Journal of Genetic Syndromes & Gene Therapy
ISSN: 2157-7412
Pandey VK et al., J Genet Syndr Gene Ther 2017, 8:2
DOI: 10.4172/2157-7412.1000321
Research Article
Open Access

Application of CRISPR/Cas9 Genome Editing in Genetic Disorders: A Systematic Review Up to Date

Vivek K Pandey[1], **Anima Tripathi**[2], **Ravi Bhushan**[1], **Akhtar Ali**[1], **Pawan K Dubey**[1*]

[1]*Centre for Genetic Disorders, Institute of Science, Banaras Hindu University, Varanasi, India*

[2]*Zoology Section, MMV, Banaras Hindu University, Varanasi, India*

[*]**Corresponding author:** Pawan K. Dubey, Centre for Genetic Disorders, Institute of Science, Banaras Hindu University, Varanasi, India, Tel: +919451890938; E-mail: pawandubey1981@gmail.com

Received date: November 15, 2017; **Accepted date:** December 2, 2017; **Published date:** December 9, 2017

Abstract

Genetic diseases in human are associated with congenital disorders and phenotypic traits. A single mutation in a gene can cause physical or mental problems, and sometimes both. Some diseases can be lethal, and there are still no cures for many of them. Socioeconomic burden of rare genetic diseases are increasing worldwide that have been tried to cure using various methods. However, they were not very successful till now. Genome editing technologies over the past few years is providing fast and effective tool to precisely manipulate the genome at specific locations. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) associated Cas9 (CRISPR/Cas9) system has been using from last few years in the field of biomedical research. CRISPR/Cas9 has advantages in terms of clinical applicability to treat genetic diseases like DMD, Hemophilia, β-Thalassemia and cystic fibrosis etc. and even in some cases this tool has already been successfully applied. Nevertheless, developed technologies for addition or deletion of genes have made notable progress in last few years and demonstrate some promising clinical results. However, several challenges still remain. Here, the latest applications of CRISPR-Cas9 technology in genetic disorders, current challenges and future directions are reviewed and discussed.

Keywords: CRISPR-Cas9; Genetic disorders; Genetic engineering; Gene therapy

Abbreviations **CRISPR:** Clustered Regularly Interspaced Short Palindromic Repeats; **ESCs:** Embryonic Stem Cells; **HDR:** Homology Directed Repair; **iPSCs:** Induced Pluripotent Stem Cells; **NHEJ:** Non-Homologous End Joining; **RNAi:** RNA Interference; **TALENs:** Transcription Activator-Like Effectors; **ZFN:** Zinc Finger Nucleases

Introduction

Treatment of genetic diseases using gene therapy has long been an aspiration for scientists, physicians and patients. The ideal approach to cure a genetic disorder is the development of an efficient method and tool to correct the mutations that causes the disease. Derivation of patient-specific induced pluripotent stem cells (iPSCs) from somatic cells and the subsequent correction of the disease-causing mutations represent a possible strategy to achieve this goal. Corrected iPSCs can undergo indefinite self-renewal without losing the ability to differentiate into all cell types and may useful for autologous transplantation [1]. However, this strategy has own limitations for the clinical use. Further, in gene therapy, a large portion of cells from related tissues or organs may need to be changed. Therefore, this approach has ethical, safety and technical challenges and may alter some other genes. If this approach is performed in germline including embryo, then all cells of body in subsequent generations may inherit that modification and it could unsafe for human welfare. Diseases like cystic fibrosis or sickle cell anemia are relatively well understood and caused by nucleotide changes in single genes. If this is the case, it might possible that faulty gene can be corrected or replaced, but gene therapy become more difficult for complex conditions such as heart disease, diabetes or many forms of cancer.

Targeted gene correction has the potential to treat many different diseases, including clotting disorders such as hemophilia A and B, muscular dystrophy, cystic fibrosis, Fabry disease, Gaucher disease, Pompe disease, von Gierke disease, and Hurler and Hunter syndromes and so on. However, number of affected individual is relatively small and the types of mutations are varies according to geographical conditions. Therefore, the cost of treatment and regulatory approval to develop safe and efficacious gene-editing tools for each of these diseases may not be permissible. Hence, in earlier days scientific community realized that there is need to develop a versatile gene editing tool which can help to manipulate the gene very precisely.

In this review, we provide overview on how CRISPR-Cas9 system may useful for the correction or deletion of faulty gene that causes genetic diseases in human. Here, we mainly focus on the application of CRISPR-Cas9 system in area of genetic diseases and to explore the possible treatments strategies using latest gene editing tool. Although a large number of genome editing have focused on treatment of monogenic disorders, here we will also discuss possible treatment strategies to apply this editing tool to other chromosomal diseases and viral mediated infections and cancer.

Methodologies

This non-systematic review was prepared using search engine: PubMed, Google Scholar and Medline for articles published from 2005 through 2017, using the following keywords: genome editing, CRISPR/Cas9, genetic disorders, DMD, Cystic fibrosa, hemophilia, Thalassemia etc. CRISPR/Cas9 mediated gene editing in cell culture lines (in vitro) and animal models (in vivo) were also reviewed and included in this

manuscript. Further, the future prospects and consequences of CRISPR/cas9 gene editing tool use in correction of genetic disorders were also reviewed based on the authors' expert opinion.

Earlier Approaches for Gene Correction

The gene manipulation technologies developed so far are viral mediated transgene expression and RNA interference (RNAi), which enables restoration of gene function and targeted gene repression respectively. However, both technologies have some limitations that prevent their therapeutic use genetic diseases. For instance, viral mediated gene therapy may cause mutagenesis at the insertion site and RNAi regularly cannot fully repress gene expression. Therefore, these technologies are unlikely to provide complete therapeutic effect where complete ablation of gene function is necessary. Further, designer DNA endonucleases such as zinc finger (ZF) nucleases based on eukaryotic transcription factors [2,3], transcription activator-like effectors (Figure 1 and 2) (TALENs) from Xanthomonas bacteria has been used to correct the mutations [4-6]. ZFN-mediated gene modification has been shown in human cells [2,7], pigs [8], rats [9,10], mice [11], zebrafish [12] and insects [13]. ZFNs have also been applied for experimental, biotechnological and therapeutic purposes. Further, it has been demonstrated that TALENs have potential to correct endogenous genes in human primary cells [14], human ESCs and iPSCs [6,15]. Furthermore, rapid gene disruption in mouse, rat and rabbit embryo by microinjection of TALENs has also been reported [16-18]. TALENs have been successfully used to correct β-thalassemia patients using a non-viral approach via efficient generation of iPSCs from the patient cells [1] demonstrating that this strategy could be used for correction of mutation.

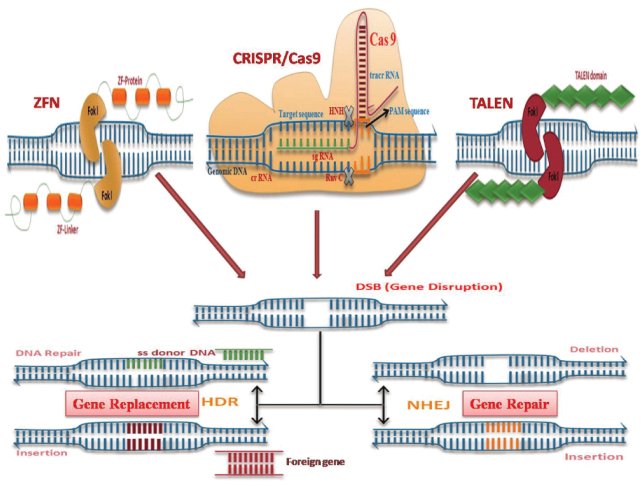

Figure 1: CRISPR–Cas9, TALEN and ZFN mediated genome editing. (A) ZFN-fok1, TALEN domain and Cas9·sgRNA-induced DSBs can be repaired by either NHEJ or by HDR pathways. NHEJ mediated repair is highly efficient but error-prone process, which causes small insertions and/or deletions (indels) at the cleave site. HDR requires a donor DNA homologous template to repair the cleavage site and this process can be used to introduce specific point mutations, correction of mutation or to knock-in of corrected DNA sequences at cleavage site.

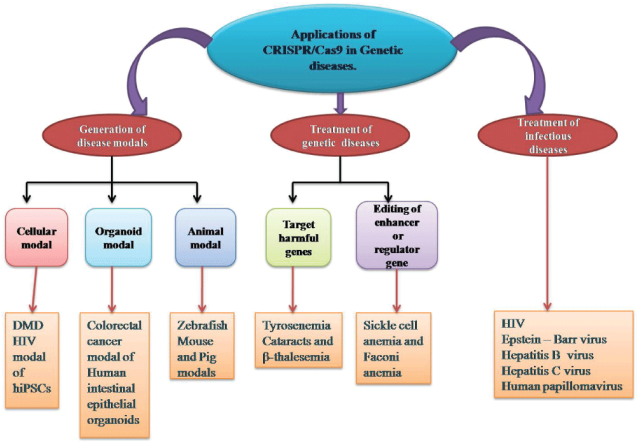

Figure 2: Applications of CRISPR/Cas9 in genetic disorders. The disease model at the level of cellular, organ and animal could be ideal targets for CRISPR-Cas9-mediated gene therapy. Monogenetic as well as non-monogenetic diseases can be treated by editing of targeted disease causing genes or enhancer and regulator genes. Further, CRISPR-Cas9-mediated gene therapy will help us to delineate the molecular mechanisms underlying the development of different genetic diseases and in near future it will become a tool to cure the diseases.

CRISPR/Cas9 and Gene Editing

CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 system, is an RNA-guided DNA nuclease (RGN) are derived from an adaptive immune system that evolved in bacteria to defend against invading plasmids and viruses. Of course, upon invading, this system transcribed and processed CRISPR RNAs (crRNAs) together with a trans-activating crRNAs (tracrRNAs) and forms a complex with CRISPR-associated (Cas) proteins to cleave nucleotide sequence by Cas nucleases at specific location 158-161. The cleaved double stranded DNA is repaired by either NHEJ (non-homologous end joining) or HDR (homology directed repair). In NHEJ, DNA ends directly join with their counterparts in a highly efficient but error-prone manner, which causes small insertions and/or deletions (indels) at the cleave site, which can disrupt the translational reading frame of a coding sequence. In other hand, HDR requires a donor DNA homologous template to repair the cleavage site. The donor template DNA can be used to introduce specific point mutations, correction of mutation or to knock-in of corrected DNA sequences at cleavage site.

In recent years, it has been seen that scientific breakthroughs have brought by correction of disease-causing genetic variants. CRISPR-Cas system has been extensively applied to edit genome in many species, such as bacteria [19], yeast [20], fruit fly [21], elegans [22], zebrafish [23], frog [24], mouse [25] and human [26]. These studies were breakthrough and taken leads to demonstrate successful in vivo gene editing in many adult tissues with CRISPR/Cas9. In a study, Song et al. successfully corrected b-thalassemia mutations in iPSCs using CRISPR/Cas9 [27]. The recent success has been made when a DMD mouse model edited by CRISPR/Cas9 restored expression of the dystrophin protein and improved muscle pathology and strength [28]. Because the rich multiplicity, CRISPR systems has been largely understudied and it is logical to expect many new CRISPR-based gene-

editing tools to emerge, including non-Cas9 based type II systems such as the recently described RNA-guided endonuclease Cpf1 and peptide nucleic acid (PNA) molecules [29,30]. Recently, Cpf1, a type V CRISPR effector has been successfully used to induce mutation in soybean genome [29]. In recent years, a series of publications demonstrated that the CRISPR/Cas9 system could be used for correction or deletion of faulty gene in mammalian cells. These translational results suggest that gene editing tool like CRISPR and its modified nucleases like Cpf1 have created new opportunities and would support the potential future clinical application for genetic diseases. Although, one major drawback of this tool is the recognition of off-target effects, which involve the deletion of nonspecific DNA sequences. There is need to develop some methods for avoiding off-target effects and non-specific sequence recognition for the effective application of CRISPR/Cas9 to cure human genetic diseases.

CRISPR/Cas9 and Genetic Disorders

Why is CRISPR/Cas9 applicable for genetic disorders?

It is well known that genetic diseases caused by alteration in DNA sequences that are responsible to perform a particular function in system. In pathogenesis, many genes have already been identified which are critically involved in the pathogenesis of genetic based diseases. In general, defective gene in diseased cells can be corrected by two ways: *ex vivo* and *in vivo.* In *ex vivo,* the diseased cell is removed, manipulated as desired using programmable nucleases and then transplanted back into the original host.

Disease	Target gene/Sequence	Delivery mode	Stem cells	Cell line	Animal modal	References
Cystic Fibrosis	CFTR	Transfection	Small (SI) and Large intestine (LI) stem cells	HEK293T	Human	Firth et al. [44] Schwank et al. [42]
Cataracts	Crygc	Microinjection Transfection and Electroporation	SSCs		Mouse	Wu et al. [77] Wu et al. [78]
Human immunodeficiency virus (HIV-1) infection and immunization	LTR loci of integrated viral genome, T10	Transfection	J-lat	CHME5,	Human	Ebina et al. [79] Hu et al. [70] Zhu et al. [80]
			T-cells	HeLa, 293T, Jurkat, TZM-bl, U1		
Duchenne muscular dystrophy (DMD)	Exon 45 of dystrophin gene	Electroporation, IM, IP and IV	hiPSCs	Immortalized patient myoblasts, HEK293T	Human	Ousterout et al. [34]
	Exon 23 of dystrophin gene	injection		C2C12 and mdx myoblasts	Mouse	Long et al. [81], Xu et al. [82], Tabebordbar et al. [38], Long et al. [28]
Sickle cell anemia	β-globin (HBB)	Transfection Electroporation	hiPSCs	HEK293T, BC1, TNC1 etc.	Human	Song et al. [27] Xie et al. [50] Huang et al. [83] Park et al. [84]
α1-antitrypsin deficiency (A1ATD)	SERPINA1	Transfection	hiPSCs	HEK293T	Human	Smith et al. [85]
Polycythemia vera (PV)	JAK2	Transfection	hiPSCs	HEK293T	Human	Smith et al. [85]
Barth syndrome	TAZ	Transfection	hiPSCs	PGP1 cell line	Human	Yang et al. [86]
Hereditary tyrosinemia type I (HTI)	Fah	Hydrodynamic injection		3T3 cells	Mouse	Yin et al. [87]
Human immunodeficiency virus (HIV-1) resistance	CCR5	Transfection	hiPSCs	HEK293T	Human	Ye et al. [66]
Epstein-Barr virus (EBV)	Multiple	Transfection		Ranji and Burkitt's lymphoma cell lines	Human	Wang and Quake. [88]

Osteosarcoma	CDK11	Transfection and electroporation		KHOS and U-20S	Human	Feng et al. [89]
Cardiovascular disease	Pcsk9	Transfection		3T3-L1	Mouse	Ding et al. [90]
Human papillomavirus (HPV) and cervical cancer	HPV16, E7 oncogenes	Transfection		SiHa and Caski	Human	Hu et al. [91]
Hepatitis B virus (HBV)	p53 and Pten gene, Multiple	Transfection Electroporation Hydrodynamic Injection		Huh7, HepG2	Mouse	Lin et al. [92], Dong et al. [68], Ramanan et al. [93], Hao et al. [94]
β- Thalassemia	HBB gene	Transfection	hiPSCs		Human	Xie et al. [50]
Huntington's disease	HTT gene	Stereotactic injection		HEK293T	Mouse	Monteys et al. [95] Yang et al. [96]
Phenylketonuria (PKU)	PAH	Transfection		c.1222C>T COS-7	Human	Pan et al. [97]
Down Syndrome	GATA 1	Transfection		K562	Human	Bloh et al. [98]
Parkinson's disease	DJ-1/parkin/PINK1 LRRK2	Microinjection	Procrine fetal fibroblasts		Pig	Wang et al. [99]
		Electroporation				
Retinitis pigmentosa	RGPR	Transfection	iPSCs		Human	Bassuk et al. [100]
		Transplantation				
Fanconi anemia	FANCC	Transfection		Patient fibroblasts	Human	Osborn et al. [101]
		Electroporation				
Cryptosporidiosis	C. parvum	Transfection		HTC8	Mouse	Vinayak et al. [102]
		Electroporation				
Colorectal cancer	APC, SMAD4, TP53, KRAS, PIK3CA	Transfection		Human intestinal epithelial organoids	Human	Matano et al. [103]
		Electroporation		HEK293T		
Urea cycle disorder	OTC	Transfection		MC57G	Mouse	Yang et al. [104]
		Intraveinously injection				
Contextual memory	Mecp2	Transfection Stereotactical injection		HEK293FT	Mouse	Swiech et al. [105]
Bronchial alveolar adenoma	Kras, p53, Lkb1	Transfection	mESCs	Neuro-2a	Mouse	Platt et al. [106]
		Electroporation				
Intestinal hyperplasia	Apc	Blastocycst injection	mESCs		Mouse	Dow et al. [107]
Cardiomyopathy	Myh6	Intraperitoneal injection		10T1/2 cells	Mouse	Carroll et al. [108]
Severe combined immune	IL2Rg	Transfection	hESCs	K-562, hCD4+ T cells,	Human	Hendel et al. [109]

deficiency X-1 (SCID						
Adrenal hypoplasia congenita and hypogonadotroic hypogonadism	DAX1	Microinjection			Monkey	Kang et al. [110]

Table 1: Studies using CRISPR mediated gene-editing approaches applied in haematological, neurological non-cancerous, cancerous and monogenic diseases.

In other hand, *in vivo* therapy modified editing tool with corrected piece of gene directly transfer into body. Therefore, these genes are might be potential target for the modified nucleases like CRISPR/Cas9 system to remove or repair the faulty gene and may lead to develop therapeutic strategies to cure genetic disorders. The each programmable nucleases has own advantages and disadvantages, and they are executed diversely to treat particular disorders. There are examples of gene-editing techniques applied in different genetic diseases in cell lines, disease models and human (Table 1).

DMD

Duchenne muscular dystrophy (DMD) is an inherited X-linked fatal genetic muscle disease, caused by in-frame deletions of dystrophin gene. The gene encoded protein dystrophin plays a crucial role in muscle development. In the absence of dystrophin, progressive muscle weakening and degeneration occur and over time the affected individual leads to premature death [31]. Presently, there is no permanent and effective treatment for DMD. Genome-editing approaches for DMD include permanent exon removal and cDNA knocking using different tool has been tried [32-34]. The exon skipping studies in patient derived myoblasts using ZFNs have also been performed and demonstrated restoration of the reading frame [34]. The mutational hotspot has also been targeted by HDR, with mega nuclease-mediated repair of exons 45-52 in immortalized patient cells [32]. Functional dystrophin gene restoration has also been demonstrated by genome editing in iPSCs derived from a patient lacking exon 44 and 45 using TALEN and CRISPR-Cas9 [33-36]. Further, recently it has been seen that researchers of different laboratories have of demonstrated that AAV-mediated CRISPR treatment enabled the function of DMD gene in cardiomyocytes and muscle stem cells [37-41].

Recently, Young et al. [35] successfully deleted human *DMD* exons 45-55, and suggest that it can be directly applied *in vivo* to restore dystrophin. Further, Bengtsson et al. [36] demonstrated that adeno-associated viral (AAV)-mediated muscle-specific gene editing has significant potential for therapy of neuromuscular disorders. Additionally, some of studies used pigs and nonhuman primates for generation of DMD phenotypes using CRISPR-Cas9 and suggested that such gene-editing may be easily switched into larger animals [37-40]. Besides that larger animal model such as rats, pigs, and primates could represent clinical appearances for DMD than commonly used mdx mouse. Recently, Cpf1-(CRISPR from *Prevotella* and *Francisella*-1), a new RNA-guided endonuclease, mediated correction of DMD mutations in human cells and in animal disease model was demonstrated that represent a noteworthy step toward therapeutic translation of gene editing for genetic diseases [41]. These encouraging results suggest that CRISPR/Cas9 tool possess the therapeutic potential to cure human genetic diseases including DMD. Further, large animal models may serve to augment our understanding about DMD at large level and may useful to develop therapeutic interventions. However, these approaches and strategies have some concerns like efficiency, applied methodology and tool, cell survival and proliferation, toxicity of injected chemicals and so on and should investigate thoroughly before clinical application.

Cystic Fibrosis

Cystic fibrosis (CF) is a chronic and progressive autosomal recessive genetic disorder, caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on the long arm of chromosome 7. CFTR encodes epithelial chloride ion channel and distributed across a wide range of organs including pancreas, kidney, liver, lungs, gastrointestinal tracts, and reproductive tracts. Mutations in CFTR causes disturbance in ion transport and fluid retention, resulting abnormal thickening of the mucus which causes obstructions in the lung airways and pancreatic ducts. CF remains the most common and lethal genetic disease among the Caucasian population and need to develop better therapeutic intervention. A non-profit cystic fibrosis foundation has announced $5 million three year agreement with Editas Medicine to develop CRISPR/Cas9-based medicines for the treatment of cystic fibrosis. This foundation in 2013 has demonstrated the functional repair of CFTR by CRISPR/Cas9 in intestinal organoids of cystic fibrosis [42]. This type of research showed potential of CRISPR/Cas9 and encouraged to researcher for correction of other genetic diseases using this tool. In cystic fibrosis, DNA, RNA, or proteins can be targeted for the modifications, but at the level of DNA, replacement of mutated CFTR gene with functional CFTR gene could better therapeutical approach. Correction of CFTR mutation in patient derived iPSCs and intestinal stem cell has been done previously using ZFN [43] and CRISPR [42,44]. In these studies, patient derived fibroblasts were reprogrammed to iPSCs and then co-nucleofected with a CRISPR/Cas9-CFTR gRNA cassette in various combinations demonstrated correction of CFTR gene with16.7% efficiency. This progress opens the perspective of undertaking the precise correction of the CFTR gene in the frame of a precision medicine approach. In 2016, Vituret et al. [45] demonstrated functional correction of the genetic defect in human CF cells by using microvesicles and exosomes as vectors that established an mRNA-mediated gene therapy approach in CF organoids. However, some concern showed that there is potential risk of permanent integration of CRISPR-Cas9 components into the host genome following viral vector transfection methods.

Hemophilia

Hemophilia, one of the most common inherited blood diseases, is caused by mutations in the human hemoglobin beta (HBB) gene. There are two variants in haemophilia-A and B. The haemophilia A is caused by different genetic mutations- in coagulation factor VIII and for hemophilia B in coagulation factor XI. In this disease it is found that decreased expression of HBB gene lead to accumulation of α-globin chains which ultimately disrupt the process of erythropoiesis and cause anaemia [46]. To date there is only one curative treatment for b-thalassaemia is allogeneic haematopoietic stem cell transplantation (HSCT). Gene therapy could be another option for treating hemophilia via correction of the defective gene. The first successful *in vivo* gene targeting of hemophilia was achieved in a hemophilia B neonate mouse [47]. Further, in 2013, Anguela et al. [48] demonstrated successful correction of defective human F9 (*hF9*) gene using a ZFN pair to target the and AAV as the delivery vector in mouse genome. Further TALENs were also used and shown to be able to correct haemophilia gene [49]. In recent years, modified nuclease like CRISPR-Cas9 technology has been successfully applied to correct b-thalassaemia mutations in patient-derived induced pluripotent stem cells (iPSCs) [27,50,51]. These corrected iPSCs thus display normal function and potentially could provide a source of cells for transplantation in patients, offering a new strategy to cure this disease. In 2015, Canver et al. [52] identified an erythroid-enhancer region within the BCL11A gene via CRISPR-Cas9-mediated and reported that this region could be an ideal therapeutic target for b-haemoglobin disorders. Hence, considering the latest development in area of gene therapy, hemoglobinopathies of hemophilia can be treated or corrected using modern gene editing approaches in patient derived iPSCs and these iPSCs can be differentiated into hematopoietic stem cells, which can be transplanted back into patients.

Sickle-Cell Anemia and β-Thalassemia

Sickle cell anemia is one of the common monogenic disorders, affecting millions of people worldwide. It is caused by mutation in the β-globin gene (*HBB*) which is a single nucleotide substitution from A to T that converts a glutamic acid to a valine that are responsible for the production of sickle hemoglobin. Till date, there is no available therapy to completely cure the sickle cell anemia except allogeneic hematopoietic stem cell transplantation (HSCT), however, blood transfusion associated with a high risk and it is possible only for a small level of patient. Manipulation of β-globin locus has been previously carried out using targeted nucleases like TALENs and ZFNs to cure hemoglobinopathies [53,54]. However, very limited success has achieved with maximum off target. Recently, one of the studies has demonstrated correction of β-globin gene in CD34+ cells using the optimized CRISPR/Cas9 system and donor template. Further, they showed that corrected cells gain the potential to develop into erythroid cells and started to produce β-globin protein [55]. This finding suggests that correction of faulty gene in monogenetic disorders is possible using CRISPR/Cas9 gene editing tool. Further, Li et al. [33] suggested that effective and efficient treatment of beta-hemoglobinopathies can be achieved via careful design and optimization of sgRNAs in gene editing technologies.

β-Thalassemia, one of the leading monogenetic disorders, is caused by mutations in the hemoglobin beta (HBB) gene. In a study, combination of CRISPR/Cas9 and the piggyBac transposon has been used to cleave the HBB gene and showed correction of two different β-thalassemia mutations in iPSCs from β-thalassemia patients [50]. Further they showed no off-target effects were detected in the corrected iPSCs, and the cells retained full pluripotency, exhibited normal karyotypes and restored expression of HBB gene compared to the parental iPSCs line [50]. This type of research offers a new strategy to cure the monogenetic disorders. Gene editing with CRISPR-Cas9 support the idea that targeting fetal haemoglobin may be safer approach to treat this devastating disease.

Huntington's Disease (HD)

Huntington's disease (HD) is a severe neurodegenerative disorder caused by the autosomal dominant mutation in the first exon of the HTT gene encoding huntingtin protein. The symptoms of HD patient are movement disorders, cognitive impairment and psychiatric disturbances due to the presence of excessive trinucleotide repeat (CAG repeats) in HTT as compared to normal person. The mutated gene consists of expanded polyglutamine stretch that directs to protein misfolding and formation of aggregates which ultimately affect molecular and cellular processes of neurons [56]. It is believed that introduction of corrected CAG repeats in normal cells or correction of the mutation in patient-derived cells could be achieved by modified nucleases [57,58]. The first patient specific iPSCs line of HD containing CAG repeats was reported in 2008 [59] and further it has expanded dramatically for research and therapeutic purposes [60]. The first successful insertion of corrected CAG repeats into the genome of HEK293 cell line using CRISPR/Cas9 is reported by Malakhova et al. [61]. Several other recent studies have given us proof of principle for the use of CRISPR/Cas9 system to correct neurodegenerative disease in cell line as wells as in small animal models. Therefore, potential of this genome-editing tool to elucidate gene function in biological and pathological conditions that leads to the correction of gene defects in disease is of great. In fact, successful correction by the CRISPR/Cas9 system of gene mutations in cell lines, iPSCs or in animal models may help to establish foundations for future gene therapy.

Other Genetic Diseases

CRISPR/Cas9 technology may also be envisioned as a tool to correct chromosomal aberrations, immunodeficiency viral diseases, cancer and other genetic diseases. The classic examples of chromosomal aberration like Robertsonian translocation, trisomy 21, Turner and Klinefelter syndrome, Y chromosome microdeletion, infertility, mitochondrial diseases and so on. These chromosomal abnormalities can be corrected by using CRISPR/Cas9 via separation of two chromosomes, restoration of centromere position and by introduction of corrected gene sequence. Although, taking into consideration of several challenges it is difficult to manipulate the chromosome and need to optimize the condition hence, therapy may be quite far from being applied. Further, combination of modern technology like whole genome sequencing and CRISPR/Cas9 may also help to identify new genes which are involved in different genetic diseases. Further, CRISPR/Cas9 could be used to correct mutations in mitochondrial DNA (mtDNA) in mammalian oocyte. It is reported that mutations in the mtDNA are transmitted exclusively via the oocyte, which can carry between 10 000 and 100 000 mtDNA copies [62]. It is also proposed that using CRISPR/Cas9 instead of TALEN may correct mutated mtDNA sequence from the oocyte or the zygote [63]. The multifactorial neurodegenerative disorders like Parkinson's disease (PD), Alzheimer's disease and amyotrophic lateral sclerosis are caused by abnormal protein folding. The gene editing tool like CRISPR/Cas9

could be able to modify or correct abnormal protein production and prevent their accumulation in pathological condition.

HIV is an immune deficiency disease which occurs due to functional loss of CD4+ cells. The successful correction of CCR5 or CXCR4 in stem cell, normal iPSCs and human CD34+ hematopoietic stem and progenitor cells (HSPCs) has been done using modified nucleases viz. ZFNs, TALENs and CRISPR/Cas9 [64-69]. In 2014, Hu et al. [70] reported that CRISPR-Cas9 mediated disruption of viral gene expression and replication in various cell lines suggest that HIV could be cure by using CRISPR/Cas9 editing tool.

Furthermore, cancer is the harbor of multiple mutations that cause cells to convert into malignant phenotypes. CRISPR-Cas9 system may highly specific tool for correcting such mutations and treating the cancers. The indels can be done in cancerous cell lines which are easily available using CRISPR-Cas9 technology. Further, to create indels, the oncogens like receptor tyrosine kinase Erb2 could be good for CRISPR-Cas9. Recently, CRISPR/Cas9 tools are applied in cancer inducing mutation in human cell line and animal models to cure lung cancer [71], acute myrloid [72], liver cancer [73] and pancreatic cancer [74] further strengthen the potentiality of CRISPR/Cas9. This technology can also be used to manipulate inherited diseases by targeting DNA regulators or enhancers of pathogenic genes (Figure 3).

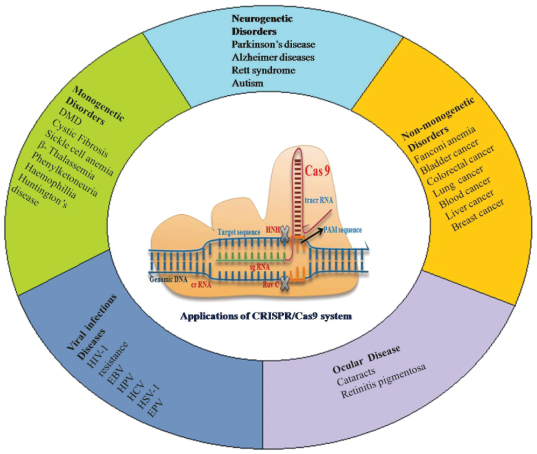

Figure 3: Summary of possible application of CRISPR/Cas9 in different genetic disorders.

Ethics

Although, CRISPR-Cas9 technology has been successfully used to cure several diseases however, it remains many things are not clear like how we should determine which disease or traits are appropriate for gene editing. In addition, could we manipulate human embryos for own interest. In these respect, we must take care these issues because these are ethical concern and need to establish some guidelines with caution and progress. It is reported that, a Chinese research group used CRISPR-Cas9 to edit nonviable human zygotes, which creates lot of controversies [75]. Certainly, there are concerns that any unexpected effect becomes heritable in the edited zygote/embryo. So, undoubtedly the gene editing will increase the risk for the health of the future offspring therefore, editing would be adopted where there are no other options to cure the disease. Likewise, other concern is delivery of both CRISPR and the repair donor DNA template into the desired tissues or cells in the body remains to be developed. Hence, gene editing technologies like CRISPR/Cas9 should be use but with care of moral and ethical issues for the wellbeing of human. The different options will have to be balanced on morally relevant criteria such as safety, efficacy and accessibility of gene editing technologies.

Future Prospective

The quick progress in the field of gene therapy is likely to continue to accept new challenges that will expand the scope of curing genetic diseases by genome editing technologies. The gene editing technology has changed the meaning of gene therapy but there are still lots of fundamental and translational work to be done to understand the full potential of CRISPR/Cas9 in particularly in area of genetic diseases. In recent years, it has been seen that increasing number of CRISPR related clinical trials and promises are being proposed worldwide. Although, we are still a long way from translational medicine. In one study, risk factor (FTO gene) of obesity has successfully manipulated using CRISPR/Cas9 technology by mutation in nucleotide bases of gene and showed this change could regulate IRX3 and IRX5 to start disperse energy and stop storage of fat [76]. This type of research in near future could lead to treatment process of obesity using latest gene editing technologies. In another study, Canver et al. [52] demonstrated that interruption of BCL11A enhancer by CRISPR/Cas9 in primary erythroid precursors has changed the level of BCL11A expression and alleviate haemoglobin disorders. This study suggests that by manipulating the gene using CRISPR/Cas9 we could determine the fate of erythrocytes. However, there is a need to improve efficiency, reduce off target effects and delivery methods of CRISPR technology to become reasonable for therapeutic application. Potential of CRISPR/Cas9 technology is remarkable, and reflect the various uses is extremely exciting. There are lots of scopes to make a promising future by correcting a disease-causing mutation in innocent patient using amazing tool like CRISPR/Cas9.

Conclusion

Genetic disorders, such as DMD, Cystic fibrosis, Thalassemia, Hemophilia and other muscular, neurological and hematological abnormalities, represent perfect targets for gene therapy. The rapid development of modified nucleases for desired genome manipulation, design of gRNA, appropriate vector construction and low cost have made CRISPR/Cas9 gene editing tool for wider applications. It has been suggested that CRISPR/Cas9 genome editing could be used in the future to correct inherited mutations in human embryos, suggesting that there is no technical barrier to manipulate embryos using genome-editing tools. Though, it is important to note that selection of the gene editing tool depends on aim of researchers, as each tool has its own characteristics and functional features. Therefore, before translating CRISPR/Cas9 technology to the clinic, some problems will need to be resolved; the main issues like ethical, legal, mosaicism and off-target effects. It is expected that in near future, genome editing technologies would help to accelerate the therapeutic directions towards understanding of molecular mechanism of disease at gene level. Scientist and clinician by using CRISPR/Cas9 tool would be able to answer the questions like which gene is responsible for causing disease and how do treat/correct that defective gene/ disease? that would become silver lining for the patients.

Funding

The funding for this research was provided by the SERB, New Delhi under Young Scientist Scheme (YSS/2014/00338).

Authorship confirmation statement:

Vivek K. Pandey and Ravi Bhushan reviewed the literature, Pawan K. Dubey, Anima Tripathi and Akhtar Ali wrote the paper and made corrections. All authors have reviewed and approved of the manuscript prior to submission and confirmed that manuscript has been submitted solely to this journal for consideration and publication.

Conflict of interest

The authors have no conflict to declare.

References

1. Ma Y, Shen B, Zhang X, Lu Y, Chen W, et al. (2014) Heritable multiplex genetic engineering in rats using CRISPR/Cas9. PLoS One 9: e89413.
2. Urnov FD, Miller JC, Lee YL (2005) Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nature 435: 646-651.
3. Miller JC, Holmes MC, Wang J, Guschin DY, Lee YL, et al. (2007) An improved zinc-finger nuclease architecture for highly specific genome editing. Nat Biotechnol 25: 778-785.
4. Christian M, Cermak T, Doyle EL, Schmidt C, Zhang F, et al. (2010) Targeting DNA double-strand breaks with TAL effector nucleases. Genetics 186: 757-761.
5. Miller JC, Tan S, Qiao G, Barlow KA, Wang J, et al. (2011) A TALE nuclease architecture for efficient genome editing. Nat Biotechnol 29: 143-148.
6. Boch J, Scholze H, Schornack S (2009) Breaking the code of DNA binding specificity of TAL-type III effectors. Science 326: 1509-1512.
7. Soldner F, Laganiere J, Cheng AW (2011) Generation of isogenic pluripotent stem cells differing exclusively at two early onset Parkinson point mutations. Cell 146: 318-331.
8. Hauschild J, Petersen B, Santiago Y (2011) Efficient generation of a bi-allelic knockout in pigs using zinc-finger nucleases. Proc Natl Acad Sci USA 108: 12013-12017.
9. Geurts AM, Cost GJ, Freyvert Y (2009) Knockout rats via embryo microinjection of zinc-finger nucleases. Science 325: 433.
10. Mashimo T, Takizawa A, Voigt B (2010) Generation of knockout rats with X-linked severe combined immunodeficiency (X-SCID) using zinc-finger nucleases. PLoS One 5: e8870.
11. Meyer M, Ortiz O, Hrabe de AM, Wurst W, Kuhn R, et al. (2012) Modeling disease mutations by gene targeting in one-cell mouse embryos. Proc Natl Acad Sci 109: 9354-9359.
12. Meng X, Noyes MB, Zhu LJ, Lawson ND, Wolfe SA, et al. (2008) Targeted gene inactivation in zebrafish using engineered zinc-finger nucleases. Nat Biotechnol 26: 695-701.
13. 1Beumer KJ, Trautman JK, Bozas A (2008) Efficient gene targeting in Drosophila by direct embryo injection with zinc-finger nucleases. Proc Natl Acad Sci 105: 19821-19826.
14. Osborn MJ, Starker CG, McElroy AN (2013) TALEN-based gene correction for epidermolysis bullosa. Mol Ther 21: 1151-1159.
15. Hockemeyer D, Wang H, Kiani S (2011) Genetic engineering of human pluripotent cells using TALE nucleases. Nat Biotechnol 29: 731-734.
16. Qiu Z, Liu M, Chen Z (2013) High-efficiency and heritable gene targeting in mouse by transcription activator-like effector nucleases. Nucleic Acids Res 41: e120.
17. Song J, Zhong J, Guo X (2013) Generation of RAG 1- and 2-deficient rabbits by embryo microinjection of TALENs. Cell Res 23: 1059-1062.
18. Tesson L, Usal C, Menoret S (2011) Knockout rats generated by embryo microinjection of TALENs. Nat Biotechnol 29: 695-696.
19. Shen B, Zhang J, Wu H, Wang J, Ma K, Li Z, et al. (2013) Generation of gene modified mice via Cas9/RNA-mediated gene targeting. Cell Res 23: 720-723.
20. Li D, Qiu Z, Shao Y, Chen Y, Guan Y, et al. (2013) Heritable gene targeting in the mouse and rat using a CRISPR-Cas system. Nat Biotechnol 31: 681-683.
21. Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA, et al. (2013) RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nat Biotechnol 31: 233-239.
22. DiCarlo JE, Norville JE, Mali P, Rios X, Aach J, et al. (2013) Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems. Nucleic Acids Res 41: 4336-4343.
23. Shan Q, Wang Y, Li J, Zhang Y, Chen K, Liang Z, et al. (2013) Targeted genome modification of crop plants using a CRISPR/Cas system. Nat Biotechnol 31: 686-688.
24. Friedland AE, Tzur YB, Esvelt KM, Colaiacovo MP, Church GM, et al. (2013) Heritable genome editing in C. elegans via a CRISPR-Cas9 system. Nat Methods 10: 741-743.
25. Wang Y, Li Z, Xu J, Zeng B, Ling L, et al. (2013) The CRISPR/Cas System mediates efficient genome engineering in Bombyx mori. Cell Res 23: 1414-1416.
26. Nakayama T, Fish MB, Fisher M, Oomen-Hajagos J, Thomsen GH et al. (2013) Simple and efficient CRISPR/Cas9-mediated targeted mutagenesis in Xenopus tropicalis. Genesis 51: 835-843.
27. Song B, Fan Y, He W, Zhu D, Niu X, et al. (2015) Improved hematopoietic differentiation efficiency of gene corrected beta-thalassemia induced pluripotent stem cells by CRISPR/Cas9 system. Stem Cells Dev 24: 1053-1065.
28. Long C, Amoasii L, Mireault AA (2016) Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. Science 351: 400-403.
29. Robert DF, Raymond HJS, Peter CF (2015) The Cpf1 CRISPR-Cas protein expands genome-editing tools. Genome Biol 16: 251.
30. Kim H, Kim ST, Ryu J, Kang BC (2017) CRISPR/Cpf1-mediated DNA-free plant genome editing. Nat Commun 8: 14406.
31. Bushby K, Finkel R, Birnkrant DJ, Case, LE, Clemens PR, et al. (2010) DMD care considerations working group. Diagnosis and management of Duchenne muscular dystrophy, part 2: Implementation of multidisciplinary care. Lancet Neurol 9: 177-189.
32. Popplewell L, Koo T, Leclerc X, Duclert A, Mamchaoui K, et al. (2013) Gene correction of a Duchenne muscular dystrophy mutation by mega nuclease enhanced exon knock-in. Hum Gene Ther 24: 692-701.
33. Li HL, Fujimoto N, Sasakawa N, Shirai S, Ohkame T, et al. (2015) Precise correction of the dystrophin gene in Duchenne muscular dystrophy patient induced pluripotent stem cells by TALEN and CRISPR-Cas9. Stem Cell Rep 4: 143-154.
34. Ousterout DG, Kabadi AM, Thakore PI, Majoros WH, Reddy TE, et al. (2015) Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy. Nat Commun 6: 6244.
35. Young CS, Mokhonova E, Quinonez M, Pyle AP, Spencer MJ, et al. (2017) Creation of a novel humanized dystrophic mouse model of Duchenne muscular dystrophy and application of a CRISPR/Cas9 gene editing therapy. J Neuromuscul Dis 4: 139-145.
36. Bengtsson NE, Hall JK, Odom GL, Phelps MP, David AR, et al. (2017) Muscle-specific CRISPR/Cas9 dystrophin gene editing ameliorates pathophysiology in a mouse model for Duchenne muscular dystrophy. Nature Commun 8: 14454.
37. Nelson CE, Hakim CH, Ousterout DG (2016) In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. Science 351: 403-407.
38. Tabebordbar M, Zhu K, Cheng JK (2016) In vivo gene editing in dystrophic mouse muscle and muscle stem cells. Science 351: 407-411.

39. Klymiuk N, Blutke A, Graf A, Krause S, Burkhardt K, et al. (2013) Dystrophin-deficient pigs provide new insights into the hierarchy of physiological derangements of dystrophic muscle. Hum Mol Genet 22: 4368-4382.
40. Chen Y, Zheng Y, Kang Y, Yang W, Niu Y, et al. (2015) Functional disruption of the dystrophin gene in rhesus monkey using CRISPR/Cas9. Hum Mol Genet 24: 3764-3774.
41. Zhang Yu, Long C, Li H, McAnally JR (2017) CRISPR-Cpf1 correction of muscular dystrophy mutations in human cardiomyocytes and mice. Sci Adv 3: e1602814.
42. Schwank G, Koo BK, Sasselli V (2013) Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell 13: 653-658.
43. Crane AM, Kramer P, Bui JH (2015) Targeted correction and restored function of the CFTR gene in cystic fibrosis induced pluripotent stem cells. Stem Cell Rep 4: 569-577.
44. Firth AL, Menon T, Parker GS (2015) Functional gene correction for cystic fibrosis in lung epithelial cells generated from patient iPSCs. Cell Rep 12: 1385-1390.
45. Vituret C, Gallay K, Confort MP, Ftaich N, Matei CI, et al. (2016) Transfer of the cystic fibrosis transmembrane conductance regulator to human cystic fibrosis cells mediated by extracellular vesicles. Hum Gene Ther 27: 166-83.
46. Finotti A, Breda L, Lederer CW, Bianchi N, Zuccato C, et al. (2015) Recent trends in the gene therapy of beta-thalassemia. J Blood Med 6: 69-85.
47. Li H, Haurigot V, Doyon Y (2011) In vivo genome editing restores haemostasis in a mouse model of haemophilia. Nature 475: 217-221.
48. Anguela XM, Sharma R, Doyon Y (2013) Robust ZFN-mediated genome editing in adult hemophilic mice. Blood 122: 3283-3287.
49. Park CY, Kim J, Kweon J (2014) Targeted inversion and reversion of the blood coagulation factor 8 gene in human iPS cells using T ALENs. Proc Natl Acad Sci 111: 9253-9258.
50. Xie F, Ye L, Chang JC, Beyer AI, Wang J, et al. (2014) Seamless gene correction of β-thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and piggyBac. Genome Res 24: 1526-1533.
51. Xu H, Xiao T, Chen CH, Li W, Meyer CA, et al. (2015) Sequence determinants of improved CRISPR sgRNA design. Genome Res 25: 1147-1157.
52. Canver MC, Smith EC, Sher F, Pinello L, Sanjana NE, et al. (2015) BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis. Nature 527: 192-197.
53. Sankaran VG, Menne TF, Xu J, Akie TE, Lettre G, et al. (2008) Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. Science 322: 1839-1842.
54. Megan DH, Gregory JC, Matthew CM (2015) Correction of the sickle cell disease mutation in human hematopoietic stem/progenitor cells. Blood 125: 2597-2604.
55. Park SH, Ciaran ML, Harshavardhan D, Gang B (2016) Therapeutic Crispr/Cas9 genome editing for treating sickle cell disease. Blood 128: 4703.
56. Reiner A, Dragatsis I, Dietrich P (2011) Genetics and neuropathology of Huntington's disease. Int Rev Neurobiol 9: 325-372
57. Liu P-Q, Tan S, Mendel MC, Murrills RJ, Bhat BM, et al. (2005) Isogenic human cell lines for drug discovery: Regulation of target gene expression by engineered zincänger protein transcription factors. J Biomol Screen 10: 304-313.
58. Freude K, Pires C, Hyttel P, Hall V (2014) Induced pluripotent stem cells derived from Alzheimer' s disease patients: The promise, the hope and the path ahead. J Clin Med 3: 1402-1436.
59. Park I-H, Arora N, Huo H, Maherali N, Ahfeldt T, et al. (2008) Disease-specific induced pluripotent stem cells. Cell 134: 877-886.
60. Tousley A, Kegel-Gleason KB (2016) Induced pluripotent stem cells in Huntington's disease research: Progress and opportunity. J Huntingtons Dis 5: 99-131.
61. Malakhova AA, Sorokin MA, Sorokina AE, Malankhanova TB, Mazurok NA, et al. (2016) Genome editing approach for generation of isogenic cell lines modeling Huntington' s disease in vitro. Genes Cells 11: 106-13.
62. Reddy P, Ocampo A, Suzuki K, Luo J, Bacman SR, et al. (2015) Selective elimination of mitochondrial mutations in the germline by genome editing. Cell 161: 459-469.
63. Wang S, Yi F, Qu J (2015) Eliminate mitochondrial diseases by gene editing in germ-line cells and embryos. Protein Cell 6: 472-475.
64. Holt N, Wang J, Kim K (2010) Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. Nat Biotechnol 28: 839-847.
65. Wilen CB, Wang J, Tilton JC (2011) Engineering HIV -resistant human CD4+ T cells with CXCR4-specific zinc-finger nucleases. PLoS Pathog 7: e1002020.
66. Ye L, Wang J, Beyer AI (2014) Seamless modification of wild-type induced pluripotent stem cells to the natural CCR5Delta32 mutation confers resistance to HIV infection. Proc Natl Acad Sci 111: 9591-9596.
67. Zhen S, Hua L, Liu YH (2015) Harnessing the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated Cas9 system to disrupt the hepatitis B virus. Gene Ther 22: 404-412.
68. Dong C, Qu L, Wang H, Wei L, Dong Y, et al. (2015) Targeting hepatitis B virus cccDNA by CRISPR/Cas9 nuclease efficiently inhibits viral replication. Antiviral Res 118: 110-117.
69. Liu X, Hao R, Chen S, Guo D, Chen Y, et al. (2015) Inhibition of hepatitis B virus by the CRISPR/Cas9 system via targeting the conserved regions of the viral genome. J Gen Virol 96: 2252-2261.
70. Hu Z, Y u L, Zhu D (2014) Disruption of HPV16-E7 by CRISPR/Cas system induces apoptosis and growth inhibition in HPV16 positive human cervical cancer cells. Biomed Res Int 612823.
71. Chen C, Liu Y, Rappaport AR (2014) MLL3 is a haplo insufficient 7q tumor suppressor in acute myeloid leukemia. Cancer Cell 25: 652-665.
72. Xue W, Chen S, Yin H (2014) CRISPR-mediated direct mutation of cancer genes in the mouse liver. Nature 514: 380-384.
73. Chiou SH, Winters IP, Wang J (2015) Pancreatic cancer modeling using retrograde viral vector delivery and in vivo CRISPR/Cas9-mediated somatic genome editing. Genes Dev 29: 1576-1585.
74. Tu Z, Yang W, Yan S, Guo X, Li XJ, et al. (2015) CRISPR/Cas9: A powerful genetic engineering tool for establishing large animal models of neurodegenerative diseases. Mol Neurodegener 10: 35.
75. Liang Puping, Yanwen Xu, Xiya Zhang, Chenhui Ding, Rui Huang et al. (2015) CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Protein Cell 6: 363-372.
76. Claussnitzer Melina, Simon N. Dankel, KyoungHan Kim (2015) FTO obesity variant circuitry and adipocyte browning in humans. N Engl J Med 373: 895-907.
77. Wu Y, Liang D, Wang Y, Bai M, Tang W, et al. (2013) Correction of a genetic disease in mouse via use of CRISPR-Cas9. Cell Stem Cell 13: 659-662.
78. Wu Y, Zhou H, Fan X, Zhang Y, Zhang M, et al. (2015) Correction of a genetic disease by CRISPR-Cas9-mediate gene editing in mouse spermatogonial stem cells. Cell Res 25: 67-79.
79. Ebina H, Misawa N, Kanemura Y, Koyanagi (2013) Harnessing the CRISPR/Cas9 system to disrupt latent HIV-1 provirus. Sci Rep 3: 2510.
80. Zhu W, Lei R, Le DY, Li J (2015) The CRISPR/Cas9 system inactivates latent HIV-1 proviral DNA. Retrovirology 12: 22.
81. Long C, McAnally JR, Shelton JM, Mireault AA, Bassel- Duby R, et al. (2014) Prevention of muscular dystrophy in mice by CRISPR/Cas9-mediated editing of germline DNA. Science 345: 1184-1188.
82. Xu L, Park KH, Zhao L (2016) CRISPR-mediated genome editing restores dystrophin expression and function in mdx mice. Mol Ther 24: 564-569.
83. Huang X, Wang Y, Yan W, Smith C, Ye Z, et al. (2015) Production of gene-corrected adult beta globin protein in human erythrocytes differentiated from patient iPSCs after genome editing of the sickle point mutation. Stem Cells 33: 1470-1479.

84. Park JY, Moon BY, Park JW, Thornton JA, Park YH, et al. (2017) Genetic engineering of a temperate phage-based delivery system for CRISPR-Cas9antimicrobials against Staphylococcus aureus. Sci Rep 7: 44929.
85. Smith C, Abalde-Atristain L, He C (2015) Efficient and allele-specific genome editing of disease loci in human iPSCs. Mol Ther 23: 570-577.
86. Yang L, Grishin D, W Gang, Aach J, Zhang CZ, et al. (2014) Targeted and genome-wide sequencing reveal single nucleotide variations impacting specificity of Cas9 in human stem cells. Nat Comm. 5: 5507.
87. Yin H, Xue W, Chen S, Bogorad RL, Benedetti E, et al. (2014) Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nat Biotechnol 32: 551-553.
88. Wang J and Quake SR. (2014) RNA-guided endonuclease provides a therapeutic strategy to cure latent herpesviridae infection. Proc Natl Acad Sci 111: 13157-13162.
89. Feng Y, Sassi S, Shen JK, Yang X, Gao Y, et al. (2015) Targeting CDK11 in osteosarcoma cells using the CRISPR-Cas9 system. J Orthop Res 33: 199-207.
90. Ding Q, Strong A, Patel KM, Ng SL, Gosis BS, et al. (2014) Permanent alteration of PCSK9 with in vivo CRISPR-Cas9 genome editing. Circ Res 115: 488-492.
91. Hu W, Kaminski R, Yang F, Zhang Y, Cosentino L, et al. (2014) RNA-directed gene editing specifically eradicates latent and prevents new HIV-1 infection. Proc Natl Acad Sci 111: 11461-11466.
92. Lin SR, Yang HC, Kuo YT, Liu CJ, Yang TY, et al. (2014) The CRISPR/Cas9 system facilitates clearance of the intrahepatic HBV templates in vivo. Mol Ther Nucleic Acids 3: 186.
93. Ramanan V, Shlomai A, Cox DB (2015) CRISPR/Cas9 cleavage of viral DNA efficiently suppresses hepatitis B virus. Sci Rep 5: 10833.
94. Hao Li, Sheng C, Wang S, Yang L (2017) Removal of Integrated Hepatitis B Virus DNA Using CRISPR Cas9. Front Cell Infect Microbiol 7: 91.
95. Monteys AM, Ebanks SA, Keiser MS, Davidson BL (2017) CRISPR/Cas9 editing of the mutant Huntington allele in vitro and in vivo. Mol Ther 25: 12-23.
96. Yang S, Chang R, Yang H, Zhao T, Hong Y, et al. (2016) CRISPR/Cas9-mediated gene editing ameliorates neurotoxicity in mouse model of Huntington's disease. JCI 127: 2719-2724.
97. Pan Y, Shen N, Jung-Klawitter S, Betzen C, Georg F, et al. (2016) CRISPR RNA-guided FokI nucleases repair a PAH variant in a phenylketonuria model. Sci Rep 6: 35794.
98. Bloh KM, Bialk PA, Gopalakrishnapillai AK (2017) CRISPR/Cas9-directed reassignment of the GATA1 initiation codon in K562 cells to recapitulate AML in Down syndrome. Mol Ther Nucleic Acids 7: 288-298.
99. Wang X, Cao C, Huang J, Yao J (2016) One-step generation of triple genetargeted pigs using CRISPR/Cas9 system. Sci Rep 6: 206-220.
100. Bassuk AG, Zheng A, Li A (2016) Precision medicine: Genetic repair of retinitis pigmentosa in patient-derived stem cells. Sci Rep 6: 19969.
101. Osborn MJ, Gabriel R, Webber BR, DeFeo AP (2015) Fanconi anemia gene editing by the CRISPR/Cas9 system. Hum Gene Ther 26: 114-126.
102. Vinayak S, Pawlowic MC, Sateriale A, Brooks CF (2015) Genetic modification of the diarrhoeal pathogen Cryptosporidium parvum. Nature 523: 477-480.
103. Matano M, Date S, Shimokawa M, Takano A (2015) Modeling colorectal cancer using CRISPR-Cas9–mediated engineering of human intestinal organoids. Nat Med 21: 256-262.
104. Yang Y, Wang L, Bell P, McMenamin D (2016) A dual AAV system enables the Cas9-mediated correction of a metabolic liver disease in newborn mice. Nat Biotechnol 34: 334-338.
105. Swiech L, Heidenreich M, Banerjee A, Habib N (2015) In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9. Nat Biotechnol 33: 102-106.
106. Platt RJ, Chen S, Zhou Y, Yim MJ (2014) CRISPR-Cas9 knockin mice for genome editing and cancer modeling. Cell 159: 440-455.
107. Dow LE, Fisher J, O'Rourke KP, Muley A (2015) Inducible in vivo genome editing with CRISPR-Cas9. Nat Biotechnol 33: 390-394.
108. Carrolla KJ, Makarewicha CA, McAnallya J (2016) A mouse model for adult cardiac-specific gene deletion with CRISPR/Cas9. PNAS 113: 338-343.
109. Hendel A, Bak RO, Clark JT, Kennedy AB (2015) Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. Nat Biotechnol 33: 985-989.
110. Kang Y, Zheng B, Shen B, Chen YC, Wang L, et al. (2015) CRISPR/Cas9-mediated Dax1 knockout in the monkey recapitulates human AHC-HH. Hum Mol Genet 24: 7255-7264.