CRISPR-Cas9 for treatment of bipolar disorder caused by gene mutation

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Abstract. Bipolar disorder is a highly debilitating psychiatric illness that is associated with great morbidity and extreme mood changes inducing emotional highs（mania) and lows (depression). Scientists have found that bipolar is a kind of complicated mental illness and difficult to fully heal. Bipolar is caused by three main factors, environmental, biological, and genetic factors. Adverse life events and environmental stress events can induce the onset of affective disorder. This bipolar disorder is one of our most heritable psychiatric disorders. It's second only to autism. The patient has abnormal central neurotransmitter metabolism and corresponding receptor function changes, neuroendocrine dysfunction, mainly the hypothalamic-pituitary-adrenal cortex axis, and hypothalamic-pituitary-thyroid axis dysfunction. Based on researches, the gene mutation is one of the pathogenic factors for bipolar disorder, and there is no therapy has been confirmed that can cure bipolar disorder so far. Thanks to the development of biotechnology, microbiologists developed a new technology. Nowadays, we try to cure bipolar disorder by CRISPR. CRISPR is a new microbiology technology by correcting mutation gene and re-editing it to reduce bipolar disorder incidence. Thus, our study provides a basis for studying gene mutation, therapy of bipolar disorder, and CRISPR technology in the future.

Keywords: Bipolar disorder, CRISPR-Cas9, gene mutation.

1. Introduction

1.1. Feature and Symptoms

Bipolar disorder is a heated health topic which more and more people focus on this complicated issue. Bipolar associated with great periodicity, mixed episodic and mood changes extremely that inducing mania (emotional highs) and depression (emotional lows) bipolar disorder, mainly due to its early onset and long-term nature during life, is associated with a greater loss of modifiable life years of labor than various types of cancer or major neurological disorders such as epilepsy and Alzheimer's disease.

Bipolar disorder is a serious disorder of mood that is associated with mental and environmental pathogenic factors. This severe psychiatric disorder, characterized by depressive, manic, and mixed episodes. Different episodes accompany individual symptoms.

Bipolar disorder is polymorphic, episodic, cyclical, mixed migratory, ebb and flows, such as three depressive periods followed by two manic periods. Intermittent period or long or short, the social function of the intermittent period is relatively normal. Still, there can also be social function damage, repeated attacks after the occurrence of fast frequency, more complex conditions.

The fourth edition of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [1] contains four main subtypes of bipolar disorder: bipolar disorder type I (episodes of depression and at least one episode of full-blown mania, this disorder can be diagnosed based on one manic episode); bipolar disorder type II (several protracted episodes of depression and at least one hypomanic episode but no manic episodes); cyclothymic disorder (many periods of hypomanic and depressive symptoms, in which the depressive symptoms do not meet the criteria for depressive episodes); and bipolar disorder not otherwise specified (depressive and hypomanic-like symptoms and episodes that might alternate rapidly but do not meet the full diagnostic criteria for any of the illnesses mentioned above.

There two main episodes of Bipolar disorder, which consisted of mania and depression.

Mania in bipolar disorder is characterized by elevated "highs" in mood and behavior in stark contrast to the depressive "lows" of the emotional cycle.

The manic episode, which the mood state is abnormally heightened and accompanied by hyperactivity, characterized by elated or irritable mood or both, and related symptoms such as hypomania, irritability, the patient has emotional upsurge, energetic performance; In severe cases, the patients have hallucinations and delusions or nervous symptoms and other psychiatric symptoms.

Clinical manifestations of a manic episode include distraction. Patients are hard to concentrate for a period; the speed of speech is accelerated, and the speech is pressed. Patients will talk more frequently and increased speech activities. Expressing excitement, patients with increased energy less fatigue, more action, and often change plant randomly and continual. They would be in a state of insomnia and eroticism exaggeration, and thoughtlessness, even compulsion and substance abuse.

Mania episodes usually last 7 days. Cases involving attacks or episodes of mania and melancholia varied from two days to one year in duration but averaged approximately six months. Transitions between relatively short-lasting mood states could occur quite suddenly, even during a single night's sleep, but that transitions were typically more gradual, with longer-lasting attacks.

Episodes of either depression characterized by low mood and related symptoms:

Loss of interest or pleasure; A sense of loss of energy or fatigue; Psychomotor retardation or agitation; Easy to have self-blame, feelings of guilt; Decreased ability to think consciously; Repeated suicidal thoughts, self-injuring behavior; Loss of appetite or significant weight loss; Hyposexuality.

Compared with mania, episodes cycles of depression (BD-II) are shorter.

In research of a composite sample of 61,392 adults from 11 countries, the overall lifetime prevalence of BP-I, BP-II, and subBP was 0.6%, 0.4%, and 1.4%, respectively. The total global incidence of BPS was estimated to be 2.4% [2].

2. Pathogenic Factors

2.1. Environmental Factor

The role of environmental risk factors in developing bipolar disorder (BD) is not well characterized. By evaluating the prevalence, duration, and predictive value of environmental exposures for BD in longitudinal studies. It is found that various environmental factors such as life stress, stressful events, weather, season, and economic status are all associated with the development of the bipolar disorder. Stress events also is related to the occurrence of a bipolar disorder. Especially, patients with depressive episode and personality and timid disposition, they lack strong ego and the sense of security, self-confidence, self-reliance. Also, they overly rely on others, care much about others, and so on. Their bad character actually is closely related to family education raise way.

Children who have been physically or sexually abused in childhood are twice as likely to have earlier onset and more severe symptoms [3]. Life experience or long-term stress are also important factors that trigger emotional changes [4].

2.2. Biological Factor

Neural biochemical, psychopharmacology, and neurotransmitter metabolism study confirmed that patients with bipolar disorder are the central neurotransmitter metabolic abnormalities and receptor function changes, such as the brain serotonin neurotransmitters content anomaly, especially serotonin (5 - HT) lack of functional activity, is the foundation of bipolar disorder, and is also easy to the quality symbol of bipolar disorder.

2.3. Serotonin

Serotonin is a kind of chemical signal called neurotransmitter cross the synapse. This process is called neurotransmission. When an action potential arrives at the axon terminal, the voltage change triggers ion channels in the membrane to open, which lets calcium ions flow into the cell. When the calcium ions bind to packages of neurotransmitter molecules called synaptic vesicles, the vesicles

fuse with the cell membrane at the axon terminal and empty their contents into the synaptic cleft. Afterwards, pieces of axon terminal membrane cycle back into the soma as new vesicles, which are refilled with neurotransmitter molecules. [5].

Serotonin impacts every part of your body, from your emotions to your motor skills. Serotonin is considered a natural mood stabilizer. It's the chemical that helps with sleeping, eating, and digesting. Serotonin also helps reduce depression, regulate anxiety; heal wounds; stimulate nausea; maintain bone strength. Serotonin is the chemical nerve cells produce. It sends signals between nerve cells. Serotonin is found mostly in the digestive system, although it's also in blood platelets and throughout the central nervous system. It is also an inhibitory neurotransmitter. In peripheral tissue, serotonin is a strong vasoconstrictor and a stimulant of smooth muscle contraction. In vivo, serotonin can be catalyzed by monoamine oxidase into 5-hydroxytryptophan and 5-hydroxyindole acetic acid and excreted in the urine. Serotonin is made from the essential amino acid tryptophan. This amino acid must enter the body through diet and is commonly found in foods such as nuts, cheese, and red meat. In the central nervous system, the synthesis of serotonin is catalyzed by TPH2. It is released in synapses, and 5-HTT controls reabsorption. Circulating serotonin does not cross the blood-brain barrier, so its activity in the brain is mediated by the synthesis, reabsorption, and adhesion of serotonin receptors. In the central nervous system, these receptors are divided into several types. Variations in the molecular structure and function of innate or acquired THP2, HTR family members, and 5-HTT can lead to changes in serotonin levels in the central nervous system. Tryptophan deficiency can lead to lower serotonin levels. This can result in mood disorders, such as anxiety or depression. A 2007 study found that people with depression often have low levels of serotonin. Serotonin deficiency has also been linked to anxiety and insomnia.

2.4. Dopamine

Dopamine is a type of catecholamine, and it is an inhibitor that inhibits body movement. Some patients with bipolar take antipsychotics, lack activity, are unemployed and live in a low-social environment, cause dopamine deficiency, manifested as anhedonia or poor mood. Patients choose substance abuse, which increases dopamine, alleviating anhedonia and poor mood. The rate of substance abuse, including alcoholism and street drug abuse, is four times higher in schizophrenia, and the rate of substance abuse is lower if subjective well-being is high while taking antipsychotics

2.5. Neuroendocrine Factor

Dysfunction of the thalamic-pituitary-adrenal axis (HPA), hypothalamic-pituitary-thyroid axis (HPT), and hypothalamic-pituitary-growth hormone axis (HPGH) is related to bipolar disorder, especially HPA dysfunction.

The hypothalamic -- pituitary -- adrenal axis (HPA or HTPA axis), also known as the limbic system, is a complex set of direct interactions and feedback interactions. The interaction between the hypothalamus (pituitary gland) and adrenal glands forms the HPA axis. The HPA axis is an important part of the neuroendocrine system that is involved in controlling the response to stress and regulating many physical activities, such as digestion, the immune system, mood and mood, sexual behavior, and energy storage and expenditure.

Many monoamine neurotransmitters play an important role in regulating the HPA axis, especially dopamine, serotonin, and norepinephrine.

2.6. Genetic Factor

Bipolar disorder (BD) is one of the most heritable mental illnesses. The heritability for bipolar disorder I is 0.75, most of which are due to common allelic variation, some of which overlap with the genes for schizophrenia [6]. Gene dominance is caused by the interaction between genes and environmental factors. According to research, the incidence of bipolar disorder in first-degree relatives of patients of bipolar disorder is several times higher than that in first-degree relatives of normal people, and the closer the blood relationship is, the higher the prevalence is.

Not only single-gene Mendelian transmission and common variant hypotheses but also multivariate threshold models and oligogenic quasi-Mendelian modes of inheritance have dominated the discussion at times.

Many genes mutations could have contributed to the disease manifestation, such as THP1, TPH2, CCL2, CXCL8, and Dynactin-1 (DCTN1) gene. Especially CCL2 and CXCL8 genes expressed at higher levels in patients with bipolar disorder as compared to healthy controls, I mainly focus on the TPH gene in these disease genes. TPH gene has two subtypes, TPH1 and TPH2 gene. The difference between these two is that TPH2 is expressed in the Peripheral region, TPH2 is expressed in the brain originally TPH1 was thought to be the only rate-limiting enzyme regulating 5-HT synthesis in vertebrates. However, Doctor Walther found that mice that had THP1 removed still expressed normal levels of 5-HT in their brainstem, hippocampus, and frontal cortex. [7]L-Tryptophan is hydroxylated by specific tryptophan hydroxylase (Tph) in a rate-limiting step to L-5-hydroxytryptophan, which is then produced by l-amino acid diphosphatase dephosphorylation. The most important enzyme that makes serotonin (rate-limiting) TPH2 is produced most significantly in the brain stem.

Variants of TPH2 with impaired enzyme activity could cause a deficiency of serotonin production and increase the risk of developing behavioral disorders.

Mutations in this gene may be associated with psychiatric diseases such as bipolar.

The mutation of the TPH gene may lead to the expression of the TPH enzyme changing, which directly affects the metabolic function of 5-HT.

3. CRISPR-Cas9 Technology

Nowadays, the usual therapy of bipolar disorder is medication; psychotherapy; electroconvulsive therapy; transcranial magnetic stimulation and managing symptoms. The CRISPR-Cas9 is a certain treatment for a bipolar disorder caused by gene mutation.

3.1. What is CRISPR

CRISPRs were first found in the Escherichia coli genome in 1987, when Ishino et al. (1987) discovered loci containing repeat sequences with an unknown function downstream from the iap gene. The CRISPR loci are observed in nearly 40 % genomes of sequenced bacteria and nearly 90 % genomes of sequenced archaea (Sorek et al. 2008). [8]

What CRISPR Cas9 does in microorganisms is to recognize an invasion, insert the virus's DNA into their genomes, and when the virus reinvades them, it cleaves the virus's DNA to eliminate the infection with its RNA. CRISPR is an immune strategy that has pitted bacteria against viruses throughout the evolutionary history of life. When the virus integrates its genes into the bacteria, it uses cellular tools to make copies of its own genes. To remove the foreign genes brought by the virus, the bacteria use the CRISPR bacterial immune system to remove the viral genes from their own chromosomes.

3.2. The Mechanism of CRISPR

CRISPR requires two components for gene editing, Cas9 nuclease and GRNA (guide RNA).

The CRISPR/Cas9 system combines CRRNA (CRISPR-derived RNA) and tracrRNA (trans-activating RNA) through base pairing to form a tracrRNA/crRNA complex. This complex directs the nuclease Cas9 protein to shear double-stranded DNA at the target site of the sequence paired with crRNA.

gRNA is an important part of the CRISPR gene knockout system. gRNA is composed of tracRNA and crRNA. crRNA guides Cas9 to the vicinity of the PAM sequence that DNA needs to edit. PAM (ProtoSpacer adjacent motif) is a sequence consisting of three pairs of bases near the 3' end of the target DNA sequence and has a sequence structure of 5 '-NGG-3'. Many targets can be found in all genes, so it has been widely used. On average, one PAM occurs every 8bp in the human genome crRNA is homologous to part of tracrRNA, and these two could be paired and bound by

complementary base pairing tracrRNA forms a linker loop and crRNA-trarcRNA chimera and binds to Cas9 CAS proteins recognize exogenous nucleotides, and invading short fragments of DNA, about 30-50 base pairs, called protospacers, are inserted into host CRISPR sites as spacer sequences separated by repeats. ProtoSpacers were derived from a region of the invading DNA with 2-5 nucleic acid structures on either side. In general, protospacers are attached to one end of the CRISPR site, and CRISPR RNA is transcribed to produce primary transcripts: Pre-crRNAs are then processed into a set of CRISPR-derived RNAs, each of which contains a sequence corresponding to the foreign DNA encountered previously.

Cas9 protein is a double-stranded DNA nuclease that can cleave target sites guided by guide RNA. Cas9 protein contains two nuclease domains that cleave two single strands of DNA. Cas9 first binds to crRNA and tracrRNA to form a complex and then binds to DNA through PAM(ProtoSpacer-Adjacentmotif) sequence and invades DNA to form an RNA-DNA complex structure, and then cleaves the target DNA double-strand and breaks the DNA double-strand. By artificial design and modification, sgRNA (SingleGuide RNA) with guiding effect was formed, which was sufficient to guide Cas9's site cleavage of DNA.

At the same time of pre-crRNA transcription, trans-activating tracrRNA (trans-activating tracrRNA), which is complementary to its repeating sequence, is also transcribed, and Cas9 and double-stranded RNA-specific RNase III nucleases are activated to process pre-crRNA. After processing and maturity, crRNA, tracrRNA, and Cas9 form a complex that recognizes and binds to the complementary sequence of the crRNA. Then, the DNA double-strand is untied to form an R-loop, making the crRNA hybridize with the complementary strand, and the other strand remains in a free single-stranded state. The complementary DNA strand of crRNA is then clipped by the HNH active site in Cas9, and the RUVC active site clips the non-complementary strand, and finally, the DNA double-strand break (DSB) is introduced. The cleavage site of CRISPR/Cas9 was located at the NGG site in the 5 '-GG-N18-NGG-3' characteristic region of the ProtoSpacer Adjacent Adjacent sequence of crRNA Adjacent to the sequence. This feature was repeated every 128bp of random DNA sequence.

The key to the success of CRISPR/Cas9 lies in the design of gRNA. There are too many non-specific results of GRNA and non-target region, and the off-target efficiency is high. Especially, 8-10bp close to PAM cannot have high homology with the non-target region. At present, to improve the accuracy of genome editing, Cas9 nuclease has been mutated, and the mutated Cas9 can only cleave one of the double strands of DNA. Two GRNAs guide Cas9 to cleave DNA, significantly improving genome editing specificity and reducing the off-target effect.

3.3. The Advantage of CRISPR-Cas9

Compared with the knockdown of the siRNA library and shRNA library, the CRISPR-Cas9 library produces phenotypic changes through the knockout of targeted genes, which can use more stringent screening criteria and remove false positivity more effectively. Moreover, it has the advantages of low cost, simple construction, and convenient operation.

3.4. The Utilization of CRISPR-Cas9 in Treatment for Bipolar

According to the DNA Learning Center, candidate genes for bipolar disorder include G72 / DAOA, DISC1, NRG1, TPH2, BDNF, 5-HTT, and DAT1.To reduce the risk of bipolar disorder, we use CRISPR to correct gene mutations. In our study, we corrected the rs78162420 mutation in the TPH2 gene. In the program, the NCBI online database was adopted to obtain the gene sequence of the TPH2 gene and the position of the mutation. Using the CRISPR-ERA website, we choose the optimal gRNA that could repair the mutation located at 72335375 on chromosome 12 by comparing criteria including E score, S score, and sequence location. Finally, we find appropriate cleavage sites for CRISPR-CAS9 and design an 80-base long DNA donor template, which assured that the broken DNA strand could be repaired. The mutant sites were splintered so that normal TPH genes synthesize normal levels of serotonin for mental health, which effectively repaired the mutation. [9]

4. Conclusion

With the rapid development and improvement of advanced biotechnology, the search for therapeutic strategies to manage bipolar disorder has become more progressive for scientists and biologists. CRISPR-Cas9 It can solve the problem of gene point mutation from the root. However, there is still some way to go to solve the problem of missing targets in reality. I believed that with more and more research on CRISPR-Cas9, the optimization of the location and method of cleaving the mutation site for the off-target problem would soon emerge.

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