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SPECIAL ARTICLE

CRISPR/Cas9 genome editing approaches for psychiatric research

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Currently, genome editing technologies, such as clustered regularly interspaced short palindromic repeats (CRISPR/Cas9), are predominantly used to model genetic diseases. This genome editing system can correct point or frameshift mutations in risk genes. Here, we analyze and discuss the advantages of genome editing, its current applications, and the feasibility of the CRISPR/Cas9 system in research on psychiatric disorders. These disorders produce cognitive and behavioral alterations and their etiology is associated with polygenetic and environmental factors. CRISPR/Cas9 may reveal the biological mechanisms of psychiatric disorders at a basic research level, translating a suitable clinical approach for use in the diagnosis and treatment of psychiatric disorders. Genetic diagnosis and treatment for these disorders have not yet been fully established in psychiatry due to the limited understanding of their heterogeneity and polygenicity. We discuss the challenges and ethical issues in using CRISPR/Cas9 as a tool for diagnosis or gene therapy.

Keywords: Autism spectrum disorder; CRISPR-Cas; gene editing; psychiatry; schizophrenia

Introduction

Genome editing (i.e., using genetic engineering to manipulate DNA sequences from one nucleotide in length to large fragments) allows the modeling of individual cells to complete organisms.[1-4] Genome editing can modify the genome of model organisms with superb resolution and includes a number of applications in basic research and biotechnology.[2-8]

This technique can be used to explore the role of genetic mechanisms of biological and medical significance. In the latter sense, genome editing tools, besides their current use to manipulate human somatic[2] and pluripotent cells,[9-13] are molecular strategies for modeling and correcting errors in mutated genes. Thus, genome editing could change the human genome to treat or develop gene-based diagnoses and therapeutics for a number of diseases and disorders.[2,14-17]

In some exceptional but paradigmatic cases, these expectations have crystallized in projects attempting to reverse or reduce the negative impact of specific mutations associated with specific diseases.[2,5,15] This interest has developed into gene manipulation/editing techniques for research on highly complex diseases or disorders.[5,14,15,18,19] This is the case in psychiatric disorders, which we will focus on in this review. We will attempt to address the scope and limitations of gene editing technologies, recognizing the inherent and vast complexity of the brain-mind relationship and the methodological challenges to be faced in research on psychiatric disorders and in potential clinical applications.

Principles of genome editing

Molecular modeling has led to the development of genome engineering techniques, such as clustered regularly interspaced short palindromic repeats (CRISPR/Cas9),[1-4,10,20-22] currently the most common gene editing system for correcting specific mutations.[15] Compared with previous techniques, the CRISPR/Cas9 editing system is simpler, cheaper,[1-4] and has greater precision and fewer off-target effects (i.e., undesired cleavages that could lead to additional mutations beyond the target region of DNA).[20] This genome editing system involves Cas9 nuclease, which produces a DNA double-strand break throughout the DNA target and complementary strand cleavage.[3,14,15] The system also uses single-guide RNA (sgRNA), which allows the binding of Cas9 nuclease

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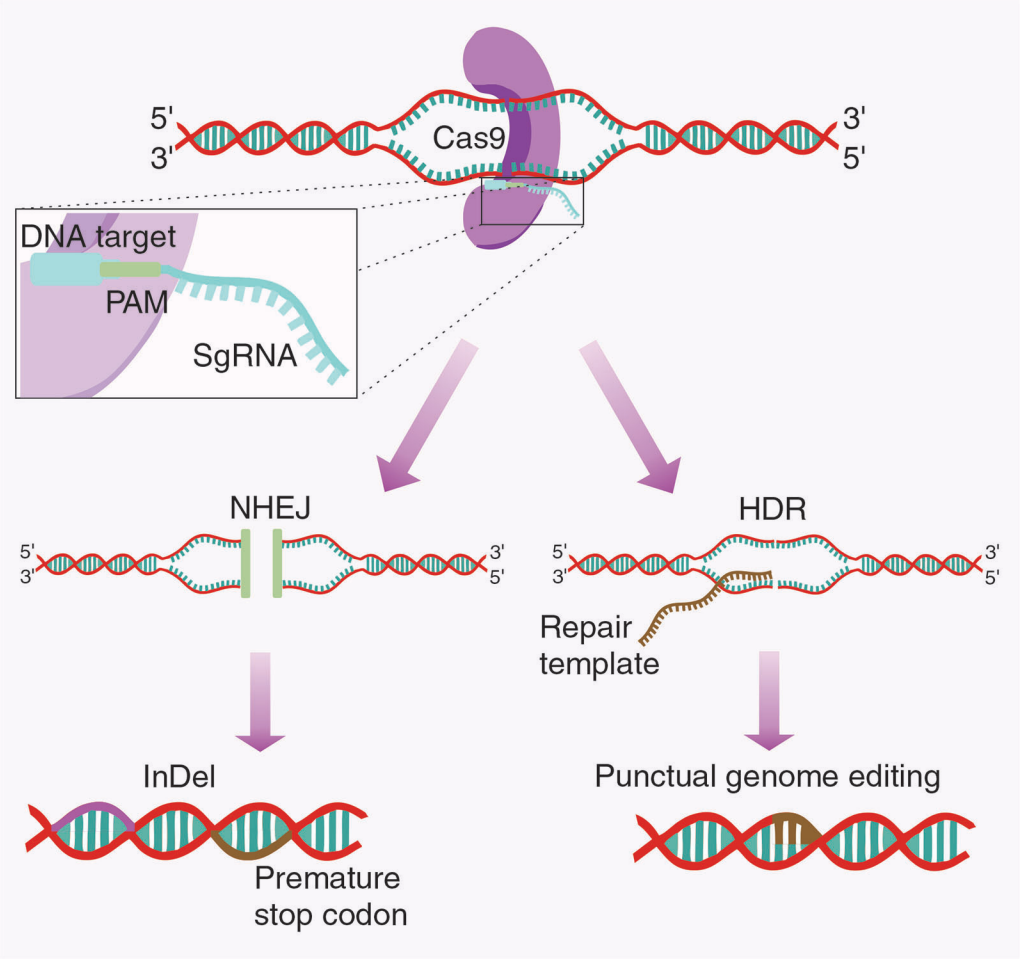

Figure 1 Mechanisms of double-strand break repair by clustered regularly interspaced short palindromic repeats (CRISPR/Cas9). The mechanism of double-strand break (DSB) is caused by Cas9 nuclease, which cleaves the target DNA. The single-guide RNA (sgRNA) and the photospacer-adjacent motif (PAM) recognize the target DNA. DSB repair can be performed through non-homologous end joining (NHEJ) or homology-directed repair (HDR). The NHEJ mechanism produces InDels (insertions and [or] deletions in the genome $<$ 1 kb) that lead to frame-shift mutations and gene knockout. The HDR mechanism precisely edits the genome only when the DNA donor template is present. This figure was created with BioRender (https://biorender.com).

to the genomic target for DNA cleavage.[1-4,14] sgRNA contains a guide sequence with a photospacer-adjacent motif at the end, which allows recognition of the DNA target sequence (Figure 1).[2-4,14,15]

The CRISPR/Cas9 system shows promise for researching and treating diseases with a considerable genetic component,[2,15,23] These diseases include polygenic illnesses, such as psychiatric disorders, in which multiple genes are affected, requiring different sgRNAs.[13] However, despite the high sensitivity and specificity of Cas9, its malfunctions, especially mismatches between sgRNA and the target DNA, could produce unpredicted off-target effects.[2,3] After the CRISPR/Cas9 system cleaves the DNA, it can be repaired by one of two mechanisms, depending on the cell type: 1) non-homologous end joining or 2) homology-directed repair (HDR) (Figure 1).[3,4,6,10] Non-homologous end joining, the most common repair method, produces InDels (insertions and (or) deletions in the genome $<$ 1 kb), leading to a loss of function in the encoded protein. The HDR mechanism is a less frequent pathway of high-fidelity DNA repair and precisely edits the genome only with an additional DNA donor template (i.e., an additional specific DNA sequence) (Figure 1).[1,2,5,15] The above- described mechanisms could have a broad range of applications, from basic research to the clinical field. However, the CRISPR/Cas9 system has mainly been used for genetic research on monogenic diseases. Nevertheless, this gene editing tool also has been used to study complex

diseases, although the focus has been on cancer rather than psychiatric disorders.

Current applications of genome editing with CRISPR/Cas9

CRISPR/Cas9 technology has aroused interest in treating genetic diseases through gain-of-function or reversion of mutations in risk genes.[8,18,24,25] Reports of clinical applications of CRISPR/Cas9 for genetic diseases have appeared in recent years, including cancer (B-cell lymphoma and leukemia, gastric carcinoma),[26] Leber congenital amaurosis,[27] thalassemia, and hematological diseases.[26] CRISPR/Cas9 allows the development of translatable animal models.[26] The most common models are of tyrosinemia, Duchenne muscular dystrophy,[2,14,28,29] Huntington's disease, Parkinson's disease,[29] or those associated with viral diseases (e.g., HIV and the hepatitis B and C viruses).[5,17,29] Likewise, the success of CRISPR/Cas9-based gene therapy in somatic cells has been described in animal models and *ex vivo* cell cultures (i.e., tissue isolated from patients for *in vitro* manipulation and reincorporation into the patient's body).[15,18] In therapeutics, aside from some exceptions, CRISPR/Cas9 has mainly been applied in the basic research area. The efficacy and safety of this DNA editing technique must be improved to translate it into the clinical field, especially in complex diseases like psychiatric disorders.[30] Studies in an animal model of Duchenne muscular dystrophy, using adeno-associated viruses as viral vehicles, have demonstrated the efficacy of CRISPR/Cas9 for gene therapy, delivered by potentially translatable methods in humans.[24,30,31] In one of those studies, the CRISPR/Cas9 system was used to revert the Duchenne muscular dystrophy gene mutation and, as a result, expression of the protein increased, partially reversing the dystrophy phenotype.[26] In recent years, there have been attempts to improve delivery in *in vivo* editing systems, including the magnetic particle/baculoviral vector complex developed to minimize the toxicity of adeno-associated viruses through CRISPR/Cas9 editing.[32] There is no evidence that delivery systems have been standardized in psychiatric research.

The efficiency and versatility of the CRISPR/Cas9 technology depend on cell type, i.e., those with a higher ability to replicate are the most appropriate.[3] This technology has been used on different cell types *in vitro*, such as induced pluripotent stem cells (iPSCs),[11,12,16,33] endothelial cells derived from the liver, lungs, and kidneys, and even neurons derived from the whole brain or specific cortical zones.[5,18] iPSCs and neuronal cells are the most relevant types for psychiatric research. As expected, most studies have focused on generating gene knockouts in monogenic diseases.[1,6,19,24,34,35] Gene editing has also been applied in polygenic diseases like cancer, using simultaneously targeted editing sites through the non-homologous end joining or HDR mechanism. For example, a combinatorial system in an *ex vivo* parallel gene editing study successfully corrected two mutations with no off-target effects in iPSCs derived from patients with β-thalassemia.[15] Another animal study on lung cancer involving HDR of genes associated with proto-oncogenes and tumor suppressor genes used a single vector with three sgRNAs to create a triple gene knockout.[18] These examples illustrate the ability of CRISPR/Cas9 to reverse or induce changes simultaneously in phenotype-associated gene mutations.[2,5,7,8] This feature could be useful in polygenic diseases such as psychiatric disorders. However, gene editing with CRISPR/Cas9 in these disorders is still challenging due to their polygenicity, as well as to environmental factors that could trigger the disorder. To apply genome editing in research on psychiatric disorders, we must first consider their heterogeneous phenotypes, complex etiology, and genotype background.

Psychiatric disorders as complex diseases

Etiology and phenotypes

Psychiatric disorders are a group of heterogeneous diseases with a high worldwide prevalence,[36,37] causing occupational and interpersonal disability[36,38] and increasing the risk of early death.[39] Schizophrenia and autism spectrum disorder (ASD), which have received the most attention from gene editing research,[40-42] involve complex phenotypes and genetic architecture. Regarding phenotype, patients with schizophrenia show positive symptoms, such as delusions, hallucinations, and disorganized behavior. Negative symptoms can also serve as diagnostic criteria, such as affective flattening, alogia, and anhedonia-asociality.[43] On the other hand, ASD patients may exhibit restrictive and repetitive behavior patterns.[44] Some symptoms include social communication and cognitive impairment, which occur in both disorders.[43-45] The phenotypic variability in schizophrenia and ASD may be due to a complex interplay of genetic and environmental components that influence symptom onset and development.[39,46,47] For example, exposure to certain environmental factors plays a decisive role in psychiatric disorders by triggering onset.[36,48,49] Thus, a critical challenge is reproducing environmental factors under controlled experimental conditions to gain insight into neurobiological mechanisms.[50,51]

Although advanced diagnostic tests have been developed over the years, psychiatric diagnosis is often only confirmed years after onset of the disorder,[36,48,49] which compromises the effectiveness of the treatment. Moreover, delayed diagnosis and partially effective treatments can negatively affect patients' personal and professional lives.[44,47,50] Pharmacological treatment of psychiatric disorders depends on various factors: 1) the stage, 2) the symptomatology, and 3) the pharmacological response. Several drugs commonly used in schizophrenia and ASD (alone or in combination) for those factors could be more costly and increase the risk of drug interactions and side effects.[9,40,50-53] Hence, treatment for schizophrenia and ASD is complex due to the disorders' phenotypic and polygenetic variability.[44,45,47,54] The latter is due to the high rate of heritability in schizophrenia and ASD (60-80%).[23,47,54] Heritability estimates show that a high percentage of the variability in schizophrenia

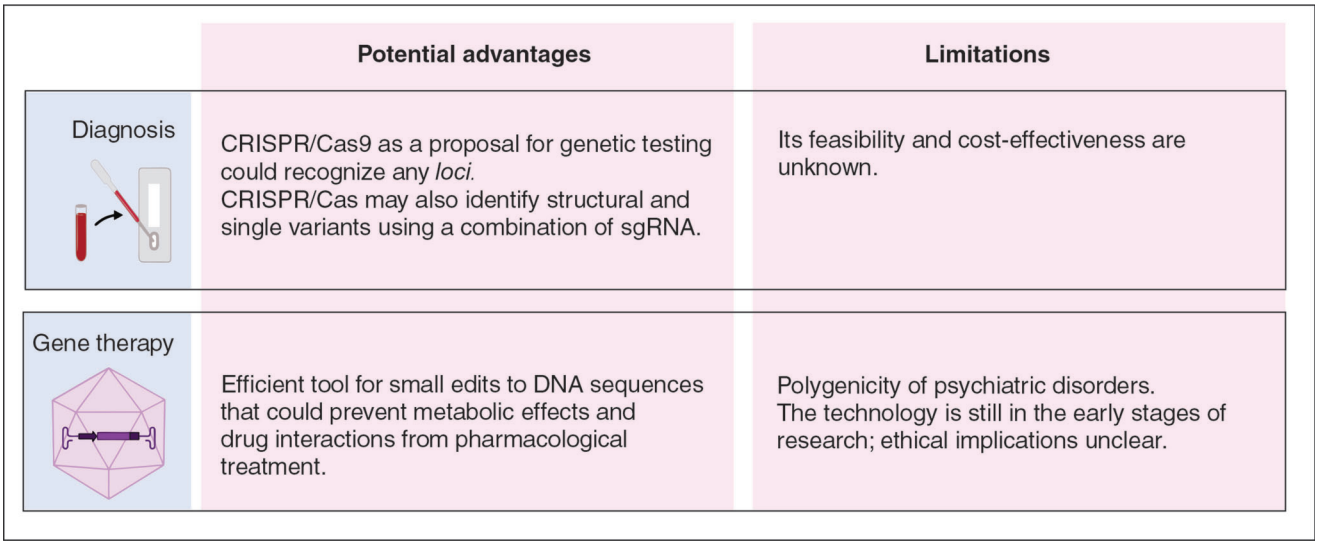

Figure 2 Potential advantages and limitations of the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) gene editing system in diagnosis and a genome editing-based therapy for schizophrenia and autism spectrum disorder. This figure was created with BioRender (https://biorender.com). sgRNA = single-guide RNA.

and ASD phenotypes could be attributed to genetic factors.[37,54,55]

Psychiatric disorders as genetic diseases

Genome sequencing or microarray-derived analysis, linked to genome-wide association studies (GWAS), identifies genetic risk variants related to psychiatric disorders.[5,23,55-57] Single nucleotide polymorphisms (SNPs), copy number variations (CNVs), and InDels[45,58] represent a significant genetic risk component associated with schizophrenia and ASD.[55,56,58] Variants may be detected early through genetic testing, thus achieving an appropriate diagnosis and a better approach to psychiatric phenotyping.[14,55] However, the polygenic nature of psychiatric disorders is a constraint, since schizophrenia and ASD share thousands of shared variants, both common and rare.[45,55,59] More critically, the functions of several genetic variants remain unknown, and a significant portion of the missing heritability that could be explained by epigenetics, gene-gene interactions, or rare variants has yet to be discovered.[45,47,59]

Recent GWAS-based evidence in schizophrenia and ASD research suggests there are variants with pathogenic relevance due to their high penetrance (i.e., a large percentage of patients with these variants present the disorder phenotype).[57,60] These relevant variants could be used for diagnostic purposes and may lead to suitable personalized treatment,[23,55] which could resolve the low efficacy and adverse effects of pharmacological approaches.[50] However, this effort is far from simple. To consider the possibility of precision medicine in psychiatry, we must first recognize the relevance of genetic testing, and a variety of commercial genetic tests are already available.[14,55] However, their use is still limited in clinical psychiatry,[55] and genetic tests require analytical and clinical validation and must predict the risk of suffering from a specific psychiatric disorder.[46,55] This risk depends on the clinical background, family medical history, and an unknown number of the patient's genetic variants. The latter is one of the greatest challenges for translating genomic information on these disorders from basic research into the clinical field.[7,55] Therefore, further studies on gene editing approaches are necessary to fully explain or detect genotype alterations in psychiatric disorders (Figure 2). For example, specific target identification by CRISPR/Cas through sgRNA would be convenient for distinguishing between variant types (CNV, SNPs, and InDels),[14] prioritizing those with the greatest pathogenic relevance.[57,60]

Gene editing technologies have been proposed and reviewed *in vitro* (e.g., in cell culture), *in vivo* (e.g., in animal models or human patients), and *ex vivo* (e.g., using tissue culture from patients) to experimentally evaluate the impact of genetic variants linked to psychiatric disorders (Figure 3).[5,15,27,30,55] The method and the model depend on the study's aims. For example, rodent models are adequate for researching gene expression variances in the brain or behavior changes.[51,52] However, to analyze modifications in higher mental and social skills, a non-human primate model is the most desirable.[5,8] Likewise, human participation is fundamental to assess drug assimilation, epidemiological research, and behavioral impairments.[51] Due to the complexity of their etiology, symptomatology, and treatment, in recent years, promising alternatives, such as CRISPR/Cas9 systems, may provide a better approach to research on psychiatric disorders (Figure 2). Such strategies may provide a better biological understanding of these disorders and could lead to new pharmacological treatments, especially for disorders those with less clinical symptomatology, raising the idea of gene therapy through precision genomic medicine.

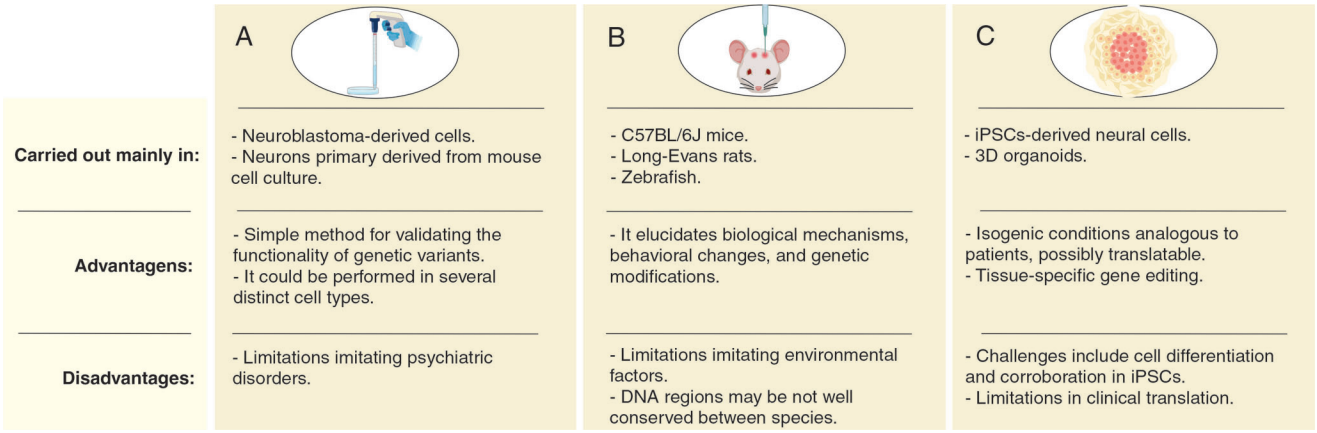

Figure 3 Generalities, advantages, and disadvantages of the clustered regularly interspaced short palindromic repeats gene editing system in disease modeling *in vitro* (A), *in vivo* (B), and *ex vivo* (C) for schizophrenia and autism spectrum disorder. This figure was created with BioRender (https://biorender.com). 3D = three-dimensional; IPSCs = induced pluripotent stem cells.

Genome editing approaches to psychiatric disorders

The polygenic nature of psychiatric disorders seems to lie mainly in several common variants with different levels of genetic penetrance. However, loci enriched with rare SNPs and CNVs may act as highly penetrant variants in psychiatric disorders, making them an adequate target for gene editing.[61] For example, current data suggest that microdeletions and microduplications of CNVs affect the expression of genes that heighten the risk of psychiatric disorders, including ASD and schizophrenia.[20,23,61,62] One of the latest approaches was reported by Kathuria et al.[21] in 2018; these researchers restructured neuronal cells from patients with ASD, rescuing the cell phenotype of *SHANK3* CNVs, which induced a decrease in synaptic signaling. The CRISPR/Cas9 system has also been adapted to simultaneously target different chromosomes to create or correct large CNVs in DNA.[22] It has been reported that CRISPR/Cas9 can efficiently edit CNVs in the 16p11.2 region, frequently found in schizophrenia. However, the efficiency of CNV editing might be compromised when longer DNA regions are targeted.[63]

Another relevant approach to the polygenic nature of psychiatric disorders might be highly penetrant SNPs that may increase the risk of psychiatric disorders.[8,18,24,31,64] Rare variants on the disrupted-in-schizophrenia-1 gene (*DISC1*) are one example of a potentially highly penetrant mutation. The *DISC1* mutation was identified in an extended family with a history of schizophrenia, with many *DISC1*-interacting proteins affected by genetic variants. Detected by GWAS, *KCTD13* is another relevant gene in psychiatry. *KCTD13* knockout by CRISPR/Cas9 in iPSCs decreased neurite formation through the NRG/ERBB pathway, which has been strongly associated with neurodevelopmental disorders like ASD and schizophrenia. Interestingly, the edited gene did not affect non-neuronal human cells.[65] Hence, the editing approach of the potential penetrant variants could produce insight into the molecular mechanisms of schizophrenia in specific tissue.[57,61,64] A final relevant example is the strong genetic association between complement component *C4* gene and schizophrenia. Sekar et al.[66] reported a striking correlation between over 7,000 SNPs related to the *C4* gene and a heightened risk of schizophrenia. This finding is also linked to the observation that during brain development the synapse pruning mechanism is impaired in mice lacking the *C4* gene.[66] This report showed that in CRISPR/Cas9-generated knockout cell lines, those that entirely lacked complement regulatory protein CD46 resulted in increased C4 deposition.[67] All of the points above encourage examination of *C4* and *C4*-related gene editing in regulatory regions.

Another promising approach to complex psychiatric disorders with variants in a number of genes could be the simultaneous editing of risk genes, which could be accomplished using different sgRNAs.[2] For this purpose, a combinatorial genetics system called CombiGEM-CRISPR was designed. The system uses a triple simultaneous gene knockout in drug targets to obtain a synergistic therapeutic effect against complex diseases (e.g., ovarian cancer and Parkinson-associated toxicity).[19] Changing the target sequence is quite simple: redesigning sgRNA is sufficient to direct genome editing to another gene, and could be derived from a sgRNA library. However, controlling and improving the specificity and efficiency of the library will be fundamental for avoiding off-target effects.[2,15] Finally, it is worth noting that, although highly specific gene editing techniques have been developed, the polygenetic nature of psychiatric disorders remains a limitation to their translation to clinical practice. For that reason, most genome editing-related studies on psychiatric disorders focus on disease modeling.

Modeling psychiatric disorders with genome editing

Animal models in psychiatry involving clustered regularly interspaced short palindromic repeats

The CRISPR/Cas9 system has been used to create or reverse phenotypes associated with complex diseases with an important genetic component, such as cancer[5,8,18]

and psychiatric disorders,[2,5,7,8] in a tissue-specific manner (Figure 3).[40,64] Approaches to psychiatric disorders have been made to imitate genetic factors.[24,50,51] Animal models may allow pharmacological testing and clarify the neuronal mechanisms of psychiatric disorders.[33,42] However, due to the role of environmental factors, animal modeling of psychiatric disorders is a challenge. Hence, these factors have not yet been included in psychiatric genome editing studies.[50,51] In zebrafish, a widely used animal model of ASD, intellectual disability, and schizophrenia,[68,69] the CRISPR/Cas9-mediated knockout of ASD-related genes, such as progranulin and *shank3b*, produced synaptic proteins, decreasing and shortening axons in motor neurons.[70,71] These neurobiological changes include motor behavior impairments,[70] reduced sociability, and repetitive behavior, all observed in patients with ASD.[71] Notably, the anatomical and behavioral data derived through gene editing in zebrafish modeling have allowed us to examine the role of neural circuits in psychiatric research.[65,69]

However, most animal studies that mimic human psychiatric diseases are conducted in rodents[8,51] due to the similarity among mammalian central nervous systems, making them a more suitable animal model in psychiatry.[8,50] According to experimental evidence, successful knock-in gene editing (i.e., substitution or addition of a gene sequence to the target gene) in *SHANK3* mutant mice showed how proteins involved in synaptic processes are recruited. Mutant mice could be a convenient model for observing the effects of *SHANK3* dysregulation, which is present in up to 15% of patients with ASD.[35] One ASD study successfully translated knockout of the human 3q29 deletion into the mouse genome, resulting in behavioral changes in the social, cognitive, and acoustic response domains. This deletion confers a higher risk for psychiatric disorders, such as ASD, intellectual disability, and schizophrenia ($>$ 40-fold).[72] In 2020, precise translational research mimicked the specific phenotype of the 22q11.2 deletion syndrome, observed mainly in patients with schizophrenia, ASD, and intellectual disability, in a region of the mouse chromosome, causing a diminished prepulse inhibition response, as observed in patients with schizophrenia.[73] To create a specific schizophrenia-like model using CRISPR/Cas9 genome editing, Lu et al.[74] produced α1,6-fucosyltransferase-deficient mice, finding increased immunoinflammatory factors in the brain,[74] which has been suggested in the phenotype observed in schizophrenia patients through positron emission tomography imaging.[75] The deletion of *RELN* (associated with schizophrenia and ASD in Asian populations) led to the creation of a mouse model with schizophrenia-like symptoms such as decreased sociability and altered neuronal migration in the cerebellum,[42] which might be a neurobiological mechanism in schizophrenia. In another knock-in study, CRISPR/Cas9 was used to rescue the pathological phenotype of gain-function in the transcription factor 4 gene, which is characterized by disrupted cortical development and is associated with a risk of psychiatric disorders and dementia.[41]

Based on this evidence, schizophrenia and ASD models may have predictive validity through behavior tests and a certain parallelism between symptoms in humans.[50,51] However, besides *in vivo* studies, *ex vivo* modeling may also be a suitable means of investigating the effects of the gene mutations observed in bipolar disorder, ASD, schizophrenia, and other developmental disorders by differentiating patient-derived iPSCs into neural stem cells, with subsequent *in vitro* study.[2,9-13,16]

Induced pluripotent stem cells and organoid models

iPSCs are widely used for disease modeling due to their properties of self-renewal and differentiation.[2,10,13] In particular, iPSC-derived neural cells from patients with various psychiatric disorders have been extended to create an approximate isogenic comparison of *in vitro* conditions analogous to those found in patients (Figure 3).[9,11-13] Genome editing of iPSCs might help explain the effect of specific variants. For example, the genetically-engineered extension of a region of the fragile X mental retardation gene through gene-by-gene editing has been tested in iPSCs to emulate Fragile X syndrome, the most common cause of ASD in males. Some of the phenotypic changes observed in this study were neurogenesis-deregulated pathways, aberrant neuronal differentiation, and high levels of inflammation glial markers.[64] However, some limitations must also be considered, such as neural differentiation, lineage corroboration, and epigenetic changes that could affect the maturation stages of iPSC cells.[11]

Applying CRISPR/Cas9 to 3D organoids[8] to model specific ASD phenotypes has also been suggested.[76] Organoids obtained through iPSC-derived neurons from psychiatric patients could emulate structural and functional features of the organs from which they were derived.[8,76] Therefore, combining 3D brain organoids and CRISPR/Cas9 may elucidate the neural mechanisms associated with psychiatric disorder phenotypes,[9] which could stimulate the development of new drugs.[11]

Limitations of gene engineering

Understanding the scope of precise genome editing in patients is complicated,[7] and cell lines and animal models can only imitate the behavior and complex physiology of psychiatric disorders in a limited way.[9,10] Moreover, the differences between *in vitro* and *in vivo* studies could lead to erroneous conclusions. One example is the deletion of glycogen synthase kinase 3 beta (*GSK3*β), whose disruption has been associated with bipolar disorder and schizophrenia. *In vitro GSK3*β deletion showed increased neurite arborization complexity in neuron cultures. In contrast, *GSK3*β deletion in mice resulted in deficient complexity and density of neurite arborization in striatal neurons.[77] Thus, corroboration at different levels is needed.

Another limitation involves methods of delivering the genome editing system to the cell due to efficacy and safety issues. Delivery is generally performed by viral vectors, such as adeno-associated viruses and baculoviral and lentiviral systems. However, these vectors may increase immune response against the editing

system.[23,24,32] Other delivery methods, such as liposomes, electroporation,[2,34] and nanotechnology,[32,78] could be used instead. A promising new technology called Prime editing, which is based on the CRISPR/Cas9 system, uses a prime editor instead of the DNA template and a prime-editing guide RNA instead of sgRNA. In this technology, unlike CRISPR/Cas9, a double-strand DNA break is not performed, which reduces the frequency of InDels.[79] Nevertheless, further discussion about this technology is beyond the scope of this review.

The challenges of gene therapy

Genetic engineering through CRISPR/Cas9 has been used to model complex illnesses, such as psychiatric disorders. However, clinical applications of mutation reversal are focused on particular diseases. Due to the considerable efficiency of CRISPR/Cas9, there is a growing interest in applying gene therapy to polygenic conditions, such as psychiatric disorders.[23,24] We propose this technology for gene therapy in psychiatry, considering the precision of CRISPR/Cas9 in HDR and with a combinatorial system. This therapy could be applied *ex vivo* to perform tissue-specific gene editing.[5,8,30] To the best of our knowledge, as a preventative treatment in psychiatry, CRISPR/Cas9 is still in the preliminary stages of experimentation in basic research (Figure 2), far from consideration as a gene therapy for complex diseases. Despite CRISPR/Cas9's high potential as a modeling and therapeutic technique, psychiatric disorders are highly polygenic, preventing the use of genome editing as a standalone tool.[69] It must be pointed out clinical use could only occur after its safety in humans had been confirmed,[30,33,80] including the prediction of adverse effects.[80] Likewise, several concerns have been raised about the use of CRISPR/Cas9 in gene therapy, such as mosaicism (i.e., two or more genetic profiles in cells of the same individual), off-target modifications,[2,3,7] and the quality of on-target editing.[7] Potential solutions include improving the efficiency of cleavage in target sites[2,3] by using minimal concentrations of Cas9 or Cas9 mutations.[13,81] Another means of increasing gene editing efficiency could be the out-crossing of cell lines or homozygous mutant animals for several generations to eliminate off-target effects.[6,82]

One final challenge to consider is the route of gene therapy administration, given the tissue-specific response and immune response to genetic engineering. Platt et al.[14] demonstrated that CRISPR/Cas9 could be used directly in mouse brains via injection in the prefrontal cortex, even in adults.[18] They used an adeno-associated virus vector containing a sgRNA with a neuronal-specific RNA-splicing factor, reducing expression of the target gene by 80% in neuronal cells.[18] Finally, Graf & Wurster[83] suggested an antisense oligonucleotide gene strategy as an alternative treatment for psychiatric disorders.[8] In this strategy, oligonucleotides are injected into the subarachnoid space of rat spinal cords. Antisense oligonucleotides bind in a complementary manner into a DNA sequence and inhibit mRNA by binding to a natural antisense transcript.[1,84] This treatment strategy has been studied for other complex diseases, such as Alzheimer's disease.[8] Considering these findings, we suggest that gene editing at a somatic level in patients, with a direct way to the brain, can outperform pharmacological treatments in crossing the blood-brain barrier, which could decrease their effects on the brain.[51] However, the mutagenic effects of genome editing in psychiatric disorders have not been sufficiently investigated at any stage of development. Moreover, we should consider the potential toxicity of gene editing in the brain or iPSC-derived neurons due to the gene delivery vehicle or the DNA-complex. Maguire et al.[85] mentioned new strategies as possible solutions, such as hybrid bacteriophage vectors or promoters of vector-mediated gene modifications, which could efficiently reduce immunity against the CRISPR/Cas9 editing-based system.[85] Given the clinical challenges and the novelty of the CRISPR/Cas9 technology in psychiatry, the risks of this system are still uncertain.[7]

Ethical considerations

In addition to the methodological and application challenges, there are ethical and moral issues related to the use of gene editing in clinical practice to be discussed. There are clear concerns about germline alterations, which could cause undesirable mutations that may transfer from generation to generation.[86,87] However, Dr. George Church reported that the modification of germlines could be considered suitable if its safety was optimized and agreed upon by the scientific community.[86] The U.S. National Institutes of Health's Somatic Cell Genome Editing program seeks to improve the safety of editing tools for different genetic diseases[7] through *ex vivo* somatic modification.[2] The U.S. Food and Drug Administration has required a series of clinical trials to be conducted before it will approve widespread use of gene therapy in psychiatric patients. Nevertheless, in a number of countries, there has been little discussion of legislation regarding genome editing for complex diseases such as psychiatric disorders.[80]

The regulation of CRISPR/Cas9 for human germline editing in embryos has developed according to different uses and purposes.[31,86] For human germline editing, it has been proposed that non-viable embryos be used to study gene editing in a more integrative and complex way.[86] However, many concerns remain about this application of CRISPR/Cas9, which involves ethical issues that are not completely clear.[7] According to Lanphier et al.,[87] genome editing in embryos could have side effects with profound repercussions due to the possibility of creating a mosaic embryo. CRISPR/Cas9 gene editing in non-viable embryos began in China in 2015,[86] following specific legislation for human research (e.g., Regulation of Human Genetic Resources).[88] Despite such regulations, in 2019 Dr. Jiankui He conducted a genome editing study in viable embryos to alter the pathway HIV uses to infect cells by knocking out the C-C Motif Chemokine Receptor 5 gene. The experiments were performed on twin embryos whose father was an HIV carrier. This study was controversial because the

embryos were implanted in the mother without regard for off-target mutations that could lead to other diseases in the newborns.[89] Based on the above, we suggest that, after corroborating the safety of gene therapy for psychiatric disorders, the CRISPR/Cas9 system could be used post-natally in humans in specific tissues.

The CRISPR/Cas9 system has been widely used to model diverse diseases *in vitro*, *in vivo*, and *ex vivo*, and applications in the medical field are currently aiming to model and treat monogenic diseases. However, the effects of this technique on complex illnesses such as psychiatric disorders are poorly understood. Our review has described advances in gene editing research on schizophrenia and ASD. CRISPR/Cas9 genome editing could contribute to the diagnosis and therapy of psychiatric disorders by providing a better understanding of their underlying biological mechanisms.

Disclosure

The authors report no conflicts of interest.

References

1 Tang XD, Gao F, Liu MJ, Fan QL, Chen DK, Ma WT. Methods for enhancing clustered regularly interspaced short palindromic repeats/Cas9-mediated homology-directed repair efficiency. Front Genet. 2019;10:551.
2 Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014;157:1262-78.
3 Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. Nat Protoc. 2013;8:2281-308.
4 Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346:1258096.
5 Eid A, Mahfouz MM. Genome editing: the road of CRISPR/Cas9 from bench to clinic. Exp Mol Med. 2016;48:e265.
6 Armstrong GAB, Liao M, You Z, Lissouba A, Chen BE, Drapeau P. Homology directed knockin of point mutations in the Zebrafish tardbp and fus genes in ALS using the CRISPR/Cas9 system. PLoS One. 2016;11:e0150188.
7 Brokowski C, Adli M. CRISPR ethics: moral considerations for applications of a powerful tool. J Mol Biol. 2019;431:88-101.
8 Feng W, Liu HK, Kawauchi D. CRISPR-engineered genome editing for the next generation neurological disease modeling. Prog Neuropsychopharmacol Biol Psychiatry. 2018;81:459-67.
9 LaMarca EA, Powell SK, Akbarian S, Brennand KJ. Modeling neuropsychiatric and neurodegenerative diseases with induced pluripotent stem cells. Front Pediatr. 2018;6:82.
10 Das D, Feuer K, Wahbeh M, Avramopoulos D. Modeling psychiatric disorder biology with stem cells. Curr Psychiatry Rep. 2020;22:24.
11 Rehbach K, Fernando MB, Brennand KJ. Integrating CRISPR Engineering and hiPSC-derived 2D disease modeling systems. J Neurosci. 2020;40:1176-85.
12 Jiang S, Wen N, Li Z, Dube U, Del Aguila J, Budde J, et al. Integrative system biology analyses of CRISPR-edited iPSC-derived neurons and human brains reveal deficiencies of presynaptic signaling in FTLD and PSP. Transl Psychiatry. 2018;8:265.
13 Michael Deans PJ, Brennand KJ. Applying stem cells and CRISPR engineering to uncover the etiology of schizophrenia. Curr Opin Neurobiol. 2021;69:193-201.
14 Uppada V, Gokara M, Rasineni GK. Diagnosis and therapy with CRISPR advanced CRISPR based tools for point of care diagnostics and early therapies. Gene. 2018;656:22-9.
15 Pellagatti A, Dolatshad H, Valletta S, Boultwood J. Application of CRISPR/Cas9 genome editing to the study and treatment of disease. Arch Toxicol. 2015;89:1023-34.
16 Hazelbaker DZ, Beccard A, Bara AM, Dabkowski N, Messana A, Mazzucato P, et al. A scaled framework for CRISPR editing of human pluripotent stem cells to study psychiatric disease. Stem Cell Reports. 2017;9:1315-27.
17 Xiao Q, Guo D, Chen S. Application of CRISPR/Cas9-based gene editing in HIV-1/AIDS therapy. Front Cell Infect Microbiol. 2019;9:69.
18 Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR, et al. CRISPR-Cas9 knockin mice for genome editing and cancer modeling. Cell. 2014;159:440-55.
19 Zhou P, Chan BKC, Wan YK, Yuen CTL, Choi GCG, Li X, et al. A three-way combinatorial CRISPR screen for analyzing interactions among druggable targets. Cell Rep. 2020;32:108020.
20 Rutkowski TP, Schroeder JP, Gafford GM, Warren ST, Weinshenker D, Caspary T, et al. Unraveling the genetic architecture of copy number variants associated with schizophrenia and other neuropsychiatric disorders. J Neurosci Res. 2017;95:1144-60.
21 Kathuria A, Nowosiad P, Jagasia R, Aigner S, Taylor RD, Andreae LC, et al. Stem cell-derived neurons from autistic individuals with SHANK3 mutation show morphogenetic abnormalities during early development. Mol Psychiatry. 2018;23:735-46.
22 Gaj T, Sirk SJ, Shui SL, Liu J. Genome-editing technologies: principles and applications. Cold Spring Harb Perspect Biol. 2016;8:a023754.
23 Zhuo C, Hou W, Li G, Mao F, Li S, Li X, et al. The genomics of schizophrenia: shortcomings and solutions. Prog Neuropsychopharmacol Biol Psychiatry. 2019;93:71-6.
24 Paquet D, Kwart D, Chen A, Sproul A, Jacob S, Teo S, et al. Efficient introduction of specific homozygous and heterozygous mutations using CRISPR/Cas9. Nature. 2016;533:125-9.
25 Genovese G, Fromer M, Stahl EA, Ruderfer DM, Chambert K, Landén M, et al. Increased burden of ultra-rare protein-altering variants among 4,877 individuals with schizophrenia. Nat Neurosci. 2016;19:1433-41.
26 Birling MC, Herault Y, Pavlovic G. Modeling human disease in rodents by CRISPR/Cas9 genome editing. Mamm Genome. 2017;28:291-301.
27 Chiu W, Lin TY, Chang YC, Lai HIAM, Lin SC, Ma C, et al. An update on gene therapy for inherited retinal dystrophy: experience in leber congenital amaurosis clinical trials. Int J Mol Sci. 2021;22:4534.
28 Long C, McAnally JR, Shelton JM, Mireault AA, Bassel-Duby R, Olson EN. Prevention of muscular dystrophy in mice by CRISPR/Cas9-mediated editing of germline DNA. Science. 2014;345:1184-8.
29 Zhang B. CRISPR/Cas gene therapy. J Cell Physiol. 2021;236:2459-81.
30 Memi F, Ntokou A, Papangeli I. CRISPR/Cas9 gene-editing: research technologies, clinical applications and ethical considerations. Semin Perinatol. 2018;42:487-500.
31 Min YL, Bassel-Duby R, Olson EN. CRISPR correction of Duchenne muscular dystrophy. Annu Rev Med. 2019;70:239-55.
32 Zhu H, Zhang L, Tong S, Lee CM, Deshmukh H, Bao G. Spatial control of in vivo CRISPR-Cas9 genome editing via nanomagnets. Nat Biomed Eng. 2019;3:126136.
33 Flaherty EK, Brennand KJ. Using hiPSCs to model neuropsychiatric copy number variations (CNVs) has potential to reveal underlying disease mechanisms. Brain Res. 2017;1655:283-93.
34 Wang P, Lin M, Pedrosa E, Hrabovsky A, Zhang Z, Guo W, et al. CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in neurodevelopment. Mol Autism. 2015;6:55.
35 Wang L, Pang K, Han K, Adamski CJ, Wang W, He L, et al. An autism-linked missense mutation in SHANK3 reveals the modularity of Shank3 function. Mol Psychiatry. 2020;25:2534-55.
36 Kahn RS, Sommer IE, Murray RM, Meyer-Lindenberg A, Weinberger DR, Canno TD, et al. Schizophrenia. Nat Rev Dis Primers. 2015;1:15067.
37 Jin H, Mosweu I. The societal cost of schizophrenia: a systematic review. Pharmacoeconomics. 2017;35:25-42.
38 Olfson M, Gerhard T, Huang C, Crystal S, Stroup TS. Premature mortality among adults with schizophrenia in the United States. JAMA Psychiatry. 2015;72:1172-81.
39 Chaste P, Leboyer M. Autism risk factors: genes, environment, and gene-environment interactions. Dialogues Clin Neurosci. 2012;14:281-92.
40 Marder SR, Cannon TD. Schizophrenia. N Engl J Med. 2019;381:1753-61.

41 Page SC, Hamersky GR, Gallo RA, Rannals MD, Calcaterra NE, Campbell MN, et al. The schizophrenia- and autism-associated gene, transcription factor 4 regulates the columnar distribution of layer 2/3 prefrontal pyramidal neurons in an activity-dependent manner. Mol Psychiatry. 2018;23:304-15.
42 Sawahata M, Mori D, Arioka Y, Kubo H, Kushima I, Kitagawa K, et al. Generation and analysis of novel Reln-deleted mouse model corresponding to exonic Reln deletion in schizophrenia. Psychiatry Clin Neurosci. 2020;74:318-27.
43 Kirkpatrick B. Understanding the physiology of schizophrenia. J Clin Psychiatry. 2013;74:e05.
44 Ivanov HY, Stoyanova VK, Popov NT, Vachev TI. Autism spectrum disorder - a complex genetic disorder. Folia Med (Plovdiv). 2015; 57:19-28.
45 Shailesh H, Gupta I, Sif S, Ouhtit A. Towards understanding the genetics of Autism. Front Biosci (Elite Ed). 2016;8:412-26.
46 Dubovsky SL. The limitations of genetic testing in psychiatry. Psychother Psychosom. 2016;85:129-35.
47 van de Leemput J, Hess JL, Glatt SJ, Tsuang MT. Genetics of Schizophrenia: historical insights and prevailing evidence. Adv Genet. 2016;96:99-141.
48 Stilo SA, Murray RM. Non-genetic factors in schizophrenia. Curr Psychiatry Rep. 2019;21:100.
49 Lai MC, Lombardo MV, Baron-Cohen S. Autism. Lancet. 2014;383: 896-910.
50 Nikvarz N, Alaghband-Rad J, Tehrani-Doost M, Alimadadi A, Ghaeli P. Comparing efficacy and side effects of memantine vs. risperidone in the treatment of autistic disorder. Pharmacopsychiatry. 2017;50:19-25.
51 Focus on psychiatric disorders. Nat Neurosci.2016;19:1381-2.
52 Cui L, Sun W, Yu M, Li N, Guo L, Gu H, et al. Disrupted-in-schizophrenia1 (DISC1) L100P mutation alters synaptic transmission and plasticity in the hippocampus and causes recognition memory deficits. Mol Brain. 2016;9:89.
53 Ocaña-Zurita MC, Juárez-Rojop IE, Genis A, Tovilla-Zárate CA, González-Castro TB, López-Narváez ML, et al. Potential drug-drug interaction in Mexican patients with schizophrenia. Int J Psychiatry Clin Pract. 2016;20:249-53.
54 Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014;511:421-7.
55 Hoehe MR, Morris-Rosendahl DJ. The role of genetics and genomics in clinical psychiatry. Dialogues Clin Neurosci. 2018;20:169-77.
56 Yuen RKC, Merico D, Bookman M, Howe JL, Thiruvahindrapuram B, Pate RV, et al. Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. Nat Neurosci. 2017; 20:602-11.
57 Trubetskoy V, Pardiñas AF, Qi T, Panagiotaropoulou G, Awasthi S, Bigdeli TB, et al. Mapping genomic loci implicates genes and synaptic biology in schizophrenia. Nature. 2022;604:502-8.
58 Modai S, Shomron N. Molecular risk factors for schizophrenia. Trends Mol Med. 2016;22:242-53.
59 Brainstorm Consortium; Anttila V, Bulik-Sullivan B, Finucane HK, Walters RK, Bras J, et al. Analysis of shared heritability in common disorders of the brain. Science. 2018;360:eaap8757.
60 Grove J, Ripke S, Als TD, Mattheisen M, Walters RK, Won H, et al. Identification of common genetic risk variants for autism spectrum disorder. Nat Genet. 2019;51:431-44.
61 Kirov G, Rees E, Walters JT, Escott-Price V, Georgieva L, Richards AL, et al. The penetrance of copy number variations for schizophrenia and developmental delay. Biol Psychiatry. 2014;75:378-85.
62 Lee KW, Woon PS, Teo YY, Sim K. Genome wide association studies (GWAS) and copy number variation (CNV) studies of the major psychoses: what have we learnt? Neurosci Biobehav Rev. 2012; 36:556-71.
63 Tai DJC, Ragavendran A, Manavalan P, Stortchevoi A, Seabra CM, Erdin S, et al. Engineering microdeletions and microduplications by targeting segmental duplications with CRISPR. Nat Neurosci. 2016;19:517-22.
64 Haggarty SJ, Silva MC, Cross A, Brandon NJ, Perlis RH. Advancing drug discovery for neuropsychiatric disorders using patient-specific stem cell models. Mol Cell Neurosci. 2016;73:104-15.
65 Kizner V, Naujock M, Fischer S, Jäger S, Reich S, Schlotthauer I, et al. CRISPR/Cas9-mediated knockout of the neuropsychiatric risk gene KCTD13 causes developmental deficits in human cortical neurons derived from induced pluripotent stem cells. Mol Neurobiol. 2020;57:616-34.
66 Sekar A, Bialas AR, de Rivera H, Davis A, Hammond TR, Kamitaki N, et al. Schizophrenia risk from complex variation of complement component 4. Nature. 2016;530:177-83.
67 Thielen AJF, van Baarsen IM, Jongsma ML, Zeerleder S, Spaapen RM, Wouters D. CRISPR/Cas9 generated human CD46, CD55 and CD59 knockout cell lines as a tool for complement research J Immunol Methods. 2018;456:15-22.
68 Wang P, Mokhtari R, Pedrosa E, Kirschenbaum M, Bayrak C, Zheng D, et al. CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in cerebral organoids derived from iPS cells. Mol Autism. 2017;8:11.
69 Sakai C, Ijaz S, Hoffman EJ. Zebrafish models of neurodevelopmental disorders: past, present, and future. Front Mol Neurosci. 2018;11:294.
70 Zhu J, Xu H, Song H, Li X, Wang N, Zhao J, et al. CRISPR/Cas9-mediated grna gene knockout leads to neurodevelopmental defects and motor behavior changes in zebrafish. J Neurochem. 2021; 157:520-31.
71 Liu CX, Li CY, Hu CC, Wang Y, Lin J, Jiang YH, et al. CRISPR/Cas9-induced shank3b mutant zebrafish display autism-like behaviors. Mol Autism. 2018;9:23.
72 Rutkowski TP, Purcell RH, Pollak RM, Grewenow SM, Gafford GM, Malone TM, et al. Behavioral changes and growth deficits in a CRISPR engineered mouse model of the schizophrenia-associated 3q29 deletion. Mol Psychiatry. 2021;26:772-83.
73 Saito R, Koebis M, Nagai T, Shimizu K, Liao J, Wulae B, et al. Comprehensive analysis of a novel mouse model of the 22q11.2 deletion syndrome: a model with the most common 3.0-Mb deletion at the human 22q11.2 locus. Transl Psychiatry. 2020;10:35.
74 Lu X, Zhang D, Shoji H, Duan C, Zhang G, Isaji T, et al. Deficiency of α1,6-fucosyltransferase promotes neuroinflammation by increasing the sensitivity of glial cells to inflammatory mediators. Biochim Biophys Acta Gen Subj. 2019;1863:598-608.
75 Meyer JH, Cervenka S, Kim MJ, Kreisl WC, Henter ID, Innis RB. Neuroinflammation in psychiatric disorders: PET imaging and promising new targets. Lancet Psychiatry. 2020;7:1064-74.
76 Choi H, Song J, Park G, Kim J. Modeling of autism using organoid technology. Mol Neurobiol. 2017;54:7789-95.
77 Kim W, Won SY, Yoon BJ. CRMP2 mediates GSK3β actions in the striatum on regulating neuronal structure and mania-like behavior. J Affect Disord. 2019;245:1079-88.
78 Duan L, Ouyang K, Xu X, Xu L, Wen C, Zhou X, et al. Nanoparticle delivery of CRISPR/Cas9 for genome editing. Front Genet. 2021; 12:673286.
79 Kantor A, McClements ME, MacLaren RE. CRISPR-Cas9 DNA base-editing and prime-editing. Int J Mol Sci. 2020;21:6240.
80 Foulkes AL, Soda T, Farrell M, Giusti-Rodríguez P, Lázaro-Muñoz G. Legal and ethical implications of CRISPR applications in psychiatry. North Carol Law Rev. 2019;97:1359-98.
81 Nishiyama J, Mikuni T, Yasuda R. Virus-mediated genome editing via homology-directed repair in mitotic and postmitotic cells in mammalian brain. Neuron. 2017;96:755-68.e5.
82 Amar M, Pramod AB, Yu NK, Herrera VM, Qiu LR, Moran-Losada P, et al. Autism-linked Cullin3 germline haploinsufficiency impacts cytoskeletal dynamics and cortical neurogenesis through RhoA signaling. Mol Psychiatry. 2021;26:3586-613.
83 Graf H, Wurster CD. Antisense oligonucleotides in psychiatric disorders. J Clin Psychopharmacol. 2018;38:651-2.
84 Zhang M, Creese I. Antisense oligodeoxynucleotide reduces brain dopamine D2 receptors: behavioral correlates. Neurosci Lett. 1993; 161:223-6.
85 Maguire CA, Ramirez SH, Merkel SF, Sena-Esteves M, Breakefield XO. Gene therapy for the nervous system: challenges and new strategies. Neurotherapeutics. 2014;11:817-39.
86 Cyranoski D. Ethics of embryo editing divides scientists. Nature. 2015;519:272.
87 Lanphier E, Urnov F, Haecker SE, Werner M, Smolenski J. Don't edit the human germ line. Nature. 2015;519:410-1.
88 Zhai X, Ng V, Lie R. No ethical divide between China and the West in human embryo research. Dev World Bioeth. 2016;16:116-20.
89 Cyranoski D. Russian biologist plans more CRISPR-edited babies. Nature. 2019;570:145-6.