CRISPR/Cas System in Human Genetic Diseases

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Abstract. Clustered regularly interspaced short palindromic repeats/CRISPR-associated CRISPER/Cas system, as the current most popular gene-editing technology, shows great advantages of simple composition, good specificity and high cutting efficiency compared with other gene editing technology. With the rapid development of CRISPR-Cas systems, such as Cas9, Cas12a and Cas12f, can be used to edit the DNA of eukaryotic cells, and then successively found that Cas13a, Cas13b and Cas13d are targeted to the RNA merons. Through various modifications, scientists also developed a new type of the CRISPR-Cas system. With higher DNA-cutting activity, greater specificity, and smaller size than the natural CRISPR system, these engineered gene-editing systems form a powerful tool set for DNA sequence knockout, replacement, epigenetic editing, and even the activation and suppression of gene expression. Despite the potential problems in the practical application of CRISPR technology to be solved, it is believed that with further improvement, the CRISPR treatment technology will play a more important role in the prevention and treatment of human diseases, more perfectly and precisely. This review introduces the structure, functional mechanism and application of CRISPR/Cas system in human genetic diseases, and the current status and development of CRISPR/Cas system are summarized and prospected.

Keywords: CRISPR/Cas, Gene editing, Human disease, Genetic disease.

1. Introduction

Genome editing (GE), transcriptional perturbation, epigenetic regulation, and genomic imaging can now utilize the CRISPR/Cas9 system as a powerful RNA-guided DNA targeting platform [1]. It has a specific mechanism of defense of bacteria against phage infection and transference of plasmids in nature. This technique can precisely manipulate almost any genome sequence appointed by a small piece of guide RNA, also clarify gene functions involved in disease development, and correct disease-causing genetic mutations [2]. According to single guided RNA (gRNA) libraries, genome-wide detection with CRISPR can be used to identify drug targets or resistance genes [3]. As a result, genomic engineering mediated by CRISPR/Cas9 is highly promising in the treatment of genetic diseases.

When a bacteriuma happened to the invasion from a virus, it could firstly recognize its specific PAM sequence, digest the viral DNA into an appropriately sized spacer sequence, and integrate it into CRISPR spacer region [4,5]. The CRISPR/Cas system's gene editing function is attributed to the specific target sequence recognition capability of crRNA, the DNA-splitting activity of Cas nuclease, and the cell's DNA repair mechanism. Cells can repair DNA damage and avoid cell death by activating their own repair mechanisms when double-strand breaks (DSBs) occur. Two repair mechanisms are available, both nonhomologous end joining (NHEJ) and homologous directed repair (HDR) [6,7].

Based on a homologous sequence, cells will usually repair the DSBs through homologous recombination via HDR mechanism activation, which is the integration of homologous fragments into the DNA. When homologous DNA is not available, the cell usually activates the NHEJ mechanism to connect the broken DNA directly. Base pair insertions or deletions (indels) are common in this mode of repair, resulting in frameshift mutations that result in gene knockout.

Genome editing (GE) is a method for editing the genomes of living organisms, such as humans, plants, animals, and microorganisms, which is modernizing the biological world. Zinc finger nucleases (ZFNs) and transcriptional activator-like effect nucleases (TALENs) are the main components of traditional GE. ZFNs can target proteins that cut DNA to cut DNA sequences from

any location. DNA damage response pathways and genome modification are triggered by TALENs, which causes double strand breaks (DSBs) in target sequences. Although zfn and TALENs are widely used in GE, there are still some limitations. The specificity of ZFN is limited, and it often introduces off-target mutations. The vector construction of zfn and TALENs is time-consuming and laborious [8-10]. Thus, since 2013, attention has turned to the use of CRISPR/Cas9. The endonuclease CRISPR/Cas9 is guided by RNA and targets DNA. Genomic engineering can be done efficiently, accurately, and easily with CRISPR/Cas9. Until now, GE has optimally selected the CRISPR/Cas9 system.

2. CRISPR/Cas system

2.1. Classification of CRISPR/Cas system

Two essential components make up CRISPR/Cas systems. The CRISPR array is a genomic region that contains short segments of foreign DNA [11]. Cas genes on both sides of the CRISPR arrays encode the Cas protein, which is an essential component of CRISPR/Cas systems [12]. Up until 2019, there was research on the classification of CRISPR/Cas systems that stated that CRISPR/Cas systems were classified into two major categories and six types, each type was divided into multiple subtypes, and the Cas proteins contained in each type are also different, as shown in Table 1.

Table 1. Classification of CRISPR-Cas systems.

Classes	Types	Subtypes	Proteins contained
Class1	I	I-A、I-B、I-C、I-D、I-E、I-F1、I-F2、I-F3	Cas1、Cas2、Cas3、Cas4、 Cas5、Cas6、Cas7、Cas8
Class1	III	III-A、III-B、III-C、III-D、III-E、III-F	Cas1、Cas2、Cas5、Cas6、 Cas7、Cas10、Cas11
Class1	IV	IV-A、IV-B、IV-C	Cas1、Cas2、Cas5、Cas6、Cas7
Class2	II	II-A、II-B、II-C1、II-C2	Cas1、Cas2、Cas4、Cas9
Class2	V	V-A、V-B1、V-B2、V-C、V-D、V-E、V-F1、V-F1、V- F2、V-F3、V-G、V-U1、V-U2、V-U4、V-K	Cas1、Cas2、Cas4、Cas12
Class2	VI	VI-A、VI-B1、VI-B2、VI-C、VI-D	Cas1、Cas2、Cas13

2.1.1 Class 1

The proteins of effector modules made up of multiple Cas proteins are contained in the first class of systems in CRISPR/Cas systems, in which the working elements make up the first class are moved and combined from different systems. The first category can be divided into three types: type I, type III and type IV [13].

At present, type I accounts for the highest proportion in CRISPR/Cas systems and contains many defective subtypes, such as I-F and I-B subtypes. Cas1 and Cas2 are the most conserved proteins that are involved in almost all CRISPR/Cas systems. The Cas1 gene usually co-exists with other Cas genes and is associated with all type I and type II systems, most III-A systems, and some III-B systems, which has important implications for the classification of CRISPR/Cas system subtypes. The protein Cas3 that is responsible for the cleavage of the target nucleic acid, is not only a hallmark of the Type I system but also a protein found in many subtypes.

The Type III system is a special type of CRISPR because it not only has resistance to invading DNA, but also shows resistance to invading RNA, and the Cas10 protein is a marker protein in type III.

The original type IV system was found in Streptococcus pneumoniae and was originally classified as U-type. Many type IV systems lack nuclease proteins similar to cutting nucleic acids, but nucleic acids from different plasmids can be detected in their spacer sequences, so the CRISPR function of type IV may be related to resistance to foreign plasmids.

2.1.2 Class2

The second category in the system contains a single, multi-domain crRNA binding protein, which are functional proteins and includes all the components needed to cut nucleic acid. This category is considered as 20% of the entire CRISPR/Cas system, and its classification includes type II, type V and type VI. The second class of systems requires only one group to do the work that many groups in the first class of systems work together to do. The current second class of CRISPR/Cas systems is simpler than those in the first class and it is the first choice for biotechnology applications.

The representative of type II system is CRISPR/Cas9 protein system, which is mainly derived from Streptococcus pyogenes and Streptococcus thermophilus. During the operation of the type II system, Cas1, Cas2 and Cas4 proteins are in charge of the establishment of the repeat interval region, and RNase III helps crRNA formation in the stage of expression, while the remaining work is accomplished by Cas9 protein. CRISPR/Cas9 system, a new type and most widely applied genitic programming technology, is gradually put into use in various fields due to its simplification, rapidity and high efficiency. The CRISPR/ Cas9 system can also be applied to the study in the relationship between epigenetic editing, chromatin patterns and cell differentiation, as well as disease progression.

In 2015, Zetsche et al. defined a new type - Type V. Although the technical research of Type V system is not mature, it has been first applied in many fields because of its low detection cost and fast reaction speed.

At the beginning, the description of the type VI system was that a CRISPR/Cas system with RNA cutting ability was found, which was different from the type III system. Compared with the type III system with multiple subunits, the protein element size of the type VI system was significantly more advantageous. However, the present application of Cas13 is still challenging, and the failure rate of the current type VI system far beyonds the use of standards. Currently, type VI has been used in many fields, and the outbreak of the new coronavirus epidemic in the past two years, to a certain extent, has also accelerated the development of type VI system. Many laboratories have begun to use the characteristics of Cas13 protein to rapidly detect the new coronavirus, and develop small molecule drugs against the new coronavirus and even other RNA viruses [14].

2.2. Molecular mechanisms of the CRISPR-Cas system

The CRISPR system has acquired immunity to the invasion of foreign DNA, and this function has self-regulating properties. Rollie et al. found that when bacteria were transferred into genes beneficial to themselves, the immune function of CRISPR system would be lost, enabling bacteria to acquire certain new biological traits. However, when the unfavorable gene is transferred, its expression is upregulated. This immune function of the CRISPR system in bacteria is called interference, and it is divided into 3 stages: adaptation, expression, and interference. The main Cas proteins involved in each stage are different, and the main proteins involved in expression are Cas9, Cas6 and RNaseIII. In addition to Cas9, there are also Cas7, Cas10 and Cas8 families of interfering proteins. Cas1 and Cas2 egg whites are mainly composed of Cas1 and Cas2 egg whites, with the exception of type III-C, III-D and IV CRISPR systems. In addition, there are some Cas proteins that play auxiliary functions, mainly Cas4, Csn2 and DinG [15].

2.2.1 Adaption

The CRISPR system identifies the protospacer sequence of the invading virus or plasmid through the PAM, and then inserts it into the host genome so that the bacteria could quickly adapt to the invaders called the "adaptation phase" of the immune function of the CRISPR system. Cas1 is an integrase that can insert new spacer sequences into CRISPR sequences by cutting specific parts of the interior. Cas2 is a homologue of mRNA interferon toxin. When foreign viruses or plasmids invade,

Cas1 and Cas2 proteins recognize the PAM sequence of the foreign DNA, and then use the PAM sequence adjacent sequences as candidate primary spacer sequences. Then, based on the action of the Cas2 protein complex, Cas1 cleaves the initial spacer sequence of the foreign DNA sequence by cutting a specific insert, and through the action of other enzymes, the initial spacer sequence is inserted into the adjacent CRISPR sequence.

2.2.2 Expression

When the invader attacks in the expression phase, the corresponding spacer sequence will be transcribed into pre-crRNA, binding to the multiple subunit crRNA effector complex or Cas9 protein, and cutting the pre-crRNA into small mature crRNA under the catalysis of endonuclease RNAIII.

2.2.3 Interference

The crRNA instructs the corresponding crRNA to find the initial spacer sequence that is complementary to the viral or plasmid crRNA during interference. In the Type I system, the HD nuclease domain is combined with Cas3. In the Type III system, different HD nuclease domains are fused into Cas10 to cut single-stranded DNA. The large protein Cas9 is required for each plan of CRISPR immunity in type II systems.

3. Applications of CRISPR-Cas system in genetic diseases

3.1. Hemoglobinopathies

Hemoglobinopathy is a genetic disorder that affects the molecular structure or production of hemoglobin. The hemoglobin molecule consists of polypeptide chains, and its chemical structure is controlled by genes. The different hemoglobin proteins are named alphabetically in the order in which they were discovered. The exception was the first abnormal hemoglobin to be discovered, sickle cell hemoglobin, which was named hemoglobin S. The standard description of a patient's hemoglobin composition puts the highest concentration of hemoglobin first. Some hemoglobinopathies cause anemia, which is severe in homozygous patients and mild in heterozygous patients. Some patients are complex heterozygotes of two different hemoglobinopathies and have varying degrees of anemia. Some of the more common anemias include sickle cell disease (HB-S disease) and thalassemia, which are caused by genetic mutations [16].

Your body has lower than normal levels of hemoglobin due to a genetic blood disorder called Thalassemia. Oxygen can be transported by red blood cells through hemoglobin. Anemia and fatigue can be caused by thalasemia. The typical signs and symptoms of thalassemia include several aspects, such as pale or yellowish skin, weakness, slow growth, swollen abdomen, dark urinefacial bone deformities. Some babies have certain symptoms when they are born, others appear in the first two years of life. Symptoms of thalassemia can be absent for individuals with only one affected hemoglobin gene [17].

The hereditary diseases of sickle cell disease include sickle cell anemia. It affects the form of hematies. The roundness and flexibility of red blood cells make them easy to circulate through blood vessels and bring oxygen to all parts of the body. Some red blood cells in sickle cell anemia have the shape of a sickle or crescent. The hardening and sticky state of these sickle cells can result in slowing or blocking blood flow [18].

Around 6 months of age, sickle cell anemia usually presents with signs and symptoms. Individuals have different variations and can change over time. Anemia, frequent infections, stunted growth, swollen hands and feet, vision problems, pain attacks, and more can be seen as signs and symptoms.

The hematopoietic system can be corrected by gene therapy, which can modify genes that cause disease in autologous hematopoietic stem cells (HSCS). The gene-editing CRISPR-Cas9 technology can increase the production of fetal hemoglobin, thereby ameliorating the pathology of sickle cell disease and beta-thalassemia. The treatment involves isolating stem cells from the patient's peripheral blood and then editing those cells with CRISPR-Cas9 to destroy BCL11A, which increases the

production of fetal gamma globin. The engineered cells are then returned to the patient after myeloablative conditioning of the bone marrow.

Study participants had an increase in circulating hemoglobin, a decrease or elimination of the need for red blood cell transfusions, and no signs of clonal advantage due to insertion mutagenesis of the therapeutic vector.

3.2. Inherited eye disease

Leber congenital amaurosis (LCA) is a rare inherited eye disease that causes severe vision loss at birth or in infancy. LCA10 is a serious form of retinal malnutrition resulting from mutations in the CEP290 gene. LCA is characterized by severe visual impairment, eye rotation or nystagmus, eye indications, poor pupil light response, and undetectable or severely abnormal full-field electroretinogram [19].

Symptoms range from severe visual impairment at birth or in the first few months after birth, eye rotation or nystagmus, poor pupil light response, eye indications, and undetectable or severely abnormal full-field electroretinogram.

Targeted genomic deletion by the CRISPR/Cas9 system is a promising treatment for LCA10 patients who carry the CEP290 splicing mutation. Guide RNA pairs coupled with SpCas9 can efficiently remove intron splicing mutations and restore wild-type CEP290 expression.

More than 30 cone rod dystrophy types are distinct based on their genetic causes and inheritance patterns: autosomal recessive, autosomal dominant, and X-linked. The retina, which is a layer of light-sensitive tissue at the back of the eye, is affected by these diseases. The gradual degeneration of light-sensitive cells in the retina results in the loss of the patient's vision. Its early symptoms generally occur in childhood and are generally reduced vision and increased sensitivity to light. Impaired color vision, blind spots in the center of the visual field, and partial lateral vision loss are often accompanied by these features [20].

The genetic causes and inheritance patterns of cone rod dystrophy are categorized into autosomal recessive, autosomal dominant, and X-linked types. Targeted genomic deletion using the CRISPR/Cas9 system is a promising treatment for LCA10 patients with a CEP290 splice mutation. Guide RNA pairs fused to SpCas9 can efficiently eliminate intron splicing mutations and restore expression of wild-type CEP290.

3.3. Muscular genetic disease

Duchenne muscular dystrophy (DMD), a fatal X-linked recessive disease, which is characterized by progressive muscular atrophy caused by DMD gene mutations of encoding dystrophin, leading to submuscular deletion of dystrophins in DMD patients. Dystrophin is essential for maintaining the integrity of muscle fibers and the stability of their membranes. In the absence of dystrophin, muscle fibers are damaged during contraction. Early on, people with DMD begin to show noticeable symptoms such as slow walking and frequent falls, and in this age range, children with DMD have larger muscles around the lower legs, pelvis, and thighs than their peers. The weakening of muscles and loss of mobility begin at the age of eight and continue to develop from then on. Patients with DMD generally die from cardiopulmonary complications between 18 and 30 years of age [21,22].

Myotrophin can bind to DGC and localize to skeletal muscle membranes in DMD patients. Recent studies have shown that additional transcriptional regulation using the small molecule SMT C1100 or esutromid, tripling myotropin RNA levels, can significantly improve malnutrition in mdx mice. Utrophin can also be stably upregulated in cells using CRISPR by fusing catalysically inactivated Cas9 or dead Cas9 into the VP160 transcriptional activation domain to target Utrophin promoters.

This experiment demonstrated that in vivo repair of full-length dystrophin by Homologous Recombination (HR) resulted in the recovery of up to 8% of full-length dystrophin by intramuscular injection. Truncated forms of dystrophin were discovered in skeletal and cardiac muscle in every case, and DGC components were recovered as well. Bengtsson and colleagues used a muscle-specific CK8 promoter to regulate Cas9 expression in other tissues to minimize its off-target effects. It also re-

established ORF DMD in mdx4cv a different strain of dystrophin-deficient mice. In vivo repair of full-length dystrophin by Homologous Recombination (HR) resulted in a recovery of up to 8% of full-length dystrophin via intramuscular injection, as demonstrated by this experiment.

Muscle weakness, abnormal myelin neuropathy, hypotonia, and white matter abnormalities are symptoms of congenital muscular dystrophy type 1A (MDC1A) that is characterized by autosomal recessive neuromuscular disease at neonatal onset. MDC1A caused by multiple mutations in the LAMA2 gene contributes to apoptosis and degeneration of muscle and Schwann cells, which in turn leads to fibrosis and deletion of muscle function.

In CRISPR/Cas9 genome editing treatments, the Cas9 endonuclease produces double-strand breaks in a specific genomic region located near the protospatial neighboring motif (PAM) and targeted by complementary single guide RNAs (SgRNAs). When DNA repair models are present, specific genomic changes can be made by homologous directed repair (HDR), while NHEJ corrects Cas9-induced breaks in the absence of foreign models. Crispr-induced upregulation of human LAMA1 compensates for lama2 deficiency in patients with congenital muscular dystrophy. Endogenous expression of Lama1 was induced in MDC1A mouse models using a CRISPR activation system with deactivated Cas9, lacking endonucase activity, VP64 transcriptional activator, and sgRNA targeting Lama1's proximal promoter. We found that MDC1A mice treated with CRISPRa component carrying AAV9 showed robust Lama1 expression and an overall reduction in malnutrition features, including reduced fibrosis and hind limb paralysis [23,24].

3.4. Genetic liver disease

Inherited Type I tyrosinemia (HTI) is a rare autosomal recessive kidney disease caused by mutations in fumarylacetoacetate hydrolase (FAH). The defects of FAH gene result in the accumulation of toxic metabolites in the liver and kidneys, including fumarylacetoacetic acid and maleylacetoacetic acid. The liver and kidneys are severely damaged by these metabolites, resulting in liver failure, cirrhosis, and hepatocellular carcinoma.

At DNA target sites, double-strand breaks are effectively generated by the programmable nuclease Cas9, guided by a single guide RNA (sgRNA). Two main mechanisms are used to repair site-specific DSBS in cells simultaneously. Non-homologous end join (NHEJ) or high-fidelity homologous directed repair (HDR) pathway are both pathways that are error-prone. In gene therapy studies, both NHEJ and HDR DNA repair pathways have been employed, with NHEJ deleting dominant active mutations and HDR repairing functionally deficient mutations. Adult animal disease models can be used to test the feasibility of CRISPR/Cas9-mediated gene therapy, such as mouse HTI, hemophilia, hyperammonemia,and other diseases, has been demonstrated by several research groups, including ours, through HDR-mediated gene correction.

Treatment delivers CRISPR-Cas9 and the donor template to newborn HT1 rabbits via adeno-associated viruses. Adult mice and rats have previously been treated with correction of the Fah gene. HT1 rabbit livers were injected with AAV 8 packaged Cas9, fah single conducting RNA (sgRNA) and donor template via ear vein in this study. In the liver of HT1 rabbits, gene correction occurred via homologous targeted repair (HDR) and non-homologous end-joining (NHEJ), with efficiencies ranging from 0.90% to 3.71% and 2.39% to 6.35%, respectively. The structure and function of liver and kidney were normal after gene correction treatment. The findings indicate that delivering Cas9, sgRNA, and donor templates through AAV can adequately induce FAH+ hepatocytes and prevent liver and kidney damage in FAH-deleted rabbits [25,26].

3.5. Congential genetic lung disease

Mutations in the cystic fibrosis transmembrane regulatory gene are responsible for the autosomal recessive genetic disease known as cystic fibrosis. CAMP-regulated chloride ion channels located in the apical membrane of epithelial cells are responsible for the catalyzing of small ions to pass through the membrane, as encoded by CFTR. The impaired salt and fluid homeostasis is caused by the

dysfunction of this mechanism, which leads to multiple organ dysfunction and ultimately death from respiratory failure.

To edit DNA in target cells, mRNAs encoding nucleases, like CRISPR-Cas9, can be used in conjunction with GRNAs as a potential treatment. Cas9 RNP containing chemically modified SgRNAs in AAV6 was delivered to upper respiratory basal stem cells and bronchial epithelial cells with ΔF508 mutation, which ultimately could achieve an editing efficiency of 30-50% and restoring CFTR function equivalent to 20-50% of wild-type controls [27].

4. Conclusion

CRISPR has become one of the most effective gene editing tools available today. It is inexpensive, relatively simple and reliable to use. Moreover, it can be used to treat genetic diseases, the genes that cause some genetic diseases can be removed by CRISPR technology, and it can be used to treat faulty genes that cause genetic diseases. The potential of CRISPR/Cas for disease modeling and mutation correction in single-gene diseases is considerable. The discovery and development of drug targets can be accelerated through the use of CRISPR disease models. The application of RNA therapy in human diseases will be facilitated by the controllability of RNA changes in time and space through Cas13-based RNA targeting tools.

However, the CRISPR system still has some shortcomings, including efficiency and off-target problems. First, in terms of delivery efficiency, AAV, as one of CRISPR-Cas9 delivery systems, is often used for in vivo research. However, one of the drawbacks of this virus is its small capacity, while the CRISPR system has larger plasmids, so in the future, CRISPR delivery systems will need to have a higher high-load capacity, or they can also be studied with a smaller size of highly efficient Cas9 variants. Second, off-target effects also bring many uncertainties to research, including sequence mutations, deletions, rearrangements, oncogene activation, and cell death during genome editing, which seriously limit the wider application of the CRISPR-Cas9 system. Therefore, while CRISPR gene editing technology is powerful, its "off-target" by cutting non-target genes can also cause unexpected harm. Therefore, the ethical issues in its application have aroused widespread concern in society, and scientists and bioethicists also need more time to evaluate and analyze the potential risks of this technology. However, it is believed that with the progress of time and the efforts of scientists, these problems will be solved in the near future, CRISPR-Cas gene editing technology will become more precise, allowing it to play a larger role in preventing and treating human diseases.

References

[1] Jiang F, Doudna JA. CRISPR-Cas9 Structures and Mechanisms. Annu Rev Biophys. 2017 May 22;46:505-529.

[2] Charpentier E, Doudna JA. Biotechnology: Rewriting a genome. Nature. 2013 Mar 7;495(7439):50-1.

[3] Dominguez AA, Lim WA, Qi LS. Beyond editing: repurposing CRISPR-Cas9 for precision genome regulation and interrogation. Nat Rev Mol Cell Biol. 2016 Jan;17(1):5-15.

[4] Zhu Y. Advances in CRISPR/Cas9. Biomed Res Int. 2022 Sep 23;2022:9978571.

[5] Shah SZ, Rehman A, Nasir H, Asif A, Tufail B, Usama M, Jabbar B. Advances In Research On Genome Editing Crispr-Cas9 Technology. J Ayub Med Coll Abbottabad. 2019 Jan-Mar;31(1):108-122.

[6] Wyman C, Kanaar R. DNA double-strand break repair: all's well that ends well. Annu Rev Genet. 2006;40:363-83.

[7] Ghaemi A, Bagheri E, Abnous K, Taghdisi SM, Ramezani M, Alibolandi M. CRISPR-cas9 genome editing delivery systems for targeted cancer therapy. Life Sci. 2021 Feb 15;267:118969.

[8] Manghwar H, Lindsey K, Zhang X, Jin S. CRISPR/Cas System: Recent Advances and Future Prospects for Genome Editing. Trends Plant Sci. 2019 Dec;24(12):1102-1125.

[9] Gaj T, Gersbach CA, Barbas CF 3rd. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 2013 Jul;31(7):397-405.

[10] Puchta H. Applying CRISPR/Cas for genome engineering in plants: the best is yet to come. Curr Opin Plant Biol. 2017 Apr;36:1-8.

[11] Chavez, M., Chen, X., Finn, P.B. et al. Advances in CRISPR therapeutics. Nat Rev Nephrol19, 9–22 (2023).

[12] Devi, V., Harjai, K. & Chhibber, S. CRISPR-Cas systems: role in cellular processes beyond adaptive immunity. Folia Microbiol 67, 837–850 (2022).

[13] Xu, Y., & Li, Z. (2020). CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. Computational and structural biotechnology journal, 18, 2401–2415.

[14] Makarova, K.S., Wolf, Y.I., Iranzo, J., Shmakov, S.A., Alkhnbashi, O.S., Brouns, S.J.J., Charpentier, E., Cheng, D., Haft, D.H., Horvath, P., Moineau, S., Mojica, F.J.M., Scott, D., Shah, S.A., Siksnys, V., Terns, M.P., Venclovas, Č., White, M.F., Yakunin, A.F. and Yan, W. (2019). Evolutionary classification of CRISPR–Cas systems: a burst of class 2 and derived variants. Nature Reviews Microbiology, [online] 18(2), pp.67–83.

[15] Chaudhuri, A., Halder, K. and Datta, A. (2022). Classification of CRISPR/Cas system and its application in tomato breeding. Theoretical and Applied Genetics, 135(2), pp.367–387.

[16] Wu, S.-S., Li, Q.-C., Yin, C.-Q., Xue, W. and Song, C.-Q. (2020). Advances in CRISPR/Cas-based Gene Therapy in Human Genetic Diseases. Theranostics, [online] 10(10), pp.4374–4382.

[17] Papizan, J.B., Porter, S.N., Sharma, A. and Pruett-Miller, S.M. (2021). Therapeutic gene editing strategies using CRISPR-Cas9 for the β-hemoglobinopathies. Journal of Biomedical Research, [online] 35(2), pp.115–134.

[18] Mayo Clinic (2022). Sickle Cell Anemia - Symptoms and Causes. [online] Mayo Clinic. Available at: https://www.mayoclinic.org/diseases-conditions/sickle-cell-anemia/symptoms-causes/syc-20355876.

[19] Kumaran, N., Pennesi, M.E., Yang, P., Trzupek, K.M., Schlechter, C., Moore, A.T., Weleber, R.G. and Michaelides, M. (1993). Leber Congenital Amaurosis / Early-Onset Severe Retinal Dystrophy Overview. [online] PubMed. Available at: https://www.ncbi.nlm.nih.gov/books/NBK531510/.

[20] Mellen, R.W., Calabro, K.R., K. Tyler McCullough, Crosson, S.M., Alejandro, Fajardo, D., Xu, E., Boye, S.L. and Boye, S.L. (2023). Development of an AAV-CRISPR-Cas9-based treatment for dominant cone-rod dystrophy 6. [online] 30, pp.48–64.

[21] Elangkovan, N., & Dickson, G. (2021). Gene Therapy for Duchenne Muscular Dystrophy. Journal of neuromuscular diseases, 8(s2), S303–S316.

[22] Happi Mbakam, C., Lamothe, G., Tremblay, G. et al. CRISPR-Cas9 Gene Therapy for Duchenne Muscular Dystrophy. Neurotherapeutics 19, 931–941 (2022).

[23] Wong, T. W. Y., & Cohn, R. D. (2017). Therapeutic Applications of CRISPR/Cas for Duchenne Muscular Dystrophy. Current gene therapy, 17(4), 301–308.

[24] Arockiaraj, A. I., Johnson, M. A., Munir, A., Ekambaram, P., Lucas, P. C., McAllister-Lucas, L. M., & Kemaladewi, D. U. (2023). CRISPRa-induced upregulation of human LAMA1 compensates for LAMA2 -deficiency in Merosin-deficient congenital muscular dystrophy. bioRxiv : the preprint server for biology, 2023.03.06.531347.

[25] Shao, Y., Wang, L., Guo, N., Wang, S., Yang, L., Li, Y., Wang, M., Yin, S., Han, H., Zeng, L., Zhang, L., Hui, L., Ding, Q., Zhang, J., Geng, H., Liu, M., & Li, D. (2018). Cas9-nickase-mediated genome editing corrects hereditary tyrosinemia in rats. The Journal of biological chemistry, 293(18), 6883–6892.

[26] Ibraheim, R., Tai, P.W.L., Mir, A. et al. Self-inactivating, all-in-one AAV vectors for precision Cas9 genome editing via homology-directed repair in vivo. Nat Commun 12, 6267 (2021).

[27] Da Silva Sanchez, A., Paunovska, K., Cristian, A., & Dahlman, J. E. (2020). Treating Cystic Fibrosis with mRNA and CRISPR. Human gene therapy, 31(17-18), 940–955.