CRISPR-based therapeutic targeting of ADHD

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Abstract:

Attention-deficit/hyperactivity disorder (ADHD) is characterized as a complicated condition that likely involves at least two genes. Hence the genetic linkage, CRISPR can be a potential cure for treating the attention deficit hyperactivity disorder. In most cases, it's believed that the genes you acquire from your parents play a key role in developing the disorder because ADHD tends to run in families. According to research, those with ADHD are more likely to have parents or siblings who also have the disorder. It is also thought that non-genetic factors including aberrant brain development, brain damage, or environmental factors may contribute to the illness. Children with ADHD are too impulsive, restless, and easily distracted, and they struggle both at home and at school. For now research suggests that treating ADHD with gene therapy and CRISPR may be possible. Besides all, there are still ethical issues researchers face, whilst using CRISPR technologies. The purpose of this research review paper is to report on the newest updates regarding the usage of CRISPR/Cas9 in the treatment of ADHD.

Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR) is a genetic modification technique that has opened the doors for many gene editing opportunities. CRISPR continues to revolutionize medicine as a growing potential solution to a range of genetic diseases such as neurodegenerative diseases and blood disorders [1]. Attention deficit hyperactivity disorder (ADHD), is a neurological disorder that is characterized by inattention and hyperactivity-impulsive behavior. In the context of ADHD, the parts of the brain that help us plan, focus and execute tasks are compromised. The symptoms of ADHD vary by subtype - inattentive, hyperactive or combined. The causes and genetic links remain elusive. However, the biological hallmarks of the disorder are dysregulated dopamine signaling. Genetic inheritance has been implicated with ADHD, with

one-third to one-half of individuals with ADHD bearing offspring with the disorder [2]. Given the genetic association, ADHD may be therapeutically targetable with CRISPR-based gene editing [3]. Currently, the most prominent medication for ADHD is called methylphenidate, a neuro stimulant [4]. Methylphenidate specifically targets the dopamine receptors. However, a drawback of methylphenidate and other ADHD medication is that it can be misused, which may lead to addiction [5]. Given this strong association with dopamine signaling, this review will focus on genes involved in this process for CRISPR targeting and the therapeutic potential of CRISPR for ADHD.

History of CRISPR

CRISPR and the endonuclease Cas9 was first identified in *E.coli*, by Japanese scientist Yoshizumi Ishino and his team in 1987 (Figure 1). They accidentally cloned an unusual series of repeated sequences interspersed with spacer sequences while analyzing a gene responsible for the conversion of alkaline phosphate [6]. The role of CRISPR as a safeguard for bacteria against bacteriophages was elucidated in 2007 (Figure 1) [7].

Taking advantage of this bacterial immune system, scientists engineered the CRISPR system for scientific use. CRISPR uses a specific sequence of DNA and its associated endonuclease Cas9 to edit the base pairs of a gene. Compared to other genetic modification methods (i.e. TALENS), CRISPR works in more precisely by cutting DNA using the Cas9 enzymes, and allowing the natural DNA repair processes to take over. To simplify, this genetic modification method consists of two main parts: the Cas9 enzyme and the guide RNA [8].

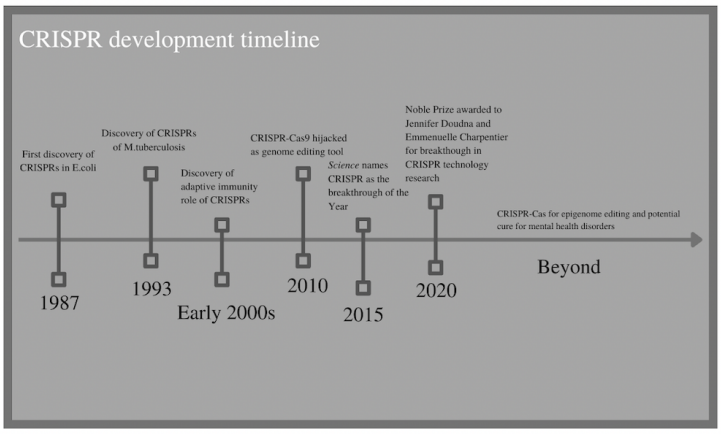

Figure 1: Historic timeline of CRISPR

Applicable uses for CRISPR today are experimentation with gene-editing mosquitos in order to reduce, and hopefully eradicate, the risk of malaria. Apart from using CRISPR in the hopes of creating malaria-resistant mosquitoes, researchers have been trying to use CRISPR to engineer agricultural crops that can withstand climate change, and in human trials to treat a range of diseases, including ADHD [9].

Therefore, CRISPR's potential for genome editing from climate change resistant crops to treating mental health disorders, make it a powerful tool. CRISPR-Cas systems have catalyzed gene

modification in the brain. In most cases, endogenous genes can be precisely edited in mitotic cells and postmitotic neurons. This genetic modification technique can and will deepen our understanding of gene functions in the brain [10]. Precise editing is only possible if the heritability rate is high enough to find the gene responsible for causing the disorder. Overall, CRISPR, compared to other gene editing methods, has been used on the brain in a number of various clinical trials. An example of using CRISPR in the brain, is with the case of Alzheimer's disease. With Alzheimer being a progressive neurodivergent health condition, the usage of a CRISPR system could be used to target the single-gene mutations associated with Alzheimer's disease, for example Amyloid Precursor Protein on chromosome 21, (APP) [11]. Even though this revolutionary gene editing tool could be used to treat those with Alzheimer and other neurological disorders, the question still remains on how to deliver CRISPR gene therapy to the brain.

Technical and biological limitations exist currently with the CRISPR system in the brain. For example, the Cas9 enzyme does not translate easily when applied to mental illnesses and the brain because stem cells and mature neurons have a strong response to DNA damage from the endonuclease Cas9, resulting in toxicity [12]. This highlights the limitations of genetic engineering and the complexity of the human response system and how far we have come from accessing the central nervous system.

CRISPR associated reagents and the Blood Brain Barrier

As mentioned, there exists limitations in implementing the CRISPR system for neuronal targeting. One of the major limitations is delivery of the proposed therapy to the brain and overcoming the blood-brain barrier (BBB), a pivotal immunological front of cells that protects the central nervous system from unwanted contents in the bloodstream. The BBB's function is to isolate and protect neural tissues, controlling the entry of molecules from blood to the brain [13]. As a result, the BBB hinders attempts to deliver molecule-based therapies.

Currently, lipid-based nanoparticlesistorically been studied and used to cross the BBB. The two most common lipid-based nanoparticles are a non-viral based nanoparticle which encompasses lipid formatted encapsulates and a viral-based nanoparticle (i.e. a modified viral particle is used). Non-viral based nanoparticles are often sufficient to cross the BBB, but lack efficiency for encapsulating the CRISPR-associated reagents and delivering them to neuronal cells. Viral-based nanoparticles, derived from plant viruses, are more efficient at crossing the BBB and delivering the encapsulated system to neuronal cells. However, the viral based method allows for the potential to be recognized and trigger the immune system. Due to the undesirable genetic mutations and immunogenicity caused by the current CRISPR-Cas9 brain delivery systems (lipid-based) [14]), we

cannot deliver CRISPR systems to the brain. Not only do current delivery systems cause undesirable genetic mutations, but they are difficult to produce on a large scale, which is necessary for clinical use. On the other hand, nonviral nanoparticle-based vectors are whereas nonviral nanoparticle-based vectors are often nonimmunogenic and allow for easier bulk production.

Outside of lipid-based nanoparticles, there has recently been development in utilizing polymer-based nanoparticles, cross-linked GOLD nanoparticles, and exosomes from cancerous cells loaded with CRISPR-Cas9 systems. Cancer derived exosomes are currently favored because they are non-invasive and efficient. Overall, the usage of nanoparticle-encapsulated CRISPR-Cas9 technology is needed to catalyze gene therapy of brain disorders. While the challenge remains in crossing the BBB, it may be helpful to take advantage of the heterogeneity of the BBB.

While some delivery systems like lipid-based nanoparticles and newer systems like cancer derived exosomes show a lot of promise for CRISPR delivery, there is still a need for further optimization. Achieving effective delivery of CRISPR reagents in the brain would not only benefit therapeutic targeting of ADHD, but many other neurologic and psychiatric disorders.

Ethical Concerns of CRISPR

With the rapid application and usage of this genetic modification technique in clinical research, we must consider accessibility and cost, the need for controlled clinical trials, as well as policies regarding the usage of CRISPR. Disregarding these aspects creates another limitation for implementation of CRISPR; ethical concerns. Controlled clinical trials are crucial; especially because the Cas9 enzyme can lead to off-target effects, including cellular toxicity and cell death [15].

Current ethical concerns about CRISPR and genetic engineering focus on the modification of human offspring and oneself for non-health or cosmetic purposes [16]. Once an aspect is genetically modified with CRISPR, there exists high chances the change is permanent and will persist the rest of the individual's life. If the individual gives rise to an offspring this modification will be passed onto the offspring - and the consequences of that, as of now, are unknown. Direct genetic manipulation of embryonic offspring does appear to be very promising in preventive care to minimize the potential for inheritable disease. However, growing concerns focus on the possibilities of turning from using CRISPR as preventive health care to manipulation of genetics for trivial purposes.

A study was performed in the context of Huntington's disease, a genetic disorder which causes degeneration of nerve cells in the brain and invariably ends in an early death, with the implementation of CRISPR for its ablation [17]. Jeff Carroll, who just married with his wife, had to make the drastic decision of not having children [18 . He had just found out he has a mutation that causes Huntington's disease [19].

Since Carroll's mother has the disease, he knew he was destined to develop it as well. Huntington's disease has a 50% probability of being inherited by the offspring, Jeff Carroll and his wife decided not to have children. Due to the process referred to as pre-implantation genetic diagnosis or PGD, Carroll and his wife were able to almost minimize the possibility of transferring the mutation to their offspring by using in vitro fertilization (IVF) and screening the embryos. They made the decision to try it, and in 2006 they gave birth to twins free of the Huntington's mutation [20].

Now, as rapid developments of CRISPR occur, Carroll is a researcher at Western Washington University and tackled the usage of CRISPR gene editing. He has not been using CRISPR in human embryos, instead he experiments with gene expression of the gene responsible for Huntington's disease in murine cell culture. Overall, CRISPR and genetic engineering remains controversial with its usage in human embryos, despite the growing evidence of its preventive care potential such as in Carroll's Huntington's case. Although using CRISPR to eradicate Huntington's disease sounds promising, using CRISPR in human embryos still raises many concerns and there must be regulations implemented in order to use it for more complex purposes in the future.

The Biology of ADHD

ADHD is characterized as dysregulated dopamine signaling and it is often treated with stimulants. CRISPR could be used to treat ADHD by targeting the same components of the central nervous system that the ADHD medication stimulates. Medication now for ADHD improves attention by helping normal brain chemicals/neurotransmitters (e.g. dopamine) work better. Therefore, it would be best to target genes relating to dopamine production and signaling. These genetic modifications may result in the same therapeutic effects, including aiding attention and cognition abilities, similar to those as medical stimulants.

With CRISPR, similarly to the medication stimulants on the market, one could target two brain chemicals, dopamine and norepinephrine. These biological chemicals are pivotal signals to the central nervous system and affect a person's attention and concentration [21].

Based on previous reports, the human D_4 dopamine receptor is most often disrupted in the context of ADHD. The D_4 dopamine receptor is a synaptic neurotransmitter receptor responsible for neural signaling in the mesolimbic system of the brain, an area of the brain that regulates emotion and complex behavior [22].

Therapeutic targeting of the D_4 dopamine receptor has received immense attention over the last couple of years, not only for its quality to treating ADHD. The D_4 dopamine receptor is also a pharmacological target for the treatment of schizophrenia, Parkinson's disease and depression [23]. - encompassing potential cures for a range of neuro-associated disorders. As mentioned, along with

dopamine we would also focus on norepinephrine. ADHD-associated brains exhibit low levels of these neurotransmitters. Norepinephrine is closely associated with dopamine. Dopamine is described as the neurotransmitter that helps control the brain's reward and pleasure center. The ADHD brain has impaired activity in four functional regions of the brain due to this dysregulation [24]. .

In addition to targeting the dopamine receptor genes, there may be potential in targeting genes associated with dopamine signaling (non-receptor). There have been non-receptor genes uncovered that are involved in dopamine production and transport: catechol-O-methyltransferase (COMT), an enzyme that produces dopamine (the neurotransmitter/ signaling chemical), and transporter SLC6A3. Targeting these genes in addition to dopamine receptors, there is potential to modulate the production and transport of dopamine-containing vesicles of the presynaptic neuron.

Hereditary of ADHD

ADHD has been demonstrated to be a highly heritable neurodevelopmental disorder with childhood onset. Children with ADHD show hyperactivity in a range of brain regions. Siblings of ADHD probands have a ninefold higher chance of developing ADHD than do siblings of controls, according to a study of over 900 ADHD probands and over 1000 of their younger siblings, aged 5 to 17 (Figure 2). A more recent study reported rates of ADHD to be higher among biological relatives of non-adopted ADHD children than adoptive relatives of adopted ADHD children (Figure 2). In conjunction, adoption studies suggest that the familial factors of ADHD are attributable to genetic factors rather than shared environmental factors. Similar to the risk in control child's family, the adoptive relatives were at risk for ADHD [25].

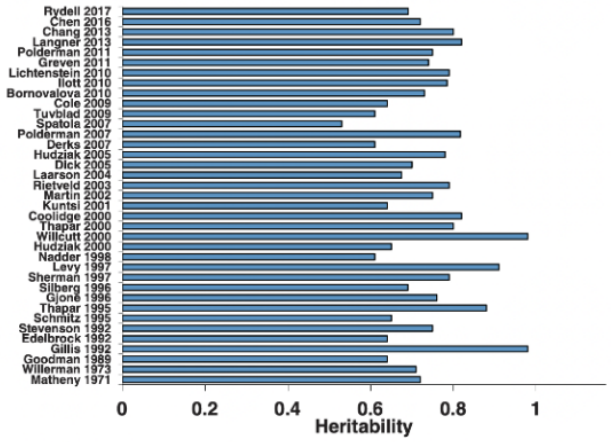

Figure 2: Heritability of ADHD in twin studies

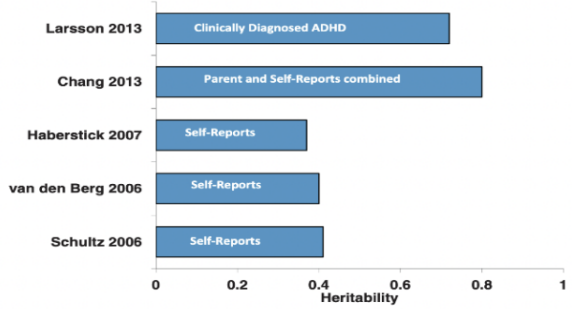

Figure 3: Assessing ADHD heritability depends on reporting strategies

High heritability estimates (70–80%) are obtained from parent and teacher reports of ADHD symptoms. Studies that used self-ratings during adolescence and adulthood, however, revealed lower heritabilities (50%) (Figure 2) .

Heritability was further investigated in two twin investigations. They demonstrated that

self-ratings and ratings from various parents and teachers within twin pairs were linked to lower heritability estimates (30–40%) than heritabilities based on ratings from the same parent and same instructor (70–80%) (Figure 2). Heritability estimates may be lower in studies of self-rated ADHD symptoms due to low reliability of self-reports. When each twin in a pair has their ADHD symptoms assessed by a different informant, rater effects (where each rater experiences and reports a different set of ADHD symptoms) or rater bias (where a rater consistently overestimates or underestimates ADHD symptoms) are introduced.

Conclusion

In conclusion, CRISPR and genetic engineering have great promise as a therapeutic intervention for mental disorders, including . Despite this revolutionary genetic modification tool facing ethical drawbacks, it still has potential to cure many mental health disorders.

Targeting the dopamine D_4 receptor and dopamine associated genes, could not only potential cure ADHD, but could also have broad implications in other neuropsychiatric disorders, such as Alzheimer's disease and depression. One of the biggest drawbacks of the CRISPR/Cas9 system to treat neurologic illnesses is delivering the CRISPR associated reagents through the blood brain barrier. With recent technological advancements, such as non-viral and viral vectors and nanotechnology, there are promising strategies to overcome that current limitation.

Overall, CRISPR has the ability to revolutionize every disease arising from DNA mutations because of its pinpoint accuracy and reasonably inexpensive production costs. This not only includes ADHD , but also curing genetic defects like sickle cell anemia and hemophilia.

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