was dropped due to poor efficacy and safety red flags, but Farabursen (1 – 3 mg/kg s.c. Injection every 2 weeks) successfully completed Ph I safety trials and IIa efficacy studies in ADPKD patients which showed it to be safe and produced dose-dependent changes in disease-related biomarkers (e.g. proteinurea, urinary PC1/2 protein levels) and reduced kidney volume growth rate.[82] Regulus had designed a Ph III study for Q3 2025 but then Novartis acquired the company for $800 million upfront in March 2025 to take over the rare kidney disease gene therapy.[83]

Renasant Bio, a spinout from UC San Francisco and Berkeley who came out of stealth in mid-2025, are taking a traditional pharmacological approach to treat ADPKD. They aim to develop small molecule 'potentiator' and 'corrector' programs for TRPP2 channels, analogous to CFTR drug discovery efforts by Vertex and others which delivered very successful and therapeutically effective drugs for cystic fibrosis patients. The corrector is in lead optimization and the potentiator is at the early discovery stage, with the company utilising Ca^{2+} imaging and both manual and automated patch clamp screening to progress these programs. Unlike CFTR and APOL1 drugs from Vertex, their strategy is to design and optimise small molecules that can modulate multiple ADPKD mutations, but like CFTR drugs these will likely be dosed as a combination of correctors and potentiators.[84]

APOL1 renal ion channel

The poster child for kidney channelopathy drug discovery is

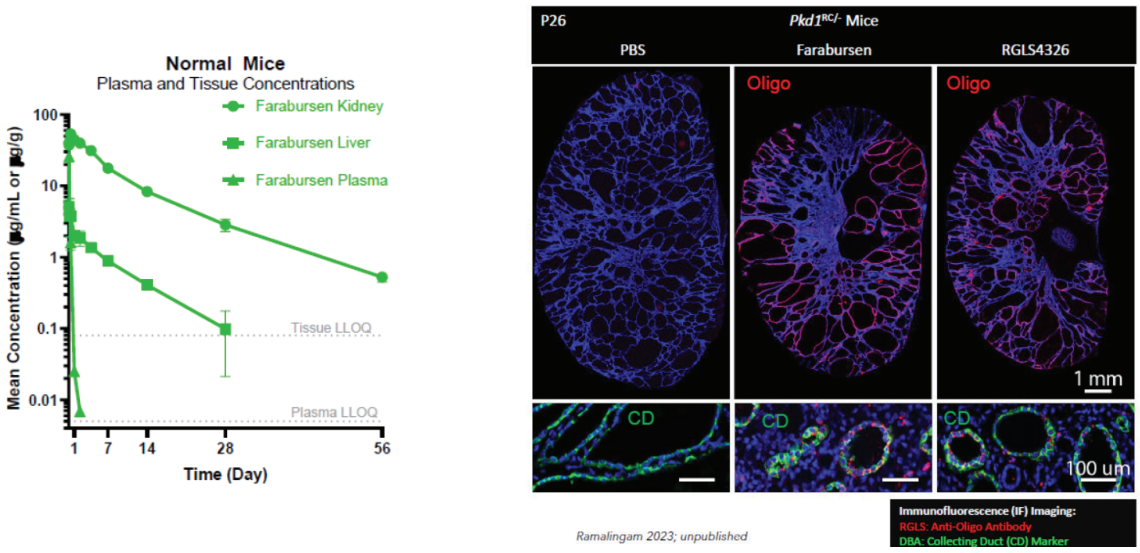
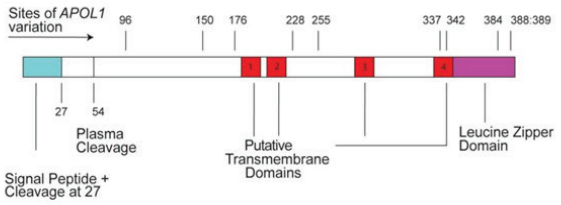


Figure 8 ***Preclinical data on Regulus Therapeutics miR-17 ASO gene therapy candidates for ADPKD.*** *Figure shows Farabursen being preferentially delivered to the kidney and localized to kidney cyst epithelium. Figure taken from Regulus corporate presentation (March 2025).*

APOL1, thanks to the industry-leading efforts of Vertex and their small molecule drug candidate VX-147 (inaxaplin) and Ionis/AZ with an ASO gene therapy (ION532, AZD2373), both of which have reached mid-stage clinical trials in recent years.

There is a fascinating target validation story behind APOL1, a little known cation ion channel predominantly expressed in the ER of primate cells where it is thought to be a cellular immune response protein, but also present on the surface of kidney podocytes.[85] APOL1 channel proteins are secreted from hepatocytes on high density lipoprotein particles, and evolutionary selection in sub-Saharan African primates and humans favoured gain-of-function variants that produced more effective protection against sleeping sickness by inducing lysis in trypanosome parasites.[86] These G1 and G2 variants are now highly prevalent in Africans (Figure 9) but the expression of over-active APOL1 cation channels in the kidney produces a high risk of chronic kidney disease and this adverse effect is most noticeable in African-Americans where 35% carry one risk allele and 10-15% carry both of the common G1 or G2 gain-of-function variants, with recessive inheritance of both increasing the occurrence of chronic kidney disease, focal segmental glomerulosclerosis (FSGS), HIV- and Covid-19-associated nephropathy, and biomarkers such as dialysis and proteinuria by 2-3 fold.[87,88] G0 is the most common allele across worldwide populations, and interestingly the Reference APOL1 sequence (G4) is a rare haplotype that may have entered our modern genome from Neanderthals. Two recent papers from US researchers also described a new APOL1 missense variant N264K that reduces the risk of CKD via a loss-of-function effect on cation currents and G1/G2 variant toxicity and disease inheritance,[89,90] further enriching the landscape of APOL1 disease-modifying channelopathies. There has been historical discussion about whether APOL1 exhibits pH-gated anion or cation permeability, but the former is mostly seen in artificial bilayers (and could occur in organelles?) whereas the latter effect is seen in native cells and can be recorded using patch clamp electrophysiology and fluorescent dye imaging (e.g. Ca^{2+} flux and membrane potential).[88, 89] However, APOL1 is unusual in needing an acidic stimulus to drive plasma membrane insertion, after which it operates as a cation channel (and cell lysis protein) at physiological pH. Also, APOL1 is the only isoform in the gene family able to escape intracellular organelles into the secretory pathway via a signal peptide (Figure 9). High throughput drug screening programs have utilised transient or stable expression of APOL1 variants to the plasma membrane of heterologous cell lines, and exploited non-selective cation channel permeability to design plate-based thallium flux, Ca^{2+} imaging and automated patch clamp assays to screen compound libraries and optimise small molecule inhibitors. Public disclosures on the preclinical discovery methods of APOL1 programs are limited to Vertex's small molecules and the Ionis/AZ ASOs (review[91]), while limited data is available on other APOL1 small molecule inhibitors from Maze Therapeutics and Podium Bio.

Vertex have the most advanced APOL1 drug discovery program (*https://www.vrtx.com/our-science/pipeline/apol1-mediated-kidney-disease/*) having started discovery work on small molecule inhibitors in the mid-2010s in response to publications on APOL1 variants linked to chronic kidney disease, and publishing on their preclinical assays and a 2021 Ph IIa clinical trial in FSGS recently[92,93] before starting a global Ph III trial in APOL1-mediated kidney disease (AMKD) patients in 2024. Their initial low potency HTS thallium flux



Global Frequency		Variation of *APOL1* Alleles									Distribution of Alleles within Populations			
Allele	Freq (%)	96	150	176	228	255	337	342	384	388/389	AFR Freq (%)	LAT AMR	ASN	EUR
G0	65.3	G	K	N	I	K	D	S	I	NY	10.4	19.6	31.6	38.4
Ref/ G4	15.6	G	E	N	M	R	D	S	I	NY	4.69	14.4	31.7	49.3
G1	4.85	G	E	N	I	K	D	G	M	NY	98.1	1.89	0	0
	3.21	G	E	N	I	K	D	S	I	NY	94.3	5.71	0	0
	3.07	R	K	N	I	K	D	S	I	NY	32.8	13.4	1.49	52.2
G2	2.01	G	E	N	I	K	D	S	I	--	95.5	4.55	0	0
	1.05	G	E	N	I	K	N	S	I	NY	95.7	4.35	0	0
	0.96	G	E	S	I	K	N	S	I	NY	90.5	9.5	0	0

Figure 9 ***APOL1 variant sequences and population frequencies; G1 and G2 kidney disease alleles.*** *From reference [88] and used under CC4/0 open license.*

assay hit (540 nM IC_{50}, 11% oral bioavailability) was optimised to increase potency and oral bioavailability and reduce metabolic liabilities and plasma clearance, resulting in an *in vivo* tool compound (4.2 nM, 28%) and the clinical candidate VX-147 (2.0 nM, 57%). Their small molecules all inhibited APOL1 variants equally, but measuring effects on whole-cell currents with APC looks challenging as current expression was not high, although they could show that non-disease G0 variant currents were smaller than those from G1 and G2 renal disease gain-of-function variants (despite lower surface antibody labelling) and that APC potencies closely matched thallium flux. Orthogonal assays including a HEK cell viability readout, a microscale thermophoresis fluorescent binding assay and a trypanosome lysis assay all delivered consistent nM potencies for VX-147. *In vivo* studies are complicated by the lack of APOL1 in rodents, so Vertex (and Ionis) developed a transgenic hAPOL1 G2 knock-in mouse to show that APOL1 inhibition reduced kidney podocyte cell death, proteinuria and glomerular pathology. Finally, VX-147 (Inaxaplin) is safe and significantly reduced proteinuria in a Ph IIa study of FSGS patients with genotyped APOL1 variants. Vertex have also disclosed a back-up compound VX-840 for their APOL1 program, which completed a Ph I safety trials in late 2022 (NCT05324410).

Maze Therapeutics recently broke cover with their own APOL1 small molecule inhibitor program, co-authoring the 2023 paper on the protective N264K variant[89] and publishing a poster abstract at the 2023 American Society of Nephrology describing a preclinical candidate MZ-301 that could inhibit G2 variant gain-of-function currents and reduce cytotoxicity in HEK cells and an immortalised podocyte cell line, reduce APOL1-mediated trypanosome lysis, and reduce proteinuria in transgenic APOL1 G2 knock-in mice.[94] The company nominated the clinical candidate MZE829 at the end of 2022 and started a Ph I safety trial in December 2023 with positive results released in October 2024, with an open label Ph II efficacy trial underway since early 2025 (see company press releases).

I can now also reveal that a small UK biotech Podium Bio (created by the Medicxi EU investment fund) is also working on APOL1 small molecule inhibitors, as their patent became public in July 2025[95] and was reported by several biopharma news websites at the time. The patent discloses structures of a thiophenyl series and the stable HEK APOL1 G1 variant cell line and HTS thallium flux assay used to identify and optimise them, revealing compound potencies of 10-1000 nM.

Another APOL1 drug discovery player may be Genentech, based on several papers describing APOL1 monoclonal antibodies (mAb) they used to determine the sub-cellular localisation and membrane topology of APOL isoforms.[96,97] This looks like a serious target validation effort as 170 rabbit and mouse mAbs were developed to show that only APOL1 (all G variants) was expressed at the cell surface, opening up 'new therapeutic targeting avenues' which presumably refers to small molecules or biologicals that could reduce APOL1 channel expression and/or activity. However, the authors suggested that large IgG mAbs would struggle to cross the kidney glomerular filtration barrier, so other antibody formats (scFv, nanobodies) might be needed to access podocyte APOL1 channel proteins. A follow-up paper showed differences in APOL1 G1 and G2 variant cytotoxicity in HEK cells and immortalised podocytes dependent on haplotype polymorphisms, which correlated with their thallium flux.[98]

Finally and not unexpectedly for a monogenic disease, APOL1 gene therapies have been developed and one has reached mid-stage clinical trials. This work is complicated by the fact that rodents do not express APOL genes so groups have generated global and podocyte-specific transgenic animals expressing human G0, G1 or G2 variants, but this approach has the advantage of testing reagents against the human sequence without the need for preclinical species-specific versions. A US academic group described APOL1 exon-targeting ASOs that reduced doxycycline-induced protein expression and protected mice from severe G2 variant-dependent albuminuria, glomerulosclerosis and renal fibrosis, inflammation and kidney failure.[99] Ionis took a similar transgenic mouse approach and published preclinical data on a 2nd generation APOL1 ASO in 2019 they variously called IONIS-APOL1Rx, IONIS-AZ5-2.5Rx or ION-532. Preclinical work used transgenic global hAPOL1 G0 and G1 mice but proteinuria, CDK transcriptome changes and podocyte damage were only seen in G1 mice after a 'second hit' of IFN-g administration.[100] Over 4000 ASOs were tested for knockdown of APOL1 expression in A431

cells *in vitro*, with the clinical candidate selected based on potency (IC_{50} of 105 nM), specificity and *in vivo* safety. Weekly sub-cutaneous administration of the ASO reduced APOL1 expression in kidney, liver and plasma in both G0 and G1 mice, and crucially prevented IFN-g induced proteinuria, reduced APOL1 mRNA and protein upregulation and normalised kidney transcriptome profiles. ION-532 was licensed to AstraZeneca in 2018 and renamed AZD-2373, and entered Ph I clinical trials in African-American volunteers in 2020 (NCT04269031) and again in 2022 (NCT05351047) where it was shown to be safe and able to dose-dependently reduce APOL1 plasma protein levels in patients carrying G1 or G2 variants receiving 6 weekly injections. In 2025 AZ initiated a Ph I safety and PK study and a Ph II efficacy study of Opemalirsen as this ASO gene therapy is now known, so this precision medicine therapy for chronic kidney diseases is progressing well.

References

1. Estévez R. et al. (2001). Nature 414:558-61. doi:10.1038/35107099
2. Janssen AG. et al. (2009). J Am Soc Nephrol. 20:145-53. doi:10.1681/ASN.2008010102
3. Coppola MA. et al. (2023). Biomolecules 13:710. doi:10.3390/biom13040710
4. Bokhari SRA, Zulfiqar H. and Mansur A. (2024) Bartter Syndrome. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK442019/
5. Mrad FCC et al. (2021). World J Pediatr. 17:31-39. doi:10.1007/s12519-020-00370-4
6. Welling PA and Ho K. (2009). Am J Physiol Renal Physiol. 297:F849-63. doi:10.1152/ajprenal.00181.2009
7. Hampton C. et al. (2016) J Pharm Expt Therap. 359:194-206. doi:10.1124/jpet.116.235150
8. Peters M et al. (2003). Kidney Int. 64:923-32. doi:10.1046/j.1523-1755.2003.00153.x
9. Fallen K et al. (2009) Channels (Austin) 3:57-68. doi:10.4161/chan.3.1.7817
10. Fang L, Li D and Welling PA. (2010). Am J Physiol Renal Physiol. 299:F1359-64. doi:10.1152/ajprenal.00257.2010
11. Nguyen NH et al. (2023). PLoS Genetics 19(11): e1011051. doi:10.1371/journal.pgen.1011051
12. Bhave G et al. (2011). Mol Pharmacol. 79:42-50. doi:10.1124/mol.110.066928
13. Walsh SP et al (2015). ACS Med Chem Lett. 7:747-52. doi:10.1021/ml500440u
14. Priest BT and Pasternak A. (2017). Future Med Chem. 9:1963-1977. doi:10.4155/fmc-2017-0083.
15. Tang H et al (2016). ACS Med Chem Lett. 12:697-701. doi:10.1021/acsmedchemlett.6b00122
16. Jiang J et al (2021). J Med Chem. 64:7691-7701. doi:10.1021/acs.jmedchem.1c00406
17. Poroca DR., Pelis RM. & Chappe VM (2017). Front Pharm 8:151. doi: 10.3389/fphar.2017.00151
18. Park E. Campbell E. & MacKinnon R. (2017). Nature 541, 500–505. doi:10.1038/nature20812
19. Louet M. et al. (2017). Sci Rep 7:7249. doi:10.1038/s41598-017-07794-5
20. Jentsch TJ & Pusch M. (2018). Physiological reviews 98:1493-1590. doi: 10.1152/physrev.00047.2017
21. Cheng CJ et al. (2017). J Physiol 595:5573-5586. doi: 10.1113/JP274344
22. Scholl U et al. (2006). Proc. Natl. Acad. Sci. U.S.A. 103:11411-11416. doi:10.1073/pnas.0601631103
23. Fahlke C. & Fischer M. (2010). Front. Physiol 1:155. doi:10.3389/fphys.2010.00155
24. Janssen AG et al. (2008). J Am Soc Nephrol. 20:145-53. doi:10.1681/ASN.2008010102
25. Gradogna A. and Pusch M. (2010). Front Pharmacol. 1:130. doi:10.3389/fphar.2010.00130.
26. Liantonio A. et al. (2006). Mol Pharmacol. 69:165-73. doi:10.1124/mol.105.017384
27. Imbrici P. et al. (2014). Biochim Biophys Acta. 1838:2484-91. doi:10.1016/j.bbamem.2014.05.017.
28. Gradogna A. et al. (2014). Biochimica et Biophysica Acta (BBA)-Biomembranes 1838:2745-2756. doi: 10.1016/j.bbamem.2014.07.021
29. Mansour□Hendili L. et al. (2015). Human Mutation 36:743-752. doi: 10.1002/humu.22804
30. 30. Smith AJ. et al. (2009). American J Physiol-Renal Physiol 296:F390-F397. doi: 10.1152/ajprenal.90526.2008
31. Lourdel S. et al. (2012). Pflugers Arch - Eur J Physiol 463: 247–256. doi:10.1007/s00424-011-1052-0
32. Gorvin CM. et al. (2013). Proc. Natl. Acad. Sci. U.S.A. 110:7014-7019. doi:10.1073/pnas.1302063110
33. Liu J, Sadeh TT, Lippiat JD, Thakker RV, Black GC, Manson F. J Cell Mol Med. 2021 Jan;25(2):1319-1322. doi: 10.1111/jcmm.16091. Epub 2020 Nov 16. PMID: 33200471; PMCID: PMC7812281.
34. Lyu P. et al. (2024). Gene Ther. 31:563-571. doi:10.1038/s41434-024-00490-w
35. Garcia-Celma J., Szydelko A. and Dutzler R. (2013). J Gen Physiol. 141:479-91. doi:10.1085/jgp.201210927
36. Cunniff, J et al. (2024). Poster on Judo Bio company website. https://judo.bio/wp-content/uploads/2024/10/JudoBio_Cunniff_OTS-poster-2024.pdf
37. Drozdzik M., Drozdzik M. and Oswald S. (2021). Biomedicines 9:426. doi:10.3390/biomedicines9040426
38. Yee SW and Giacomini KM. (2021). Drug Metab Dispos. 50:1193–210. doi:10.1124/dmd.121.000702
39. Ding, S et al. (2024). Poster on Judo Bio company website https://judo.bio/wp-content/uploads/2024/10/2024-Judo-ASN-poster_Final.pdf
40. Ding WY. et al. (2023). Sci. Transl. Med. 15:eabc8226. doi: 10.1126/scitranslmed.abc8226

41. Pablo JL and Greka A. (2019). Trends Pharmacol Sci. 40:911-918. doi:10.1016/j.tips.2019.10.001

42. Hall G., Wang L. and Spurney RF. (2019). Cells 9:44. doi: 10.3390/cells9010044

43. Szabó T. et al. (2015). Acta Physiol Hung. 102:241-51. doi: 10.1556/036.102.2015.3.2

44. Englisch CN., Paulsen F. and Tschernig T. (2022). Int J Mol Sci. 24:181. doi: 10.3390/ijms24010181

45. Staruschenko A. et al. (2023). Physiological Rev 103:787-854. doi: 10.1152/physrev.00013.2022

46. Schaldecker T. et al. (2013). J Clin. Invest. 123:5298-5309. doi: 10.1172/JCI71165

47. Nilius B. et al. (2007). Physiol Rev 87:165-217. Doi: 10.1152/physrev.00021.2006

48. Heeringa SF. et al. (2009). PLoS ONE 4:e7771. https://doi.org/10.1371/journal.pone.0007771

49. Minard, A., Bauer, C. C., Wright, D. J., Rubaiy, H. N., Muraki, K., Beech, D. J., & Bon, R. S. (2018). Cells, 7(6), 52. Doi: 10.3390/cells7060052

50. Wang H. et al. (2020). Pharmacol Ther. 209:107497. doi:10.1016/j.pharmthera.2020.107497

51. Gao YY et al. (2021). Arch Pharm Res. 44:354-377. doi: 10.1007/s12272-021-01319-5

52. Bon RS. et al. (2022). Annual Rev Pharm and Toxicol 62:427-446. doi: 10.1146/annurev-pharmtox-030121-122314

53. Sharma SH. et al. (2019). Bioorg Med Chem Lett. 29:155-159. doi: 10.1016/j.bmcl.2018.12.007

54. Baradaran-Heravi A. et al. (2022). J Biol Chem. 298:101546. doi: 10.1016/j.jbc.2021.101546

55. Richter JM., Schaefer M. and Hill K. (2014). Mol. Pharm. 86:514-521. doi: 10.1124/mol.114.093229

56. Rubaiy HN. et al. (2017). J Biol Chem. 292:8158-8173. doi:10.1074/jbc.M116.773556

57. Bai, Y et al., (2020). eLife 9:e5331. doi: 10.7554/eLife.53311

58. Song, K. et al. (2021). eLife 10:e63429. doi: 10.7554/eLife.63429

59. Qiao, X. et al. (2025). Med. Res. Rev.. doi: 10.1002/med.70007

60. Xu, X. et al. (2013). Biophysical J 104:454a. doi: 10.1016/j.bpj.2012.11.2513

61. McPate M. et al. 2014). Assay Drug Dev Technol. 12:282-92. doi: 10.1089/adt.2014.574

63. Xu SZ. et al. (2008). Nature 451:69–72. doi: 10.1038/nature06414

64. Sharma SL. et al. (2022). ChemMedChem, 17: e202200151. doi: 10.1002/cmdc.202200151

65. Maier T. et al. (2015). Br J Pharm. 172:3650-60. doi: 10.1111/bph.13151

66. Yu M. et al. (2019). ACS Med Chem Lett. 10:1579-1585. doi: 10.1021/acsmedchemlett.9b00430

67. Westerling-Bui, AD. et al (2022). Science Advances, 8:eabj5633. doi: 10.1126/sciadv.abj5633

68. Just S. et al. (2018) PLoS ONE 13: e0191225. doi: 10.1371/journal.pone.0191225

69. Lin BL. et al. (2019). Proc. Natl. Acad. Sci. U.S.A. 116:10156-10161. doi: 10.1073/pnas.1815354116

70. Motoyama K. et al (2018). Bioorganic & Med. Chem. Letters, 28:2222-2227. doi: 10.1016/j.bmcl.2018.03.056

71. Qamar S., Vadivelu M. and Sandford R. (2007). Biochem Soc Trans. 35:124-8. doi: 10.1042/BST0350124

72. Brill AL. & Ehrlich BE. (2020). Cellular Signalling, 66:109490. doi: 10.1016/j.cellsig.2019.109490

73. Su Q. et al. (2018). Science 361:eaat9819. doi: 10.1126/science.aat9819

74. González-Perrett S. et al. (2001). Proc. Natl. Acad. Sci. U.S.A. 98:1182-1187. doi: 10.1073/pnas.98.3.1182

75. Giamarchi A. et al. (2006). EMBO Reports 7:787-793. doi: 10.1038/sj.embor.7400745

76. Hansen JN. Et al. (2025). Cell. Doi: 10.1016/j.cell.2025.08.039

77. Vassilev PM. Et al 2001). Biochem Biophys Res Commun. 282:341-50. doi: 10.1006/bbrc.2001.4554

78. Delmas P. et al. (2004). FASEB J. 18:740-2. doi: 10.1096/fj.03-0319fje

79. Pavel MA. et al. (2016). Proc. Natl. Acad. Sci. U.S.A. 113:E2363-E2372. doi:10.1073/pnas.1517066113

80. Dong K. et al (2021). Nat Genet 53:1649–1663. doi:10.1038/s41588-021-00946-4

81. Patel V. et al. (2013). Proc Natl Acad Sci U S A. 110:10765-70. doi: 10.1073/pnas.1301693110

82. Yu, ASL. et al. (2024). J American Soc. Nephrology 35(10S). doi:10.1681/ASN.2024w1ps7ssb

83. Taylor, NP (2025). Fierce Biotech. https://www.fiercebiotech.com/biotech/novartis-pays-800m-buy-regulus-phase-3-ready-kidney-drug?

84. Grinstein, JD (2025). Inside Precision Medicine https://www.insideprecisionmedicine.com/topics/translational-research/renasant-bio-launches-to-solve-complex-kidney-disease-adpkd/

85. Bruggeman LA., Sedor JR. and O'Toole JF. (2021). Curr Opin Nephrol Hypertens. 30:317-323. doi: 10.1097/MNH.0000000000000704

86. Cooper A. et al. (2017). eLife 6:e25461. doi:10.7554/eLife.25461

87. Bajaj A. et al. (2020). Kidney international, 97:1032-1041. doi: 10.1016/j.kint.2020.01.027

88. Giovinazzo JA. et al. (2020). eLife 9:e51185. doi: 10.7554/eLife.51185

89. Hung, AM. et al. (2023). J American Soc Nephrology 34:1889-1899. doi:10.1681/ASN.0000000000000219

90. Gupta Y. et al. (2023). Nat Commun 14:7836. doi:10.1038/s41467-023-43020-9

91. Vasquez-Rios, G. et al. (2023). Kidney Intern. Rep. 8:2226-2234. doi: 10.1016/j.ekir.2023.08.028

92. Egbuna O. et al. (2023). N Engl J Med. 388:969-979. doi: 10.1056/NEJMoa2202396

93. Zimmerman B. et al. (2025). Nat Commun 16:167. doi:10.1038/s41467-024-55408-2

94. Assimon V. et al. (2023). American Society of Nephrology conference abstract FR-PO318. https://www.asn-online.org/education/kidneyweek/archives/

KW22Abstracts.pdf

95. Lightfoot, A. et al (2024). APOL1 INHIBITORS. WO/2025/163336

96. Scales SJ. et al. (2020). J Amer. Soc. Nephrol. 31:2044-2064. doi: 10.1681/ASN.2019080829

97. Gupta N. et al. (2020). J Amer. Soc. Nephrol 31:2065-2082. doi: 10.1681/ASN.2019080830

98. Gupta N. et al. (2024). Sci Rep. 14:3765. doi: 10.1038/s41598-024-53298-4

99. Yang YW. et al. (2022). Molecular Therapy 30:2491-2504. doi: 10.1016/j.ymthe.2022.04.007

100. Aghajan M. et al. (2019). J Clin Invest. Insight 4:e126124. doi: 10.1172/jci.insight.126124

Contact Information

Dr. Alison Obergrussberger
Director of Scientific Sales and Customer Engagement
Nanion Technologies GmbH
Ganghoferstr 70a
DE - 80339 München
ali@nanion.de
www.nanion.de

Dr. Marc Rogers
Founder and Director
Albion Drug Discovery Services Ltd
channelogist@gmail.com