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Cell and gene therapy for kidney disease

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Abstract

Kidney disease is a leading cause of morbidity and mortality across the globe. Current interventions for kidney disease include dialysis and renal transplantation, which have limited efficacy or availability and are often associated with complications such as cardiovascular disease and immunosuppression. There is therefore a pressing need for novel therapies for kidney disease. Notably, as many as 30% of kidney disease cases are caused by monogenic disease and are thus potentially amenable to genetic medicine, such as cell and gene therapy. Systemic disease that affects the kidney, such as diabetes and hypertension, might also be targetable by cell and gene therapy. However, although there are now several approved gene and cell therapies for inherited diseases that affect other organs, none targets the kidney. Promising recent advances in cell and gene therapy have been made, including in the kidney research field, suggesting that this form of therapy might represent a potential solution for kidney disease in the future. In this Review, we describe the potential for cell and gene therapy in treating kidney disease, focusing on recent genetic studies, key advances and emerging technologies, and we describe several crucial considerations for renal genetic and cell therapies.

Introduction

Chronic kidney disease (CKD) is estimated to affect approximately 13% of the global population and is associated with high levels of morbidity and mortality[1-3]. For instance, among patients with end-stage kidney disease caused by diabetes, the 5-year mortality rate for a patient is almost as high as that for lung cancer and worse than that for colon cancer[4]. Nonetheless, cancer receives a huge investment from federal funding agencies and

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biotechnology and pharmaceutical companies in the cell and gene therapy fields, whereas kidney-targeted cell and gene therapy clinical trials are almost nonexistent[5]. Despite its severity and a continuously evolving understanding of kidney disease pathophysiology, however, end-stage treatments are limited. Dialysis and renal transplantation are required for patients with end-stage kidney disease, although these interventions involve risk of major complications such as cardiovascular disease and immunosuppression[6]. In addition, dialysis provides only 5–10% of normal kidney function and necessitates considerable lifestyle changes involving three times-per-week or daily, multiple-hour appointments for dialysis treatment. Moreover, the number of patients with end-stage kidney disease far outstrips the supply of organs available for transplant, meaning that many patients can wait years for an organ and die while waiting. There is therefore an urgent need for new and innovative therapies for CKD, with the aim to lessen the burden on patients and the wider health-care ecosystem.

Recent advances in cell and gene therapy have translated into successful therapies for a range of clinical diseases, but none that targets the kidney. Cell and gene therapies aim to prevent, treat or even cure diseases by introducing cells or genetic material into a patient, and they often require an understanding of the underlying disease pathophysiology. Notably, many of the genetic causes of kidney disease are known[7], with up to 30%of CKD attributed to inherited monogenic disorders[8,9], including polycystic kidney disease (PKD), Alport syndrome, cystinosis, Fabry disease, tuberous sclerosis, Gitelman syndrome and cystinuria[10,11] (Table 1). Clinicians can frequently diagnose these monogenic renal diseases in childhood before the onset of CKD, sometimes allowing for intervention before kidney damage becomes irreparable[12]. Genetic testing can also identify affected patients before onset of symptoms or irreversible kidney damage[13,14]. Unfortunately, the few effective treatments for monogenic diseases affecting the kidney, such as enzyme replacement therapy for Fabry disease, are often expensive, have short-term effects and are inaccessible to a large number of patients[15-17]. The clear treatment gap for patients with monogenic renal disorders therefore needs to be addressed with innovative strategies, such as cell and gene therapy. Of note, recent successes in the cell and gene therapy field have occurred in cells and organs more easily targeted and less complex than the kidney; nonetheless, as technologies advance with improved vector design and gene delivery, hope for cell and gene therapy for kidney disease seems just on the horizon.

This Review discusses the potential for cell and gene therapies to treat kidney disease. We do not describe induced pluripotent stem cell-based regenerative medicine therapy or extracellular vesicles for kidney disease as those are covered elsewhere[18-20]. Herein, we focus on approved cell and gene therapies and those of similar technology being developed in the kidney field. We discuss the basics of cell and gene therapy including its technical aspects and history. This is followed by a discussion of the potential for cell and gene therapy of kidney disease based on successes within other organs and diseases, as well as genetic experiments in animal models that reveal the potential for gene therapy of kidney disease. Cell therapies for kidney disease are also reviewed. Finally, we discuss structural and vector considerations for targeting kidney disease and review recent advances and future directions.

A brief introduction to cell and gene therapy

Gene therapy is broadly defined as the use of genetic material to treat or prevent disease. This process includes but is not limited to the insertion or deletion of entire genes, resulting in gain or loss of gene function, or the editing of endogenous genes. Critically, gene therapies target somatic cells rather than germ cells to circumvent germline mutations and subsequent inherited genetic changes. Cell therapy, by contrast, involves the transfer of entire cells into a patient with goals including but not limited to replacing or repairing damaged tissue or cells or even for targeted destruction of pathological cells. Cell therapies cover a spectrum that ranges from simple transfusions of unmodified cells to the delivery of T cells genetically modified ex vivo to target specific cancers. In this Review, we restrict discussion to cell therapies that involve genetic modification.

Cell and gene therapy approaches

For cell and gene therapy to be successful, genetic cargo must be delivered to a target cell population. This target cell population is determined by knowledge about the pathological basis of the disease of interest. There is a wide range of approaches for designing gene therapies, allowing investigators to tailor the delivery, integration and method of editing the genome. Genetic material can be delivered through several vectors, including naked nucleic acids or those encapsulated in viruses or nanoparticles. Genetic cargo may then integrate into the genome or remain unintegrated and therefore episomal, or serve as a template to edit the genetic sequence.

The targeted cells can be modified in vivo or ex vivo. Both types of modification have been approved for use in patients. For instance, chimeric antigen receptor (CAR)-T cell-based therapies for cancer involve ex vivo modification of T cells, which are then adoptively transferred back into patients. Alternatively cells can be modified in vivo, instead of ex vivo. For example, adeno-associated virus (AAV) vectors have been used for gene delivery to both the retina and liver for inherited blindness and haemophilia, respectively. Therefore, cells can be genetically modified ex vivo and then transferred in vivo to modify a disease, or one can genetically modify cells directly in vivo.

Delivery to target cells is key for successful therapy. As such, viral vectors have gained prominence for use in gene therapy and are currently being used in the clinic for a wide range of diseases primarily owing to their intrinsic ability to more efficiently transduce cells compared with non-viral vectors. Adenovirus was used early in humans for gene therapy and soon after produced tragic results, involving immune response and death[21]. Because of this, adenovirus has not gained traction in clinical trials for treatment of genetic disease. It was hoped that helper-dependent adenovirus[22], or gutless adenovirus devoid of immunogenic adenoviral genes, would overcome immunogenicity issues with adenovirus but this too has faced difficulties for treatment of inherited diseases, primarily owing to dose-dependent immune response to capsid proteins[23]. Lentivirus is a type of retrovirus that has been used for years for developing CAR-T cell immunotherapies. Aside from CAR-T cell development, lentivirus-mediated gene therapy has been approved for cerebral adrenoleukodystrophy and β-thalassaemia, wherein haematopoietic stem cells are modified with virus ex vivo and then infused.

AAV of the *Parvoviridae* family was discovered in the 1960s[24], contains a DNA genome and has been shown to efficiently transduce various human cells, although it is not known to cause disease in humans. AAV-mediated gene therapy was first approved in the West as Glybera (alipogene tiparvovec) for lipoprotein lipase deficiency. This was followed by AAV gene therapy for retinal disease and haemophilia in the USA.

Cell and gene therapy approaches depend on understanding the genetic basis of the disease, having a vector capable of delivering genetic components to the target cells and the ability of the therapeutic agent to prevent or reverse pathology. For now, viruses are used overwhelmingly for approved gene therapy approaches. In time, novel technologies may overcome the use of viruses as delivery vehicles.

Pre-approval era

The history of cell and gene therapy includes both successes and failures. In 1990, the first gene therapy trial was conducted in which a 4-year-old child with severe combined immunodeficiency (SCID) received the adenosine deaminase gene through a viral vector[25]. Despite success of this trial and other gene therapy trials early on, progress in the field halted in 1999 with the death of an 18-year-old patient from a severe immune reaction to an adenovirus designed to treat a urea cycle disorder[21]. This tragic death prompted the field to strengthen safety measures surrounding the pre-existing clinical trials for gene therapies. The field slowly regained momentum in the early 2000s, with apparent curative therapy for X-linked SCID by retrovirally mediated delivery of the γc gene into $CD34^+$ cells[26]. However, in subsequent years, 40% of treated patients developed leukaemia, and subsequent research into the underlying cause concluded that the vector led to oncogene activation[27,28]. More recently, a patient undergoing CRISPR gene editing for Duchenne muscular dystrophy died[29]. Although the cause of death is unclear, the FDA has placed a hold on this phase I study. These trials demonstrate the difficulties associated with gene therapies and paved the way for research into newer therapies that were eventually agency approved for treatment of patients. To date, 22 gene therapies, 21 RNA therapies and 59 non-genetically modified cell therapies have been approved globally for clinical use covering the gamut of ex vivo and in vivo therapies[30]. An additional ~4,000 cell and gene therapies are currently in development for clinical use[30].

Approved cell and gene therapies

Approval of the first gene therapy outside of clinical trials occurred in Europe in 2012, with the authorization of Glybera[31]. This therapy, which involves the AAV-mediated delivery of the *LPL* gene into muscle, was approved for lipoprotein lipase deficiency[31], but was later withdrawn in 2017 owing to commercial failure. Subsequently, in 2017, the FDA approved the first gene therapies in the USA, which included those targeting the eye and modification of T cells for cancer immunotherapy. CAR-T cell therapy involves genetic modification of T cells to redirect them against tumour antigens[32]. This form of therapy showed promise for many years in clinical trials and was finally approved by the FDA in 2017 under the name Kymriah(tisagenlecleucel), which used CD19-directed CAR-T cells to treat acute lymphoblastic leukaemia. The potential for genotoxicity exists with any integrating gene delivery vector. CAR-T cells were thought to be safe until recently two of ten patients

developed CAR-T lymphoma in a clinical trial in Australia using a transposon system[33]. The cause of this adverse outcome remains to be definitively proved but may be related to the way in which the CAR-T cells were manufactured[34]. AAV-mediated gene therapies have expanded since Glybera, with Luxturna(voretigene neparvovec-rzyl) approved in 2017 for AAV-mediated gene therapy for *RPE65* mutation-associated retinal dystrophy. This was later followed by approval for the use of AAV-delivered genes to treat spinal muscular atrophy type I and haemophilia B.

Several cell and gene therapies are now available, although they are mostly limited to certain tissues, including the retina, liver, muscle and the haematopoietic system (Fig. 1). This limited diversity in targetable tissue types is largely due to few available routes of delivery, risk of immunogenicity and poor specificity of cellular targeting. For example, retinal gene therapies have been developed owing to the advantageous immune privilege of the retina — a phenomena in which the eye limits inflammatory responses to preserve vision — as well as accessibility of delivery. Additionally, the liver is a relatively easily targetable organ owing to the predominance of only one cell type (hepatocytes) and high rates of transduction of viral vectors; for these reasons, therapies for several inherited hepatic diseases are currently in development. Finally, many blood-related disorders are prime targets for cell and gene therapy and have already seen success in clinical trials for leukaemia, lymphoma, myeloma, β-thalassaemia and haemophilia. In these diseases, a patient's own cells can be modified ex vivo and then adoptively transferred back into the patient. The diversity in therapeutic approaches and diseases targeted for these more easily accessible tissues has provided hope for the gene therapy field of applying these insights to other tissues. A new range of treatments for kidney disease could be implemented if the field could adapt these strategies.

The potential for gene therapy for kidney disease

The number of genetic targets for cell and gene therapy of kidney disease is not lacking (Table 1). Congenital nephrotic syndrome most commonly results from mutations in *NPHS1* or *NPHS2* and often leads to severe disease[35]. In 2010, researchers showed that inducibly expressing nephrin (encoded by *NPHS1*) in nephrin-deficient mice could prevent perinatal death[36], which might have implications for gene therapy in patients with congenital nephrotic syndrome. However, incomplete phenotypic rescue was reported, as mostly normal kidney pathology reported at 1 week progressed to damage and proteinuria by week 6. Candidate reasons for this finding include the timing of transgene expression or use of rat nephrin in the mouse[36]. Nonetheless, congenital nephrotic syndromes remain potential targets for gene therapy[35]. Given the severity of this disease, such a novel therapeutic approach would be highly desirable, although perinatal or infant gene therapy will carry with it a high bar for safety.

Alport syndrome results from a defect in production of α3α4α5(IV) collagen in the glomerular basement membrane[37], caused by a mutation in the *COL4A3, COL4A4* or *COL4A5* gene. Transfer of any of these genes to restore normal collagen expression in the glomerular basement membrane has not yet been achieved in humans. However, in a mouse model of Alport syndrome, an inducible transgenic system — which reactivated

the *COL4A3* gene — restored α3α4α5(IV) collagen expression from podocytes, thereby improving kidney function and extending lifespan[38]. These results demonstrate that restoration of collagen expression using gene therapy might therapeutically treat Alport syndrome in vivo. Slowing the progression of inherited renal diseases such as Alport syndrome is essential, as patients will progressively accumulate kidney damage until the point of end-stage kidney disease. Therefore, improvements in renal function even after glomerular basement membrane development may hold potential for patients with Alport syndrome.

In 2021, a similar study was conducted using a mouse model of autosomal dominant polycystic kidney disease (ADPKD), a disease that results when kidney tubule cells harbour mutations of either *PKD1* or *PKD2* (ref. 39). ADPKD is the most common monogenic kidney disorder, affecting more than 12 million patients worldwide[40]. Therefore, novel therapies that target *PKD1* or *PKD2* are a high priority for the kidney cell and gene therapy field. In that study, the authors conditionally inactivated *Pkd1* or *Pkd2* to induce ADPKD development, but subsequently induced re-expression of the inactivated gene. This model led to rapid reversal of key ADPKD features including proliferation, inflammation, extracellular matrix deposition and cell lining metaplasia. In animals that had developed cystic kidneys, the re-expression of PKD genes was able to reverse ADPKD in vivo, demonstrating that gene therapy could potentially reverse ADPKD even after cystic disease has developed. These findings hold promise for the use of gene therapy before patients develop key disease characteristics, but also for reversing disease pathogenesis.

Cell therapy for kidney disease

Although the use of mesenchymal stem cells and regenerative cell populations has been proposed for cell therapy of kidney disease[20,41], herein we focus on gene-modified cell populations. Cell populations can be genetically modified and adoptively transferred to affect the kidney directly or to provide therapy for complications of kidney disease.

Haematopoietic stem and progenitor cell therapy

Haematopoietic stem and progenitor cells (HSPCs) are a promising avenue within renal cell therapy. One notable study investigated the use of HSPCs with a mouse model of cystinosis[42], a lysosomal storage disorder characterized by the widespread accumulation of the amino acid cystine. Cystinosis affects several tissues and organs of the body, including the kidney, potentially leading to permanent kidney damage and failure. Through lentiviral transduction of HSPCs to express cystinosin — the pathogenically deficient cystine transporter in cystinosis — the authors demonstrated that transduced HSPCs differentiate and subsequently integrate into the kidney and other tissues. Importantly, such therapy led to a reduction in global cystine content and improved renal function. Thus, although not currently kidney specific, HSPC therapies hold therapeutic potential for diseases that affect multiple tissues, such as lysosomal storage disorders.

The use of HSPCs for kidney-targeted enhancements, such as to promote collagen deposition within the glomerulus, remains controversial. Researchers previously reported that bone marrow transplantation from wild-type mice could lead to collagen deposition

in mouse models of Alport syndrome[43,44]. These results were challenged by a later publication suggesting that irradiation, which preceded transplantation, was responsible for improved renal histology and survival in these mice, rather than the effect of bone marrow transplantation[45]. The mechanisms that underlie this effect of irradiation are unknown. A subsequent study investigated the use of several cell therapies in a mouse model of Alport syndrome (*Col4A3* knockout)[46]. Specifically, these researchers investigated the infusion of three cellular therapies, including wild-type bone marrow, wild-type unfractionated blood and human embryonic stem cells, and assessed their effects on kidney disease progression. All three cell therapies reportedly increased de novo α3(IV) collagen expression in the mice, as well as reduced proteinuria and histological damage. Importantly, the observed therapeutic effect occurred without irradiating or conditioning the mice before cell therapy infusions, indicating the promise of possible translation to the clinic. Overall, although the field remains controversial in this area, these studies raise the possibility of HSPC therapy as a treatment strategy for kidney disease.

Ex vivo modification and subsequent delivery of gene-modified autologous cells is another exciting avenue for cell therapies, as this strategy reduces the risk of immunogenicity and rejection. To assess the clinical effects of ex vivo modification of autologous cells, a recent study used lentivirus-mediated transduction in patients with Fabry disease[47], which results from α-gal A deficiency and causes kidney disease over time. HSPCs were collected from five patients, genetically modified to express α-gal A and then injected back into the respective patients. Within 1 week after the therapy, all patients were noted to have reduced disease severity, including near-normal α-gal A activity and reductions in levels of both plasma and urine globotriaosylceramide (Gb3), which accumulates in Fabry disease. This early evidence of ex vivo cell therapy in the clinic for a lysosomal storage disorder that affects many organs, including the kidney, indicates that similar approaches may be effective for other renal diseases.

T lymphocyte-mediated cell therapy

Another large subclass of cell therapies centres around the modification of lymphocytes for cytotoxic or therapeutic effects. One application of this type of therapy is the delivery of specific proteins, as demonstrated by a 2017 study that used non-viral, transposon-mediated modification of T cells to act as a sustained peptide delivery platform[48]. In that study, mouse T cells were modified using the *piggyBac* transposon system to express erythropoietin and subsequently injected into mice. Sustained increases in haematocrit were observed for more than 20 weeks after injection, an effect that could be perpetuated by vaccination. This non-virally modified T cell approach has the potential to be applied to similar hormone or peptide-deficient diseases that affect the kidney, such as Fabry disease, or for therapy of complications of kidney disease.

Viral modification of T cells may similarly be an effective approach for delivering essential enzymes or peptides. A recent study generated cellular delivery vehicles — termed ‘micropharmacies’ — composed of $CD4^+$ T cells modified ex vivo with a lentiviral vector[49]. These modified T cells were conditioned with rapamycin, an immunosuppressant that enhances survival of T cells and their memory capacity and reduces inflammation. The

authors investigated the therapeutic potential of this ‘micropharmacy’ approach in several lysosomal storage disorders, including Fabry disease. Specifically, when investigating delivery of α-gal A in a mouse model of Fabry disease, they reported α-gal activity above wild-type levels and a systemic reduction in Gb3 upon delivery of modified micropharmacies from a patient with Fabry disease. Overall, ex vivo modification of T cells for therapeutic delivery appears to be a promising strategy for targeting systemic disorders such as lysosomal storage disorders. However, more research into the long-term effects of T cell therapy needs to be examined for use outside of the immunotherapy realm.

Both HSPCs and T cells offer vehicles for expression of therapeutic proteins to be delivered via the blood. Such approaches can act through bystander effects whereby therapeutic proteins can be expressed elsewhere and then taken up by target cells. Delivery and expression of structural proteins may not be amenable to such an approach.

Cell therapy for implantable devices

Implantable devices have the potential to replace organs or important organ functions. A notable example of an implantable device is an artificial kidney, although bioengineering functional kidneys is not without challenges[50]. An artificial kidney aims to recapitulate normal kidney function, requiring functional cells for reabsorption of salt and water. However, tubular cells — which serve to reabsorb salt and water — lose their ability to mimic in vivo function when removed from their in vivo niche and cultured, owing to loss of expression of key transporters[51]. To overcome this obstacle, researchers have used genetic engineering to enhance volumetric transport of renal epithelial cells[52]. Specifically, kidney cells have been engineered to overexpress sodium hydrogen exchanger 3 and aquaporin 1, resulting in increased volumetric transport, measured through a functional assay of water transport. Such modification of cells would be of great benefit in an implantable artificial kidney by improving reabsorption of salt and water much like the native kidney in vivo. Similarly, researchers have modified cultured proximal tubular cells to stably express organic ion transporters (OAT1 and OAT3), improving their ability for drug toxicity screening[53]. Therefore, cultured kidney tubular cells can be gene modified to better mimic their in vivo niche. Importantly, the ability to modulate renal transport is desirable not only for inherited renal diseases but also for acute and chronic kidney injuries in which transport is altered. Cell therapies that target renal transport could be applied to a broad range of diseases and patients, so further enquiry into transport modulation is essential for kidney cell therapy.

Technical considerations

Targeting

The nephron is a highly complicated structure that contains multiple specialized cell types, which in part explains why renal gene therapy has been difficult to achieve compared with other tissues. In contrast to the liver, where hepatocytes comprise more than 80% of the tissue, there are at least 26 unique cell types within the kidney[54]. Delivery of therapy products to a specific cell type within a specific region of the kidney has thus been the primary roadblock to kidney gene therapy. Notably, genetic kidney diseases arise from all

parts of the nephron, meaning that any one of these cell types might need to be targeted to treat kidney disease (Fig. 2). Historically, viruses have not transduced the kidney efficiently, implying a lack of known viral receptor expression or accessibility, providing increased difficulty for viral targeting of key parts of the nephron. Engineering new viral capsids or particles to target specific kidney cell types could overcome this obstacle.

Genetics

There are many considerations when deploying a gene therapy modality. One must consider promoter elements — deciding between ubiquitous and tissue-or cell-specific promoters or enhancers — whether the transgene cDNA will be codon optimized, whether additional elements will be added to enhance or stabilize expression such as the woodchuck hepatitis virus post-transcriptional regulatory element, and all of this must fit within the packaging capacity of the vector. Another important consideration is whether integration of genetic cargo is needed or preferred. Integration is needed for a dividing target cell population whereas episomal genetic cargo may suffice for quite some time for quiescent non-dividing target cells.

Accessibility

The size exclusion of the glomerulus remains another hurdle to overcome for renal therapeutic design. Particles travelling from the blood through the glomerulus into the urinary space have to pass through glomerular endothelium with 80–100-nm pores[55], the glomerular basement membrane with a reported pore size of 3 nm (refs. 56,57) and interdigitating podocyte foot processes separated by 32 nm (ref. 58). Because of this, it is generally believed that particles above 10 nm and 50 kDa in size are actively excluded by the glomerular barrier[59].

As described above, viral vectors are a frequently used delivery modality for many current gene therapies, but the size limitations of the three most common vectors must be considered (Fig. 3). The smallest of the viral vectors — adeno-associated virus (AAV) — is around 25 nm in size, whereas the largest viral vector — adenovirus — can reach a size of 100 nm. In addition to size exclusion, few serotypes of viral vectors have been shown to target the kidney, and as a result, higher viral doses are needed to achieve noticeable effects. Common viral receptors appear to be expressed in the kidney, such as AAVR/KIAA0319L for AAV[60] and LDLR for the common VSV-G pseudotyped lentivirus[61], implying that subcellular localization of the receptor (or receptors) or kidney architecture may contribute to a lack of kidney transduction. With these considerations in mind, nanoparticles are a promising route of delivery, given their variable size of between 1 nm and 400 nm, and because their modifiable shape and exterior could allow for enhanced targeting of a specific renal cell type.

Alternative routes for vectors to enter the kidney are important for overcoming the filtration limitations of the glomerulus. The nephron is widely accessible through several routes of injection, including anterograde delivery through the renal artery, retrograde delivery through the ureter, systemic administration and intraparenchymal administration. Each route of delivery has its own advantages, such that targeting of a specific cell type within the

kidney might be enhanced by differential injection techniques. For instance, the tubular epithelium could be targeted on the apical side from the urinary space via retrograde ureteral or renal pelvis injection, but also via the basolateral side from particles traversing the blood endothelium. Additionally, the high rate of blood flow to the kidney may allow for increased exposure to vectors introduced through the circulation compared with tissues that receive relatively lower blood flow. Finally, although local injection to the kidney may improve specific targeting of renal gene and cell therapy, systemic administration is more accessible for translation to the clinic. Innovations in renal targeting of therapeutic vectors as well as optimizing the administration route of an individual therapy will eventually be required to maximize therapeutic benefit while minimizing toxicity and off-target effects. Nonetheless, many different delivery methods have been attempted over the years with none generating high enough transfection or transduction efficiency to gain traction for gene therapy of kidney disease[59].

Vector type

One must also choose between viral and non-viral vectors, and if non-viral, whether delivery agents such as nanoparticles or liposomes will be used (Fig. 3). An additional consideration is whether pre-existing immunity to the gene transfer vector exists within the target patient population. If so, many patients may be excluded from receiving the therapy. All of these considerations have advantages and disadvantages, and by the time a gene therapy makes it to the clinic these various options have been extensively evaluated.

Vector choice involves consideration of packaging limits, differences in immunogenicity and whether cargo will remain episomal or integrate into the genome (Fig. 3). Adenovirus vectors have been in use for years in research for kidney gene transfer, with mixed results[59]; the clinical application of such a vector for kidney disease seems questionable. The use of adenovirus for kidney gene therapy has been reviewed elsewhere[59]. Here, we focus on vectors for kidney therapy that have reached clinical application in other tissues.

Lentivirus and retrovirus vectors are common tools for gene delivery. Although lentiviral transduction of the kidney has been poor[62], recent work showed several months of symptomatic improvement in a mouse model of Dent disease — a kidney disorder caused by mutations in the *CLCN5* gene — using retrograde ureteral injection of lentivirus carrying *CLCN5* (ref. 63). Lentiviral and retroviral vectors transduce dividing cells, which may limit transduction of kidney tissue given that most renal cell types are postmitotic. Integration into the genome is part of the life cycle of these vectors, which, although being a useful attribute for sustained transgene expression, raises the possibility of genotoxicity. Nonetheless, integration could be favourable for certain kidney cell types that turn over over time, such as tubular cells. In particular, off-target toxicity could result from vector integration in cells outside of the kidney, unless kidney targeting specificity can be achieved. Pseudotyping with altered envelopes[64,65], attaching monoclonal antibodies[66] or other targeting moieties[67] are strategies that have been used to retarget lentivirus to other cell types. Future research should aim to improve retargeting of lentivirus to the kidney for enhanced transduction.

AAV has emerged as a prominent viral vector for in vivo human gene therapy, even achieving approval for patient use. Although AAV serotypes have been identified that

efficiently target tissues such as the liver, retina and muscle, few serotypes have shown kidney localization. Accordingly, AAV has not achieved widespread use in the kidney owing to variable transduction efficiency in multiple reports[68-77]. For instance, delivery of AAV9 in a mouse model of acrodysostosis demonstrated up to 70% transduction in kidney cortex tubular cells with subsequent restoration of disease symptoms[78], but such success with AAV9 in the kidney has not been reported by others. In a separate AAV delivery study testing six AAV serotypes, researchers demonstrated transduction of kidney mesenchymal cells, including pericytes, fibroblasts and mesangial cells with a novel AAV serotype; however, they observed no kidney transduction with AAV9 (ref. 79). A recent report examined the biodistribution after injection of ^{124}iodine-labelled AAV in non-human primates and found no uptake of AAV9 or AAVrh.10 in the kidney[80]. Re-engineering of AAV capsids may be necessary for efficient kidney transduction by AAV.

AAV is often preferred for its favourable viral safety profile, although the small packaging capacity compared with adenovirus and lentivirus is an important consideration for its use. The DNA within AAV can be made as single-stranded or self-complementary, resulting in a packaging capacity of 4.7 kb or 2.3 kb, respectively[81]. This limit is smaller than transgenes for the more common genetic kidney diseases such as PKD or Alport syndrome, which are too large for single AAV packaging (Table 1). To circumvent this issue, trans-splicing or the use of recombination-prone nucleic acid sequences can be used to stitch together larger transgenes from smaller pieces in cells. Researchers have successfully developed trans-splicing AAVs to reconstitute larger transgenes in cells[82-85]. However, success of trans-splicing AAVs in vivo in intact animals has been limited[86,87]. Integration of recombinant AAV is thought to be rare, although tumour development has been reported in mice after AAV-mediated gene delivery[88-92]. Although the native serotypes of AAV appear to be somewhat inefficient for kidney transduction, highly sensitive assays to detect transduction have suggested that AAV transduction of the kidney may be under-reported through using a highly sensitive assay to detect transduction[93]. Some researchers have reported transduction of tubular cells via retrograde injection of the ureter producing apparently higher transduction efficiency compared with intravenous injection of virus[73]. High dosage of AAV may be necessary for kidney transduction owing to current limitations on viral targeting. However, recent reports of patient deaths at high AAV dosage raise concerns[94]. AAV capsid engineering has enabled retargeting of AAV to various tissues and cell types[95-99]. Capsid engineering may offer new kidney-targeted AAVs, opening avenues towards a wide variety of gene therapy opportunities for kidney researchers. Overall, viral vectors are a leading choice for gene therapy approaches for several tissues and diseases. Identifying viral serotypes that better target the kidney and understanding the underlying mechanism behind those increased transduction rates are essential for pursuing viral vector-based therapies in the future.

Non-viral vectors, including nanoparticles, might be advantageous for kidney delivery owing to their modifiable size and reduced immunogenicity compared with viral vectors (Fig. 3). Their low manufacturing cost and flexibility in structural components are additional benefits. Several kidney-targeted non-viral gene delivery approaches have been attempted over the years, including liposomes[100,101], ultrasound with microbubbles[102-104], various vascular methods of naked DNA injection[105-107] and hydrodynamic renal pelvis

injection[108]. Promising nanoparticle approaches have been developed in recent years to overcome the hurdle of delivery to the kidney in vivo. For example, proximal tubule-targeted mesoscale nanoparticles have been developed[109], with a reported renal uptake that was 26- to 94-fold higher than other tissues. Researchers have also developed proximal tubule-targeted nanocarriers that bind to megalin, a large receptor that mediates proximal tubule protein uptake[110]. Mesoscale nanoparticles that targeted proximal tubular nuclear factor-κB essential modulator were beneficial in a mouse model of acute kidney injury[111]. Diseases of the proximal tubule epithelium could benefit from this nanoparticle approach, as the renal specificity and lack of toxicity of this vector hold translational potential. In theory, nanoparticles can be used to deliver drugs, RNA, protein or DNA to target cells in vivo, although delivery of DNA has been less successful to date.

Emerging technologies and future advances

The future of effective kidney targeting and sustained phenotypic correction by cell and gene therapies remains hopeful. In the past year, several efforts have highlighted the field's technological improvements and enhanced understanding of renal pathophysiology.

ADPKD is the most common monogenic renal disorder (Table 1). Recent insights into its disease pathology have offered hope for new avenues for treatment, including gene therapy. A 2022 paper investigated the inhibitory effect of the primarily mutated genes in ADPKD: *PKD1* and *PKD2* (ref. 112). Using a mouse model of ADPKD, it was found that a non-inactivated copy of *Pkd1* produces mRNA that becomes repressed owing to cis binding in the 3′ untranslated region (UTR). Consequently, when that portion of the 3′ UTR motif is removed through CRISPR–Cas9 editing or is inhibited with an oligonucleotide, cyst size and growth are reduced. This attenuated disease pathogenesis was also seen with inhibition of the 3′ UTR in the non-inactivated copy of *Pkd2.* The robust mechanisms demonstrated here indicate multiple avenues for development of ADPKD gene therapy, as both gene editing and oligonucleotide delivery modified disease progression.

Both viral and non-viral approaches for kidney gene therapy have also gained traction recently. One study developed a novel nanoparticle coated with non-inhibitory plasminogen activator inhibitor 1R (PAI-1R) that selectively targets glomerular mesangial cells[113]. This nanoparticle was packaged with small interfering RNA to silence transforming growth factor-β1 (TGFβ1) in a rat model of human mesangial proliferative glomerulonephritis. Systemic TGFβ1 inhibitors are efficacious at slowing CKD progression, but harbour substantial risks of inflammation, as TGFβ1 is also a key anti-inflammatory factor[114]. Importantly for renal targeting, the PAI-1R-coated nanoparticles were able to target mesangial cells through glomerular vascular fenestrations, thereby bypassing the size restrictions of the glomerular filtration barrier. The authors observed that a single dose of the nanoparticle improved renal function, including significantly reducing urinary protein excretion and glomerular matrix accumulation 5 days after injection. Additionally, although TGFβ1 protein and mRNA levels were significantly inhibited in the glomerulus and the liver, no changes were observed in lung, spleen, arterial or renal medullary tissue. Although this study highlighted a model of glomerulonephritis, glomerulus-targeted nanoparticles

could be modified for a wide range of glomerular diseases including both inherited and acquired pathologies.

Programmable nucleases can be used for various genomic manipulations that might have clinical implications. These include CRISPR–Cas9, transcription-activator-like effector nucleases and zinc-finger nucleases that induce targeted double-stranded breaks in the genome, leading to activation of the error-prone non-homologous end-joining pathway or template-dependent homologous directed repair[115]. Base editing can enable user-directed change of DNA or RNA bases to correct mutations or expression[116]. Prime editing uses a catalytically impaired Cas9 enzyme fused to reverse transcriptase wherein the guide RNA encodes targeting and the template for editing[117]. There are now several ongoing CRISPR clinical trials targeting various diseases[118]. Ultimately, the use of CRISPR–Cas for kidney gene delivery will depend upon effective delivery to the kidney.

Technologies have also become available for modulating gene transcription or protein translation without transgene delivery per se. For instance, anti-sense oligonucleotides have been used to enable exon skipping in an X-linked mouse model of Alport syndrome, resulting in improved collagen expression and animal survival[119]. AAV has been used to deliver suppressor tRNAs, enabling readthrough of nonsense mutations in vivo[120]. Time will tell whether such approaches are translatable for clinical application.

Notable advancements in viral vector design have improved feasibility for renal gene therapy. Viral pseudotyping — the process of producing viral vectors using viral envelope proteins from a different virus — allows investigators to alter the existing specificity of viral serotypes[121,122]. To improve renal targeting of lentivirus, a recent study designed lentivirus pseudotyped with the envelope from Zika virus, chosen for its affinity to renal tubular epithelial cells[123]. Interestingly, the pseudotyped virus, named ZIKV-E, demonstrated 100-fold higher transduction efficiency in renal tubular epithelial cells compared with the lentivirus-envelope control. ZIKV-E also demonstrated high transduction within the liver, brain, heart and spleen, indicating that further modification to the pseudotyped envelope would be necessary for selective renal targeting. In addition to pseudotyping of viral envelopes, capsid engineering of viruses also provides an avenue for enhanced renal specificity of cell and gene therapies[124,125]. High-throughput screening of diverse capsid libraries could allow for identification of kidney-targeted viruses. Additionally, identification of key peptides that facilitate renal transduction will be essential for understanding how transduction occurs within the kidney and therefore how the field could enhance renal cell and gene therapy efforts. Both viral and non-viral approaches for renal cell and gene therapy have drastically improved in the past few decades, but there are still major hurdles to overcome in targeting, efficiency and longevity before cell and gene therapies could be seen in the clinic.

Technological advances in cell and gene therapies may prove futile if costs are prohibitive. Costs of currently marketed products range from hundreds of thousands to millions of dollars per dose[126]. The hope would be that improved manufacturing and more available products would drive down costs. Much work needs to be done on many levels, from basic

science and translational researchers through to drug companies and policy-makers, to make these therapies accessible and equitable for patient populations.

Conclusions

Cell and gene therapy for kidney disease is in its infancy. Although gene delivery to the kidney has been attempted for many years in various animal models, delivery of genetic cargo to the kidney remains the main obstacle for cell and gene therapy of kidney disease. The field will benefit from agency-approved cell and gene therapies for nonkidney diseases as well as the vectors used. However, breakthrough research needs to be carried out to target diseases of this complex but very important organ. Currently, almost all kidney diseases are treated with supportive care without the use of molecular therapies to target the underlying cause. This is unacceptable given the high burden, morbidity and mortality associated with kidney disease. Many patients are waiting for cell and gene therapy of kidney disease to improve and lengthen their lives. It is hoped that, in the near future, a toolbox of safe and effective cell and gene therapies for kidney disease will exist, revolutionizing the war against the various causes of CKD and its complications.

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Key points

- Despite the use of cell and gene therapies in the clinic for other tissues, no such interventions are available that target the kidney.
- Approximately 30% of chronic kidney diseases are inherited, and the genetic basis is well understood, meaning that they are suitable for targeting by cell or gene therapy before development of irreparable renal failure.
- Genetic studies in mouse models have revealed the potential of gene therapy for kidney disease.
- Delivery of therapeutic material to the kidney is the main hurdle to cell and gene therapy development.
- Innovations in vector technology, delivery and an enhanced understanding of kidney disease pathogenesis provide hope for future kidney cell and gene therapies.

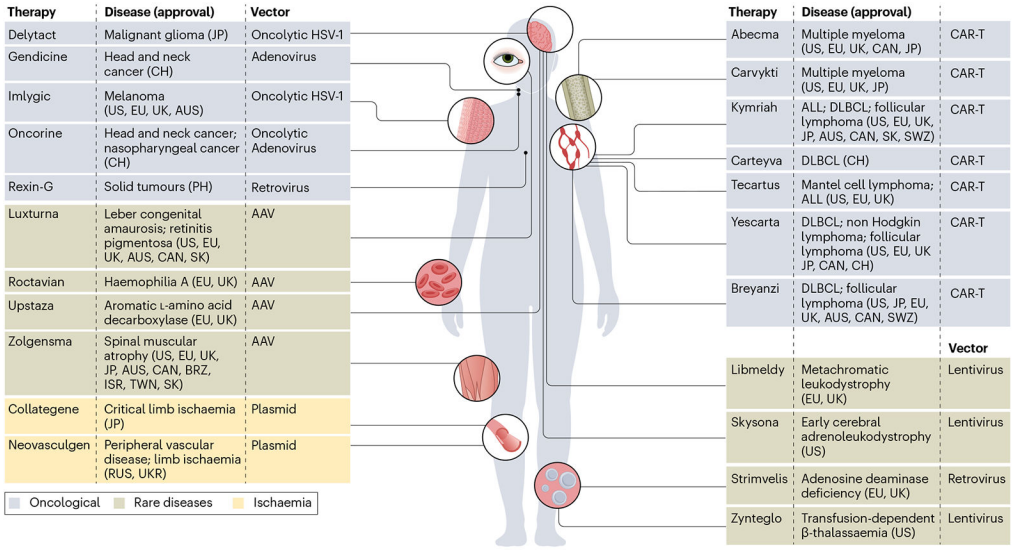

Fig. 1 |. Approved cell and gene therapies worldwide.

Approved gene therapies involve gene transfer in vivo (left) or modification of cells ex vivo that are transferred to patients (right). Various vectors are used with chimeric antigen receptor (CAR)-T cells generated using lentiviral or retroviral vectors. AAV, adeno-associated virus; ALL, acute lymphoblastic leukaemia; AUS, Australia; CAN, Canada; CH, China; DLBCL, diffuse large B cell lymphoma; EU, European Union; HSV-1, herpes simplex 1; ISR, Israel;JP,Japan; PH, Philippines; RUS, Russia; SK, South Korea; SWZ, Switzerland; TWN, Taiwan; UK, United Kingdom; UKR, Ukraine; USA, United States. Delytact (teserpaturev (Daiichi Sankyo and the University of Tokyo)); Gendicine (recombinant human p53 adenovirus (Shenzhen SiBiono GeneTech Co)); Imlygic (talimogene laherparepvec (BioVex Inc)); Oncorine (H101 (Shanghai Sunway Biotech)); Rexin-G (retroviral vector carrying mutant form of cyclin G1 gene)); Luxturna (voretigene neparvovec-rzyl (Spark Therapeutics, Inc.)); Roctavian (valoctocogene roxaparvovec (BioMarin)); Upstaza (eladocagene exuparvovec (PTC Therapeutics)); Zolgensma (onasemnogene abeparvovec (Novartis Gene Therapies, Inc.)); Collategene (HGF plasmid (AnGes, Inc.)); Neovasculgen (cambiogenplasmid (Human Stem Cell Institute)); Abecma (idecabtagene vicleucel (Bristol-Myers Squibb Pharma EEIG)); Carvykti (ciltacabtagene autoleucel (Janssen Biotech, Inc.)); Kymriah (tisagenlecleucel (Novartis Pharmaceuticals Corporation)); Carteyva (relmacabtagene autoleucel (JW Therapeutics)); Tecartus (brexucabtagene autoleucel (Kite Pharma, Inc)); Yescarta (axicabtagene ciloleucel (Kite Pharma Inc.)); Breyanzi (lisocabtagene maraleucel (Bristol-Myers Squibb Pharma EEIG)); Libmeldy (atidarsagene autotemcel (Orchard Therapeutics)); Skysona (elivaldogene autotemcel (Bluebird bio, Inc.)); Strimvelis (CD34+ cells transduced with retroviral vector that encodes for the ADA gene (GlaxoSmithKline)); Zynteglo (betibeglogene autotemcel (Bluebird bio, Inc.)).

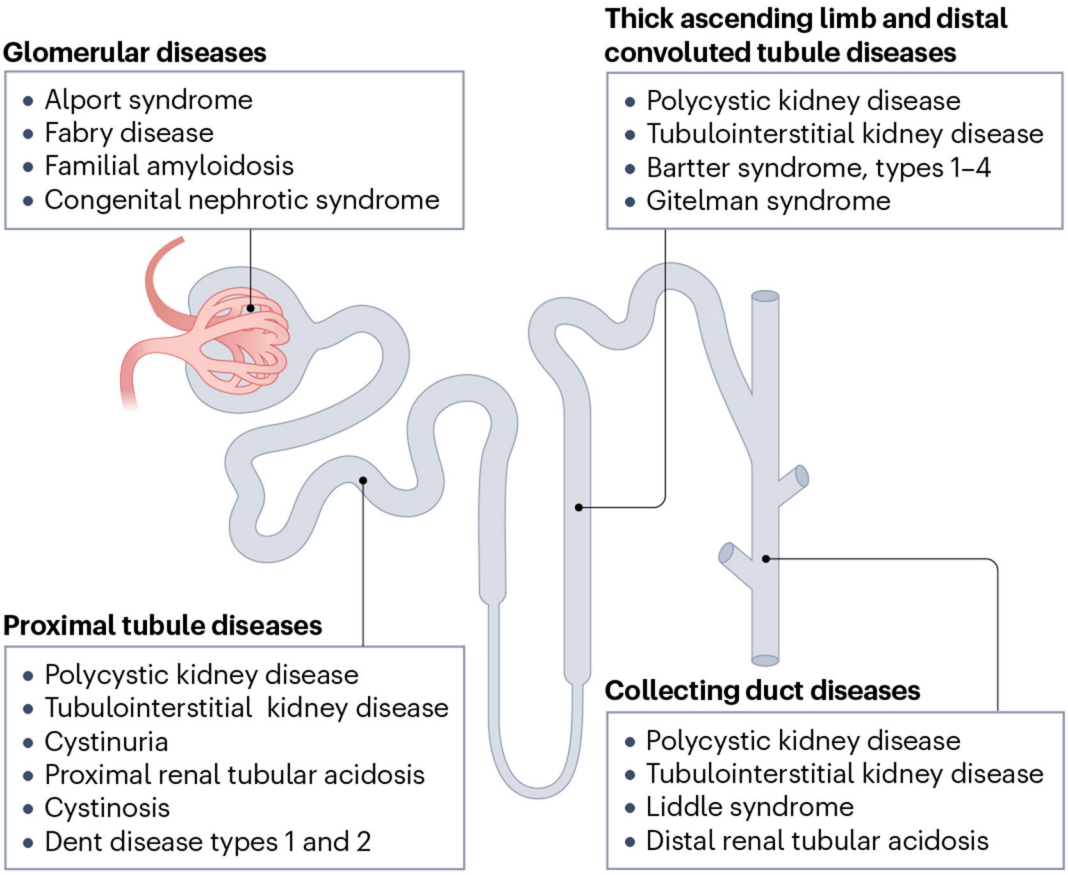

Fig. 2 |. Genetic kidney disease target location within the nephron, the basic functional unit of the kidney.

The major genetic kidney diseases for consideration of cell and gene therapy manifest disease in various cell types throughout the nephron structure.

	AAV	Adenovirus	Lentivirus	Plasmid DNA	Nucleotides	Nanoparticle
Size	26 nm	80–100 nm	80–130 nm	>50 nm	~0.15 kb	30 to >~400 nm
Genome	DNA (ss)	DNA (ds)	RNA	DNA	RNA or DNA	RNA or DNA
Genome size	4.6 kb	36 kb	9 kb	Varies	Varies	Varies
Targeting	✓	✓	✓	–	–	✓
Immune response	Low-moderate	✓	Low	Low	Low	Low
Integration	Rare	–	✓	Possible	Possible	Possible

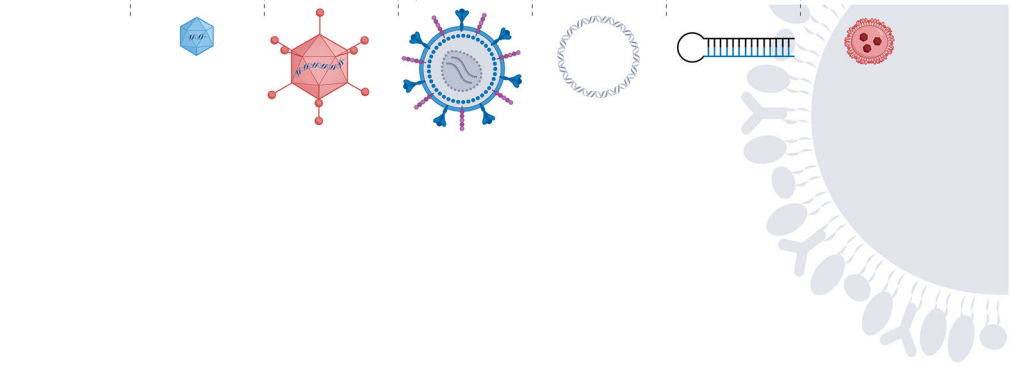

Fig. 3 | Vector considerations for cell and gene therapy of kidney disease.
Viral and non-viral vectors range in size and can package DNA or RNA with different capacity. Some can be engineered to target specific cells or tissues via capsid, envelope or particle modification. Immune response varies between vectors. Any time nucleic acids are delivered to cells, integration is a possibility, although the chance of integration can vary depending on vector type. Integration is part of lentivirus-mediated delivery but is possible, although rare, with other vector methodologies. AAV, adeno-associated virus; ds, double-stranded; ss, single-stranded.

Table 1 |

Prevalence and pathogenesis for genetic kidney diseases

Disease group	Disease	Gene	cDNA size (bp)	Prevalence
Glomerular diseases	Alport syndrome	*COL4A3*	8,097	1 in 5,000–10,000 in the USA
		COL4A4	9,895	
		COL4A5	6,483	
	Fabry disease	*GLA*	1,290	1 in 15,000 worldwide
	Familial amyloidosis	*FGA*	2,110	50,000 patients worldwide
		APOA1	1,009	
		LYZ	447	
		B2M	360	
	Congenital nephrotic syndrome	*NPHS1*	3,762	1 in 100,000 worldwide
		NPHS2	1,152	
Proximal tubule diseases	Autosomal dominant PKD	*PKD1*	14,138	1 in 1,000 worldwide
		PKD2	5,056	
		GANAB	3,906	
	Autosomal recessive PKD	*PKHD1*	16,282	1 in 20,000 worldwide
	Tubulointerstitial kidney disease	*MUC1*	Variable	500 families in the USA; 2–5% of CKD due to monogenic disorders
		UMOD	2,477	
		REN	1,462	
		HNF1B	2,790	
		SEC61A1	1,871	
	Cystinuria	*SLC3A1*	1,737	1 in 7,000 in the USA
		SLC7A9	1,752	
	Proximal renal tubular acidosis	*SLC4A4*	3,108	<1 in 1,000,000 worldwide
	Cystinosis	*CTNS*	2,866	1 in 100,000–200,000 live births
	Dent disease	*CLCN5* (type 1)	10,108	250 families worldwide
		OCRL (type 2)	5,138	
Thick ascending limb and distal convoluted tubule diseases	Bartter syndrome	*SLC12A1* (type I)	4,707	1 in 1,000,000 worldwide
		KCNJ1 (type II)	4,074	
		CLCNKB (types III and IV)	1,544	
		BSND (type IV)	3,472	
		CLCNKA (type IV)	2,581	
		MAGED2 (type V)	2,066	
	Gitelman syndrome	*C12A3*	3,119	1 in 40,000 worldwide
		CLCNKB	1,544	
Collecting duct diseases	Liddle syndrome	*SCNN1A*	3,481	80 families worldwide
		SCNN1B	2,597	
		SCNN1G	3,507	

Disease group	Disease	Gene	cDNA size (bp)	Prevalence
	Distal renal tubular acidosis	*ATP6V0A4*	2,523	<1 in 100,000 worldwide
		ATP6V1B1	1,542	
		FOXI1	1,137	
		SLC4A1	2,736	
		WDR72	267	

Select genetic kidney diseases with respective causative gene, cDNA size and prevalence. Some of the information is adapted from ref. 7. CKD, chronic kidney disease; PKD, polycystic kidney disease.