Targeted gene therapy for rare genetic kidney diseases

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Chronic kidney disease is one of the leading causes of mortality worldwide because of kidney failure and the associated challenges of its treatment including dialysis and kidney transplantation. About one-third of chronic kidney disease cases are linked to inherited monogenic factors, making them suitable for potential gene therapy interventions. However, the intricate anatomical structure of the kidney poses a challenge, limiting the effectiveness of targeted gene delivery to the renal system. In this review, we explore the progress made in the field of targeted gene therapy approaches and their implications for rare genetic kidney disorders, examining preclinical studies and prospects for clinical application. *In vivo* gene therapy is most commonly used for kidney-targeted gene delivery and involves administering viral and nonviral vectors through various routes such as systemic, renal vein, and renal arterial injections. Small nucleic acids have also been used in preclinical and clinical studies for treating certain kidney disorders. Unexpectedly, hematopoietic stem and progenitor cells have been used as an *ex vivo* gene therapy vehicle for kidney gene delivery, highlighting their ability to differentiate into macrophages within the kidney, forming tunneling nanotubes that can deliver genetic material and organelles to adjacent kidney cells, even across the basement membrane to target the proximal tubular cells. As gene therapy technologies continue to advance and our understanding of kidney biology deepens, there is hope for patients with genetic kidney disorders to eventually avoid kidney transplantation.

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Editor's Note

Genomic medicine is an emerging discipline empowered by the technological advances in sequencing, computation, and bioinformatics. These advances have accelerated the characterization of the genetic architecture of human disease and allowed the development of accurate and cost-effective molecular diagnostic modalities and prognostic tools. These tools, from targeted gene panels to genome sequencing, are now becoming broadly available to clinicians, enabling accurate molecular diagnosis of hundreds of inherited kidney disorders and implementation of precision medicine. However, the true realization of genomic medicine will ultimately depend on the availability of targeted treatments of the newly diagnosed gene defects. In this issue of *Kidney International*, Khare and Cherqui provide a concise overview of the current approaches and techniques used to develop new gene therapies for inherited kidney disorders. This is the first article in our new mini review series titled "Kidney Genomic Medicine." Subsequent articles will cover diverse topics, ranging from new research methods in genetics and genomics to clinical genetic testing, pharmacogenomics, exposomics, polygenic risk scores, prenatal genetic diagnostics, reproductive planning, and ethical, legal, cost, and privacy issues related to kidney genomic medicine. See the Kidney Genomic Medicine series at https://www.kidney-international.org/content/kidney-genomic-medicine.

Chronic kidney disease is a major cause of morbidity and mortality, affecting >10% of the population worldwide.[1] The current standard of care for nephropathies advancing to kidney failure involves dialysis and kidney transplantation. Dialysis is associated with numerous complications including long-term cardiovascular disease.[2]

Kidney replacement therapy also presents many challenges, including severe shortage of donor organs with an average waiting time of 3 to 5 years and lifelong immunosuppression that can result in infections and post-transplant lymphoproliferative disorders.[3] Nearly 30% of chronic kidney disease with onset at <25 years of age are linked to inherited monogenic factors with ∼150 recognized genetic kidney diseases caused by mutations in ∼80 genes.[4–6] Advances in genomic technologies are expected to add to the current list. Therefore, gene therapy emerges as a valuable alternative to improve the management of chronic kidney disease.

Over the past 30 years, there have been significant advancements in gene therapy–based treatments, marked by a rising number of commercially available gene therapy products. However, when compared to organs such as the brain, eyes, or liver, success of gene therapy studies specifically targeting the kidneys has been limited. Several factors contribute to this lag, including the complex nature of the kidney being composed of >18 to 26 different cell types making specialized compartments,[7–9] and its rigorous filtering function performed by the glomeruli, which exclude any particle >50 kDa and 10 nm in size.[4,10] Intravenous (i.v.) injection of gene therapy products has been mostly used for kidney-targeted gene delivery. However, numerous studies have also been conducted to optimize the most effective mode of *in vivo* delivery of genetic products to the kidney, including delivery via the renal vein,[11,12] retrograde ureteral injection,[13] subcapsular injection,[4,14] renal pelvis,[15] renal artery injection,[16] and renal parenchymal delivery.[17] In addition, viral and nonviral vehicles have been used. This review will describe different gene therapy approaches for inherited kidney disorders, covering *in vivo* delivery of targeted genes as well as small nucleic acids to modulate gene expression. We will also explore the potential of using *ex vivo* hematopoietic stem cell gene therapy and examine the mechanisms involved in genetic transfer to kidney cells.

Direct *in vivo* gene addition for kidney-targeted gene delivery in inherited kidney diseases

Nonviral vectors offer multiple advantages with low immunogenicity, cost-effectiveness, and accommodation of various gene sizes.[18] However, their effect is transient and with lower transfection efficacy. Researchers are actively addressing this limitation through innovative strategies, and promising results have been demonstrated for genetic kidney diseases (Figure 1 and Table 1[12,13,15,16,19–44]). The first successful gene therapy for an inherited kidney disease was for carbonic anhydrase II deficiency characterized by renal tubular acidosis, cerebral calcification, and osteopetrosis. Correction of renal tubular acidosis in carbonic anhydrase II–deficient mice for up to 3 weeks was achieved by cationic liposome–mediated delivery of a plasmid containing human *CAII* cDNA via renal pelvis injection.[15] Expression of carbonic anhydrase II was observed in the corticomedullary junction and tubular cells of the outer medulla. This successful gene therapy marked a significant advancement in the field, showcasing the potential for targeted genetic interventions to address specific genetic renal diseases. Fabry disease (FD) is an X-linked systemic disorder caused by mutations in the *GLA* gene encoding the lysosomal enzyme α-galactosidase A (α-Gal A), resulting in systemic accumulation of globotriaosylceramide (Gb3) and globotriaosylsphingosine. FD is associated with life-threatening complications such as progressive kidney failure, heart failure, and stroke. Administration of naked plasmid DNA containing human *GLA* cDNA in a mouse model of FD via hydrodynamics-based retrograde renal vein injection led to expression of α-Gal A in the injected kidney for 1 week with a reduction in Gb3 persisting for 2 weeks.[12]

The use of nonviral vector–based solid lipid nanoparticles carrying plasmid DNA containing *GLA* demonstrated higher efficacy compared to naked plasmid DNA with greater α-Gal A activity detected in the kidneys after i.v. administration once a week for 3 weeks.[34]

To improve kidney gene delivery efficiency, viral vectors have been used (Figure 1 and Table 1), with the adeno-associated virus (AAV) emerging as the predominant choice for *in vivo* gene therapy. AAV is a nonpathogenic parvovirus with a 4.7 kb DNA genome, which is predominantly episomal and transduces both proliferating and differentiated cells. AAV has different serotypes, each having preferential tropism for different tissues. The comparison of AAV2, AAV6.2, AAV8, and AAVrh10 vectors delivered by subcapsular injection reveals that the highest delivery by AAV6.2 and AAV8 is valuable information that can guide the selection of vectors for effective gene delivery to the kidney.[4] Another comparative study of the recombinant AAV serotypes 5, 6, 8, and 9 showed that AAV9 was optimal for kidney-targeted gene delivery via the renal vein route.[11] Several studies using i.v. injection of AAV carrying human *GLA* under the control of ubiquitous promoters have been conducted in mouse models of FD. Because α-Gal A is a secreted enzyme, it is possible to obtain a beneficial effect in the kidney without direct gene delivery in that tissue.[45–52] However, the AAV1-*GLA* vector injected into both adult and neonatal mice resulted in a significant decrease in Gb3 levels in neonatal mice with up to 55% reduction compared with untreated mice but limited decrease was observed in the kidney of adult mice despite high levels of circulating α-Gal A.[29] In contrast, using other AAV serotypes than AAV1, α-Gal A expression was detected within the kidney correlating with the reduction in Gb3 levels. Indeed, Choi *et al.* reported a sustained elevation of α-Gal A enzyme activity for 60 weeks with expression observed in the proximal tubules and glomeruli in the kidney post i.v. injection of pseudotyped AAV2/8-*GLA*.[30] Similar results were obtained with AAV9-*GLA* resulting in detectable levels of α-Gal A expression and a concomitant reduction in Gb3 levels in the kidney.[31,32] Steroid-resistant nephrotic syndrome ranks the second most common contributor of kidney failure in the first 2 decades of life.[53] One-third of childhood steroid-resistant nephrotic syndrome is inherited, with the most common mutations being reported in the *NPHS2* gene, which

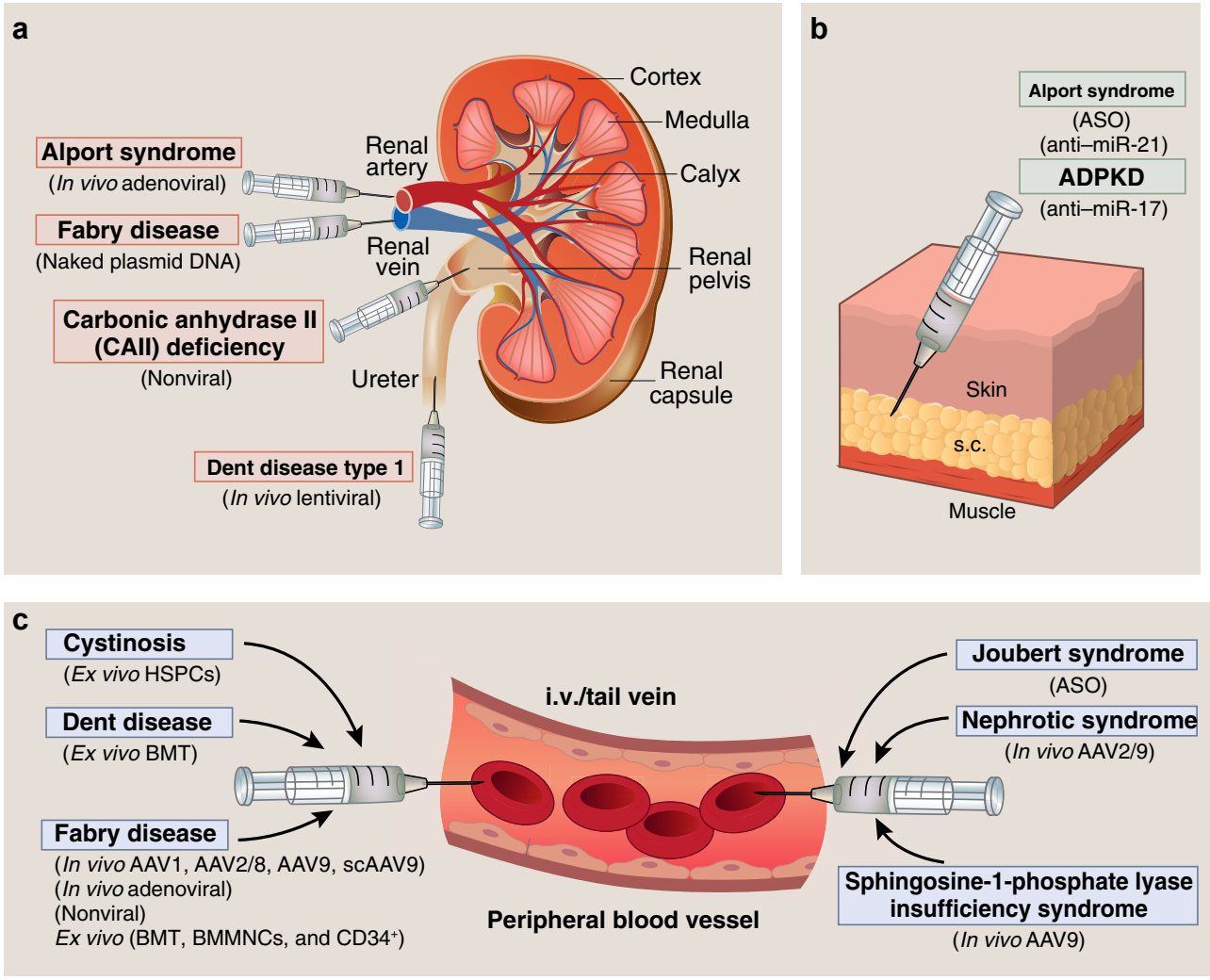

Figure 1 | Schema illustrating the methods for kidney-targeted gene delivery in genetic kidney diseases. The diagram depicts distinct routes used to attain therapeutic outcomes in the renal system. (**a**) Direct kidney gene delivery via the renal artery, renal vein, ureter, and renal pelvis used to achieve therapeutic effects in the context of 4 diseases. (**b**) The administration of small nucleic acid therapy is primarily facilitated through s.c. injection. (**c**) The systemic route is used for both *in vivo* gene therapy using adeno-associated virus (AAV) and antisense oligonucleotide (ASO) and *ex vivo* hematopoietic stem cell gene therapy applications. ADPKD, autosomal dominant polycystic kidney disease; BMMNC, bone marrow mononuclear cell; BMT, bone marrow transplantation; HSPC, hematopoietic stem and progenitor cell; miR, microRNA; scAAV, self-complementary adeno-associated virus.

encodes podocin. Prophylactic i.v. injection of AAV2/9 carrying hemagglutinin-tagged *Nphs2* cDNA under the control of the minimal human or mouse nephrin (podocyte-specific) promoter in *Nphs2* conditional knockout mice resulted in improved kidney function/damage as measured by plasma creatinine and urea, urine albumin, as well as long-term survival. AAV-inverted terminal repeats were detected in the kidney cortex and hemagglutinin-tagged podocin colocalized with nephrin and endogenous podocin in the podocytes. Furthermore, injection of AAV2/9-*Nphs2*-hemagglutinin 2-weeks post-disease induction demonstrated improvements in albuminuria and plasma albumin.[42] This research highlights the potential of AAV-based gene therapy as a therapeutic approach for nephrotic syndrome. Sphingosine-1-phosphate lyase insufficiency syndrome is an ultrarare metabolic disorder caused by loss-of-function mutations in *SGPL1*, which encodes sphingosine-1-phosphate lyase 1. Sphingosine-1-phosphate lyase insufficiency syndrome is manifested clinically by steroid-resistant nephrotic syndrome progressing rapidly to kidney failure as well as impairment of endocrine, neurological, and hematopoietic systems. Temporal vein injection of AAV9 containing the human *SGPL1* in *Sgpl1*-knockout newborn mice led to the prevention of nephrosis and prolonged survival, 137 ± 114 days versus untreated mice 11 ± 7 days.[43] Human sphingosine-1-phosphate lyase 1 was widely expressed in the kidneys of treated mice, detectable in both tubular and glomerular cells. A follow-up study using AAV9-*SGPL1* 2.0 that contained the cytomegalovirus early enhancer/chicken β-actin promoter instead of the original cytomegalovirus promoter led to newborn *Sgpl1* knockout mice surviving for at least 5 months with a delayed onset of proteinuria.[44] However, treated mice

Table 1 | List of rare genetic kidney diseases with their defective gene, protein, and kidney-directed gene therapy clinical trial

Disease	Defective protein (gene)	Clinical manifestation: renal/extrarenal	Gene therapy	Mode of delivery (injection)	Kidney-directed clinical trial (current status)
AS	Collagen type IV alpha 5 chain (*COL4A5*) Collagen type IV alpha 3 chain (*COL4A3*) Collagen type IV alpha 4 chain (*COL4A4*)	*Renal:* hematuria, proteinuria, progression to kidney failure *Extrarenal:* sensorineural hearing loss, ocular defects, leiomyomatosis of the esophagus and genitalia	*In vivo* (adenovirus)[16] ASO/exon skipping[19] Anti–miR-21[20] Anti–miR-21 + ACE inhibitor[21]	Renal artery s.c. s.c. s.c.	NCT03373786 (phase 1) (completed) NCT02855268 (phase 2) (discontinued)
ADPKD	Polycystin 1 (*PKD1*) Polycystin 2 (*PKD2*)	*Renal:* moderately increased albuminuria, hypertension, proteinuria, hematuria, kidney failure *Extrarenal:* hepatic and pancreatic cysts, intracranial aneurysms, cardiac disease, diverticular disease, hernias	RGLS4326 (anti–miR-17)[22–24]	s.c.	NCT04536688 (completed) NCT05521191 (active/recruiting)
CAII deficiency	Carbonic anhydrase 2 (*CAII*)	*Renal:* renal tubular acidosis *Extrarenal:* osteopetrosis, cerebral calcification	Nonviral vector[15]	Renal pelvis	
Cystinosis	Cystinosin (*CTNS*)	*Renal:* early Fanconi syndrome and then progresses to kidney failure *Extrarenal:* eye involvement (photophobia, retinal depigmentation, visual impairment), hypothyroidism, portal hypertension, muscle weakness, bone defects, incomplete pubertal development, encephalopathy	Autologous *ex vivo* HSPCs[25–27]	Systemic (i.v.)	NCT03897361 (active)
Dent disease (type 1)	Chloride voltage-gated channel 5 (*CLCN5*)	*Renal:* Fanconi syndrome with kidney failure (in men), radiopaque calcium stones, hypercalciuria, nephrocalcinosis *Extrarenal:* rickets	*Ex vivo* BMT[28] *In vivo* (lentivirus)[13]	Systemic (i.v.) Retrograde ureter	

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Table 1 | (Continued)

Disease	Defective protein (gene)	Clinical manifestation: renal/extrarenal	Gene therapy	Mode of delivery (injection)	Kidney-directed clinical trial (current status)
Fabry disease	α-Galactosidase A (*GLA*)	*Renal:* renal parapyelic cysts, proteinuria, progressive kidney failure; sometimes tubule dysfunction (polyuria, Fanconi syndrome) *Extrarenal:* eye (cornea verticillata), auditory defects, pain (acromelalgia), skin (angiokeratomas, anhydrosis), neurological defects, strokes (hearing loss, ataxia, vascular dementia), heart (left ventricular hypertrophy, conduction anomalies, valve anomalies, angina)	*In vivo* (AAV1)[29]	Systemic (i.v.)	
			In vivo (AAV2/8)[30]	Systemic (i.v.)	
			In vivo (AAV9)[31]	Systemic (i.v.)	
			In vivo (scAAV9)[32]	Systemic (i.v.)	
			In vivo (Ad2)[33]	Systemic (.v.)	
			Naked plasmid DNA[12]	Retrograde renal vein	
			Nonviral vectors (SLN)[34]	Systemic (i.v.)	
			Ex vivo (BMT)[35]	Systemic (i.v.)	
			Ex vivo (BMMNCs)[36,37]	Systemic (i.v.)	
			Ex vivo transduced $CD34^+$ cells[38–40]	Systemic (i.v.)	NCT04999059 (discontinued) NCT02800070 (completed) NCT03454893 (discontinued)
Joubert syndrome	Multiple genes, one of them is centrosomal protein 290 (*CEP290*)	*Renal:* renal cystic disease, typical features of nephronophthisis *Extrarenal:* abnormal eye movements, developmental delay, cerebellar vermis hypoplasia, congenital hepatic fibrosis, hypotonia, polydactyly	ASO/exon skipping[41]	Systemic (series of i.v.)	
Nephrotic syndrome	Podocin (*NPHS2*)	*Renal:* proteinuria, hypoalbuminemia *Extrarenal:* hyperlipidemia, thromboembolism, edema	*In vivo* (AAV2/9)[42]	Systemic (i.v.)	
Sphingosine-1-phosphate lyase insufficiency syndrome	Sphingosine-1-phosphate lyase 1 (*SGPL1*)	*Renal:* SRNS, progressing to kidney failure *Extrarenal:* adrenal insufficiency, neuronal defects, ichthyosis, lymphopenia with or without immune deficiency	*In vivo* (AAV9)[43]	Systemic (i.v.)	
			In vivo (AAV9) with the CAG promoter[44]	Systemic (i.v.)	

AAV, adeno-associated virus; ACE, angiotensin-converting enzyme; Ad, adenovirus; ADPKD, autosomal dominant polycystic kidney disease; AS, Alport syndrome; ASO, antisense oligonucleotide; BMMNC, bone marrow mononuclear cell; BMT, bone marrow transplantation; CAG, chicken β-actin; CAII, carbonic anhydrase II; HSPC, hematopoietic stem and progenitor cell; miR, microRNA; scAAV, self-complementary adeno-associated virus; SLN, solid lipid nanoparticle; SRNS, steroid-resistant nephrotic syndrome.

eventually developed nephrosis and glomerulosclerosis, contributing to their death. Administering a single dose of AAV-*SGPL1* in the very early days of mice (1–5 days of life) could be a factor contributing to the study's shortcomings because of the episomal nature of AAVs and cell division during growth. Also, the detection of anti-AAV antibodies in the first study suggests that immune response may affect the longevity and efficacy of gene therapy. Although AAVs have several clinical trials and approved drug products for genetic disorders, there has been no successful attempts at treating human monogenic kidney disease using this approach thus far. Lentiviral vectors (LVs) have also been used for kidney-targeted gene delivery. These vectors integrate into the genome allowing long-term transgene expression, have a high packaging capacity of up to 9 kb and low immunogenicity, and can transduce both dividing and nondividing cells. Dent disease type I is caused by mutations in the *CLCN5* gene encoding for an endosomal chloride voltage-gated channel 5 (ClC-5), leading to severe proximal tubule dysfunction and defective receptor–mediated endocytosis. Affected individuals develop proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, and eventually kidney failure. Retrograde ureter injection of LVs containing codon-optimized human *CLCN5* cDNA in a Dent disease type I mouse model resulted in detectable chloride voltage-gated channel 5 in kidney tubules and glomeruli and improvements in proteinuria, hypercalciuria, and diuresis for all treated mice at different ages of injection, ranging from 53 to 196 days.[13] However, the therapeutic benefits observed were sustained only for a duration of 3 months. Immune response to the chloride voltage-gated channel 5 protein was likely the cause of the loss of therapeutic effects. This underscores the importance of addressing immune reactions for successful gene therapy outcomes. Using promoters specific to proximal tubular cells may avoid expression in dendritic cells that play a role in adaptive immune responses and minimize the immune reaction. These factors add to the complexity of gene therapy, highlighting the need for a comprehensive understanding of various elements influencing its success.

Adenoviral vectors are one of the oldest platforms to be used for *in vivo* gene therapy. Their packaging capacity (∼8–36 kb) and ability to transduce various cell types with minimal integration make them appealing for gene therapy applications. However, adenovirus generates immune response, limiting their long-term therapeutic efficiency. Therapeutic correction in the kidney was observed after tail vein injection of recombinant adenoviral vector type 2 carrying human *GLA* cDNA in a mouse model of FD.[33] *In situ* histochemical staining of kidney sections revealed α-Gal A expression in the outer cortex, primarily within the glomeruli, and focally in the inner portions of the medulla. Alport syndrome (AS) is the second most common monogenic cause of kidney failure and is caused by mutations in any 1 of the 3 glomerular basement membrane collagen IV chain genes: *COL4A3*, *COL4A4*, or *COL4A5*.[54] Heikkilä *et al.* described renal artery perfusion of adenoviral vector carrying human *COL4A5* cDNA with a Flag tag (Ad-A5FLAG) along with red blood cells in pigs.[16] The study reports successful *in vivo* perfusion of pig kidneys with expression of *COL4A5* cDNA observed in the glomeruli and efficient deposition of COL4A5 in the glomerular basement membrane. This study demonstrates the feasibility of using this strategy for treating AS.

Perspectives for clinical translation. The preclinical studies presented underscore the potential of a direct *in vivo* gene addition approach for treating inherited kidney disorders. However, the transition to clinical application remains incomplete. Many of these studies involve systemic injection of viral particles to target the kidney, but this method requires a high viral vector dose, with much of it getting trapped in the liver. Because of the cost and risks associated with a high dose of viral particles, optimal kidney gene delivery could be achieved through local delivery methods, such as renal vein or arterial injections. These procedures would be minimally invasive and well established in humans, being the clinical equivalent of renal venography[55] and renal arteriography.[56,57] In addition, developing a viral vector with high specificity for the kidney would greatly enhance safety and efficacy. Although lentiviral and adenoviral vectors have shown some efficacy for gene delivery, they are rarely used for *in vivo* gene therapy because of the risks of random integration with LVs and high immunogenicity with adenoviral vector. AAV remains the primary viral vector for *in vivo* gene therapy in clinics. Therefore, discovering AAV capsids with high kidney specificity would be optimal for treating inherited kidney diseases. Advances in AAV vector biology and capsid design have improved transduction efficiency, immune system evasion, and kidney-targeted specificity. As such, Anc80 has shown high-efficiency transduction of kidney stroma and mesangial cells.[58] Also, the AAV9 vector, along with segment-specific gene promoters administered through retrograde infusion via the ureter, achieves renal nephron segment–specific gene expression.[59] Therefore, combining kidney-specific AAV capsids with local delivery methods could enable the use of lower viral particle doses, maximizing safety and efficacy as well as lowering the cost, facilitating the clinical translation of kidney-targeted gene delivery for treating inherited kidney diseases.

Small nucleic acid for kidney-targeted gene modulation and regulation for inherited kidney diseases

Antisense oligonucleotides (ASOs) have been explored as a targeted gene therapy approach for genetic kidney disorders (Figure 1 and Table 1). They are synthetic short single-stranded RNA or DNA molecules that bind to complementary nucleic acid sequences, typically mRNA, affecting their function by causing RNA degradation, RNA splicing, or translation inhibition.[60] Exon skipping therapy using ASOs targeting truncating variants in exon 21 of the *Col4a5* gene showed promising results for X-linked Alport syndrome, enabling the generation of functional collagen IV α345 trimers.[19] Subcutaneous (s.c.) injection of the ASO in the X-linked Alport syndrome mouse model resulted in their

delivery to podocytes and tubular epithelial cells, leading to expression of Col4α5 in the glomerular and tubular basement membranes as well as prolonged survival. The effect of the ASO persisted for at least 2 weeks in the kidney, eliminating the need for frequent administration. This strategy also holds promise as a therapeutic approach for Joubert syndrome, which is a ciliopathy syndrome with the most common causal gene being *CEP290*, leading to a retinal-cerebello-renal phenotype. i.v. injections of ASOs promoted alternate splicing in a murine model of Joubert syndrome containing a gene trap insertion within intron 25 of *Cep290*, leading to exon skipping and resulting in the production of functional centrosomal protein 290 (CEP290), successfully rescuing both the ciliary and kidney disease phenotypes.[41]

MicroRNAs (miRs), which are small non-coding RNAs that posttranscriptionally regulate gene expression, have been reported to be dysregulated in several kidney diseases including AS (Figure 1 and Table 1).[61] Expression of miR-21 was reported to be elevated in kidney's glomeruli and tubulointerstitium of AS mice. Administration of anti–miR-21 by s.c. injection in *Col4a3*$^{-/-}$ mice led to a beneficial kidney phenotype including reduced blood urea nitrogen and albuminuria, reduced glomerulosclerosis, interstitial fibrosis, tubular injury, and inflammation correlating with preservation in uremic toxin secretion and prolonged survival.[20] Based on these promising results, the HERA clinical trial (ClinicalTrials.gov identifier NCT02855268) enrolled 43 patients who received anti–miR-21 s.c. every week for 48 weeks. However, the study was terminated 3 years later, as no significant improvement in kidney function was observed. Interestingly, Rubel *et al.* highlighted the synergistic effect of anti–miR-21 administered s.c. twice a week, along with the standard-of-care angiotensin-converting enzyme inhibitor in delaying kidney failure and prolonging life in *Col4a3*$^{-/-}$ mice, proposing the use of this combination for achieving long-term therapy for AS.[21] Autosomal dominant polycystic kidney disease is caused by mutations in either the *PKD1* or *PKD2* genes and characterized by the growth of numerous fluid-filled cysts in the kidneys. Autosomal dominant polycystic kidney disease is among the most common human monogenetic disorders and accounts for the leading genetic cause of kidney failure.[22,62] Both *Pkd1* and *Pkd2* contain miR-17 binding motif in their 3'-untranslated region, and inhibiting their expression using the anti–miR-17 oligonucleotide RGLS4326 administered via s.c. injection has demonstrated a beneficial effect in attenuating autosomal dominant polycystic kidney disease, even after the onset of cyst.[22,24] s.c. administration of RGLS4326 showed preferential distribution in the kidney tubules and cysts in mice and in the kidneys of cynomolgus monkeys as well as attenuated growth of cysts in multiple polycystic kidney disease mouse models.[22–24] The phase 1b clinical trial demonstrated that treatment with RGLS4326 administered via s.c. injection resulted in a statistically significant increase in urinary exosome levels of polycystin-1 and polycystin-2, which are encoded by the *PKD1* and *PKD2* genes, respectively (ClinicalTrials.gov identifiers NCT04536688 and NCT05521191). These examples demonstrate the potential of small nucleic acids, which have significant strengths, in treating genetic kidney disorders when applicable.

Perspectives for clinical translation. Because of their notable efficiency, favorable safety profiles, and ability to target the kidneys after systemic or s.c. administration, ASOs and miRs emerge as promising therapeutic tools for treating inherited kidney diseases, as evidenced by the preclinical and clinical studies discussed. More than 15 ASO drugs have already gained Food and Drug Administration approval for addressing various genetic disorders.[60,63,64] However, certain ASOs have exhibited significant hepatotoxicity[65–67] and nephrotoxicity.[68,69] Therefore, comprehensive safety studies are required for each drug product. In addition, synthetic antisense nucleic acids are applicable to a limited range of diseases characterized by mutations resulting in overexpression, alternative splicing, or toxic protein. They also require regular injections. These characteristics limit their clinical application for inherited kidney diseases.

Ex vivo gene therapy for targeted gene therapy for kidney diseases

Ex vivo gene therapy involves the genetic modification of a patient's cells in a cultured environment that are subsequently reintroduced into the patient to accomplish therapeutic objectives. Interestingly, hematopoietic stem cells have been shown as an efficient vehicle to bring gene therapy products to the kidney (Table 1). Transplantation of wild-type hematopoietic stem and progenitor cells (HSPCs) in the mouse model of cystinosis after myeloablation led to the long-term preservation of kidney function and structure along with tissue cystine decrease.[25,26] Cystinosis is a multisystemic disease caused by defects in a lysosomal cystine transporter, cystinosin (*CTNS* gene), with the kidney being the primary tissue to be affected. This is manifested by renal Fanconi syndrome early in infancy and chronic kidney disease leading to kidney failure.[70] Considering that cystinosin is a lysosomal transmembrane protein, the mechanism of rescue of the kidney involved vesicular cross-correction after the differentiation of HSPCs into phagocytic cells that have a paracrine effect via exosome shedding and generation of long membrane protrusions called tunneling nanotubes (TNTs).[71] In *Ctns*$^{-/-}$ mice, HSPC-derived macrophages engrafted in cystinotic kidneys generate TNTs that were even able to cross the tubular basement membrane and deliver cystinosin-bearing lysosomes into diseased proximal tubular cells (Figure 2[71]). In addition, TNTs have a bidirectional transport capability, enabling cystine-loaded lysosomes to undergo processing in cystinosin-expressing macrophages, further reducing cystine levels in tissues of transplanted *Ctns*$^{-/-}$ mice. Altogether, this bidirectional organelle transport could correct lysosomal storage and prevent cell degeneration, even the proximal tubular cells, thus contributing to the long-term preservation of kidney structure and function.[25] These preclinical

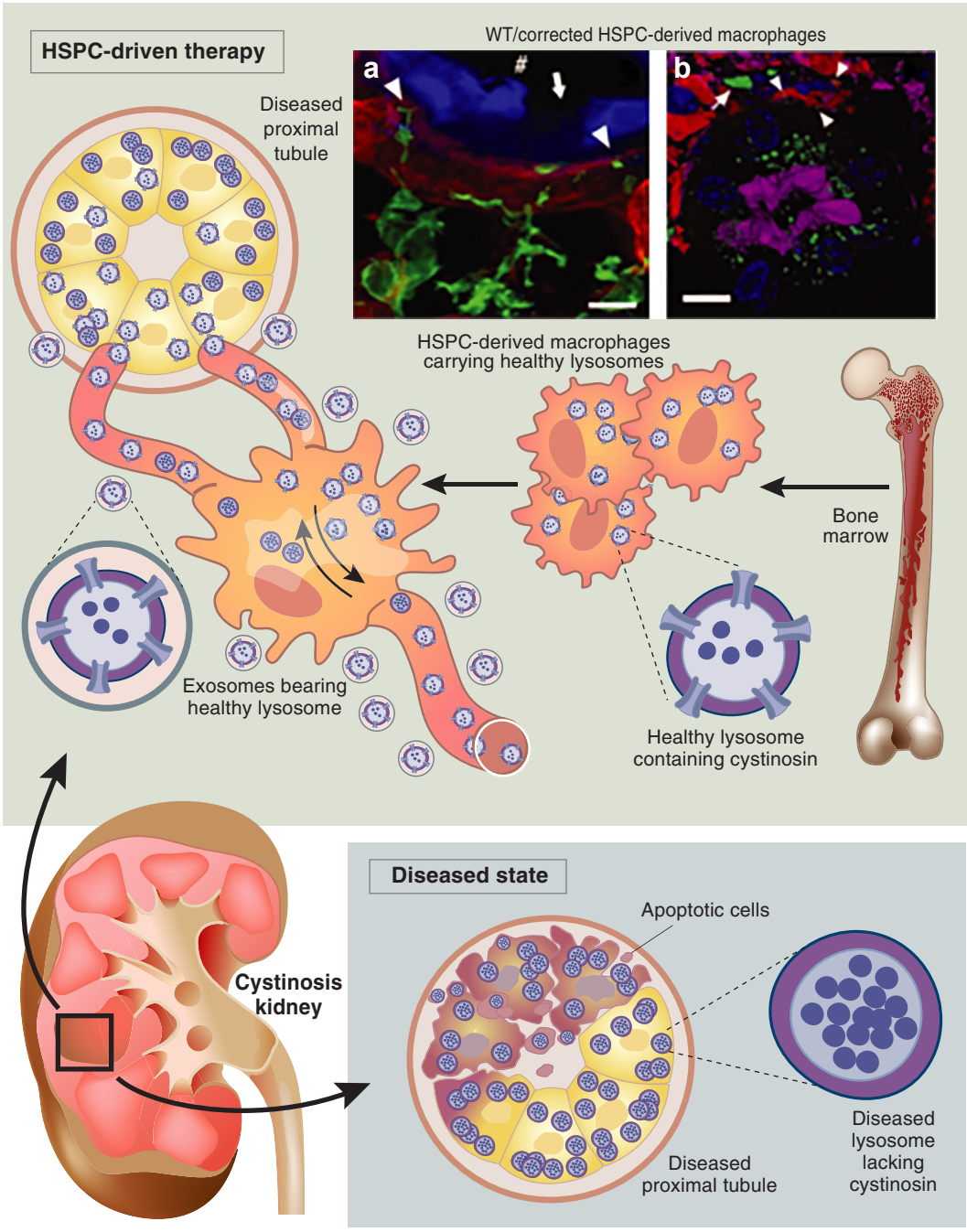

Figure 2| Schema illustrating the proposed mechanism of rescue of the proximal tubular cells (PTCs) in cystinosis after hematopoietic stem and progenitor cell (HSPC) transplantation. Cystine accumulation caused by the absence of cystinosin induces cell stress and subsequent apoptosis of PTCs, as depicted in the lower panel. In the top panel, transplantation of *CTNS*-expressing HSPCs lead to their differentiation in macrophages in the kidney interstitium surrounding the proximal tubules. HSPC-derived macrophages generate multiple tubular protrusions known as tunneling nanotubes (TNTs) that can extend across the tubular basement membrane (TBM) of PTCs, facilitating the bidirectional transfer of lysosomes. In addition, extracellular exosomes may serve as another mechanism for transferring cystinosis to injured kidney cells. (**a**) HSPC-derived macrophage TNTs shown in green (enhanced green fluorescent protein [eGFP]; arrowheads) crossing the TBM shown in red (laminin); PTCs are shown in blue (Lotus Tetragonolobus Lectin staining) (lumen; #). (**b**) Representative confocal image of the kidney showing the transfer of cystinosin-eGFP (green) from the Discosoma red fluorescent protein (DsRed)–HSPC–derived macrophages surrounding the kidney tubules. (**a**) Bar = 5 μm. (**b**) Bar = 10 μm. WT, wild type. (**a**) and (**b**): Adapted from our publication after transplantation of *Ctns*$^{-/-}$ mice.[71] To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

investigations and the newly elucidated mechanism of action for HSPC-mediated kidney repair underscore the therapeutic potential of HSPC transplantation in inherited nephropathies.

Given the considerable risk of morbidity and mortality associated with allogeneic HSPC transplantation,[72,73] it is not recommended for treating kidney disorders. Allogeneic HSPC transplantation performed in a 16-year-old patient affected with cystinosis resulted in severe graft-versus-host disease and eventual death 35 months after transplantation.[74] Despite the occurrence of graft-versus-host disease and other serious adverse events due to allogenic transplantation, the efficacy of HSPC transplantation in cystinosis was demonstrated in this patient with stabilization of kidney function, reduction in polyuria, decrease in photophobia, reduction in cystine crystal accumulation, and evidence of cystinosin expression in proximal tubular cells.[74] This dramatic case report underscores the potential therapeutic benefits of HSPC transplantation in cystinosis, but also emphasizes the necessity for developing an autologous HSPC gene therapy strategy to circumvent the risks of graft-versus-host disease and immune rejection. Thus, we developed an autologous transplantation approach of HSPCs modified *ex vivo* using a self-inactivated LV to introduce a functional version of the *CTNS* cDNA and showed its efficacy in the mouse model of cystinosis.[27] A phase 1/2 clinical trial is currently ongoing at the University of California San Diego with 6 adult patients treated (ClinicalTrials.gov identifier NCT03897361).[75]

Other studies showed the potential of HSPC transplantation for treating kidney diseases. *As such,* transplantation of wild-type bone marrow cells in a mouse model of Dent disease type 1, *Clcn5*$^{Y/-}$ mice, significantly improved proximal tubule dysfunction such as proteinuria, calciuria, glycosuria, polyuria, and elevated endocytic capacity. Similarly, kidney-engrafted cells were mononuclear phagocytes found in the interstitium, surrounding the proximal tubules and extending TNTs. *In vitro* experiments showed that cell/cell contact was also mandatory to rescue defective endocytosis, suggesting that not only lysosomes but also endosomes could be transferred to diseased cells via TNTs.[28] Two independent studies showed that wild-type bone marrow transplantation improved kidney function and podocyte regeneration in the *Col4a3*$^{-/-}$ mouse model for AS.[76,77] However, another study did not observe differences in *Col4a3*$^{-/-}$ mice that received bone marrow cells from either wild-type or *Col4a3*$^{-/-}$ mice.[78] Thus, a beneficial effect of *Col4a3*-expressing bone marrow cells on AS remains to be demonstrated.

The study by Ohshima *et al.* demonstrated the beneficial effect of bone marrow transplantation in FD disease mice after systemic injection.[35] They observed restoration of α-gal A enzyme activity in the kidney, reaching a level of 7% compared to wild type. Transplantation of α-gal A–deficient bone marrow mononuclear cells genetically modified using retroviral vectors or LVs carrying human *GLA* and i.v. injected in myeloablated α-gal A$^{-/-}$ mice resulted in sustained genetic correction correlating with augmentation in enzyme activity and leading to the reduction in Gb3 levels in several tissues including kidneys.[36,37] Follow-up studies in immune-deficient nonobese diabetic/severe combined immunodeficiency Fabry disease mice systemically injected with LV-*GLA*–transduced Fabry patients' CD34$^+$ cells resulted in α-gal A activity and lipid reductions in several tissues and limited Gb3 level decreased in kidneys.[38,39] Three clinical trials are registered for FD involving the transplantation of *ex vivo* transduced CD34$^+$ cells with LV-*GLA* (ClinicalTrials.gov identifiers NCT03454893, NCT04999059, and NCT02800070[40]). Although these trials no longer enroll new patients, preliminary data for treated patients suggest stable kidney function. These results support the use of *ex vivo* gene therapy for the treatment of inherited kidney diseases.

Perspectives for clinical translation. As described here, clinical trials are currently ongoing for *ex vivo* HSPC gene therapies for kidney diseases. However, it is important to emphasize that because of myeloablation necessary for this method, it should be reserved solely for diseases affecting multiple organs.

Conclusion

The studies reported in this review underscore the tremendous potential of gene therapy for inherited kidney diseases. The most commonly used approach being the direct injection of viral vectors containing the gene of interest via the i.v. route. However, the genetic cargo delivered through systemic injection suffers from off-target effects and is subjected to the filtering of the glomerulus, usually leading to a low level of gene delivery to the kidney cells. Thus, for clinical application, a local route of injection should be considered, such as renal vein or arterial injection, that may lead to better kidney-specific gene delivery. Kidney-specific AAV capsid would also greatly enhance the safety, efficacy, and cost. An interesting avenue is the use of small nucleic acids such as ASOs or anti-miRs for mediating gene modulation or regulation; their efficiency has led to several clinical trials. An unexpected route of delivery for kidney gene therapy is the use of HSPCs via their differentiation into macrophages in the kidney and formation of TNTs that can deliver genetic material and organelles to adjacent kidney cells and even cross the basement membrane to target the proximal tubular cells. This opens new avenues for treating inherited kidney diseases when associated with other organ complications. With ongoing advancements in gene therapy technologies and a deeper understanding of kidney biology, gene therapy holds hope for patients with genetic kidney disorders, offering the possibility of preventing the need for kidney transplantation.

DISCLOSURE

SC is a coinventor on a patent titled "Methods of treating lysosomal disorders" (#20378-101530) and is a cofounder, shareholder, and a member of both the scientific board and the board of directors of Papillon Therapeutics Inc. SC serves as a member of the scientific review board and the board of trustees of the Cystinosis Research Foundation. The terms of this arrangement have been reviewed and

approved by the University of California San Diego in accordance with its conflict-of-interest policies. All the other authors declared no competing interests.

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