

Applications of CRISPR/Cas9 in Modeling and Investigating Schizophrenia

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ABSTRACT: Schizophrenia is an impairing psychiatric disorder that causes patients to experience distorted perceptions of reality, altering their regular thoughts, emotions, actions, and feelings. This disease currently lingers as an unknown condition whose precise biological mechanisms and genetic links remain incompletely understood due to a lack of research, and therefore has no decisive cure. This lack of knowledge has intensified the need for biomedical tools that are capable of examining schizophrenia at a genetic level. CRISPR/Cas9 is a 21st-century genome-editing technique that offers the potential to investigate the cellular processes underlying many under-researched diseases. This tool can accurately cut DNA at specific locations, enabling scientists to replicate or modify targeted genetic regions. In this context, CRISPR/Cas9 provides a formidable approach for studying complex, polygenic disorders such as schizophrenia. Using CRISPR/Cas9, scientists can develop biomedical technologies, such as animal models, epigenome-editing tools, and induced pluripotent stem cells, that can model specific genetic variants implicated in schizophrenia. These tools will magnify and isolate these mutations, providing scientists with a clearer view of the intricacies of this mental disorder. However, this genome-editing tool is still in the research stage and has recently been discovered, making it essential to carefully consider its scientific challenges, limitations, and ethical implications.

KEYWORDS: Medical and Health Sciences, Psychiatric Diseases, Schizophrenia, Genetics, Polygenic Risk Variants, CRISPR/Cas9 Genome Editing.

■ Introduction

Schizophrenia is a crippling mental disorder that affects 1 percent of the world's population.¹ This illness affects an individual's thoughts, emotions, actions, and feelings by altering their perception of reality.² This psychiatric disease is polygenic, meaning that thousands of genetic mutations are suspected of contributing to the risk. As of now, schizophrenia remains a challenging mental disorder; a definitive cure that erases damage on a genetic level has yet to be discovered. While current treatment options can help manage symptoms and improve quality of life, they cannot remove the root cause of this disease, which lies in the patient's genes. The large number and complexity of the genetic factors that bring about schizophrenia make it so difficult for researchers to study; each patient can be diagnosed with a different combination of mutations, triggering different effects. However, an emerging genome-editing tool, CRISPR/Cas9, offers an opportunity to correct and replicate genetic mutations associated with schizophrenia. Studies in animal models and cell culture systems are already providing valuable insights into how CRISPR/Cas9 can be applied safely and effectively. By precisely targeting and modifying genes, researchers could use CRISPR/Cas9 to develop mouse models, epigenetic editing tools, and induced pluripotent stem cells (iPSCs) that incorporate genetic variants identified in patients with schizophrenia, thereby providing valuable insights into disease mechanisms.³ These CRISPR/Cas9-based mechanisms will allow researchers to isolate specific linked mutations and test their effects on neural function on a larger scale. With a better understanding of schizophrenia, genome editing could potentially revolutionize the way schizophre-

nia and other complex diseases are treated, shifting the focus from symptom control to true cure and prevention in the future. Although this emerging technology is still grappling with ethical concerns and scientific limitations, increased research and funding in the future could further develop this tool into a highly effective method for studying and potentially treating schizophrenia. In this review, I will provide an overview of the CRISPR/Cas9 genome-editing tool, followed by a discussion of its applications for modeling and studying schizophrenia. Finally, I will examine the therapeutic potential of CRISPR/Cas9 in schizophrenia, along with the current limitations and challenges associated with its use.

The overview of schizophrenia:

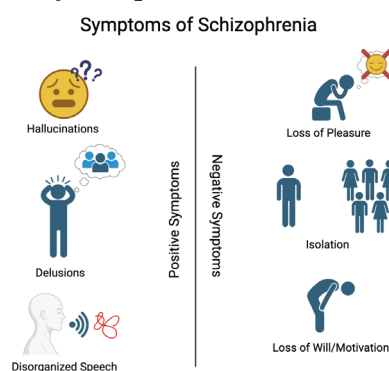


Figure 1: Symptoms of Schizophrenia. The figure above depicts common symptoms that are indicative of schizophrenia. The left side classifies positive symptoms: hallucinations, delusions, and disorganized speech; and the right side classifies negative symptoms: loss of pleasure, isolation, and loss of will/motivation, of schizophrenia. Created in Biorender.

Schizophrenia is characterized by positive and negative symptoms that can influence a patient's thoughts, perceptions, speech, affect, and behaviors, as depicted in Figure 1.² Positive symptoms include hallucinations, particularly auditory hallucinations involving voices that communicate with or about the patient, and paranoid delusions. Negative symptoms include reduced emotional expression, loss of pleasure, loss of will and motivation, and social withdrawal.⁴ Schizophrenia is also characterized by disorganized thoughts, which are highlighted in speech and behavior. Disorganized speech may range from loose associations and rapid movement among multiple topics to speech so muddled that it resembles schizophrenia (commonly referred to as “word salad”) (add any paper?). This disorganized behavior that schizophrenia causes may lead to difficulties in performing daily living activities, such as preparing meals or maintaining personal hygiene. It can also manifest as childlike silliness or outbursts of unpredictable anger and irritation. Schizophrenia is also associated with death rates that are two to three times higher than those seen in the general population. This surplus in deaths is accounted for by a combination of an increased risk of suicide, unhealthy lifestyles, and inadequate healthcare that schizophrenia patients go through.⁵ Many individuals with schizophrenia experience mood-related symptoms such as depression and anxiety, which may contribute to the 10% lifetime incidence of suicide in schizophrenia.⁴

Mutation	Main Function	Relation to Schizophrenia
DISC1	helps regulate brain development, neuron growth, and neuron connection	can disrupt communication between neurons involved in memory, emotion, and reasoning
FMR1	controls synapse development and protein production at synapses	changes in the gene may affect neuron communication linked to schizophrenia
NRG1	involved in brain development, myelination, and neuron communication	can cause abnormal brain wiring and disrupted neuron signaling
3q29 deletion	contains genes involved in mitochondrial and neuron function	linked to mitochondrial inflexibility and increased schizophrenia risk due to malfunctioning neurons
22q11.2 deletion	contains multiple genes important for dopamine regulation, neuron migration, and synaptic communication	disrupts many aspects of brain function, greatly increasing the risk of schizophrenia
CASKIN1	sets up signaling complexes at synapses	may disrupt communication between neurons
15q11.2 microdeletion	affects genes involved in synapse regulation and neuronal signaling	can increase the risk of schizophrenia by affecting synapse development and brain signaling

Figure 2: Table of Genetic Mutations Associated with Schizophrenia. The table above lists some genes and chromosomal deletions that are associated with schizophrenia. It explains how each gene functions normally in the brain, along with the reason why variations in these genes contribute to schizophrenia. Created in Google Sheets.

Schizophrenia is a complex mental disorder with no single known cause. However, among the various contributing factors - environmental, genetic, and psychosocial - genetics appears to be the most significant one. An individual's likelihood of developing schizophrenia is six times higher if they have a close relative with the genetic variations of this disease.² A combination of genetic mutations called Single Nucleotide Polymorphisms (SNPs) contributes to the development of schizophrenia. Some of these SNPs are outlined in Figure 2. For example, Callicott *et al.* studied 12 genetic variants in Disrupted-in-Schizophrenia 1 (DISC1), which plays a crucial role in brain development.⁶ Some DISC1 mutations can

disrupt the growth and connectivity of neurons in the hippocampus and prefrontal cortex, which play crucial roles in memory, emotion, and reasoning.¹¹ The study found that a common variant named Ser704Cys in DISC1 was found in patients with schizophrenia more often.⁶ The FMR1 gene works closely with the DISC1 gene to control how neurons connect and communicate. A disruption in this gene can influence the development of schizophrenia, as many patients have an altered amount of FMRP (a protein produced by FMR1).⁷ Another candidate gene is Neuregulin 1 (NRG1), which encodes a protein involved in multiple brain processes, including brain development, myelination (insulating layers around nerve fibers that speed up electrical signal transmission), and neuronal communication.⁸ Certain variants in the NRG1 gene have resulted in changes in brain development, causing abnormal neural wiring and disrupted neuron communication and myelination, which are distinct characteristics of schizophrenia. Problems with some neurotransmitters, such as dopamine and glutamate, which allow neurons to communicate with each other, may also contribute to the pathophysiology of schizophrenia.² The 3q29 deletion is another strong indicator of an increased risk in many psychiatric disorders, including schizophrenia.⁹ Specifically, researchers Purcell *et al.* found associated dysregulation of mitochondrial function in mouse models. Tests showed that cells lacked mitochondrial flexibility, meaning they were unable to adapt their energy use when conditions changed.¹⁰ Additionally, patients with a small deleted region on chromosome 22, called the 22q11.2 deletion, have 30% increased risk of developing schizophrenia, as it gets rid of many genes important for brain development. The genes in this deleted region help break down dopamine in the brain, regulate microRNAs (which control gene expression), play important roles in neuronal communication, and influence how neurons assemble during brain development.¹¹ A protein called CASKIN1 helps set up signaling pathways at synapses (the connection points between neurons). A mutation in the gene that codes for this protein can pose a great risk to brain function and can aid in the development of schizophrenia.¹² Lastly, a small deletion on chromosome 15 called the 15q11.2 microdeletion is closely related to schizophrenia as it erases genes that aid in brain development and neuron communication.¹³ Overall, the role that these mutations play in increasing schizophrenia risk further solidifies that this psychiatric disease is polygenic.

As of now, there is no definitive cure for schizophrenia, but it can be treated and managed in a few ways, such as antipsychotic medications and psychotherapy. Psychotherapy is a form of treatment for emotional, behavioral, and mental problems in which a trained therapist works with a patient to help them understand themselves better and develop healthy coping strategies.¹⁴ One type of psychotherapy that has been proven to be effective for schizophrenia is Cognitive-Behavioral Therapy (CBT). CBT for schizophrenia is adapted to address psychotic symptoms, especially delusions, hallucinations, and thought disorders, and to help patients develop insight into their condition, reduce distress from symptoms, and improve coping skills.¹⁵ Antipsychotics are medications primarily used

to manage psychosis, especially in conditions including schizophrenia and bipolar disorder. They are used to reduce positive symptoms such as hallucinations and delusions and help stabilize mood. Since dopamine overactivity is linked to psychotic symptoms, antipsychotics work by blocking dopamine D2 receptors in the brain.¹⁶ However, despite some of these available treatment options, schizophrenia remains a condition that can not be fully cured. These therapies primarily focus on controlling symptoms and improving daily functioning rather than studying and targeting the root genetic factors responsible for the disease. The exact cause of schizophrenia is still not understood, involving a complex mix of genetics, which makes developing a definitive cure challenging as of now.⁶

The overview of the CRISPR/Cas9 system:

CRISPR/Cas9 consists of a DNA array and Cas proteins that cut DNA at specific sites. The CRISPR/Cas9 array consists of short, palindromic repeats separated by unique spacer sequences derived from foreign DNA.⁷ Different regions of the CRISPR/Cas9 array are copied into RNA, which eventually become CRISPR/Cas9 RNA (crRNA) and trans-activating CRISPR/Cas9 RNA (tracrRNA). These two molecules guide the CRISPR/Cas9 system to cut specific DNA sequences.⁸ The crRNA is a complementary strand to the spacer retrieved from foreign DNA stored in the CRISPR/Cas9 array. The crRNA, tracrRNA, and Cas9 counter the DNA of attacking viruses. The guide RNA (gRNA) is made up of crRNA and tracrRNA.⁸

CRISPR/Cas9 is an immune system found in many bacteria and archaea.⁷ When a bacterium is infected by a bacteriophage, the CRISPR/Cas9 system cuts a segment of viral DNA and stores it in the CRISPR/Cas9 array as a new spacer. Each time the same bacteriophage reinfects, the bacterium can destroy it using its guide RNA and Cas9. The CRISPR/Cas9 system compares the virus's DNA with all of its spacers. If the DNA matches one of the spacers, the bacterium can identify the invader as a threat. The guide RNA guides Cas9 to the bacteriophage, where it cleaves the DNA on both strands, creating a double-strand break (DSB). DSBs eliminate the virus because it lacks its own DNA repair mechanisms.⁹

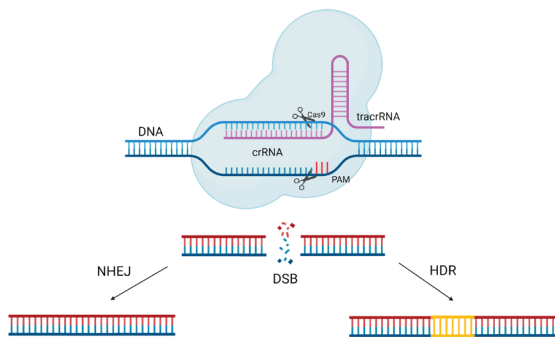


Figure 3: CRISPR/Cas9 DNA Cutting and Repair Pathways. Cas9, guided by crRNA and tracrRNA, binds to a target DNA sequence next to a PAM site and creates a double-strand break (DSB). The DNA is then repaired either by non-homologous end joining (NHEJ), which joins the broken ends together, or by homology-directed repair (HDR), which allows the deleted DNA to be replicated. Created in Biorender.

The CRISPR/Cas9 system, originally discovered as a bacterial immune response mechanism, has been adapted into a genome-editing tool to treat genetic diseases. To apply the CRISPR/Cas9 system in genetic engineering, scientists artificially modify the CRISPR's guide RNA to match the targeted cell in an organism's genome.¹⁰ The guide RNA directs Cas9 to the location of the mutation in the organism's DNA, where it searches for the protospacer adjacent motif (PAM), a short sequence downstream of the target site.⁷ When it recognizes PAM, Cas9 checks the region above. If it locates the target provided by the gRNA, it will create a DSB. The DNA will then replace the missing mutated sequence with the correct sequence, using the second strand to make a copy. DSBs are repaired by either homologous direct repair (HDR) or non-homologous end joining (NHEJ).⁸ NHEJ is simple and random, while HDR is much more accurate and complex.⁹ HDR uses identical donor DNA to repair DNA damage, and NHEJ joins broken DNA ends, often resulting in many missing or added DNA segments.¹¹

Gene therapy has long shown potential to correct a variety of human diseases and defects. The discovery of CRISPR/Cas9 and its use as a gene-editing tool have revolutionized the field of gene therapy. Much of this enthusiasm stems from the accuracy and specificity with which CRISPR/Cas9 can treat human disease and edit the human genome.⁷

Application of CRISPR/Cas-9 in Schizophrenia Research:

Schizophrenia is a polygenic disorder, meaning hundreds of genetic variants contribute to the risk together but have small impact sizes individually.¹⁸ The sheer number and size of these mutations hinder researchers' ability to examine their effects and learn more about this obscure disease. The CRISPR/Cas9 system offers a solution to this problem, enabling precise and accurate editing of genetic sequences through multiple approaches, including animal models, induced pluripotent stem cells, and epigenome-editing tools.³ With the help of these genetically engineered tools, researchers can segregate specific genes that are suspected to increase schizophrenia risk and study their implications individually.

Mice are commonly used to model the human genome because their genes are highly similar to those of humans, and their brain structure closely resembles that of humans. To replicate a schizophrenia-associated mutation in a mouse, scientists use the CRISPR/Cas9 genome editing tool.

They insert a Cas9 protein, a guide RNA, and a repair template into a fertilized mouse egg. Cas9 cuts the DNA to produce the genetic variant, and the cell repairs it using the repair template. The edited egg is implanted into a surrogate female mouse to be carried to term. The first generation may contain a mixture of edited and unedited cells, so they are often bred together to produce stable experimental outcomes. As these mice develop, scientists carefully examine the changes in brain structure, protein production, gene expression, and behavior.¹⁹ In 2019, Baba *et al.* used CRISPR/Cas9 to delete the 3q29 region in mice to replicate the schizophrenia-associated 3q29 deletion.²⁰ These edited mice showed 50% lower expression of most genes in the 3q29 region. They also exhibited a

significant reduction in body weight, consistent with growth deficiencies observed in humans with the same deletion. Social interaction, brain function, and reaction were also skewed within these mice.³ With these engineered mouse models, researchers were able to isolate the 3p29 deletion and pinpoint its exact effects. By observing changes in behavior and function, they were able to study the direct connection between the deletion and schizophrenia symptoms. In 2020, Saito *et al.* conducted the same experiment using a mouse model with the 22q11.2 deletion.²¹ With this deletion, the mice had trouble filtering out unnecessary sensory information, remembering cues in fear-based tests, and processing their vision. These behavioral changes are characteristic of individuals who have schizophrenia.³ Again, the researchers were able to narrow their focus on the 22q11.2 deletion and directly observe its contribution to schizophrenia on a larger scale. Mouse models like these give researchers a better understanding of schizophrenia, helping them test how specific mutations contribute to this disorder in a controlled environment. These studies demonstrate that CRISPR/Cas9-engineered mouse models can effectively replicate human genetic mutations associated with schizophrenia, thereby providing valuable tools for studying the effects of specific genes.

Epigenetics is the study of how changes in gene expression occur without altering the DNA sequence itself. These changes often involve chemical marks on DNA, such as DNA methylation, which can turn off a gene, or histone modifications, which can make DNA easier or harder for the cell to process.²² Epigenome editing enables precise targeting of these switches to turn specific genes on or off. Instead of using traditional CRISPR/Cas9, scientists employ a dead CRISPR/Cas9 tool, dCas9, for epigenetic editing. dCas9 can still be guided to a specific DNA sequence by its gRNA, but it can not cut a nucleotide. Instead, it is fused to an epigenetic effector protein that alters the chemical marks on the target DNA. For example, dCas9 could be fused to an activator, which turns a gene on; a repressor, which turns a gene off; or an epigenetic enzyme, which fine-tunes genetic activity. Since schizophrenia is linked to SNPs that cause genes to be turned on or off at the wrong times rather than to broken genes, scientists could use epigenome editing to study how a gene's activity affects neurons and to fix abnormal gene activity in lab-grown cells, which could lead to therapies in the future. In 2018, Liu *et al.* conducted an experiment using CRISPR/Cas9 to remove the chemical tags on the FMR1 gene.²³ The chemical tags on this gene silence it, preventing it from making the protein the brain needs to function properly. The researchers used the Tet1 enzyme, which can remove these chemical tags, and aimed it directly at the silenced FMR1 gene. Soon, the chemical tags on these genes were removed, and it began producing its protein again.²⁴ This study showed that epigenetic editing tools can successfully be used to reverse errors in gene regulation and restore normal neural function. By comparing neurons before and after editing, researchers can study how disrupted gene expression contributes to schizophrenia symptoms. This evidence also suggests that CRISPR/Cas9 epigenetic tools can

one day be used to correct irregular gene activity contributing to schizophrenia on a much larger scale.

Induced pluripotent stem cells (iPSCs) are lab-created stem cells derived from skin or blood adult cells by reprogramming them into pluripotent cells (cells that can specialize into any type of cell in the body).²⁶ Scientists can derive iPSCs from individuals with or without schizophrenia. Using CRISPR/Cas9, they can precisely modify a single base pair in DNA, either by targeting a specific SNP or by introducing a new SNP. This approach generates two cell populations: one with the variant and one without, both of which differentiate into neurons.²⁷ Scientists observe the differences in neuron behavior, and because everything else in the DNA is the same, any differences can be attributed specifically to that single genetic variant.¹⁸ In 2023, Wahbeh *et al.* used CRISPR/Cas9 to introduce a mutation in the CASKIN1 gene (suspected of contributing to schizophrenia) into iPSCs, which were then differentiated into neurons. When they compared the neuron with the mutation with the control, they found that the expression of 368 other genes had changed significantly. These mutant neurons fired electrical signals at rates different from those of normal neurons, affecting their development over time.³ These findings uncover the substantial effects that a single mutation can have on otherwise healthy neurons. It solidifies our understanding of schizophrenia as a genetic disorder, largely influenced by errors in DNA sequences disrupting brain function. Additionally, in 2014, Yoon *et al.* generated iPSCs from individuals with the 15q11.2 microdeletion, a known genetic variation associated with schizophrenia.²⁸ The 15q11.2 microdeletion contains a missing copy of a protein called CYFIP1, which controls the cytoskeleton. This is especially important in neurons, which have precise shapes and connections. Scientists observed that, in the absence of the CYFIP1 protein, patients' cells failed to adhere properly and were disorganized, resulting in many neurons being mislocalized.³ Again, with investigations such as these, researchers are able to deepen their comprehension of how specific mutations contribute to schizophrenia. These genetic variations need to be individually dissected to obtain a better grasp on the mechanisms of this psychiatric disorder and the changes that need to be introduced to explore better treatment options in the future.

These studies show that using CRISPR/Cas9 genome editing in iPSCs can elucidate the effects of specific schizophrenia-related genetic mutations.

Therapeutic Potential and Challenges:

The CRISPR/Cas9 gene-editing tool can be directly applied to human patients for therapeutic purposes. With its ability to precisely modify a mutated DNA sequence, CRISPR/Cas9 has the potential to eliminate inherited genetic disorders right at the source. It also has the potential to combat viral infections with a higher success rate by targeting and cleaving viral genetic material within human cells. With further research, CRISPR/Cas9 can be leveraged to precisely correct human DNA, thereby enabling the development of effective treatments for many incurable genetic disorders.²⁹

As promising as animal models, iPSCs, and epigenome editing seem, scientists cannot overlook the potential limitations and challenges these tools may entail. Self-evidently, animal models can only represent the complex behaviors of psychiatric disorders such as schizophrenia in a limited way. The modeled neural responses of these cells and animal models are not guaranteed to replicate the responses observed in patients with schizophrenia exactly.³⁰ Another challenge involves finding a safe way to deliver the Cas9 protein into cells. Researchers typically use modified viruses, such as adeno-associated viruses, baculoviral, and lentiviral vectors, to deliver the editing tools to target cells.²⁴ However, the body's immune system may recognize these viruses as threats, triggering an immune response that rejects the treatment.³¹ The potential for the complete treatment of psychiatric diseases like schizophrenia that CRISPR/Cas9 invites is still quite far from development. This advancement is relatively new and remains in the early stages of research; it has been tested primarily on laboratory animals.³² And because psychiatric diseases are influenced by many genes, it would be almost impossible for CRISPR/Cas9 to edit every single one of these genes at the stage it is right now.³³ Researchers also have concerns about the CRISPR/Cas9 editing tool, including mosaicism, off-target effects, and incomplete edits. Mosaicism can occur when not all mutated cells are edited the same way, resulting in different genetic profiles within the same individual.³⁴ Off-target effects occur when CRISPR/Cas9 accidentally cuts or edits the DNA at the wrong location. These unintended edits can disrupt essential genes, potentially leading to adverse effects.³⁵ And the edits implemented on the target can be incomplete or low-quality.³⁶

In addition to the scientific challenges posed by the CRISPR/Cas9 genome-editing tool, there are important ethical and moral concerns regarding its use in human patients. A major point of debate among researchers concerns the ethics of germline alterations, which involve making genetic changes in an embryo's DNA that can be passed down to future generations.³⁶ These changes could cause unexpected and unwanted mutations in subsequent generations, as researchers still do not fully understand the associated risks. According to Lanphier *et al.*, embryo editing could result in mosaic embryos, in which not all cells share the same genetic modifications.³⁷ In 2015, scientists in China began using CRISPR/Cas9 on non-viable embryos under strict human research regulations, such as the Regulation on Human Genetic Resources for Experimental Purposes.³⁸ However, in 2019, Dr. Jiankui He went against these regulations and edited the genes of viable embryos to disable the CCR5 gene, which HIV uses to infect cells. These edited embryos were implanted despite concerns about potential additional health risks.³⁹ This event sparked major controversy. Overall, we still do not fully understand how the CRISPR/Cas9 gene-editing tool might affect complex diseases, such as schizophrenia, making ethical considerations even more crucial.

■ Conclusion

Schizophrenia remains a complex psychiatric disorder with thousands of genetic links and no definite cure. This review

highlights how the CRISPR/Cas9 gene-editing tool provides a powerful new approach to studying the genetic factors that contribute to schizophrenia. Specifically, researchers can use CRISPR/Cas9 in animal models, induced pluripotent stem cells, and epigenome editing to closely examine how specific genetic mutations affect brain development and function. Although we still need to consider major limitations, such as off-target effects, immune reactions, and the polygenic nature of schizophrenia, ongoing advances in this tool suggest a promising future for its research contributions. The future direction of this developing technology should include improving the precision and safety to ensure its accuracy and reliability. Specifically for the CRISPR-based mouse models, epigenetic editing, and iPSCs, a larger number of genetic mutations should be tested to map out the contributors to schizophrenia. Overall, CRISPR/Cas9 holds the potential to advance schizophrenia research, improve our understanding of its intricate genetic connections, and pave the path for future therapeutic breakthroughs.

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