CRISPR's Promise for Rett Syndrome and Beyond

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Rett Syndrome (RTT) is a devastating neurodevelopmental disorder caused by mutations in the MECP2 gene, affecting approximately 1 in 10,000 girls (Liebertpub, 2022). The disease primarily affects girls since the MECP2 gene is located on the X chromosome, and it is passed down as an X-linked trait. (Coenraads, 2022) Since girls have two X chromosomes and boys have an X and Y chromosome, it is more likely that girls develop Rett Syndrome lasting beyond infancy (Coenraads 2022). However, when boys develop Rett Syndrome, it results in more severe impacts since they don't have a backup X chromosome to do the same function as the original one (Coenraads 2022). The MECP2 gene plays a crucial role in brain function and regulates gene activity (Panayotis et al. 2023) The gene provides instructions for creating the MECP2 protein, which is essential for brain development and function. MECP2 acts as a genetic switch that controls the activity of many other genes, especially in neurons, where it regulates when and how certain genes are turned on or off (Panayotis et al. 2023). When the MECP2 gene is mutated, the MECP2 protein doesn't function correctly or is missing, leading to widespread neurological symptoms including motor, sensory, cognitive, and behavioral decline (International Rett Syndrome Foundation 2023). Though the disease starts subtly, it follows four stages: Stage 1, early onset, which often begins between 6–18 months, and includes reduced eye contact and slowed development. In stage 2, symptoms worsen with loss of motor skills, abnormal hand movements, and speech difficulties (MayoClinic 2022). Stage 3, the plateau, can last many years with motor issues but improved behavior as individuals grow older. Finally, Stage 4, or late motor deterioration, involves muscle weakness, scoliosis, and decreased mobility (Mayo Clinic 2022). RTT is part of a group of neurodegenerative disease conditions caused by inherited gene mutations that progressively damage the brain and nervous system. (International Rett Syndrome Foundation 2023). Despite 60 clinical trials that emerged out of necessity for a cure or treatment, current therapies are symptom-based, such as medication, physical, occupational, and speech language therapy, behavioral intervention, and regular medical care (Mayo Clinic Staff 2022). These therapies and treatments don't provide a solution for the genetic cause of the disease. However, CRISPR offers hope by providing a genetic solution. Gene editing is a powerful technology that allows scientists to customize genetic makeup, making specific and precise changes to an organism's DNA itself. In the case of Rett Syndrome, CRISPR provides a potential solution by directly fixing the mutation in the MECP2 gene, helping to restore normal gene and protein function (Sargent).

Since RTT is caused by a single-gene mutation on the X chromosome, CRISPR offers a direct solution to fix the root of the problem. CRISPR allows scientists to target this mutated gene and edit it back using gene editing (base and prime editing) and X-chromosome reactivation. CRISPR uses a molecule called Cas9 and guide RNA to find and cut the faulty DNA. However, newer versions like base and prime editing allow for even more precise corrections. Base editing is a technique that chemically modifies the DNA bases of an organism without cutting the DNA. It is primarily used for single-base mutations, emphasizing the advantage of avoiding double-strand DNA breaks. Prime editing is a mechanism where the genetic information of a longer DNA sequence is rewritten (Sargent 2024). These techniques allow scientists to edit the mutated MECP2 gene, restoring function to the MECP2 protein which regulates gene activity in

the brain. In one study, male RTT mice treated with AAV9-delivered MECP2 lived nearly four times longer, with lifespans extended from around 12 weeks to up to 37 weeks, while also showing improved motor coordination and breathing patterns (Palmieri et al. 2023). These results give scientists hope that gene replacement therapy could become one of the most promising cures for RTT and MECP2-related disorders. However, translating this success from mice to humans is a major step. Since mice have simpler brains and shorter lifespans, treatments may not work the same. Humans also have a more complex immune system and a wider range of possible symptoms. Since both organisms are so different, possible clinical trials on humans must be carefully tested, as small differences in gene regulation could have big impacts. Another significant challenge in gene therapy for RTT is MECP2 dosage control. Too little MeCP2 protein leads to RETT symptoms, while too much can result in MECP2 Duplication syndrome (where the MECP2 gene is duplicated, leading to many more similar neurological disadvantages and intellectual disabilities). MECP2 Duplication primarily affects males as they don't have a second X chromosome to compensate for the duplication, while females typically have a second, normal X chromosome that can mitigate the effects (Coorey 2022). Newer CRISPR strategies are being carefully designed to match the natural levels of MECP2 in the brain. Another promising approach benefiting females with RTT is X-chromosome reactivation. Since females carry two X chromosomes, one of which is usually inactive, CRISPR can be used to reactivate the healthy copy of the MECP2 gene that is normally silent (Panayotis et al. 2023). This method avoids altering DNA sequences, instead using chemical actions that switch the gene back on. It allows for a more natural restoration of MeCP2 protein levels and avoids the risk of overexpression (Panayotis et al. 2023). Although this method only works in females since males carry an X and Y chromosome, it offers a precise and potentially safer alternative to gene editing. These strategies show that CRISPR-based approaches could correct genetic mutations at its source and restore neurological function.

Although CRISPR holds a promise for treating Rett Syndrome, one of the biggest challenges is delivery. The most important question is how we get gene-editing tools to enter the brain. The blood-brain barrier (BBB) is a tightly regulated shield that protects the brains' bloodstream from harmful substances, but it also blocks many therapies, including CRISPR components (Palmieri et al. 2023). A major breakthrough in this area has been the usage of adeno-associated viral vectors (AAV), a mechanism that allows scientists to transform a naturally occurring virus into a mechanism for the delivery of gene therapy (Palmieri et al. 2023). The viral genes are replaced by therapeutic genes that target specific cells or tissues (Palmieri et al. 2023). This method has shown promise in crossing the BBB and delivering CRISPR elements, but it comes with its own risks, including immune system reactions, uneven distribution in the brain, and the risk of off-target effects if the virus infects tissues outside the brain (Palmieri et al. 2023). To address these issues, researchers are exploring nanoparticle-based delivery systems as an alternative to viral vectors. These systems use nanoparticles attached with antibodies, peptides, or proteins that can target specific receptors or cells in the brain. This method allows scientists to cross the BBB more efficiently and reduce immune responses. Some are even being designed to target specific types of brain cells, increasing precision and safety (Palmieri et al. 2023). Another approach to cross the BBB is the combination of CRISPR with stem cell therapy. In this Ex Vivo method, stem cells are edited outside the body to correct MECP2 mutations, and then reintroduced into the patient’s system. These modified, corrected cells can then integrate into

neural tissue, develop into functional neurons, and help repair or replace damage caused by the disease in many areas of the brain (Palmieri et al. 2023). The biggest advantage of this strategy is that editing is completed outside the body, making it easier to ensure the CRISPR worked correctly, reducing incorrect effects. Although his method is still in its early stages and not yet tested in clinical trials for RTT, it opens many new possibilities for precise and long-lasting treatment options, especially when combined with other delivery methods such as nanoparticles or AAVs.

While CRISPR provides a powerful way to correct the root genetic causes of Rett Syndrome, it may not be able to reverse all the disease's effects on its own, especially since treatments are usually started later in life. Rett Syndrome affects the development of neurons and the regulation of gene activity, causing long-term damage in the brain. Once this damage has occurred, simply correcting the MECP2 gene mutation may not be enough to fully restore brain function (Singh et al.). One of the most promising strategies is the combination of CRISPR with Trofinetide, the first FDA-approved drug for Rett Syndrome (Palmieri et al. 2023). Trofinetide helps improve brain function by boosting neurotransmission and reducing inflammation. While Trofinetide doesn't help target the genetic root of the disorder, it helps ease symptoms and stabilize brain activity, allowing CRISPR gene editing more time and support to work. This type of "dual therapy" has the potential to stop diseases from getting worse but also improve cognitive and motor function rather than using CRISPR alone. Another promising combination is CRISPR with RNA editing (Panayotis et al. 2023). Instead of editing DNA permanently, RNA editing temporarily corrects the MECP2 mutation in the RNA, offering a reversible and more controlled method. This is especially useful when scientists attempt to fine-tune MECP2 expression to avoid the harmful effects of overexpression and underexpression. Studies have shown that combining RNA editing with CRISPR mainly provides better dosage control, reducing the risk of MECP2 toxicity (Palmieri et al. 2023). Since RNA editing can be conveniently adjusted, it offers more flexibility to personalize treatment based on a patient's symptoms, age, or type of mutation. Finally, if CRISPR-based therapies are introduced into the body after symptoms have developed, restoring brain function would require rehabilitation to reverse impairments (Singh et al.). Once the genetic issue is corrected, the brain still needs to rebuild neural pathways and relearn lost functions such as cognitive, motor, and sensory abilities. Scientists suggest that combining CRISPR (molecular correction) with behavioral therapies such as physical, occupational, or speech treatments could help patients regain more skills and independence. Together, these combined treatments offer a more complete strategy for treating Rett Syndrome and improving quality of life.

CRISPR provides an effective method to repair the MECP2 mutation on the X chromosome, potentially correcting the root cause of the neurological disorder Rett Syndrome, restoring the MECP2 protein's role in regulating gene expression in the brain. Techniques like base and prime editing offer precise ways to fix single mutations, while X-chromosome reactivation opens new possibilities for female patients with a healthy but silenced part of the gene (Panayotis et al. 2023). Despite these developments, safely delivering CRISPR across the blood-brain barrier remains a significant challenge. Advancements such as adeno-associated viral vectors and nanoparticle delivery systems offer potential solutions to transport these therapies into the brain

more effectively (Palmieri et al. 2023). However, just editing the MECP2 gene may not be enough, especially if treatments begin later in life. This leads to developments in therapeutic combinations with CRISPR, such as combining Trofinetide, RNA editing, or neurorehabilitation with genetic editing, helping stabilize brain function and support recovery. These combinations offer helpful strategies for treatment of symptoms and ease of life for patients who already have brain damage caused by the disease. Research continues to focus on dosage control, ensuring that MECP2 levels are not under or overexpressed, which is essential for safety and effectiveness (Coorey et al.). Since Rett Syndrome is a rare disease, it highlights the power of CRISPR. Treatments developed for RTT can help researchers better understand how to treat other rare monogenic diseases (Vidal et al.). This creates an emphasis on how investments in RTT research have effects that can speed up treatments for other neurological and genetic conditions, showing the value of research in rare diseases. Ultimately, Rett syndrome remains a neurodevelopmental disorder with no cure, but CRISPR technology offers the first real chances at reversing its effects at a genetic level. As gene editing research advances, there is growing hope that Rett Syndrome could become one of the first neurodegenerative diseases successfully treated with CRISPR-based therapies. These treatments lay the foundation for many other approaches to neurological disorders, making a shift and a big impact in precision medicine.

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