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CRISPR-mediated Ophthalmic Genome Surgery

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Abstract

Purpose of review—Clustered regularly interspaced short palindromic repeats (CRISPR) is a genome engineering system with great potential for clinical applications due to its versatility and programmability. This review highlights the development and use of CRISPR-mediated ophthalmic genome surgery in recent years.

Recent findings—Diverse CRISPR techniques are in development to target a wide array of ophthalmic conditions, including inherited and acquired conditions. Preclinical disease modeling and recent successes in gene editing suggest potential efficacy of CRISPR as a therapeutic for

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Compliance with Ethical Guidelines

Conflict of Interest

Sally Justus, Vinit Mahajan, Alexander Bassuk, Jesse Sengillo, Yazeed Abdulla, Galaxy Cho, and Kellie Schaefer declare no conflict of interest.

Human and Animal Rights and Informed Consent

All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

Informed consent

Patient images and genetic information are not identifiable to the patient.

inherited conditions. In particular, the treatment of Leber congenital amaurosis with CRISPR-mediated genome surgery is expected to reach clinical trials in the near future.

Summary—Treatment options for inherited retinal dystrophies are currently limited. CRISPR-mediated genome surgery methods may be able to address this unmet need in the future.

Keywords

CRISPR-Cas; induced pluripotent stem cells; genome surgery; inherited retinal dystrophy

Introduction

Inherited retinal dystrophies cause irreversible visual impairments, rendering many affected persons blind (1, 2). Recent improvements have been made in DNA modification technologies by repurposing the clustered regularly interspaced short palindromic repeats (CRISPR) system, which is derived from bacteria and archaea immune systems and has been shown to be an effective and precise DNA targeting mechanism with great potential for clinical applications in inherited conditions (3). This review provides an overview of the role of various CRISPR systems in treating retinal diseases.

CRISPR-Cas9

Originally, CRISPR was identified in bacteria as an adaptive immune strategy to defend against bacteriophages (4). In response to infection, the bacterium cuts the viral genome into smaller segments and incorporates them into its own genome in between short, highly repetitive sequences, called CRISPR (5–7). The viral genome is then transcribed into CRISPR RNAs (crRNA), which associate with trans-activating crRNA (tracrRNA). The next time the virus attempts to infect the bacteria, the crRNA and tracrRNA direct CRISPR-associated ‘Cas’ proteins to degrade the invading viral genome, thus rendering it incapable of causing subsequent infections (8).

The primary action of CRISPR relies heavily on Cas, an endonuclease that induces double strand breaks (DSBs) in the DNA, which stands as a serious assault to the integrity of the genetic code. DSBs are readily repaired by non-homologous end-joining (NHEJ), in which direct, albeit error-prone, ligation of the DNA strand occurs. Alternatively, DSBs can be resolved by homology-directed repair (HDR), in which the homologous strand of DNA is used as a template for the counterpart strand that was cleaved (9, 10). HDR occurs much less frequently than NHEJ, since homologous recombination is naturally confined to the late S and G2 phases of cellular division (11). In the laboratory, a gene of interest that harbors a pathogenic mutation can be corrected by introducing a modified wild type donor template homologous to the target site. When Cas induces a DSB in the DNA near the mutation, the donor template can be used to remodel the sequence of the cleaved strand during HDR. The resulting genetic sequence will no longer contain the mutation, but the template sequence, thus restoring the genotype to wild type. In this manner, efficient genome modifications can be permanently introduced to correct pathogenic mutations.

Previously, genetic modification strategies in the lab relied on other endonucleases, such as meganuclease (MN), zinc-finger nuclease (ZFN), and transcription activator-like effector nuclease (TALEN), to introduce double-strand breaks (DSBs) (12). With the repurposing of CRISPR, genome engineering is now easier to execute. ZFNs and TALENs are guided by protein-DNA binding, which limits their broad application due to difficulties in engineering new proteins for each target (3). On the other hand, the RNA-mediated CRISPR system offers efficient and versatile gene-modifying capabilities, allowing for diverse applications including deletions, insertions, knockouts, transcriptional control, epigenetic alterations, and more (3, 5, 13). These CRISPR strategies not only allow an expanded toolkit for studying implicated genes in relation to retinal development and physiology, but also may find success in treating inherited retinal dystrophies (5, 14–16).

Since its inception, CRISPR has been further optimized to enhance efficiency in the laboratory setting. For example, the crRNA and tracrRNA have been combined into a single chimeric guide RNA (sgRNA) that can be designed to target a wide breadth of sites in the genome, thereby enhancing the ease with which CRISPR can be employed (8, 17). The most commonly utilized Cas protein today is spCas9, derived from *Streptococcus pyogenes* (18), which requires a proto-spacer adjacent motif (PAM) adjacent to the targeted site in order for the nuclease to cleave the DNA (17, 19–21). The PAM sequence for spCas9 is NGG, in which N can be any nucleotide. Consequently, a primary consideration when determining targetable sites for CRISPR is whether there is a PAM sequence adjacent to the desired cleavage point. Outside of this restriction, CRISPR can be widely and easily applied to a variety of genetic backgrounds, and to address mutations causing any number of conditions.

The CRISPR system has been further customized to perform other genome engineering functions (22). For example, Cas9 has been altered to function as a nickase, named as such because it ‘nicks’ the DNA by inducing single-strand breaks instead of DSBs. Two sgRNAs targeting sites that are close together can direct separate Cas9 nickases to induce breaks on each DNA strand (23). By requiring two different cleavage events to induce recombination, this strategy decreases the likelihood of off-targeting effects, wherein mutagenesis occurs at unintended sites by the non-specific action of Cas9.

Another interesting deviation from the standard implementation of CRISPR is through the inactivation of Cas9 endonuclease activity, creating a catalytically deactivated Cas9 (dCas9). dCas9 can bind but not cleave DNA, thus preventing the transcription of the target gene by creating a physical barrier to the action of transcription factors (24). This rendition of CRISPR works at the transcription level in a reversible fashion and may therefore be more readily approved for use in patients by regulatory boards such as the Food and Drug Administration (FDA). This strategy has been termed CRISPR interference, or CRISPRi, and was further enhanced by fusing Cas to the Kruppel-associated box repression domain (KRAB), which augments the repressive effects of dCas9 (25).

Yet another repurposed use of CRISPR comes in the form of CRISPR deletion (CRISPRd). CRISPRd capitalizes on the tendency of DNA repair strategies to default towards NHEJ, and does not require a donor template to repair the cleaved strand. Instead, Cas9 creates a DSB in the gene harboring a mutation first, then NHEJ occurs, and insertions and deletions

(INDELs) are introduced that corrupt the sequence, thus either preventing the gene from being expressed or proper protein folding from occurring. This strategy may be particularly applicable for dominant conditions, in which case knocking out the mutated, dominant allele and leaving the wild type allele intact may, theoretically, be sufficient to restore the phenotype to wild type. To compare each of these strategies with the traditional CRISPR nuclease (CRISPRn) system, Figure 1 provides a visual representation.

Dominant Mutations

Conditions inherited in a dominant fashion were previously thought to be unapproachable. Although targeting inherited retinal conditions by gene therapy exists, current gene therapies utilize adeno-associated virus (AAV) as a vector to deliver additional wild-type genes by subretinal or intravitreal injections. As such, traditional approaches of gene therapy do not directly target the pathogenic gene and can only be applied to recessive and haploinsufficiency conditions (15). Thus, there is a need for CRISPR application in the treatment of dominant genetic mutations.

Targeting of a dominant-negative allele utilizing CRISPR/Cas9 was demonstrated by Courtney et al. in the *KRT12* gene (26). The C395T mutation in the *KRT12* gene is a dominant-negative mutation which causes Meesmann epithelial corneal dysplasia (MECD). The disease-causing mutation creates a novel PAM sequence which can be recognized by Cas9. Since a PAM sequence is crucial for targeting of Cas9, this allows for Cas9 to recognize the mutated allele, but not the wildtype (27). Courtney et al. targeted the SNP in a mouse model of MECD by intrastromal injection of sgRNA and SpCas9-GFP plasmids. In the 13 sequences that were analyzed, four resulted in inactivation of the pathogenic allele by CRISPRd causing early termination (26).

In vivo Approach

Delivering the CRISPR system directly into the retina of patients as a therapeutic tool is still in pursuit (5). Efficacy of such a method was examined in adult *Thy1::YFP* transgenic mice via intravitreal injections using dual AAV delivery of SpCas9 and yellow fluorescent protein (YFP)-targeting gRNA (28). CRISPR-Cas9 cleavage was repaired by NHEJ in order to decrease YFP expression. Hung et al. used a YFP-sgRNA construct and a noncutting sgRNA control which showed an 84% reduction in YFP expression in the YFP-sgRNA construct containing cells. AAV2 transduction was the greatest in the retinal ganglion cells (RGCs). Another significance of Hung et al.'s finding was the electroretinography taken five-weeks post-transduction; no significant retinal changes were observed suggesting that no toxicity from CRISPR components could be seen (28–34). The potential to utilize *in vivo* approaches for genome surgery clinically is seen in this genome engineering of adult mouse retina.

Disease Models

Retinitis Pigmentosa

Diverse CRISPR technologies have been applied to a variety of preclinical models contributing to furthering our understanding of disease models and to approaching feasible

treatment options (5, 15). One disease which has been approached with CRISPR applications in the pre-clinical setting is retinitis pigmentosa (RP). RP is characterized by progressive retinal degeneration that corresponds to a decreasing visual field. It is genetically heterogeneous, as it is known to be caused by mutations in over 70 different genes. Autosomal recessive, dominant, and x-linked inheritance patterns are observed in RP depending on the gene implicated. Figure 2 shows retinitis pigmentosa caused by a heterozygous mutation in *RHO*. Current dietary modifications slow the condition's progression but gene-editing strategies may prove to be a more efficacious treatment (15, 35).

The *rd1* mouse, a preclinical model of RP, harbors two homozygous variants in *Pde6b* leading to rod cell death (36–38). In this model, there is a nonsense mutation (Y347X, C to A) in exon 7 and a murine leukemia virus insertion (*Xmv-28*) in intron 1 of *Pde6b* (36, 39). After nearly a century long debate concerning the origin of this phenotype, a CRISPR-mediated homology-directed recombination (HDR) of the nonsense mutation (Y347X) by Wu et al. resulted in disease model rescue, demonstrating the Y347X mutation to be pathogenic (36).

In another preclinical model of RP, the transgenic S334ter-3 rat holds the mutation *Rho*^{S334}, which yields a truncated RHO^{S334} protein lacking the signal sequence for RHO trafficking resulting in rapid degeneration of photoreceptors (40–42). The PAM sequence in *Rho*^{S334} (5'-TGG-3') differs from the PAM sequence in *Rho*^{WT} (5'-TGC-3') by one nucleotide. Bakondi et al. were able to disrupt *Rho*^{S334} without disrupting the *Rho*^{WT} by utilizing the difference in PAM sequence (42). Subretinal injection of CRISPR/Cas9 and a sgRNA yielded photoreceptor rescue in transfected regions with retinal synapse preservation. Bakondi et al. reported a 53% increase in visual acuity measured by optokinetic response and a nine-fold increase in photoreceptor nuclei density.

Retinitis Pigmentosa: Stem Cell Modification

Another approach to treating retinal degeneration is stem cell transplantation. Recent clinical trials of subretinal transplantation of retinal pigment epithelium (RPE) derived from human embryonic stem cells (hESCs) have shown potential efficacy and safety (43). Due to the ethical controversy surrounding the use of hESCs and potential immune rejection, the use of patient-derived induced pluripotent stem cells (iPSCs) is a topic of interest (44). Of important consideration is that iPSCs contain the same genetic mutation that caused the degeneration in the patient and the disease model would persist; CRISPR-mediated correction of patient-derived iPSCs could theoretically yield a useful cell-based treatment method that does not require immunosuppression (14, 44). In 2016, Bassuk et al. were the first to report CRISPR correction of a pathogenic mutation causing photoreceptor degeneration in patient-derived iPSCs. The iPSCs were derived from patients with X-linked RP caused by a retinitis pigmentosa GTPase regulator (RPGR) mutation (c.3070G > T, pGlu1024X). Figure 3 shows retinitis pigmentosa caused by a mutation in *RPGR*. The iPSCs were induced from fibroblasts collected through a skin-punch biopsy. The iPSCs were transfected with spCas9, sgRNA g58, and a WT RPGR anti-sense single-stranded

oligonucleotide donor template. Correction was seen in 13% of the cells establishing proof of concept of utilizing iPSCs for subretinal transplantation in the future (44).

Age-related Macular Degeneration

Age-related Macular Degeneration (AMD) is associated with over-expression of vascular endothelial growth factor A (*VEGFA*). Although AMD is not caused by a monogenic mutation, Kim et al. sought to harness the many potentials of the CRISPR systems by utilizing *Vegfa* gene-specific Cas9 ribonucleoproteins (RNPs), a preassembled Cas9 protein complexed with *in vitro* transcribed gRNA, to inactivate the expression of *Vegfa*. AMD was modeled by laser-induced choroidal neovascularization (CNV) in adult mice. Sub-retinal injection of Cas9 RNPs led to a reduction in *VEGFA* expression followed by a reduction of CNV. This preclinical model is of particular significance because of the expansion in potential treatment methods utilizing CRISPR to non-genetic diseases and to the adult eye (45).

Leber Congenital Amaurosis

Leber congenital amaurosis (LCA) is a genetically heterogeneous retinal dystrophy which can manifest within the first months of life (46, 47). One of the mutations associated with LCA is in the *KCNJ13* gene which encodes a RPE-specific potassium channel subunit Kir7.1. Traditionally, scientists would have needed to employ Cre-lox systems to study this disease model as a homozygous null mutation of *Kcnj13* is lethal (48). Although a useful method, generation of Cre-lox systems can be time-consuming. In seeking a faster method for disease model generation, Zhong et al. utilized CRISPR/Cas9 with NHEJ to introduce mutations in the *Kcnj13* locus to generate a LCA disease model. Mouse zygotes were injected with sgRNA and spCas9 mRNA. Tail genotyping of the developed mice exhibited tissue mosaicism in the *Kcnj13* locus as expected from heterogeneous mutations that arise from NHEJ (48, 49). Study of the generated disease model showed that loss of function of KCNJ13 corresponded with loss of photoreceptors and rhodopsin mislocalization. As demonstrated by Zhong et al., CRISPR is useful not only in correction of disease, but also study of disease as well (48).

Another LCA causative mutation is an intronic mutation in the *CEP290* gene (c. 2991+1655A>G) known to cause LCA10; it is the most frequently detected mutation causing LCA in patients. Targeting *CEP290* proves a challenge due to its large size exceeding the capacity of adeno-associated virus (AAVs) delivery (50, 51). Ruan et al. reported successful deletion of *CEP290* in 293FT cells utilizing a dual AAV delivery approach of pAAV-SpCas9 plasmid and pAAV-sgRNA plasmid, circumventing the carrying capacity limitation (50). Another approach to circumvent the carrying capacity limitation of AAV vectors was reported by Maeder et al. utilizing the smaller *S. aureus*-derived CRISPR/Cas9 which allowed a single AAV vector delivery of two guide RNAs to perform a dual-cut excision of the *CEP290* gene in LCA10 patient-derived primary fibroblasts (51).

Whether CRISPR will truly be a viable treatment option for ophthalmic conditions has not yet been tested in clinical trials. This may soon change. A press release in March 2017 from Editas and Allergan announced plans for a research and development alliance to develop

CRISPR-utilized treatment of LCA10. Editas's pipeline for 2018 includes plans to test treatment of LCA10 via NHEJ small deletion via AAV local injection delivery of the treatment product.

Potential Limitations to using the CRISPR system in patients

Although the CRISPR system has shown promising preliminary results for a variety of ophthalmic conditions, limitations still exist. One such limitation is the possibility of unintended DNA alterations. Because CRISPR modifies DNA (as opposed to incorporating extra copies of the wild-type gene as gene therapy does), off-targeting mutations— that is, the unintended editing of other regions in the genome— could lead to the formation of secondary phenotypes.

A variety of methods to test for off-target mutations have been developed (52–54). The most common method for determining potential off-targeting of a sgRNA is to use algorithms to computationally detect homologous regions in the genome that are most likely to be mis-targeted. Although most studies subsequently sequence the predicted off-target sites to test for mutation, it is currently not clear if Cas9 has the potential to target other, non-homologous sequences.

Additionally, some studies have begun to perform whole exome sequencing (WES) in CRISPR-treated cells and organisms (55, 56). While these studies are the most accurate and comprehensive to-date, they still only detect approximately 1% of the genome, and it is known that non-coding regions also play crucial roles in the cell (57).

In the past decade, the role of the CRISPR-system for disease gene editing has been realized and developed into a potential therapeutic tool. The final, and perhaps most important, step before translating CRISPR to clinical use is to develop a complete, non-biased method for detecting potential off-targeting mutations at the whole organism level.

Conclusion

The reprogrammable ability of CRISPR allows versatility in approaching disease treatment. In combination with the eye's easy accessibility and relative immune-privileged status, CRISPR-Cas therapeutic strategies show great promise in the field of ophthalmology. CRISPR technologies have furthered our understanding of ophthalmic diseases in significant ways. Given the successes of CRISPR techniques in preclinical models and plans for clinical trials, therapeutic uses of CRISPR in ophthalmology may become available to patients in the near future.

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References

Papers of particular interest, published recently, have been highlighted as:

•Of importance

••Of major importance

1. Scanga HL, Nischal KK. Genetics and ocular disorders: a focused review. *Pediatr Clin North Am*. 2014; 61(3):555–65. [PubMed: 24852152]
2. Graw J. The genetic and molecular basis of congenital eye defects. *Nat Rev Genet*. 2003; 4(11):876–88. [PubMed: 14634635]
- 3•. Barrangou R, Doudna JA. Applications of CRISPR technologies in research and beyond. *Nat Biotechnol*. 2016; 34(9):933–41. This review provides a comprehensive update on CRISPR systems in various models and species as well as an excellent summary of CRISPR mechanism. [PubMed: 27606440]
4. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science*. 2014; 346(6213):1258096. [PubMed: 25430774]
5. Cabral T, DiCarlo JE, Justus S, Sengillo JD, Xu Y, Tsang SH. CRISPR applications in ophthalmologic genome surgery. *Curr Opin Ophthalmol*. 2017
6. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex genome engineering using CRISPR/Cas systems. *Science*. 2013; 339(6121):819–23. [PubMed: 23287718]
7. Horvath P, Barrangou R. CRISPR/Cas, the immune system of bacteria and archaea. *Science*. 2010; 327(5962):167–70. [PubMed: 20056882]
8. Barrangou R. Diversity of CRISPR-Cas immune systems and molecular machines. *Genome Biol*. 2015; 16:247. [PubMed: 26549499]
9. Symington LS, Gautier J. Double-strand break end resection and repair pathway choice. *Annu Rev Genet*. 2011; 45:247–71. [PubMed: 21910633]
10. Pardo B, Gomez-Gonzalez B, Aguilera A. DNA repair in mammalian cells: DNA double-strand break repair: how to fix a broken relationship. *Cell Mol Life Sci*. 2009; 66(6):1039–56. [PubMed: 19153654]
11. Heyer WD, Ehmsen KT, Liu J. Regulation of homologous recombination in eukaryotes. *Annu Rev Genet*. 2010; 44:113–39. [PubMed: 20690856]
12. Yanik M, Muller B, Song F, Gall J, Wagner F, Wende W, et al. In vivo genome editing as a potential treatment strategy for inherited retinal dystrophies. *Prog Retin Eye Res*. 2017; 56:1–18. [PubMed: 27623223]
- 13•. Hess GT, Fresard L, Han K, Lee CH, Li A, Cimprich KA, et al. Directed evolution using dCas9-targeted somatic hypermutation in mammalian cells. *Nat Methods*. 2016; 13(12):1036–42. This study reports a novel application of genetic engineering, CRISPR-X. [PubMed: 27798611]
14. Sengillo JD, Justus S, Cabral T, Tsang SH. Correction of Monogenic and Common Retinal Disorders with Gene Therapy. *Genes (Basel)*. 2017; 8(2)
- 15••. Sengillo JD, Justus S, Tsai YT, Cabral T, Tsang SH. Gene and cell-based therapies for inherited retinal disorders: An update. *Am J Med Genet C Semin Med Genet*. 2016; 172(4):349–66. This review provides a summary of gene therapy clinical trials in the retina and introduces developing technologies of CRISPR applications in the retina. [PubMed: 27862925]
16. Wang S, Sengel C, Emerson MM, Cepko CL. A gene regulatory network controls the binary fate decision of rod and bipolar cells in the vertebrate retina. *Dev Cell*. 2014; 30(5):513–27. [PubMed: 25155555]
17. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012; 337(6096):816–21. [PubMed: 22745249]

18. Wright AV, Nunez JK, Doudna JA. Biology and Applications of CRISPR Systems: Harnessing Nature's Toolbox for Genome Engineering. *Cell*. 2016; 164(1–2):29–44. [PubMed: 26771484]
19. Zhang Y, Ge X, Yang F, Zhang L, Zheng J, Tan X, et al. Comparison of non-canonical PAMs for CRISPR/Cas9-mediated DNA cleavage in human cells. *Sci Rep*. 2014; 4:5405. [PubMed: 24956376]
20. Sternberg SH, Redding S, Jinek M, Greene EC, Doudna JA. DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. *Nature*. 2014; 507(7490):62–7. [PubMed: 24476820]
21. Jinek M, Jiang F, Taylor DW, Sternberg SH, Kaya E, Ma E, et al. Structures of Cas9 endonucleases reveal RNA-mediated conformational activation. *Science*. 2014; 343(6176):1247997. [PubMed: 24505130]
22. Sternberg SH, Doudna JA. Expanding the Biologist's Toolkit with CRISPR-Cas9. *Mol Cell*. 2015; 58(4):568–74. This brief yet comprehensive review of CRISPR provides an introduction to its discovery in bacteria and how it has subsequently been repurposed for use in research and biopharmaceutical settings. [PubMed: 26000842]
23. Mali P, Aach J, Stranges PB, Esvelt KM, Moosburner M, Kosuri S, et al. CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. *Nat Biotechnol*. 2013; 31(9):833–8. [PubMed: 23907171]
24. Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*. 2013; 152(5):1173–83. [PubMed: 23452860]
25. Gilbert LA, Larson MH, Morsut L, Liu Z, Brar GA, Torres SE, et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell*. 2013; 154(2):442–51. [PubMed: 23849981]
26. Courtney DG, Moore JE, Atkinson SD, Maurizi E, Allen EH, Pedrioli DM, et al. CRISPR/Cas9 DNA cleavage at SNP-derived PAM enables both in vitro and in vivo KRT12 mutation-specific targeting. *Gene Ther*. 2016; 23(1):108–12. [PubMed: 26289666]
27. Liu X, Homma A, Sayadi J, Yang S, Ohashi J, Takumi T. Sequence features associated with the cleavage efficiency of CRISPR/Cas9 system. *Sci Rep*. 2016; 6:19675. [PubMed: 26813419]
28. Hung SS, Chrysostomou V, Li F, Lim JK, Wang JH, Powell JE, et al. AAV-Mediated CRISPR/Cas Gene Editing of Retinal Cells In Vivo. *Invest Ophthalmol Vis Sci*. 2016; 57(7):3470–6. [PubMed: 27367513]
29. Ran FA, Cong L, Yan WX, Scott DA, Gootenberg JS, Kriz AJ, et al. In vivo genome editing using *Staphylococcus aureus* Cas9. *Nature*. 2015; 520(7546):186–91. [PubMed: 25830891]
30. Swiech L, Heidenreich M, Banerjee A, Habib N, Li Y, Trombetta J, et al. In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9. *Nat Biotechnol*. 2015; 33(1):102–6. [PubMed: 25326897]
31. Yang Y, Wang L, Bell P, McMenamin D, He Z, White J, et al. A dual AAV system enables the Cas9-mediated correction of a metabolic liver disease in newborn mice. *Nat Biotechnol*. 2016; 34(3):334–8. [PubMed: 26829317]
32. Long C, Amoasii L, Mireault AA, McAnally JR, Li H, Sanchez-Ortiz E, et al. Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. *Science*. 2016; 351(6271):400–3. [PubMed: 26721683]
33. Tabebordbar M, Zhu K, Cheng JK, Chew WL, Widrick JJ, Yan WX, et al. In vivo gene editing in dystrophic mouse muscle and muscle stem cells. *Science*. 2016; 351(6271):407–11. [PubMed: 26721686]
34. Chew WL, Tabebordbar M, Cheng JK, Mali P, Wu EY, Ng AH, et al. A multifunctional AAV-CRISPR-Cas9 and its host response. *Nat Methods*. 2016; 13(10):868–74. [PubMed: 27595405]
35. Berson EL. Nutrition and retinal degenerations. Vitamin A, taurine, ornithine, and phytanic acid. *Retina*. 1982; 2(4):236–55. [PubMed: 6101131]
36. Wu WH, Tsai YT, Justus S, Lee TT, Zhang L, Lin CS, et al. CRISPR Repair Reveals Causative Mutation in a Preclinical Model of Retinitis Pigmentosa. *Mol Ther*. 2016; 24(8):1388–94. [PubMed: 27203441]
37. Keeler CE. The Geotropic Reaction of Rodless Mice in Light and in Darkness. *J Gen Physiol*. 1928; 11(4):361–8. [PubMed: 19872404]

38. McLaughlin ME, Ehrhart TL, Berson EL, Dryja TP. Mutation spectrum of the gene encoding the beta subunit of rod phosphodiesterase among patients with autosomal recessive retinitis pigmentosa. *Proc Natl Acad Sci U S A*. 1995; 92(8):3249–53. [PubMed: 7724547]
39. Bowes C, Li T, Danciger M, Baxter LC, Applebury ML, Farber DB. Retinal degeneration in the rd mouse is caused by a defect in the beta subunit of rod cGMP-phosphodiesterase. *Nature*. 1990; 347(6294):677–80. [PubMed: 1977087]
40. Mendes HF, van der Spuy J, Chapple JP, Cheetham ME. Mechanisms of cell death in rhodopsin retinitis pigmentosa: implications for therapy. *Trends Mol Med*. 2005; 11(4):177–85. [PubMed: 15823756]
41. Sung CH, Makino C, Baylor D, Nathans J. A rhodopsin gene mutation responsible for autosomal dominant retinitis pigmentosa results in a protein that is defective in localization to the photoreceptor outer segment. *J Neurosci*. 1994; 14(10):5818–33. [PubMed: 7523628]
42. Bakondi B, Lv W, Lu B, Jones MK, Tsai Y, Kim KJ, et al. In Vivo CRISPR/Cas9 Gene Editing Corrects Retinal Dystrophy in the S334ter-3 Rat Model of Autosomal Dominant Retinitis Pigmentosa. *Mol Ther*. 2016; 24(3):556–63. [PubMed: 26666451]
43. Schwartz SD, Regillo CD, Lam BL, Elliott D, Rosenfeld PJ, Gregori NZ, et al. Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies. *Lancet*. 2015; 385(9967):509–16. [PubMed: 25458728]
44. Bassuk AG, Zheng A, Li Y, Tsang SH, Mahajan VB. Precision Medicine: Genetic Repair of Retinitis Pigmentosa in Patient-Derived Stem Cells. *Sci Rep*. 2016; 6:19969. This study is the first to report CRISPR-mediated correction of photoreceptor degeneration in patient-derived iPSCs. [PubMed: 26814166]
45. Kim K, Park SW, Kim JH, Lee SH, Kim D, Koo T, et al. Genome surgery using Cas9 ribonucleoproteins for the treatment of age-related macular degeneration. *Genome Res*. 2017 This study demonstrates a proof of concept for the potential application of CRISPR to non-inherited diseases.
46. den Hollander AI, Koenekoop RK, Yzer S, Lopez I, Arends ML, Voesenek KE, et al. Mutations in the CEP290 (NPHP6) gene are a frequent cause of Leber congenital amaurosis. *Am J Hum Genet*. 2006; 79(3):556–61. [PubMed: 16909394]
47. Hanein S, Perrault I, Gerber S, Tanguy G, Barbet F, Ducroq D, et al. Leber congenital amaurosis: comprehensive survey of the genetic heterogeneity, refinement of the clinical definition, and genotype-phenotype correlations as a strategy for molecular diagnosis. *Hum Mutat*. 2004; 23(4): 306–17. [PubMed: 15024725]
48. Zhong H, Chen Y, Li Y, Chen R, Mardon G. CRISPR-engineered mosaicism rapidly reveals that loss of Kcnj13 function in mice mimics human disease phenotypes. *Sci Rep*. 2015; 5:8366. [PubMed: 25666713]
49. Yen ST, Zhang M, Deng JM, Usman SJ, Smith CN, Parker-Thornburg J, et al. Somatic mosaicism and allele complexity induced by CRISPR/Cas9 RNA injections in mouse zygotes. *Dev Biol*. 2014; 393(1):3–9. [PubMed: 24984260]
50. Ruan GX, Barry E, Yu D, Lukason M, Cheng SH, Scaria A. CRISPR/Cas9-Mediated Genome Editing as a Therapeutic Approach for Leber Congenital Amaurosis 10. *Mol Ther*. 2017; 25(2): 331–41. [PubMed: 28109959]
51. Maeder ML, Mepani R, Gloskowski S, Skor M, Collins M, Shen S, et al. 124. Therapeutic Correction of an LCA-Causing Splice Defect in the CEP290 Gene by CRISPR/Cas-Mediated Gene Editing. *Molecular Therapy*. 2016; 24(Supplement 1):S51–S2.
52. Zhang XH, Tee LY, Wang XG, Huang QS, Yang SH. Off-target Effects in CRISPR/Cas9-mediated Genome Engineering. *Mol Ther Nucleic Acids*. 2015; 4:e264. [PubMed: 26575098]
53. Peng R, Lin G, Li J. Potential pitfalls of CRISPR/Cas9-mediated genome editing. *FEBS J*. 2016; 283(7):1218–31. [PubMed: 26535798]
54. Yee JK. Off-target effects of engineered nucleases. *FEBS J*. 2016; 283(17):3239–48. [PubMed: 27208701]

55. Cho SW, Kim S, Kim Y, Kweon J, Kim HS, Bae S, et al. Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. *Genome Res.* 2014; 24(1):132–41. [PubMed: 24253446]
56. Nakajima K, Kazuno AA, Kelsoe J, Nakanishi M, Takumi T, Kato T. Exome sequencing in the knockin mice generated using the CRISPR/Cas system. *Sci Rep.* 2016; 6:34703. [PubMed: 27698470]
57. Mattick JS, Makunin. Non-coding RNA. *Hum Mol Genet.* 2006; 15(Spec No 1):R17–29. [PubMed: 16651366]

Figure 1. CRISPR therapeutic editing has been customized to serve a diverse array of genome engineering needs. (A) CRISPRn (n = nuclease), or the traditional CRISPR system, uses homologous recombination to restore a mutation to wild type; (B) CRISPRd (d = deletion) uses non-homologous end joining to ablate a mutant allele; and (C) CRISPRi (i = interference) uses an inactive Cas9 to bind but not cleave DNA and prevent transcription mechanisms from producing gene expression.

Figure 2.

56-year-old man with a heterozygous mutation in *RHO*, specifically c.800C>T, p. (Pro267Leu). Digital color fundus photography of the right (A) and left (B) eyes illustrate intraretinal pigment migration, attenuated blood vessels, pale discs, and a bulls-eye atrophy. Short wavelength fundus autofluorescence (SW-FAF) of the right (C) and left (D) eyes reveals extensive RPE atrophy outside of the arterial arcades with an inferior predominance. A bulls-eye atrophy in both eyes is more evident with SW-FAF imaging.

Figure 3.

12-year-old boy with a hemizygous mutation in *RPGR*, specifically c.1307G>A, p. (Gly436Asp) in exon 11. Digital color fundus photography of the right (A) and left (B) eyes shows mottling outside of the macula. SW-FAF reveals a typical hyperautofluorescent ring of RP which corresponds to the boundary of an intact and absent ellipsoid zone (EZ) layer. On SD-OCT (E), peripheral thinning is seen outside of the parafovea with preservation of the EZ-line within the central macula only.