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Genome Editing for Rare Diseases

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Abstract

Purpose of the review—Significant numbers of patients worldwide are affected by various rare diseases, but the effective treatment options to these individuals are limited. Rare diseases remain underfunded compared to more common diseases, leading to significant delays in research progress and ultimately, to finding an effective cure. Here, we review the use of genome-editing tools to understand the pathogenesis of rare diseases and develop additional therapeutic approaches with a high degree of precision.

Recent findings—Several genome-editing approaches, including CRISPR/Cas9, TALEN and ZFN, have been used to generate animal models of rare diseases, understand the disease pathogenesis, correct pathogenic mutations in patient-derived somatic cells and iPSCs, and develop new therapies for rare diseases. The CRISPR/Cas9 system stands out as the most extensively used method for genome editing due to its relative simplicity and superior efficiency compared to TALEN and ZFN. CRISPR/Cas9 is emerging as a feasible gene-editing option to treat rare monogenic and other genetically defined human diseases.

Summary—Less than 5% of ~7000 known rare diseases have FDA-approved therapies, providing a compelling need for additional research and clinical trials to identify efficient treatment options for patients with rare diseases. Development of efficient genome-editing tools

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Compliance with Ethics Guidelines

Conflict of Interest

Arun Pradhan, Tanya V. Kalin and Vladimir V. Kalinichenko declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

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capable to correct or replace dysfunctional genes will lead to novel therapeutic approaches in these diseases.

Keywords

Genome editing; rare diseases; gene therapy; CRISPR/Cas9

Introduction

A rare disease (also known as orphan disease) affects a relatively small percentage of patients in the general population. There are approximately 7000 different types of rare diseases worldwide, which altogether account for significant numbers of patients and substantial healthcare cost [1–4]. There is no universal definition for rare diseases. For example, a rare disease in the USA is defined as a condition that affects fewer than 200,000 people, whereas the European Union uses a frequency of <1 patient per 2000 people as a definition for rare diseases [3]. Although individual occurrences of these diseases are rare, they cumulatively affect a significant proportion of the human population, accounting for approximately 20–30 million patients in the US [5]. There are several factors that often complicate the development of new therapeutic approaches for patients with rare diseases. For example, rarity of their occurrences hampers the advances in scientific research and delays medical referrals to disease experts who can correctly diagnose the disorder through specialized biochemical and genetic testing. Rare diseases often have limited clinical information and inadequate numbers of patients to calculate statistical significance and design clinical trials. Lack of biomarkers to evaluate the disease progression contributes to delays in treatments. Many rare diseases have early developmental or neonatal onsets resulting in poor prognosis without immediate therapeutic intervention.

Rare diseases are diverse and affect multiple organ systems. For example, Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACDMPV) causes fatal lung disease in newborns and infants but is also associated with developmental abnormalities in cardiovascular, gastrointestinal and urogenital systems [6]. Recently, more than 70 pathogenic mutations in the Forkhead Box F1 (*FOXF1)* gene were identified in ACDMPV patients [6, 7]. Approximately 1700 mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene are associated with Cystic fibrosis (CF), a severe, progressive disorder affecting the lung, pancreas, liver, kidney and other organs [8]. Niemann–Pick disease type C1 is a neurovisceral lipid storage disorder associated with over 200 mutations in the *NPC1* gene [9]. Many rare diseases require organ transplantation, which is dependent on the availability of compatible donors and is associated with high healthcare costs [10–12]. Known mutational status in rare diseases provides a unique opportunity to use gene-editing approaches capable of achieving specific genomic modification within endogenous cells and tissues to repair the mutated gene itself.

Genome editing approaches have been used for several rare diseases with varied levels of success, aiming to correct or replace dysfunctional (mutated) genes [4, 13]. Specific nucleases, such as Transcription Activator-like Effector Nucleases (TALEN), meganucleases, Zinc Finger Nucleases (ZFN) and the Clustered Regularly Interspaced Short

Palindromic Repeats (CRISPR) / CRISPR associated protein 9 (Cas9) complex, allow the addition, replacement and removal of DNA fragments to correct mutated gene in genome. CRISPR/Cas9 system is the most commonly used method among genome editing approaches due to its simplicity, accuracy, and relatively low cost [14–17]. CRISPR/Cas9-mediated genome editing is useful to repair non-functional genes as well as to correct pathogenic mutations encoding mutant proteins that are harmful or toxic. In the following four sections we summarized recent applications of CRISPR/Cas9 technology to rare diseases and discuss improvements required for its clinical translation.

Diversity of methods for genome editing

Genome editing methods use bioengineered nuclease enzymes to modify the organism's genome in which DNA is inserted, deleted, or replaced in a precise and predetermined manner. Nuclease enzymes, also known as molecular scissors, cut genomic DNA to create site-specific double-strand breaks (DSBs) which later can be repaired through either Non-Homologous End Joining (NHEJ) or Homologous Recombination (HR), resulting in precise changes in genome (Figure 1). The main genome editing techniques include ZFN, TALEN and CRISPR/Cas9, and each method has its advantages and disadvantages. Both ZFN and TALEN use artificial fusion proteins consisting of various DNA-binding domains linked to the nuclease domain of *FokI* to make DNA breaks. ZFN uses a combination of zinc finger DNA binding domains to recognize specific DNA sequences, however, the 3-base pair requirement for DNA-binding for each zinc finger and the low efficiency of the method are the main challenges for ZFN. The TALEN system works similarly to ZFN but relies on the TAL effector DNA-binding domain and the *FokI* nuclease to cut DNA. Compared to ZFN, the TAL effector DNA-binding domain uses 33–35 amino acid repeats as recognition sites, therefore increasing the specificity of genome editing. TALEN is easier to engineer than ZFN, the latter of which is technically challenging and time-consuming to design. Disadvantages of TALEN are related to low editing efficiency in heavily methylated DNA regions and significant difficulties with gene delivery due to large sizes of TALEN constructs. Overall, bioengineering and testing of TALEN and ZFN constructs for the correction of individual mutations are often time-consuming and expensive [14–16].

CRISPR/Cas9, a two-component genome editing system [18, 19], consists of a guide RNA and the CRISPR associated protein 9 (Cas9), which function as nuclease (Figure 1). Guide RNA of the CRISPR system can be designed to bind specific DNA sequences in genome. Compared to ZFN and TALEN, the CRISPR/Cas9 system is a simple, cost-effective, and highly efficient method. CRISPR/Cas9, like TALEN, was originally discovered in bacteria, where it functions as a part of internal defense mechanisms to protect the bacterial host from invading viruses [18, 20]. The main characteristic feature of CRISPR/Cas9 is that it uses RNA sequences instead of DNA-binding proteins to recognize specific DNA motifs (Figure 1). Guide RNAs can be synthesized to target any specific DNA sequence in the genome, and specialized CRISPR/Cas9 algorithms have been developed to predict the possibility of off-target effects. CRISPR/Cas9 system has completely revolutionized the genome-editing field, opening multiple possibilities for development of new therapies for rare diseases.

Generation of animal models for rare diseases using CRISPR/Cas9 genome editing

CRISPR/ Cas9 has been extensively used to generate animal models for rare human diseases [21] (Table 1). Niemann-Pick disease type C1 (NPC1) is a rare lysosomal storage disease caused by inactivating mutations in the *NPC1* gene. This disease is characterized by abnormal accumulation of cholesterol and glycolipids in late endosomes and lysosomes. Both mouse and feline models of NPC1 have been generated and used in preclinical studies to model the disease progression in humans [22–23]. However, the use of these models for high throughput screens to identify novel therapeutic agents is not economically feasible due to large time and labor costs to maintain and screen these animals. Recently, Tseng and coworkers [22] have generated two Zebrafish *Npc1*-null mutants using CRISPR/Cas9-mediated gene targeting. The *Npc1* mutants exhibited the early hepatic and the late neurological abnormality, both phenotypes being similar to human NPC1 [22]. These Zebrafish models of NPC1 can be used for rapid *in vivo* screening of potential therapeutic agents for this lysosomal storage disease. In another study, a mouse model of Desbuquois dysplasia (DBQD) type 1, a rare skeletal dysplasia characterized by short stature, round face and skeletal abnormalities, has been generated by CRISPR/Cas9 through disruption of the *Cant1* gene encoding Calcium-Activated Nucleotidase 1 enzyme, which preferentially hydrolyzes UDP to UMP and phosphate [24]. Newly generated $Cant1^{-/-}$ mice exhibited multiple skeletal defects, recapitulating clinical features seen in patients with DBQD type 1 [24]. Recently, Dreano and coworkers [25] reported the characterization of two rat models of cystic fibrosis (CF) using CRISPR/Cas9 genome editing. Rats homozygous for the F508del mutation in the transmembrane conductance regulator (*Cftr*) gene were compared with $Cftr^{-/-}$ rats. *F508del* and $Cftr^{-/-}$ rats exhibited abnormalities that are typical to CF [25]. Authors concluded that *F508del* mutant rats represent a novel CF model, which can be useful for development of CF therapeutics.

The generation of new mouse models by CRISPR/Cas9 has been recently reported for Hyperoxaluria Type 1 (PH1) [26] and X-linked Retinitis Pigmentosa (XLRP) [27]. Our laboratory has created a clinically relevant mouse model of Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACDMPV) by introducing the S52F FOXF1 mutation (found in ACDMPV patients) [7] into the mouse *Foxf1* gene locus using CRISPR/Cas9 technology (Figure 2). ACDMPV is a severe congenital disorder characterized by lung hypoplasia, a paucity of alveolar capillaries and gallbladder agenesis in newborns and infants [6]. All phenotypes were recapitulated in genetically engineered $Foxf1^{WT/S52F}$ knock-in mice [7] and previously described mice heterozygous for the *Foxf1 null* allele ($Foxf1^{+/-}$) [28, 29]. Similar to ACDMPV patients, $Foxf1^{WT/S52F}$ and $Foxf1^{+/-}$ mice exhibited reduced endothelial cell proliferation, decreased lung angiogenesis, and increased mortality after birth [7, 30]. FOXF1, a transcription factor from the Forkhead Box (FOX) family, is expressed in mesenchyme-derived cells such as endothelial cells, fibroblasts, pericytes, and visceral smooth muscle cells [31–34]. Published studies demonstrated that FOXF1 is critical for embryonic development [35–38], carcinogenesis [39, 40], organ regeneration [35, 41, 42], and lung repair after various injuries [43–46]. The $Foxf1^{WT/S52F}$ and $Foxf1^{+/-}$ mutant mice were recently used to develop two potential therapeutic approaches for ACDMPV patients. These approaches are based on increasing perinatal lung angiogenesis and endothelial proliferation through either nanoparticle delivery of

proangiogenic genes directly into endothelial cells [7, 47, 48] or cell transplantation with pulmonary endothelial progenitor cells [49].

Using CRISPR/Cas9 to correct pathogenic mutations in patient-derived primary cells and iPSCs

CRISPR/Cas9 genome editing has been extensively used to correct pathogenic mutations in primary cells and induced pluripotent stem cells (iPSCs) generated from patients with rare diseases [21] (Table 2). Recently, an intronic variant of Structural Maintenance of Chromosomes Flexible Hinge Domain Containing 1 gene (*SMCHD1*) was repaired by CRISPR/Cas9 method in primary and immortalized myoblasts obtained from patients with Facioscapulohumeral dystrophy type 2 (FSHD2), a rare autosomal dominant muscular disorder [50]. This genome modification restored expression of SMCHD1 and inhibited transcription of the *DUX4* gene encoding highly cytotoxic myogenic protein, which is increased in Facioscapulohumeral dystrophy type 1 (FSHD1). These studies suggest that CRISPR/Cas9-mediated targeting of *SMCHD1* can be explored in the development of new therapeutics for patients with FSHD 1 and FSHD2. CRISPR/Cas9 has been also used for disruption of the *NPC1* gene in human cell lines, modeling cellular defects in cholesterol storage that occur in patients with Niemann-Pick disease [51]. Multiple groups have performed CRISPR/Cas9-mediated disruption of *GLA* gene (which encodes alpha-galactosidase A) in either immortalized patient-derived cells or human embryonic stem cells (ESCs) to model cellular abnormalities that occur in Fabry disease (FD), a rare lysosomal storage disorder. Specifically, Lenders and co-workers [52] have used *GLA*-deficient cells to examine a possibility of chaperone therapy to correct FD defects, whereas *GLA*-deficient human ESCs were used to model autophagic dysfunction and exosome secretion in FD [53, 54]. CRISPR/Cas9-mediated disruption of *GLA* in human iPSCs led to the identification of additional molecular mechanisms critical for FD pathogenesis [55].

Several research groups have reported the use of CRISPR/Cas9 genome editing to target *β-globin* gene (*HBB*) in Sickle Cell Disease (SCD) and β-thalassemia [4]. SCD is a monogenic disorder caused by a point mutation in the human *β-globin* gene. Using a short single-stranded oligonucleotide template, the sickle mutation in *β-globin* was repaired in hematopoietic stem and progenitor cells obtained from peripheral blood and bone marrow of patients with SCD [56]. Compared to *β-globin* mutants, erythrocytes derived from gene-edited cells showed fewer sickle cells after transplantation into non-obese diabetic mice *in vivo* [56]. Efficient targeting of the human *β-globin* gene to repair SCD point mutations by CRISPR/Cas9 genome editing was also performed by *Kalkan et al* [57]. Erythroid cells generated from biallelic-corrected iPSCs from SCD patients showed normal expression of *β-globin* [58]. Interestingly, CRISPR/Cas9-mediated genome editing of the γ-globin repressor binding site in patient-derived hematopoietic stem and progenitor cells restored fetal hemoglobin synthesis and corrected the SCD phenotype [59]. Activation of fetal hemoglobin in erythroid cell lineage was also achieved using CRISPR/Cas9-mediated de-repression of γ-globin genes HBG1 and HBG2 [60]. These studies provide novel and promising therapeutic approaches for hemoglobinopathies.

Genome editing methods have been used to study Chronic Granulomatous Disease (CGD), a rare immunodeficient disorder caused by defective NAPDH oxidase enzyme and mutations in either of five genes: *CYBA*, *CYBB*, *NCF1*, *NCF2*, or *NCF4*. Patient-specific point mutations in the Cytochrome b-245 heavy chain gene (*CYBB*) have been repaired in hematopoietic cells using non-integrating lentiviral vectors carrying RNA-guided Cas9 endonucleases [61]. In another study, iPSCs with patient-specific c.75_76delGT (ΔGT) mutation in the *NCF1* gene were generated by CRISPR/Cas9 and later used to develop a gene-correction approach. Functional analysis of iPSC-derived cells revealed a restoration of NADPH oxidase activity and increased anti-bacterial properties in corrected phagocytes [62]. Recently, Benati *et al.* [63] reported the use of CRISPR/Cas9-mediated *in situ* correction of the *LAMB3* gene in keratinocytes derived from a patient with Junctional Epidermolysis Bullosa (JEB), a rare disorder characterized by fragile skin and formation of blisters. CRISPR/Cas9 genome editing of the *ERCC6* (*CSB*) gene was successfully used to correct iPSCs from a patient with Cockayne syndrome (CS), a rare autosomal recessive disorder characterized by short stature, progeria, severe photosensitivity and learning disabilities [64]. Likewise, Iyer *et al.* [65] employed CRISPR/Cas9 for precise correction of pathogenic mutations in cell lines derived from patients with Limb-Girdle Muscular Dystrophy 2G and Hermansky-Pudlak Syndrome Type 1. These published studies emphasize the importance of precise genome editing tools to repair pathogenic mutations in patient-derived primary cells and iPSCs.

The use of CRISPR/Cas9 to correct pathogenic mutations *in vivo*

CRISPR/Cas9 has been used to correct or replace dysfunctional genes in rare monogenic and other diseases [4, 13] (Table 3). In the case of recessive diseases, allogeneic cell transplantation can compensate for the mutated gene [14, 16]. This approach was explored in animal models and patients with muscular dystrophy [66–69]. Furthermore, a specifically designed viral vector can deliver normal exogenous gene (exogene) into cells or tissues to compensate for the mutated gene. Lentiviruses and adeno-associated viruses (AAV) are the most commonly used vectors for the delivery of exogenes. In dominantly inherited diseases, in which the correction of pathogenic gene variants has to be done precisely, genome editing approaches have become methods of choice.

Tay-Sachs and Sandhoff diseases are rare lipid storage disorders associated with inactivating mutations in *HEXA* and *HEXB* genes and are characterized by progressive loss of neurons in the brain and spinal cord. Several groups used CRISPR/Cas9 to generate *HEXA*- or *HEXB*-deficient immortalized cell lines as well as to perform gene corrections in patient-derived cells [70, 71]. Recently, Qu and co-workers [72] developed the CRISPR/Cas9-mediated *in vivo* strategy, which is based on the expression of the exogenous lysosomal enzyme in hepatocytes of neonatal Sandhoff mice. This approach decreased the disease severity in Sandhoff mice, demonstrating the future potential for the treatment of patients with Tay-Sachs and Sandhoff diseases through *in vivo* genome editing [72]. AAV vector delivery of CRISPR/Cas9 components repaired compound heterozygous recessive mutations and rescued the disease phenotype in mouse models of Hereditary Tyrosinemia Type I and Mucopolysaccharidosis Type I [73], whereas delivery of AAV vector carrying the *iduronate-2-sulfatase* gene into cerebrospinal fluid significantly attenuated brain disease in a

mouse model of Mucopolysaccharidosis Type II [74]. *In vivo* genome editing partially restored expression of *dystrophin* and improved muscle function in a mouse model of Duchenne Muscular Dystrophy (DMD) [75, 76]. CRISPR/Cas9-mediated correction of DMD in mice was also performed using a self-complementary AAV delivery system [77]. In a landmark study for Interstitial Lung Disease, the delivery of CRISPR/Cas9 genome-editing components directly into the amniotic fluid led to *in utero* inactivation of the pathogenic *SftpcI73T* mutant allele, improving survival and decreasing lung fibrosis in *SftpcI73T* transgenic mice [78]. This study raises the possibility that in utero gene correction can be beneficial for monogenic lung diseases such as Interstitial Lung Disease, Cystic Fibrosis, and Alpha-1 Antitrypsin Deficiency.

Closing/ Conclusions

In summary, CRISPR/Cas9 genome editing technology has shown great potential for developing clinically relevant animal models that will be useful for testing new therapeutics for human rare diseases. While the correction of pathogenic mutations in cultured cells and patient-derived iPSCs are routinely performed in laboratory settings, CRISPR/Cas9 genome editing is emerging as a feasible option to target somatic cells in rare monogenic and other genetically defined human diseases. Recent progress in CRISPR/Cas9 technology has also led to clinical trials in the area of gene therapy. Several commercially available products that are based on CRISPR/Cas9 are on different stages of clinical testing to gain FDA approval [4, 79]. The global gene editing market is expected to expand at a high rate to accommodate the clinical needs of many rare diseases lacking efficient treatment options. While this technology accelerates the development of novel therapeutics for rare diseases, many important questions about the use of CRISPR/Cas9 genome editing in patients, such as efficacy, safety, and off-target effects, remain to be addressed. Furthermore, potential clinical applications of genome editing involve ethical and regulatory hurdles among other challenges [80–83]. Gene therapy applications mostly focus on targeting somatic cells, such as blood and bone marrow cells, so the genome changes cannot be inherited and passed to the next generations. Potential therapies targeting germ cells are considered to be controversial due to unknown long-term consequences that the germline gene therapy could cause.

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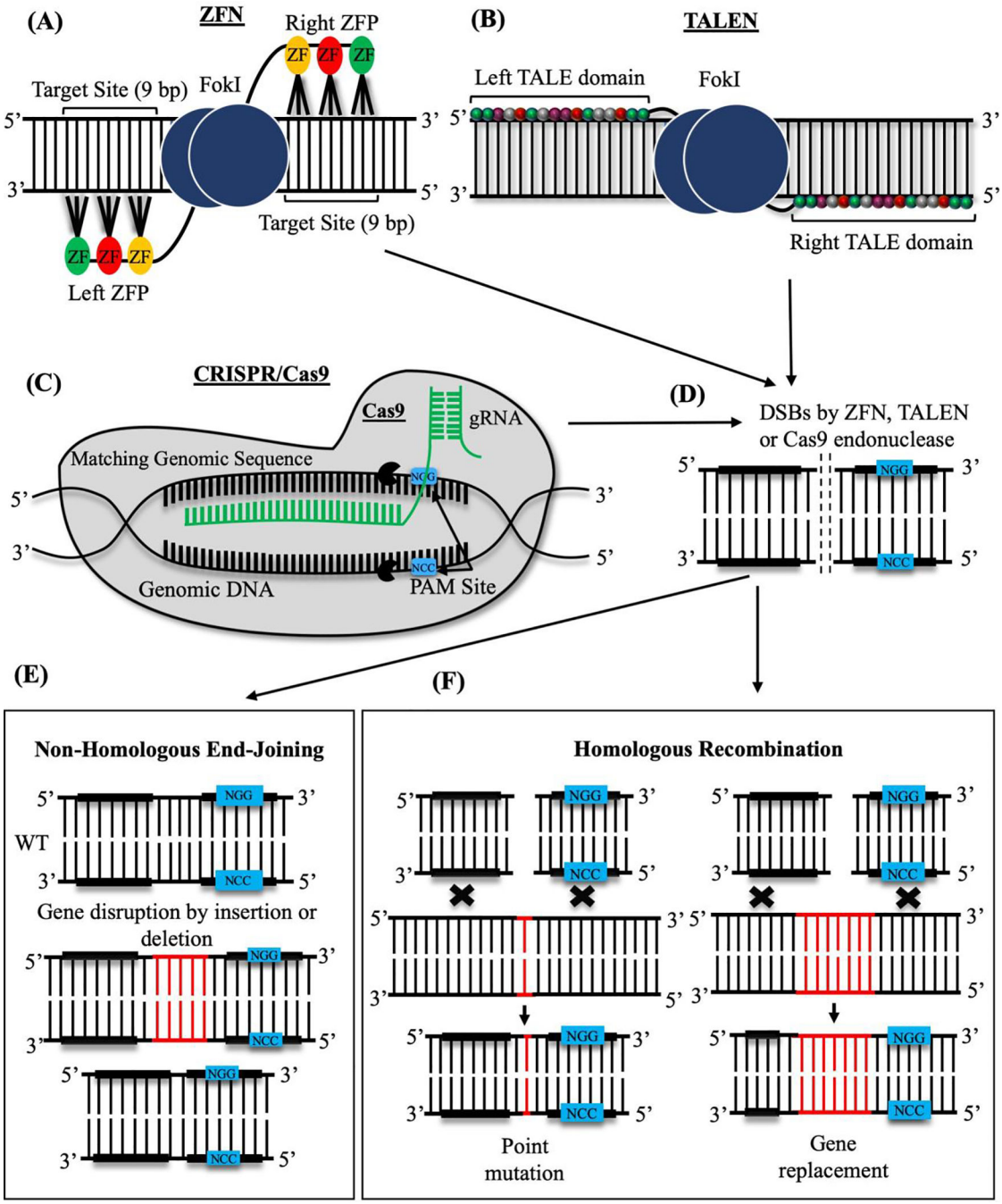

Figure 1: Site-specific genome editing methods that use endonuclease activity.

(A) The Zinc-Finger Nuclease complex (ZFN) consists of the FokI endonuclease domain (blue) and several Zinc finger (ZF) DNA-binding domains (yellow, red and green). Each ZF domain recognizes a 3-base pair (bp) DNA sequence. (B) Transcription Activator-Like Effector Nuclease (TALEN) is shown as a fusion protein consisting of FokI endonuclease domain and TALE DNA-binding domains. Sixteen colored subunits in each TALE domain recognize a single base pair of DNA sequences. (C) CRISPR/Cas9 system is comprised of Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR) and CRISPR

associated (Cas) protein 9. Guide RNA (gRNA, green) directs Cas9 endonuclease (grey) to specific DNA sites, which are located close to short Protospacer Adjacent Motifs (PAM, blue). Cas9 cleaves DNA upstream of PAM. (D) FokI and Cas9 endonucleases cause double-strand DNA breaks (DSBs). DSBs are repaired through either NHEJ or HR mechanisms. (E) NHEJ repair is based on nucleotide deletions or insertions into cleaved DNA. (F) Repair through HR mechanism occurs in the presence of the donor DNA template, which contains homology regions on both sides of the template. Exogenous DNA is specifically inserted into the target site to modify the gene.

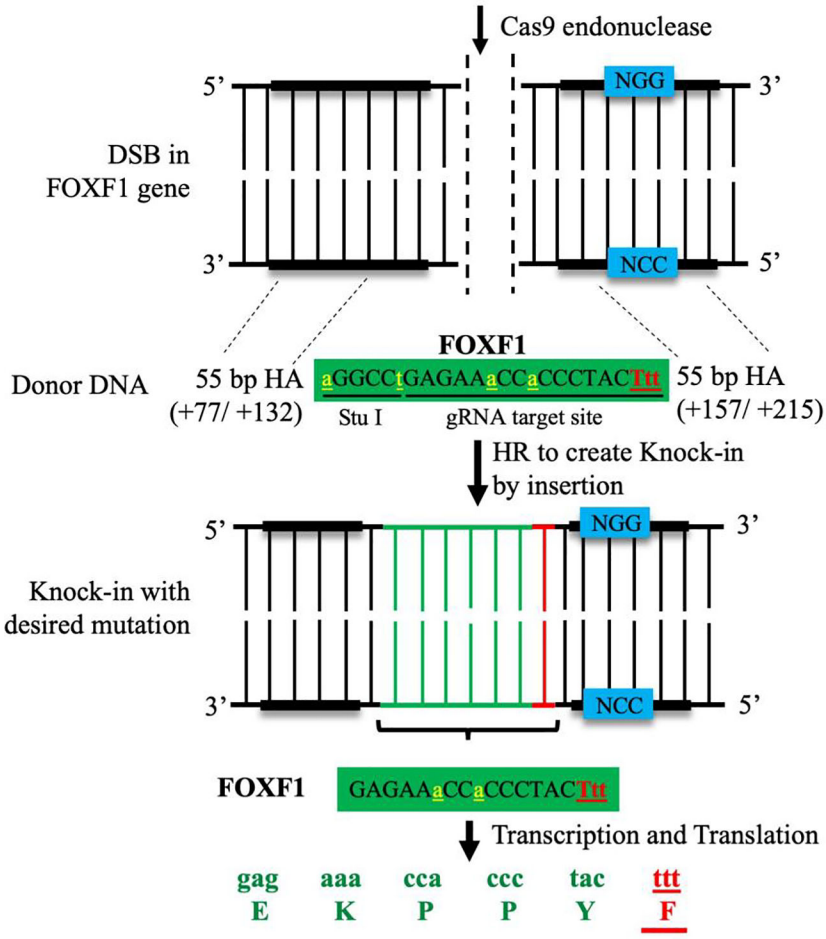

Figure 2: Generation of *Foxf1*$^{WT/S52F}$ *knock-in* mouse model of ACDMPV using CRISPR/Cas9 genome editing.

The schematic diagram shows the generation of *Foxf1*$^{WT/S52F}$ knock-in mice by homology-directed repair method. Single amino acid substitution of *serine-52* (S52) to *phenylalanine* (F) is performed using a donor DNA template containing 55 nucleotide base pairs (bp) homology arms (HA) on either side of the template. HAs allow the donor DNA template to recombine into the host genome through homologous recombination (HR), inserting the *S52F Foxf1* mutation. The mutated amino acid and triplet nucleotide codon (F/Ttt) are labeled in red, whereas endogenous nucleotides are labeled in green. Silent mutations (yellow) are inserted into the donor DNA template to create a new *Stu I* restriction site needed for identification of the *S52F Foxf1* mutant allele.

Table 1:

Animal models of rare diseases generated using CRISPR/Cas9 genome editing approach

	Disease	Species	Type of mutation	Reference
1.	Niemann-Pick disease type C1 (NPC1)	Zebrafish	Null mutant of N*pc1* gene	22
2.	Desbuquois dysplasia (DD) type 1	Mouse	Knockout of *Cant1* gene	24
3.	Cystic fibrosis	Rat	Knockout/ deletion of *Cftr* gene	25
4.	Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACDMPV)	Mouse	Knock-in mutation in *Foxf1* gene	7
5.	Primary hyperoxaluria type I (PH1)	Mouse	Gene correction in *Agxt1* gene	26
6.	X-linked retinitis pigmentosa (XLRP)	Mouse	Transgenic expression of *Rpgr* gene	27
7.	Bronchopulmonary dysplasia (BPD)	Mouse	Expression of exogenes *Foxf1* and *Foxm1* from nanoparticle vector	47

Table 2:

Use of CRISPR/Cas9 to correct pathogenic mutations in patient-derived primary cells and iPSCs.

	Disease	Species/ Cell type	Type of mutation	Reference
1.	Facioscapulohumeral dystrophy	Human / myoblasts	Pseudogene *SMCHD1* deletion	50
2.	Niemann-Pick Type C	Human/ HeLa cells	*NPC1* gene disruption	51
3.	Fabry disease	Human - HEK293T and primary urinary cells	Knockout of *GLA* gene	52
4.	Fabry disease	Human Embryonic Stem Cell	Knockout of *GLA* gene	53
5.	Fabry disease	Patient-derived fibroblasts and HEK-293T cells	Knockout of *GLA* gene	54
6.	Fabry disease	Human-induced pluripotent stem cells/ Fibroblast	Knockout of *GLA* gene	55
7.	Sickle cell disease	Human/ hematopoietic stem and progenitor cells from peripheral blood or bone marrow	*HBB* gene correction	56
8.	Sickle cell disease	Human cell lines	*HBB* gene correction	57
9.	Sickle cell disease	Induced pluripotent stem cells (iPSCs)	*HBB* gene correction	58
10.	Sickle cell disease	Patient-derived hematopoietic stem/ progenitor cells	Insertion/ deletion in *HBG* gene	59
11.	Sickle cell disease	Human hematopoietic stem and progenitor cells	*HBG1/HBG2* gene disruption	60
12.	Chronic granulomatous disease	Human hematopoietic cells and induced pluripotent stem cells (iPSCs)	*CYBB* and *NCF1* gene correction	61, 62
13.	Junctional epidermolysis bullosa	Mouse/ Keratinocytes	*LAMB3* gene correction	63
14.	Cockayne syndrome	Human induced pluripotent stem cells (iPSCs) from fibroblasts	*ERCC6* gene correction	64
15.	Limb-Girdle Muscular Dystrophy 2G (LGMD2G) and Hermansky-Pudlak Syndrome Type 1 (HPS1)	LGMD2G iPSC-derived myotubes and HPS1 patient-derived B-lymphoblastoid cell line	*TCAP* gene correction	65

Table 3:

Correction of pathogenic mutations *in vivo* using CRISPR/ Cas9

	Disease	Species	Type of mutation	Reference
1.	Duchenne muscular dystrophy (DMD)	Mouse	*Dmd* gene correction	68, 69, 76, 77
2.	Tay-Sachs or Sandhoff disease	Mouse	*HEXA* and *HEXB* gene correction	70
3.	Sandhoff disease	iPS cells/ Organoids	*HEXB* gene correction	71
4.	Tay-Sachs and Sandhoff Diseases	Mouse	*HEXA* and *HEXB* gene correction	72
5.	Tyrosinemia type I and mucopolysaccharidosis type I	Mouse	Allelic exchange in *Fah* and *Idua* genes	73
6.	Mucopolysaccharidosis type II (MPS II)	Mouse	*Ids* gene correction	74
7.	Duchenne muscular dystrophy (DMD)	Mouse	Permanent exon skipping in *Dmd* gene	75
8.	Interstitial lung disease	Mouse	Inactivation of *Sftpc* gene	78