ChiKD CHILDHOOD KIDNEY DISEASES

Review Article

Child Kidney Dis 2025;29(1):4-11
pISSN 2384-0242 · eISSN 2384-0250
https://doi.org/10.3339/ckd.25.008

CRISPR-Cas9 system in autosomal dominant polycystic kidney disease: a comprehensive review

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Genetic kidney diseases are caused by mutations in specific genes that significantly affect kidney development and function. Although the underlying pathogenic genes of many kidney diseases have been identified, an understanding of their mechanisms and effective treatments remains limited. Gene editing, particularly using clustered regularly interspaced short palindromic repeats (CRISPR), has recently become a promising approach for studying genetic diseases and the CRISPR/CRISPR-associated protein 9 (CRISPR-Cas9) method has become a prominent research method. It has been shown that CRISPR-Cas9 can be targeted to knock out specific genomic sites, which enables researchers to correct gene mutations, prevent inheritance, and better understand the function of genes and the effectiveness of drugs. However, the application of CRISPR-Cas9 technology in the development of therapeutic agents against genetic kidney disease has been overlooked compared with other genetic diseases. In this paper, we provide an overview of the current research advancements in genetic kidney diseases using CRISPR technology, as well as the diverse preclinical research methods implemented, with particular emphasis on autosomal dominant polycystic kidney disease.

Keywords: Autosomal dominant polycystic kidney disease; Clustered regularly interspaced short palindromic repeats; CRISPR-associated protein 9; Gene editing; Kidney

Introduction

Genetic kidney diseases (GKDs) are a group of rare diseases with a prevalence of approximately 60 to 80 per 100,000 individuals [1]. GKD is one of the leading causes of early-onset chronic kidney disease, affecting 10% of adults globally [2,3]. It ranks fifth among the causes of end-stage kidney disease [4]. Furthermore, it is responsible for approximately 10% to 15% of adult kidney replacement therapy cases [5].

Most GKDs are monogenic, which means that the disease is caused by a single gene mutation [6]. This characteristic of GKDs enables the use of site-specific nuclease systems to study

Received: January 24, 2025; Revised: February 19, 2025; Accepted: February 20, 2025

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the genetic characteristics of the disease. In addition, as mutated gene expression occurs *in vivo*, gene editing technology is an effective method to control the development of diseases at the genetic level [7]. Clustered regularly interspaced short palindromic repeats (CRISPR) is a site-specific nuclease gene editing system that can permanently correct destructive base mutations and disease-causing genes, and it has emerged as a new technology for future genetic disease treatment and research. The potential of CRISPR as a genome-editing tool was first recognized in 2012 and was further conventionalized in 2013 [8-11]. Applications of CRISPR technology are of considerable interest because of their diverse possibilities as therapeutic approaches for genetic diseases [12,13]. The ability of CRISPR technology to edit gene mutations and generate *in vivo* and *in vitro* models that can reflect genetic abnormalities has expanded our knowledge of the mechanisms and pathogenesis of genetic diseases.

CRISPR is a powerful tool for understanding the pathogenesis of diseases, the pathological characteristics of cells and tissues, and the constructive environment of diseases. Insights gained from these characteristics will enrich the development of gene-targeted therapeutic drugs and offer further knowledge of genetic disease treatments. This review provides a comprehensive understanding of the topic by examining current advances in our knowledge of the genetic basis of autosomal dominant polycystic kidney disease (ADPKD) and potential therapeutic strategies using CRISPR.

The CRISPR system

CRISPR is part of an adaptive immune system that protects against foreign invasive materials in bacterial and archaeal genomes [14]. In 2012, two studies discovered the potential of CRISPR as a genetic editing tool by programming it with RNA to target specific genomic sites [8,9]. In 2013, several studies further demonstrated the ability of CRISPR as a genetic engineering tool using small guide RNAs (sgRNAs) [9-11].

The CRISPR/CRISPR-associated protein 9 (CRISPR-Cas9) system comprises a Cas9 protein and a sgRNA. Typically, sgRNAs are 100 nucleotides long and the Cas9 protein binds to the last 80 nucleotides to form a ternary complex [10,11,15]. The remaining 20 nucleotides are in a free state, and thus, they bind to eukaryotic DNA based on their complementary sequences [9,16]. Through this mechanism, the first 20 nucleotides of the sgRNA guide the Cas9 protein to a specific target site in the DNA. By manipulating the first 20 nucleotide sequences, the CRISPR system can be programmed to target different genomic sites. After binding to the genome, the DNA-cutting enzyme cuts DNA at the third and fourth nucleotides upstream of the protospacer adjacent motif, introducing a double-stranded break (DSB) at the target site [8,9]. These DNA DSBs need to be rapidly repaired because they are fatal to the genome. This is accomplished through an endogenous DSB repair system involving non-homologous end joining (NHEJ) and homology-directed repair (HDR) [8,9]. NHEJ trims and ligates DNA, causing insertion and deletion mutations that can interfere with the open reading frames [8,9]. HDR repairs DNA based on a template strand within the genome or supplied in an experiment. Although HDR is more precise and accurate, the primary repair method is NHEJ because HDR is inefficient for practical use [15,10,17].

Due to its simplicity in targeting the genome by altering the sgRNA nucleotide sequence, CRISPR-Cas9 has emerged as a convenient and cost-effective platform. Current research on its therapeutic applications in the treatment of genetic diseases has demonstrated its wide applicability to diverse disease phenotypes.

ADPKD and CRISPR

ADPKD is one of the most common genetic conditions, affecting an estimated 12.5 million people globally [18]. In ADPKD, the microscopic tubules progressively expand into fluid-filled cysts that replace the normal parenchyma [19]. Furthermore, the cysts can cause bilateral kidney enlargement and renal failure. ADPKD is inherited via heterozygous loss-of-function mutations in *PKD1* and *PKD2*, which encode polycystin-1 (PC1) and polycystin-2 (PC2), respectively, and in rare circumstances, it is caused by other mutations [20,21]. Mutations in *PKD1* and *PKD2* account for approximately 78% and 13% of ADPKD cases, respectively [22]. It has been hypothesized that cyst initiation occurs from germline inactivation mutations in one allele and somatic inactivation of *PKD1* or *PKD2* [23,24]. However, recently, a gene dosage (haploinsufficiency) model hypothesized that complete loss of the *PKD* gene is unnecessary and that renal cyst formation occurs when the *PKD1* dosage falls below a critical threshold [25,26].

Applications using stem cells

The potential of the use of stem cells in research is widely understood because of their ability to self-renew and differenti-

ate into various somatic cell types and tissues under different culture conditions [27-30]. Somatic cells from human patients are conventionally converted into induced pluripotent stem cells (iPSCs), which are then subjected to patient-specific or gene-targeted mutations. The most common protocol for introducing the CRISPR-Cas9 system into iPSCs involves the use of viral vectors to deliver the guide RNA and Cas9 protein. CRISPR-Cas9 technology has been applied to stem cells for research on GKDs [31].

Many studies have implemented CRISPR in stem cells to accurately recapitulate kidney organoids and genetically model ADPKD for future research. Using human pluripotent stem cell (hPSC)-derived kidney cells, Freedman et al. [32] found that loss-of-function PKD mutations resulted in cyst formation, using CRISPR to introduce *PKD1* and *PKD2* mutations in hPSC-derived kidney cells. They also found that PKD-specific cystogenesis in the tubules is a cell-intrinsic phenomenon; thus, human kidney disease can be recapitulated *in vitro*. Their study provided a three-dimensional (3D) culture system that establishes innovative cellular systems for studying human renal physiology and pathophysiology *in vitro*. Cruz et al. [33] established a genetic model of PKD cystogenesis that implicates the microenvironment in the early stages of ADPKD. They developed the PKD model by introducing biallelic mutations in PC1 and PC2 to hPSC, using CRISPR. The successful removal of adherent cues increased cystogenesis 10-fold, leading to the production of cysts resembling PKD, which expand to a diameter of 1 cm. Defects in PC1 expression and collagen compaction were observed after stromal removal, which enabled the outgrowth of PKD cell lines. These findings provide critical insights into the modeling of PKD using human cellular systems. Furthermore, it was found that cyclic adenosine monophosphate (cAMP) induces cysts in both PKD and non-PKD kidneys. This suggests that strengthening stromal or scaffolding components may provide a cue favoring migratory repair and that PC1 may function as an adhesion regulator to maintain tubular architecture through its interactions with the microenvironment. Huang et al. [34] demonstrated that the inhibition of p38 mitogen-activated protein kinase activity leads to long-term expansion of mouse and human nephron progenitor cells (NPCs) *in vitro* in a two-dimensional (2D) culture setting, proposing a new rapid, efficient, and scalable organoid model of PKD. This finding was made by identifying experimental observations from *PKD2* gene-knocked human NPCs, using CRISPR. The activation of yes-associated protein signaling programs in hPSC-derived induced NPCs to the primary state of NPCs allows enhanced modeling of diseases that affect the distal nephron of the kidney, including ADPKDs. In addition, PTC-209 and other B-cell-specific Moloney leukemia virus insertion site 1 inhibitors have been shown to be potential candidates for future PKD treatments.

Further research into potential therapeutics is possible using stem cells and data from previous studies on the generation of ADPKD kidney organoids. Vishy et al. [35] reported eukaryotic ribosomal-selective glycosides (ERSGs) as potential PKD therapeutics that enable ribosomal readthrough of nonsense mutations. CRISPR has been used to introduce four common nonsense mutations into iPSCs, which were then differentiated into kidney organoids and treated with ERSGs. ERSGs may prevent cyst initiation and limit the growth of preformed cysts by partially restoring PC expression.

Many studies on the use of pluripotent stem cells have focused on the generation of kidney organoids (nephron organoids) [36]. Cyst formation in ADPKD involves the formation of nephron progenitors and ureteric buds [37]. Large renal cysts originate from ureteric buds rather than from nephron progenitors. Thus, modeling cyst formation in ureteric bud organoids is critical for the future treatment of ADPKD. Kuraoka et al. [38] evaluated cyst formation in nephron progenitors and ureteric buds by developing nephron progenitor and ureteric bud organoids with *PKD1* gene mutations for iPSCs using CRISPR. Upon stimulation with cAMP, enhanced cystogenesis was observed in organoids lacking *PKD1* or *PKD2*. Moreover, consistent with previous studies, it has been shown that cAMP signaling is required for *in vitro* cystogenesis in both nephron and ureteric bud organoids. After observing the expression of arginine vasopressin receptors, only ureteric organoids responded to vasopressin to form cysts. This provides a valuable basis for successful ADPKD modeling and drug screening. Observations of cyst formation in organoids with heterozygous *PKD1* mutations suggest that epithelia may impact cyst formation, with contributions toward cystogenesis via exogenous signals.

Applications using cells

CRISPR-Cas9 can be directly used in specific renal cells. Establishing cellular models of disease has long been effective at testing the hypotheses of disease pathogenesis. In addition, they may serve as potential targets for disease treatment. Huang et al. [39] modeled ADPKD using $SIX2^{+}$ NPCs to identify small-molecule inhibitors. They differentiated induced NPCs and human NPCs in 2D and 3D culture formats using human

https://doi.org/10.3339/ckd.25.008

neuropeptide S receptor-v2 medium to generate nephron organoids. CRISPR was then applied to introduce Pkd1$^{-/-}$ and Pkd2$^{-/-}$ mutations to generate cystic nephron organoids. Incubation of the organoids with PTC-209 resulted in a dose-dependent cyst-inhibitory effect without cellular toxicity. Pkd2$^{-/-}$ mutated organoids showed co-expression of lotus tetragonolobus lectin and cadherin 1 in the cyst-lining cells, increased cellular proliferation, enhanced expression levels of cell-cycle-related genes, and increased activity of the mammalian target of rapamycin pathway and MYC7. Additionally, the oxygen consumption and extracellular acidification rates were elevated. Treatment of organoids with cystic fibrosis transmembrane conductance regulator inhibitor 172, metformin, AZ505, and tubacin inhibited cyst formation, which is consistent with the results of previous studies [40-44]. Chumley et al. [45] evaluated extracellular acidification and glucose metabolism in human embryonic kidney cell lines using *PKD2*. Their findings revealed that mutations in *PKD2* (introduced by CRISPR) increased the overall extracellular acidification over time and altered mitochondrial morphology to resemble PC1 deficiency. These findings suggest the potential application of targeted energy metabolism as a therapeutic approach for PKDs. Another approach used by Porath et al. [46] showed that mutations in the *GANA* gene cause ADPKD and defects in PC1 maturation, resulting in cystogenesis. After knocking out the *GANAβ* gene using CRISPR-Cas9, loss or reduction of GIIα was observed, leading to PC1 and PC2 maturation and localization defects that caused ADPKD.

Applications using animal studies

Despite ongoing debates regarding the ethical considerations of genome editing in animals, it is an effective method for understanding the pathology of human diseases [47,48]. Moreover, it can be used to elucidate the mechanisms underlying disease progression and development [49,50]. Watanabe et al. [51] induced a PKD phenotype by generating *PKD1*-heterozygous-knockout (*PKD1*$^{insG/+}$) pigs using CRISPR-Cas9 and somatic cell cloning techniques. They found that *PKD1*$^{insG/+}$ pigs developed many pathological conditions similar to those of patients with heterozygous mutations in *PKD*, with pathological similarities in the formation of macroscopic kidney cysts at the neonatal stage, the number and cystogenic dynamics of the kidney cysts formed, interstitial tissue fibrosis, and the presence of a premature asymptomatic stage. These findings demonstrate the potential of *PKD1*$^{insG/+}$ pigs to be used to study early interventions in pediatric patients with ADPKD, to verify the effects of prophylactic treatment, and to test long-term treatment. Further research using animal models will reveal new insights into the disease and future treatment methods. Tsukiyama et al. [52] generated cynomolgus monkey models with ADPKD *PKD1* mutations to demonstrate how animals recapitulate the key pathological features of human diseases. They used CRISPR-Cas9 to generate *PKD1*-knockout or mosaic monkeys with different degrees of cyst formation. Their research identified the lineage of identities of cyst epithelia and found that most cysts in heterozygotes were derived from the distal tubules, which reflects the initial stages of cystogenesis. They also found that the formation of cysts in the collecting ducts was significantly associated with the severity of the cysts. These results suggest that distal tubules may be a potential drug target for patients with *PKD1*. Soomro et al. [53] reported that discoidin domain receptor 1 (DDR1) is not a viable drug target for ADPKD after *in vivo* genetic deletion of DDR1 using CRISPR-Cas9 in a mouse model. Mice with ADPKD showed no decrease in cyst growth or preservation of kidney function, suggesting that the pathogenesis of PKD does not involve DDR1 expression. Table 1 shows the previous findings of ADPKD models developed using CRISPR-Cas9 technology.

Conclusions

CRISPR-Cas9 technology is a new, promising technique that opens new frontiers for diverse research into the genetics of diseases. The recent introduction of this technology has greatly increased the number of studies on GKD. Methods utilizing stem cells, podocytes (cells), and animals have been used in research on GKDs involving CRISPR-Cas9. These studies have aimed to construct an accurate disease model that demonstrates human disease progression and development and to understand the mechanism of diseases to identify possible treatment methods. This comprehensive review focuses on the application of CRISPR technology in research on GKDs, especially ADPKD, and explains the methods used in detail. It summarizes robust preclinical data and identifies effective approaches that have not been considered in studies using other gene editing technologies. Moreover, it provides an updated overview of the research conducted by other scientists on GKDs. Although studies on using CRISPR for *in vivo* gene therapy for ADPKD are yet to be continued, emerging approaches for investigating the genetic and phenotypic characteristics of

Table 1. Summary of findings in the previous studies regarding ADPKD using CRISPR-Cas9 systems

Author (year)	Disease	Method	Target mutation area	Study findings
Vishy et al. (2024) [35]	ADPKD	Human pluripotent stem cell (kidney organoids)	*PKD1-R2430X, PKD1-Q3838X, PKD2-R186X, PKD2-R872X, PKD1-R2430X,* and *PKD2-R872X*	- Eukaryotic ribosomal-selective glycosides: cyst initiation and growth of preformed cysts ↓ by restoring polycystin expression. - Aminoglycoside drugs: cysts ↓ via ribosomal readthrough. - Fluorescent aminoglycosides: accumulate in kidneys and PKD cysts in mice *in vivo*. - Heterozygosity: cyst formation ↓ and base-editing gene therapy.
Freedman et al. (2015) [32]	ADPKD	Human pluripotent stem cell (kidney organoids)	*PKD1* and *PKD2*	- Loss-of-function PKD mutations → cyst formation. - PKD-specific cystogenesis from tubules: cell-intrinsic phenomenon.
Huang et al. (2023) [34]	ADPKD	Human-induced pluripotent stem cell	*p38* and *YAP*	- Inhibition of p38 MARK activity → long-term expansion of mouse and human NPCs *in vitro* in the 2D culture setting. - PTC-209 or other BMI-1 inhibitors: prospective candidates for PKD treatment.
Cruz et al. (2017) [33]	ADPKD	Induced pluripotent stem cell (kidney organoids)	*PKD1* and *PKD2*	- Removal of adherent cues: cystogenesis ↑. - Removal of stroma → outgrowth of PKD cell lines.
Kuraoka et al. (2020) [38]	ADPKD	Induced pluripotent stem cell (UB organoids)	*PKD1*	- Both nephron and UB organoids: cyst formation upon forskolin treatment. - UB organoids (not nephron organoids): responding to vasopressin to form cysts.
Chumley et al. (2019) [45]	ADPKD	Cell (HEK-293)	*PKD1* and *PKD2*	- Mutations in PKD genes → changing mitochondrial energy metabolism. - Mutations in *PKD1* and *PKD2* : overall extracellular acidification ↑. - *PKD1* mutations: non-glycolytic acidification rates and tricarboxylic acid cycle activity ↑ or breakdown intracellular glycogen, and basal and ATP-linked oxygen consumption rates ↑. - *PKHD1* and *PKD2* mutations: altering mitochondrial morphology (resembling PC1 deficiency).
Porath et al. (2016) [46]	ADPKD	Cell (RCTE)	*GANAβ*	- Knockout of *GANAβ* → loss or reduction of GIIα, and PC1 and PC2 maturation, and localization defects that cause ADPKD.
Huang et al. (2024) [39]	ADPKD	Cell (NPCs)	*PKD1* and *PKD2*	- Cystic organoids: responding to CFTRinh172, metformin, AZ505, and tubacin, but not tolvaptan. - Incubation of organoid with PTC-209 → dose-dependent cyst inhibitory effect without cellular toxicity.
Tsukiyama et al. (2019) [52]	ADPKD	Monkey model	*PKD1*	- Cyst formation in the collecting ducts: associated with cyst severity. - Abnormal function of *PKD1*: relate to high rate of abortion.
Watanabe et al. (2022) [51]	ADPKD	Pig model	*PKD1*	- Heterozygous *PKD1* pigs → many pathological conditions similar to ADPKD patients.
Soomro et al. (2019) [53]	ADPKD	Mouse model	*DDR1*	- DDR1: no playing a role in PKD pathogenesis. - DDR1: not a viable drug target for ADPKD.

CRISPR, clustered regularly interspaced short palindromic repeats; Cas9, CRISPR-associated protein 9; ADPKD, autosomal dominant polycystic kidney disease; PKD, polycystic kidney disease; MARKs, mitogen-activated protein kinases; NPCs, nephron progenitor cells; 2D, two dimensional; BMI-1, B-cell-specific Moloney leukemia virus insertion site 1; UB, ureteric bud; HEK-293, human embryonic kidney 293; PC1, polycystin-1; RCTE, renal cortical tubular epithelial; GIIα, glycoprotein α-subunit of glucosidase II; PC2, polycystin-2; CFTRinh172, cystic fibrosis transmembrane conductance regulator inhibitor 172; DDR1, discoidin domain receptor tyrosine kinase 1.

https://doi.org/10.3339/ckd.25.008

diseases using CRISPR may be promising for future research on new potential treatments for inherited diseases.

Conflicts of interest

Jae Il Shin is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

Funding

This work was supported by the Yonsei Fellowship, funded by Lee Youn Jae (JIS). This study was performed as the research internship program of Yonsei International Summer School (SK).

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Conceptualization: SK, SJP, JIS
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Writing-original draft: SK, SJP
Writing-review & editing: SK, SJP, MHL, AK, JIS
All authors read and approved the final manuscript.

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