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Future Possibilities for the use of CRISPR on Mutations in Three Eye Disorders

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Abstract

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology is a useful tool to insert, delete, and substitute DNA in the genome. This is done by separating DNA via double-stranded breaks and can be performed through two different mechanisms: Homology-directed Repair (HDR) and Non-Homologous End Jointing (NHEJ). Double strand breaks would be used for a tracrRNA:crRNA to guide the enzyme cas9 to insert, delete, or substitute the desired DNA. CRISPR has limitations on which DNA sequences it can work with. Other concerns include ethical questions and base-pair limitations. X-linked Congenital Night Blindness, Snowflake Vitreoretinal Degeneration, and Cataract Microcornea syndrome are three genetic diseases caused by mutations in the genes CACNA1F, KCNJ13, and ABCA3 respectively. This research paper will discuss the possibilities of CRISPR and decipher specific substitutions for all three eye disorders.

Keywords: X-Linked Congenital Night Blindness, Snowflake Vitreoretinal Degeneration, Microcornea Cataract Syndrome, CRISPR

1. Introduction

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) gene-editing technology in a form of gene therapy in which the scientist inserts an artificial guide-RNA (gRNA) that attracts CRISPR-Cas9 to a desired piece of DNA. Double strand breaks separate the DNA strand for the artificial gRNA, while Homology-directed Repair (HDR) and Non-Homologous End Jointing (NHEJ) correct the genome. HDR is usually the most preferred method.

CRISPR has advantages that make it a higher preference than other technologies. While CRISPR can substitute DNA in a genome and insert/delete base pairs, one crucial benefit is that CRISPR is higher affordability (Redman, et al.,2016). Other methods can cost upward of $1,000, but CRISPR is relatively more affordable, costing less than $100 per treatment for one patient (Doudna and Charpentier, 2014). Before 2007, it would take three months to administrate CRISPR gene therapy, but that time has now been shortened to 1 to 2 hours (Doudna and Charpentier, 2014). These benefits allow CRISPR treatments that are currently in trials to cure a variety of diseases including Sickle Cell Disease (SCD) and cancer (Cong and Zhang, et al., 2021). The deadly inherited SCD has already been treated in mice by using CRISPR (Newby, et al., 2021). A single letter of DNA in red blood cells caused the formation of a specific non-pathogenic variant, which conveniently multiplies healthy copies of itself (Newby, et al., 2021)! The initial mutation, caused by the SCD

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allele, reduced the red blood cells' ability to carry oxygen and curve into a crescent/sickle shape. The only current treatment is bone marrow transplant, but the treatment is difficult to obtain with specific conditions that might be deadly (Newby, et al., 2021).

The injection treatment in trials focuses on using an adenine base editor to target specific gene sequences for targeting specific sequences. On a more molecular level, the A-T base pairs are converted to G-C base pairs, as the SCD mutation is a consequence of the A base pair altering to a T (Siliezar, 2021). Previous human trials with the CRISPR treatment have determined that a maximum of 80% of the dangerous mutation were edited, providing hope for patients across the world (Siliezar, 2021). Researchers at the University of Pennsylvania are focusing on genetically modifying cancerous immune cells to fight a variety of different cancers via CRISPR. Currently, trials have only resulted in 10% success rate, but no harmful off-target effects have arisen. Before the drug can be introduced to the market, long-term effects need to be monitored: one such limitation of CRISPR.

Some of CRISPR's limitations include off-target effects, short base pair lengths, and potential unethical practices. Off-target effects include base pair changes that occur outside the targeted region on DNA; these base-pair changes can be transcribed into different proteins, creating more mutations in the genome (Plumer, et al., 2018). CRISPR technology has an off-target frequency higher than 50%, creating a need for engineering more CRISPR/Cas9 variants (Plumer, et al., 2018). One way to reduce off-target effects is to optimize the guide RNA (Plumer, et al., 2018). This, however, can now be performed using current technologies including E-CRISPR, CRISPR-design, and others (Heigwer, et al., 2014). Another limitation is the number of base pairs that CRISPR/Cas 9 can cut (Plumer, et al., 2018). The Cas9 enzyme is capable of cutting~ 20 base pairs in length (Zhang, et al., 2015). This property can be useful for diseases and disorders that have insertions and deletions that are under this limitation. X-Linked Congenital Night Blindness, Snowflake Vitreoretinal Degeneration, and Cataract Microcornea Syndrome all include mutations that are single substitutions. While ethical questions are raised on how much gene editing should we do, CRISPR has a limit of 20 base pairs, and therefore cannot be used for every disease or disorder.

By collecting data of mutations from different sources for X-linked Congenital Night Blindness, Snowflake Vitreoretinal Degeneration, and Cataract Microcornea Syndrome, I have analyzed past research papers for substitutions that can potentially be fixed by CRISPR for researchers to target with future treatments. This paper discusses, the impact of the potential corrected mutation on the disorder as a whole and addresses ethical questions that may arise.

2. Findings

2.1 The KCNJ13 and ABCA3 genes with mutations defined

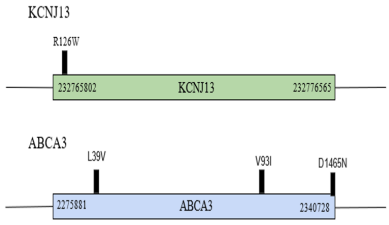

Figure 1. KCNJ13 and ABCA3 genes with mutations defined. The figure shows the R162W mutant located on the KCNJ13 gene. The R162W mutant is the result of a C to G base pair transition. The ABCA3 gene has multiple single substitution mutations: L39V-115C>G, V931-277G>A, and D1465N-4393G>A. All three mutations for the ABCA3 gene result in amino acid changes (Hejtmancik, et. al., 2008).

2.2 X-Linked Congenital Night Blindness

X-Linked Congenital Night Blindness is a recessive disorder, which is characterized by mutations in the retina, which helps to detect light and color (Boycott, et, al., 2001). Symptoms of X-Linked Congenital Night Blindness include night blindness, reduced acuity, myopia, and nystagmus (Boycott, et, al., 2001). The gene CACNA1F, responsible for the disease phenotype, is located on the retina on the surface of photoreceptors cells (Boycott, et, al., 2001). CACNA1F is responsible for encoding a retina-specific voltage-gated L-type calcium channel a1-subunit, that functions as the pore and voltage sensor (Beck-Hansen and

Pearce,1993). Both CACNA1F and another gene, NYX, make proteins that help in the process of passing visual signals from rods to cones in bipolar cells which helps with the transmission of visual information from the eyes to the brain (Beck-Hansen,1998). In the CACNA1f gene, 14 mutations have been discovered in 36 patients; six of which caused premature protein truncation (Boycott, et, al., 2001). Studies have concluded that mutations can cause amino acid substitutions/deletions and premature protein truncation (Boycott, et, al., 2001). CACNA1F has 48 exons and a predicted amino acid length of 1977 (b.Beck-Hansen,1998) (Figure 2).

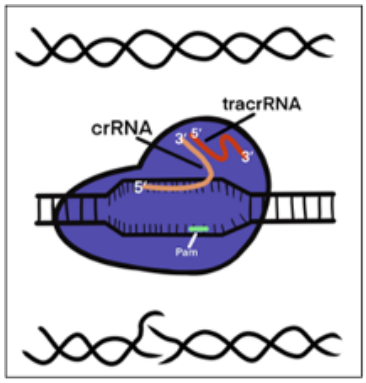

Figure 2. The process of inserting a genetically modified strand using CRISPR. CRISPR RNA (crRNA) and trans-encoded small crRNA (tracrRNA) direct the Cas9 nuclease with the help of the PAM sequence (Plumer, et. al., 2018).

2.3 Snowflake Vitreoretinal Degeneration

Snowflake Vitreoretinal Degeneration is characterized by fibrillar degeneration of the vitreous humor, early-onset cataract and minute crystalline deposits among others (Edwards and Robertson, 2006). A mutation in the KCNJ13 gene is one of the causes. In a previous study, the KCNJ13 gene was analyzed and showed a c.484C > T transition, which changed the CGG codon to a UGG codon resulting in the R162W mutant (Hejtmancik, et al., 2008). In the 210 unaffected family members present in this trial, none of them contained this R162W mutant, providing further evidence that the R162W mutant could be the cause of Snowflake Vitreoretinal Degeneration. The R162W mutant then causes the production of the transmembrane protein Kir7.1 to cause major structural damage (Hejtmancik, et al., 2008). Kir7.1 is a Kþ-selective inward-rectified channel, located between 20 genes sequenced from D2S2158 to D2S2202 (Hejtmancik, et al., 2008). The R162W mutant is located in the Kir7.1 gene (Hirose,1974).

2.4 Cataract Microcornea Syndrome

Cataract Microcornea Syndrome is characterized by a corneal diameter below 10 mm in both eyes and inherited cataracts (Chen, et al. 2014). Cataracts are defined as blurred or dim vision, which can develop into a total cataract when the eyes reach visual maturity (Chen, et al. 2014). Myopia, iris coloboma, and sclerocornea are also possible additional symptoms (Chen, et al. 2014). Corneal dystrophy and Corneal Opacity are other common eye disorders that may occur with Cataract Microcornea Syndrome (Klintworth, et al., 2009).

Before the discovery of the impact of the ABCA3 gene, 9 genes led to Cataract Microcornea Syndrome: some being CRYAA, CRYBA4, CRYBB1, CRYBB2, CRYGC, and CRYGD (a.Hansen, et al., 2019). This study also concluded that the gene ABCA3 is another cause of Cataract Microcornea Syndrome (Chen, et al. 2014). In two Chinese families, the missense mutations were c.115C>G, c.277G>A, c.4393G>A, and c.2408C>T (Chen, et al. 2014). There were also two splice-site mutations, c.4053+2T>C, c.2765-1G>T later identified (Chen, et al. 2014). The ABCA3 gene spans over 80,000 nucleotides and can be transcribed into 6500 base pair mRNA (Mulugeta, et al.,2002). This synthesizes a 1704 amino acid protein (Mulugeta, et al.,2002). ABCA3 is also predicted to be a glycoprotein that could hydrolyze ATP to provide energy for substrate transport involved in eye development (Chen, et al. 2014). Therefore, a mutation in ABCA3 might cause an impact on eye development.

3. Discussion

3.2 CRISPR

HDR can only be used on multiplying cells. Cells like the liver, neuron, and muscle which do not multiply, cannot perform HDR double-strand breaks

(Uddin, et al., 2020). While HDR is the most preferred method, it is less prone to mistakes (Uddin, et al., 2020). Double strand breaks enable editing for deletions, insertions, and substitutions (Uddin, et al., 2020). The CRISPR/Cas9 system includes guide RNA (gRNA) which directs a special Cas9 nuclease to create double-strand breaks in the desired segment of DNA (Uddin, et al., 2020). CRISPR RNA (crRNA) and trans-encoded small crRNA (tracrRNA) are used to direct the Cas9 nuclease to target precise locations (Uddin, et al., 2020). This is called the tracrRNA:crRNA complex (Uddin, et al., 2020). The PAM sequence is located immediately after the target sequence and helps the Cas9 nuclease cut the sequence (Uddin, et al., 2020). The steps mentioned before are how a genetically modified strand of DNA is substituted in the DNA strand. The steps below are how bacteria integrate spacer DNA - a memory system of past infections.

There are three stages in which the CRISPR-Cas9 system is divided into Spacer integrations, processing of the primary transcript of pre-crRNA, and DNA/RNA interference (Makarova and Koonin, et al., 2015). In the first step, Cas 1 and Cas 2 enzymes insert the spacer DNA in between DNA repeats (Bolotin, et al., 2005). Both these proteins form a complex, where Cas 1 integrates the Spacer DNA, and Cas 2 performs a non-enzymic function (Makarova and Koonin, et al., 2015). In the second step, pre-crRNA is processed into guide-crRNA via RNA endonuclease complex or RNase III (Makarova and Koonin, et al., 2015). Then the now mature crRNA can be bound by Cas proteins resulting in either type I, type II, or type III effector complex (Chylinski, et al., 2014). In the third step, the effector complex is used to target DNA or RNA known as Cascade (CRISPR-associated complex for antiviral defense) (Wang, et al., 2018) (Figure 1).

3.2 X-Linked Congenital Night Blindness

CRISPR can be used to fix certain base pairs. 14 other mutations were found from another 16 patients tested who had incomplete CSNB. With a total of 20 mutations identified, 14 were predicted to cause protein transaction, and 6 were predicted to cause amino acid substitution/deletion (Boycott, et, al., 2001). One example of such is truncating mutation, located in the 3' splice site intron 40 (Boycott, et, al., 2001). One of the mutations was an A to G transition which was predicted to cause the loss of a splice site mutation, which resulted in the loss of exon 41 (Boycott, et, al., 2001). This then resulted in a premature stop codon in exon 42 and premature truncation of the a1F protein (Boycott, et, al., 2001). The A-G transition would be one base pair in length, so CRISPR technology could be used to fix this mutation (Boycott, et, al., 2001). CRISPR could also be used to fix the 6 amino acid substitutions and deletions (Boycott, et, al., 2001). It would depend on each protein mutations for the other 14 mutations identified. Since, however, there were 20 mutations identified in 16 patients, it is reasonable to conclude that there could be more mutations that exist but that have not been documented. Since CRISPR can be used to correct 6 out of the 20 mutations because they are substitutions, it is worth brainstorming on the idea of experimenting with CRISPR on the CACNA1F gene.

3.3 Snowflake Vitreoretinal Degeneration

The KCNJ13 gene mutation was a result of the CGG codon changing to a UGG codon (Hejtmancik, et al., 2008). CRISPR technology can be used to fix single mutations, so the UGG codon could be fixed. This results in the Arginine amino acid is changed into a Tryptophan amino acid. Tryptophan is a stop codon that results in a nonsense mutation leading to the production of the R162W mutant. The R162W mutant modifies the channel selectivity of the Kir7.1 protein making it permeable to Naþ ions. CRISPR technology would be beneficial as it would create significant results by correcting the sequence for the R162W mutant. Therefore, CRISPR technology could be used to treat Snowflake Vitreoretinal Degeneration.

3.4 Cataract Microcornea Syndrome

The gene ABCA3 has multiple missense mutations and two splice-site mutations. The missense mutations are c.115C>G, c.277G>A, c.4393G>A, and c.2408C>T, and the splice-site mutations being c.4053+2T>C, c.2765-1G>T.

CRISPR could be used to treat these genes; however, it is important to note that not all genes for Cataract Microcornea Syndrome are known (Chen, et al. 2014). Therefore, complete treatment of all symptoms might not be accomplished. Another reason to keep in mind is that CRISPR might not be a feasible treatment for every patient. Depending on their ethnicity, patients will have different mutations.

4. Conclusions

While it is important to understand the concerns, not every disease can have significant effects with using CRISPR. Out of all three diseases examined in this paper, Snowflake Vitreoretinal Degeneration with the KCNJ13 gene has the highest potential for creating a feasible treatment for this disorder using CRISPR compared to X-Linked Congenital Night Blindness and Cataract Microcornea Syndrome. Since, however, each patient has a different set of mutations, the predictability of the usefulness of CRISPR is variable. CRISPR technology has its benefits and its concerns. CRISPR is relatively cheap, easy to use, and one of the most popular forms of gene editing. Some limitations are set, including the length of nucleotide bases, and having off-target effects as mentioned above (Uddin, et al., 2020). Ethical questions that may be raised mainly stem from embryonic editing compared to somatic editing because it happens after the offspring is born (Uddin, et al., 2020). While consent can be taken from the individual, it cannot be taken in Embryonic editing (Uddin, et al., 2020). Embryonic editing can also cause permanent problems for future generations as well as result in premature high off-target rates or death from the procedure (Uddin, et al., 2020). Although CRISPR can make progress in all three eye diseases (X-Linked Congenital Night Blindness, Snowflake Vitreoretinal Degeneration, and Cataract Microcornea Syndrome), future studies should heavily consider the side-effects and ethical concerns.

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