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OPINION REVIEW

Modernising autism spectrum disorder model engineering and treatment *via* CRISPR-Cas9: A gene reprogramming approach

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Abstract

A neurological abnormality called autism spectrum disorder (ASD) affects how a person perceives and interacts with others, leading to social interaction and communication issues. Limited and recurring behavioural patterns are another feature of the illness. Multiple mutations throughout development are the source of the neurodevelopmental disorder autism. However, a well-established model and perfect treatment for this spectrum disease has not been discovered. The rising era of the clustered regularly interspaced palindromic repeats (CRISPR)-associated protein 9 (Cas9) system can streamline the complexity underlying the pathogenesis of ASD. The CRISPR-Cas9 system is a powerful genetic engineering tool used to edit the genome at the targeted site in a precise manner. The major hurdle in studying ASD is the lack of appropriate animal models presenting the complex symptoms of ASD. Therefore, CRISPR-Cas9 is being used worldwide to mimic the ASD-like pathology in various systems like *in vitro* cell lines, *in vitro* 3D organoid models and *in vivo* animal models. Apart from being used in establishing ASD models, CRISPR-Cas9 can also be used to treat the complexities of ASD. The aim of this review was to summarize and critically analyse the CRISPR-Cas9-mediated discoveries in the field of ASD.

Key Words: Autism spectrum disorder; CRISPR-Cas9; Cellular models; Organoids; Animal models; Therapeutic strategies

Core Tip: There are several reviews in the literature explaining the underlying mechanisms contributing to the pathophysiology of autism spectrum disorder by performing several preclinical experiments. Given the significant role of genetics (de novo or inheritable) in the development of autism spectrum disorder, disease specific models should be established for investigating the mechanism involved. Therefore, this review specifically focused on the use of an emerging genomic editing tool, clustered regularly interspaced palindromic repeats/Cas9, for generating different types of preclinical models as well as new therapeutic options, providing a novel insight into the disease.

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INTRODUCTION

Identifying the double helix DNA structure and finding technologies to manipulate it ultimately led to an extensive investigation of genomic structure[1]. Manipulation of genomic structure requires various genomic editing techniques including homing-endonucleases or mega nucleases, zinc finger nucleases, transcription activator like effector nucleases and clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9)[2]. Advancement in this field has permitted researchers to alter the DNA of model organisms to obtain the model of interest. In this context, the discovery of the CRISPR-Cas9 system has greatly and enormously expanded the field of study related to the genetic underpinnings of complex and heterogeneous disorders like autism spectrum disorder (ASD)[3]. From bacterial defence systems to genomic engineering tools, CRISPR-Cas9 has been proven beneficial in providing a novel insight into a possible genetic mutation in ASD[4].

The spectrum of disorders under ASD is pervasive. Due to the complexity of this medical condition, it is challenging to determine the diagnostic threshold, making diagnosis difficult. Despite the apparent difficulties connected with identification of ASD aetiologies, intensive genetic investigations have shown that ASD has a substantial genetic basis. Genetic analysis has revealed many susceptibility genes [5]. In addition to this, ASD has been found to be associated with several other disorders such as anxiety, depression, attention-deficit hyperactivity disorder and obsessive-compulsive disorder[6] and with genetic syndromes like Rett syndrome, Angelman syndrome, Timothy epilepsy and Fragile-X syndrome[7,8]. Depending upon the origin of the condition, ASD is diagnosed as syndromic if it is due to specific genetic syndromes with well-defined aetiology, such as Rett syndrome, and it identified as non-syndromic if ASD is diagnosed as the first diagnosis in patients having less-characterized aetiology [9,10].

There are multiple factors involved that contribute to the development of ASD in cases of non-syndromic ASD[10]. Therefore, due to the several aetiologies involved, ASD is considered a heterogeneous group of highly heritable disorders[11,12], and their risk factors could be genetic as well as environmental. A significant role of genetics in the development of the disorder has been known for a long time as confirmed by a meta-analysis of twin studies, which stated that ASD is inherited nearly 64%-91% in monozygotic twins and around 30% in dizygotic twins[13-15].

Modelling of disease at *in vitro, in vivo* and organoid levels are major avenue of research for investigation of abnormal early brain development because several ASD-associated genes have been found to be highly expressed during prenatal brain development of patients[16]. CRISPR-Cas9 has been successfully used to generate genetically engineered models that could mimic the disorder. At the same time, gene therapies are one of the emerging fields in recent years aimed at curing a wide range of diseases including ASD. Moreover, based on the available genetic information, novel gene therapies have also been created, which may help identify the potential ASD therapy candidates. The advent of CRISPR-Cas9 in gene therapy has been helpful in either silencing the gene using non-homologous end joining or correcting the genetic mutation using non-homologous recombination[17]. These developments have given patients new hope regarding rational treatment against the disease. This article provided an overview of the potential use of CRISPR-Cas9 technology for the establishment of appropriate ASD models along with its application in therapeutic strategies at the genomic level.

GENETIC ARCHITECTURE OF ASD

The genetic background contributing to autism aetiology involves copy number variations, somatic mutations, *de novo* mutations, single nucleotide variations, insertions, deletions and chromosomal

abnormalities[18,19]. These factors interfere with the protein-coding genes involved in neuronal development and several other ASD candidate genes related to critical processes like DNA binding, transcription, postsynaptic density and neuroprotection[20]. Any alteration in well-known ASD-associated genes can ultimately result in impaired working of brain areas responsible for cognitive functions[21,22]. Forkhead box protein 1 (*FOXP1*) and fragile X messenger ribonucleoprotein 1 (*FMR1*) are transcription factors and regulating genes. Others, like methyl CpG binding protein 2 (*MECP2*), tuberous sclerosis 1 (*TSC1*), SH3- and multiple ankyrin repeats protein 1 (*SHANK1*), ubiquitin protein ligase E3A and contactin-associated protein-like 2 (*CNTNAP2*), are involved in a wide range of functions like chromatin remodelling, cell proliferation, maintaining synaptic activity, protein ubiquitination and cell adhesion, respectively. Moreover, mutations in *MECP2* and *FMR1* are related to genetic syndromes such as Rett syndrome and fragile X syndrome, respectively[3].

The latest advancements in the development of next-generation sequencing have offered opportunities for genetic analysis to elucidate the underlying genetic mechanisms of ASD[23]. Whole exome sequencing has revealed that some biallelic mutations in proximal assembly proteins, phenylalanine hydrolyses and spectrin repeat containing nuclear envelope protein 1 are associated with familial ASD[24]. These genes also include those that are known to control or be controlled by synaptic activity (*e.g.*, *MECP2*, spectrin repeat containing nuclear envelope protein 1). Genetic analysis using whole genome sequencing has shown that copy number variations and single nucleotide variations result in missense mutations with an overall increase in missense variants, including some ASD risk genes[25]. In addition to this, genome-wide association studies have been able to identify a few potential variants being implicated in the pathogenesis of ASD[26]. Altogether, mutations in specific genes, known to regulate the important biological pathways, neuronal networks, synaptic activity and plasticity, *etc*, contribute to development of ASD and associated clinical symptoms (Figure 1).

STRUCTURE AND FUNCTION OF CRISPR-Cas9

CRISPR-Cas9 is used to cut DNA at predetermined target locations. Although the method has already been revolutionised as a gene editing tool, researchers are constantly exploring new applications. Since being discovered as a bacterial immune system against invading viruses, CRISPR-Cas9 has been adapted as a powerful tool in genomic research. Repeat elements in CRISPR were initially noticed in *Escherichia coli* by Ishino *et al*[27]. Contrary to conventional tandem repeats in the genome, the CRISPR repeat clusters were interestingly separated by non-repeating DNA sequences known as spacers. Complete genome sequencing of bacteria and archaea led researchers to determine that these CRISPR elements are adjacent to well-conserved CRISPR-associated genes (Cas)[28]. This whole structure including palindromic repeats, spacer DNA and Cas gene is known as the CRISPR array. After a decade of research, scientists have finally discovered that the spacer DNA sequences belong to viruses[29,30].

The study by Gasiunas *et al*[31] provided the most significant experimental data about the potential utility of CRISPR systems for bacteria. The concept that the Cas9 enzymes in bacteria can be reprogrammed to target a specific DNA sequence has been the key discovery, which signalled the beginning of CRISPR as a biotechnological gene-editing tool[31,32]. CRISPR RNA and transactivating CRISPR RNA are both vital parts of guide RNA (gRNA) and are required for the functioning of the CRISPR system. Notably, Jinek *et al*[32] demonstrated that CRISPR-Cas9 could also be guided by single gRNA, a chimeric RNA created by joining transactivating CRISPR RNA and CRISPR RNA[32]. These studies were the reason for adopting CRISPR-Cas9 as a gene editing tool.

The ability of the CRISPR-Cas9 system to produce an autism model and its therapeutic potential are the main topics of this review. In 2012, Doudna and Charpentier[32] found that by using the appropriate template, CRISPR-Cas9 could be used to edit any desired DNA. Depending on how Cas proteins act, the CRISPR-Cas9 system has been divided into type I, type II and type III systems. Type II is the most well-studied and simplest for application in genetic engineering[33].

The Cas9 protein performs the function of genetic scissors in the type II system by producing a double-stranded break (DSB) in the DNA[34]. The Cas9 protein contains two structural lobes, one that aids in recognition (REC) and the other that aids in nuclease activity. The REC lobe consists of REC1 and REC2, which are involved in the recognition of gRNA. The nuclease also has a protospacer adjacent motif (PAM) interacting domain responsible for the binding of Cas9 to targeted DNA. The gRNA is used to target viral DNA in prokaryotes, but when utilised as a gene-editing tool, it can be synthetically constructed to target virtually any gene that needs to be changed.

The three phases of the CRISPR-Cas9 genome editing system are recognition, cleavage and repair [35]. Single gRNA binds to a complementary area on the targeted DNA to begin the recognition process. PAM is a 2–5 base pair sequence that has an "NGG" pattern, where "N" stands for any nucleotide followed by two guanine nucleotides. Once the PAM site is identified, double stranded DNA starts melting at the target site followed by an RNA-DNA hybrid formation. Now, the Cas9 protein is ready to make a DSB at the targeted DNA 3 base pairs upstream to PAM[36]. In the last step, the double stranded blunt ended breaks are repaired by non-homologous end joining and homology directed repair by cellular machinery[34,37,38]. By inserting a donor DNA template with sequence homology at the

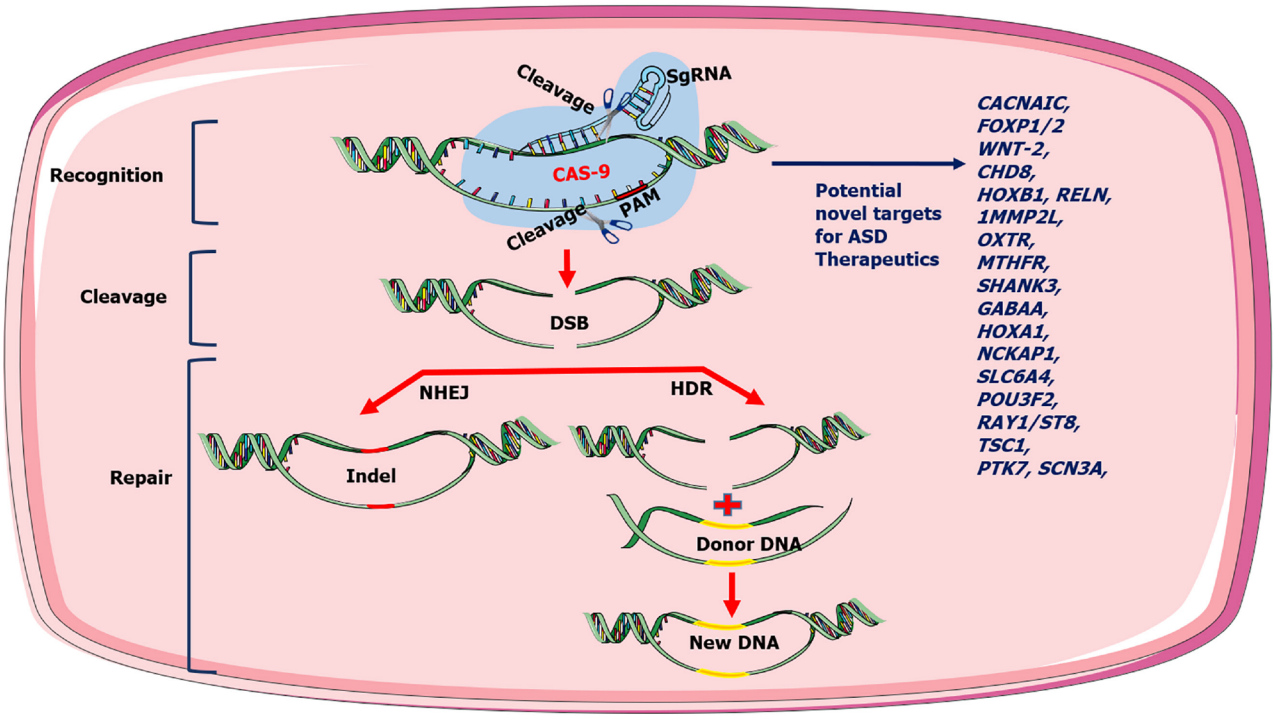

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Figure 1 Schematic diagram describing the structure and functioning of clustered regularly interspaced short palindromic repeats-associated protein 9 technique in autism spectrum disorder. In this schematic, we highlighted the mechanism of clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9) in recognizing a target using protospacer adjacent motifs sequencing and causing cut at specific point. Following cleavage and forming double stand breaks, repair systems like non-homologous end joining and homology directed repair come into play for avoiding any unspecific mutations. Diverse application of CRISPR-Cas9 has been explained in this diagram for investigating the mechanism involved in autism spectrum disorder pathophysiology. Various potential therapeutic targets for autism spectrum disorder could be investigated using CRISPR-Cas9 technology. sgRNA: Single guide RNA; ASD: Autism spectrum disorder; PAM: Protospacer adjacent motifs; DSB: Double strand breaks; NHEJ: Non-homologous end joining; HDR: Homology directed repair.

anticipated DSB site, homology directed repair carries out the precise gene insertion or replacement[39]. The property of CRISPR-Cas9 to either activate genes or to repress genes has been utilised to regulate the transcriptional level of gene expression.

CRISPR-Cas9 MEDIATED GENETIC ENGINEERING OF ASD

Most cases of ASD are idiopathic, with illusive aetiology[40]. The heterogeneous molecular nature of ASD makes it really difficult to understand the associated risk factors and the underlying mechanisms. Modelling ASD is notably challenging due to its multigenic aetiology. Only pertinent and validated disease-specific models could be helpful in discovering novel biomarkers and related therapeutic targets [41].

CRISPR-Cas9 engineered cellular models of ASD

Numerous neurodevelopmental diseases, including ASD are studied using cellular models because of the short experimental period and no ethical concerns and are less expensive. Researchers can create early human brain development, alterations in ASD or any other neurological disorders using *in vitro* models. Induced pluripotent stem cells (iPSCs), which can grow indefinitely *in vitro*, can be created by reprogramming somatic cells. Patient-derived cellular models have been validated and are realistic while preserving the genetic makeup of the donor and are an effective tool for deciphering the pathophysiology of ASD. The emergence of the genomic editing tool, CRISPR-Cas9, is helpful in facilitating more efficient *in vitro* models of ASD considering its genetic background. Using this technique, the researcher can edit primary cultured neural cells or isogenic cell lines by either introducing mutations derived from ASD patients or correcting them. Moreover, this technology reduces genetic background variation and directly correlates the observed symptoms and the associated mutation[42], which further provides information about the role of the particular ASD risk gene in neurodevelopment.

It is known that aberrant neurogenesis and synaptogenesis lead to functional impairments in brain networks in ASD[41]. Therefore, early molecular events during ASD development can be replicated in a model system in neurogenin 2-directed induced iPSCs (for excitatory neurons) that are further differentiated into forebrain glutamatergic neurons. Using that information and based on the whole exome sequencing results of some selected ASD-associated risk genes, the CRISPR-Cas9 approach was used to generate knockout (KO) iPSCs for the functional studies of the following genes: Anosmin 1; *FMR2*; calcium voltage-gated channel subunit alpha1 C; astrotactin 2; alpha-thalassemia/mental retardation, X-linked; chromodomain helicase DNA binding protein 8 (*CHD8*); disks large-associated protein 2; teneurin transmembrane protein 1; potassium voltage-gated channel subfamily q member 2; and sodium voltage-gated channel alpha subunit 2 (*SCN2A*). They revealed that ASD genes could result in similar electrophysiological phenotypes and transcriptional rewiring in the human iPSC-derived excitatory neurons model system[43].

Apart from the role of the *SHANK3* gene in synaptogenesis, one of the other consequences of its haploinsufficiency is hyperpolarization-activated cation channelopathy, which contributes to ASD pathogenesis. This impairment was analysed by generating *SHANK3* deletion by CRISPR in human embryonic stem cells[44]. Findings also highlighted that iPSC-derived glutamatergic neurons deficient in at least one allele of *CNTN5*/euchromatic histone lysine methyltransferase 2 resulting in ASD-associated phenotypes presented the increased synaptic activity of excitatory neurons *in vitro*[45]. In addition, CRISPR mediated inactivation of euchromatic histone lysine methyltransferase 1 in human neurons, which is directly associated with n-methyl-D-aspartate receptor hyperfunction and is implicated in ASD pathophysiology[46].

The major obstacle in the treatment of ASD is testing different drug candidates because of its aetiological heterogeneity. Therefore, an *in vitro* study has been done using the CRISPR tool for introducing mutations in activity-dependent neuroprotective protein, dead-box helicase 3 X-linked and *FOXP1* genes to create a relevant ASD model[47]. Similarly, hemizygous *CHD8* (*CHD8*+/−) iPSC lines were designed to investigate the role of *CHD8* in embryo development at the molecular and cellular levels. According to transcriptomic profiling, *CHD8* regulates several other genes connected to the development of ASD[48]. In addition to ASD-associated genes, the role of long noncoding RNAs, such as patched domain containing 1-antisense RNA[49]and molybdenum cofactor sulfurase[50], in ASD development was studied using CRISPR technology in human induced pluripotent stem cells (hiPSCs). Cellular models are briefly summarized in Table 1. These aforementioned findings indicate that these ASD associated genes may be a therapeutic target for the treatment of ASD.

CRISPR-Cas9 engineered organoids of ASD

The lack of suitable ASD models has always been a hindrance in ASD research because neither 2D cell culture nor animal models can accurately mimic the aetiology of ASD. Therefore, 3D *in vitro* models like organoids have recently emerged in the field of research. They have been shown to reproduce the gene expression profile, transcriptome, epigenome and disease dynamics of both idiopathic and syndromic ASD[51]. Like other cellular models, iPSC-derived organoids are being used because of no ethical concerns and are preferred over 2D culture and animal models as they can generate more disease-specific models.

This methodology has become even more reliable due to the integration of CRISPR-Cas9 to produce isogenic controls, significantly reducing genetic background differences. Idiopathic ASD has been connected to abnormalities in several genes, and genetic research has found multiple mutations that are linked to this condition[52]. Enhanced neurogenesis in idiopathic ASD has been studied through CRISPR engineered organoid models to create mutations in histone methyltransferase *SUV420H1*, the tumour suppressor phosphatase and TENsin homolog[53], *CHD8* and the GTPase-encoding RAS-related protein Rab-39B[54]. These genes are linked to macrocephalic ASD, and CRISPR-mediated deletion resulted in larger haploinsufficient cerebral organoids in comparison to isogenic control due to overactivation of the P13K-AKT-mTOR pathway[54].

Modelling of syndromic ASD is also being achieved using cerebral organoids to investigate the underlying genetic mechanism. One of the important ASD-associated genes *MECP2* is considered critical for early brain development, but its loss-of-function mutations are a common underlying aetiology of Rett syndrome[55], causing severe impairment in human interneurons and ultimately neurogenesis. Human *MECP2*-KO neurons and cortical organoids were used using CRISPR to investigate its neuropathological function[56,57]. Mutation (deletion) in *UBE3A* is also related to the pathology of syndromic ASD, and an organoid model derived from human iPSCs demonstrated hyperexcitability in brains contributing to network dysfunction[58].

Similarly, cerebral organoids are used for studying other syndromic ASD, such as a mutation in *TSC1/TSC2* genes in CRISPR-engineered human cortical spheroid model[59]. It caused synaptic imbalances, with an increase in γ-aminobutyric acid synapses[60]. Human corticostriatal organoids were studied using CRISPR-generated *SHANK3* gene deletion for modelling autism[61]. hiPSC-derived brain organoids with CRISPR-Cas9 induced *FMR1*-KO, which caused an abnormal increase in astrocyte number, was utilized to model FXS, a syndromic ASD[62]. Various organoid models of syndromic as well as idiopathic ASD is summarized in Table 1.

Table 1 Summary of clustered regularly interspaced palindromic repeats-associated protein 9 engineered models of autism spectrum disorder

Ref.	Model	Gene mutation/syndrome	Observed alterations
Cellular			
[32]	ES cells	*SHANK3*/Phelan-McDermid syndrome	Altered neuronal morphology and synaptic connectivity; impaired Ih channels
[33]	iPSCs	*CNTN5* or *EHMT2*/idiopathic ASD	Increased synaptic excitatory neuron activity
[34]	iPSCs	*EHMT1*/Kleefstra syndrome	Upregulation of NMDAR1; neuronal network impairments
[35]	iPSCs	*ADNP, DDX3X* and *FOXP1*/idiopathic ASD	Increased neurogenesis
[36]	iPSCs	*CHD8*/idiopathic ASD	Dysregulated expression of genes associated with human brain volume or head size
[37]	iPSCs	*PTCHD1-AS*/idiopathic ASD	Impaired excitatory synaptic function (NMDAR hypofunction); synaptic impairment
[38]	iPSCs	*COSMOC*/idiopathic ASD	Destabilization of lipid and energy metabolism; affected neuronal maturation
Organoids			
[41]	Cerebral	*PTEN*/idiopathic ASD, *CHD8*/idiopathic ASD, *SUV420H1*/ idiopathic ASD	Increased upper layer colossal neurons, cycling progenitor neuron; high outer radial glial cells; increased cortical interneurons; increased newly born deep layer projection neurons
[42]	Cerebral	*RAB39b*/ idiopathic ASD	Increased NPC proliferation
[44]	Cortical and neurons	*MECP2*/Rett syndrome	Dysregulation in genes of neuronal and glial cells
[46]	Cortical	*UBE3A*/ Angelman syndrome	Dysfunction in big potassium channel dysfunction causing increased neuronal excitability
[47]	Cortical	*TSC1 or 2*/Tuberous sclerosis complex	Affected cortical neurons and glial cell development
[49]	Cortico-striatal organoids	*SHANK3*/ Phelan-McDermid syndrome	Enhanced neuronal excitability; dysregulated expression of protocadherins and zinc-finger genes
[50]	Cortical	*FMR1*/Fragile X syndrome	Increased number of glial cells and bigger organoid size
Animal Models			
[52]	Cynomolgus macaques	*SHANK3*/ Phelan-McDermid syndrome	Sleep disturbances; increased repetitive behaviour, motor deficit; social and learning impairment; aberrant neural circuit connectivity
[53]	Mice	*ARID1B*/idiopathic ASD	Social behaviour impairment; altered vocalization; anxiety-like behaviour; neuroanatomical abnormalities; growth impairment
[54]	Mice	*CHD8*/idiopathic ASD	Cognitive impairment; disrupted pathways involved in neurogenesis, neuroimmune signalling, synaptic processes
[55]	Mice	*ASH1L*/idiopathic ASD	Dysregulated epigenetic modification; upregulation of neurexin-1α
[56]	Rat	*CYFIP1*/idiopathic ASD	Extensive changes in white matter; myelin sheath thinning in corpus callosum; abnormal oligodendrocytes; behavioural inflexibility
[57]	Rat	*TCF4*/idiopathic ASD	Attenuated action potential output; alteration in electrophysiological properties in neurons
[58]	Rat	*UBE3A*/idiopathic ASD	Deficits in motor coordination as well as learning and memory
[59]	Zebra fish	*CHD8*/idiopathic ASD	Increased head size; reduction in post mitotic enteric neurons
[60]	Zebra fish	*FMR1*/Fragile X syndrome	Abnormal behaviour; learning memory deficits; impaired craniofacial cartilage development
[61]	Zebra fish	*NR3C2*/idiopathic ASD	Disruption in sleep and social functions

[62]	Zebra fish	*SHANK3*/ Phelan-McDermid syndrome	Reduced social nitration and locomotory activity; repetitive swimming behaviour; reduced levels of post synaptic homer1 and presynaptic synaptophysin

ADNP: Activity-dependent neuroprotective protein; *ARID1B*: AT-rich interaction domain 1B; ASD: Autism spectrum disorder; *ASH1L*: ASH1-like histone lysine methyltransferase; *CHD8*: Chromodomain helicase DNA binding protein 8; *CNTN5*: Contactin-associated protein-like 5; *COSMOC*: Molybdenum cofactor sulfurase; *CYFIP1*: Cytoplasmic FMR1 interacting protein; *DDX3X*: Dead-box helicase 3 X-linked; *EHMT1/2*: Euchromatic histone lysine methyltransferase 1/2; *FMR1*: Fragile X messenger ribonucleoprotein 1; *FOXP1*: Forkhead box protein 1; ES: Embryonic stem; Ih: Hyperpolarization-activated cation; iPSC: Induced pluripotent stem cell; *MECP2*: Methyl CpG binding protein 2; *NMDAR1*: N-methyl-D-aspartate receptor 1; NPC: Neural progenitor cell; *NR3C2*: Nuclear receptor subfamily 3 group c member 2; *PTCHD1-AS*: Patched domain containing 1-antisense RNA; *PTEN*: Phosphatase and TENsin homolog; *RAB39b*: RAS-related protein Rab-39B; *SHANK3*: SH3- and multiple ankyrin repeats protein 3; *TSC1/2*: Tuberous sclerosis 1/2; *TCF4*: Transcription factor 4.

CRISPR-Cas9 engineered animal models of ASD

Despite the capabilities of *in vitro* models to recapitulate the basic aetiology of ASD, animal models are preferred as a more fundamental tool to fully understand the complexity involved in ASD. Animal models allow a researcher to investigate behavioural and developmental features in addition to molecular parameters. However, generating an ASD animal model is a time-consuming procedure and involves ethical concerns, but it is helpful in studying neurodevelopmental disorders. Moreover, in the case of ASD, it is helpful in validating the implication of critical genes in the development of ASD.

The emerging CRISPR-Cas9 approach has been a great help in creating various genetic animal models (KO, Knock-in, overexpression and point mutation) to study various ASD-associated genes identified in an individual with ASD. ASD models can be studied in multiple species like rodents including mice, rats, monkeys, fruit flies and zebrafish, depending upon the requirement and purpose of the experiment [63]. CRISPR-mediated generation of mutations in the *SHANK3* gene by creating insertions and deletions (indels) in exon 21 led to the development of an ASD model in monkeys and their F1 offspring, showing atypical autistic phenotypes like increased repetitive behaviour along with social and learning deficits[64].

Studies have reported that a CRISPR-mediated mutation in ASD-associated genes such as AT-rich interaction domain 1B[65], *CHD8*[66] and ASH1-like histone lysine methyltransferase[67] showed ASD-like symptoms in mice. To investigate genes implicated in ASD such as cytoplasmic FMR1 interacting protein[68], transcription factor 4[69] and *UBE3A*[70] in a rat model created with CRISPR engineered technology was studied. The rats showed autistic phenotypes like alteration in behavioural flexibility, learning ability and memory difficulties.

Similarly, a zebrafish model of ASD using the CRISPR strategy has been used to study the functional role of genes in the development of ASD such as *CHD8*[71], *FMR1*[72], nuclear receptor subfamily 3 group c member 2[73]and *SHANK3*[74]. Major ASD-linked phenotypes observed in these zebrafish models are macrocephaly, hyperactivity, anxiety, impaired social behaviour, sleep disturbances and altered neuronal development (summarized in Table 1).

CRISPR-Cas9-BASED THERAPEUTIC STRATEGIES AND POTENTIAL TARGETS

Over the years, the CRISPR-Cas9 genome editing tool has evolved as a specific delivery tool for delivering genes to the target cells including neural and brain cells. One such benchmark was set by Staahl *et al*[75], where the engineered variants of the Cas9 ribonucleoprotein complex were delivered to the mice hippocampus, striatum and cortex region and demonstrated the *in vivo* neuronal gene editing [75]. The advances in the genome editing tool have opened the door for eradicating the genetic mutations underlying severe neurological diseases like ASD.

Several genes that are linked to ASD can be targeted for correction using the CRISPR-Cas9 approach to reduce the disease burden (summarized in Figure 1). The genes that undergo mutations in ASD and ASD-associated monogenic syndromes include calcium voltage-gated channel subunit alpha1 C, *FOXP1/2*, wingless-related integration site-2, *CHD8*, homeobox B1, reelin, inner mitochondrial membrane peptidase subunit 2, oxytocin receptor gene, methylenetetrahydrofolate reductase, *SHANK2/3*, γ-aminobutyric acid type A receptor subunit, homeobox A1, *UBE3A*, NCK associated protein 1, human serotonin transporter gene, POU class 3 homeobox 2, reduced arabinose yariv1/suppression of tumorigenicity 8, *FMR1*[76-77], *MECP2, TSC1, PTK7, SCN3A* and *CNTNAP2*[78-82]. Some of these genes for monogenic syndromes associated with ASD are targeted using the CRISPR-Cas9 tool *in vitro* and *in vivo*; however, many others remain to be explored.

The lack of target specificity or the polygenic form of ASD limits the use of the CRISPR-Cas9 tool as a therapeutic strategy in ASD. The CRISPR-Cas9-based therapeutic strategies that had been explored are summarized in Table 2; They primarily consist of the monogenic form of ASD. One of the studies by Lee *et al*[83] demonstrated that gold nanoparticle delivery of CRISPR-Cas9 ribonucleoprotein rescued the

Table 2 Summary of clustered regularly interspaced palindromic repeats-associated protein 9 edited therapeutic targets of autism spectrum disorder

Ref.	*In vitro*/*in vivo*	Gene mutation/editing method	Observed alterations
[80-82]	BTBR T + tf/J (BTBR), *Fmr1* knockout, C57BL/6 mice	*mGluR5*	Rescued the exaggerated repetitive behaviours in mice caused by fragile X syndrome
[83]	HEK293 cell and Human iPSC (BCRT cell line)	*MECP2*	Reversal of ASD-associated Rett syndrome-like symptoms
[84]	RX41X iPSC and NOD/SCID female mice	*SHANK2*	Positive impact on nerve cells was reported like an increase in synapse number, dendritic complexity and length
[85]	C57BL/6 mice, Ube3a$^{m-/p+}$ mice and Ube3a$^{m-/pYFP}$ mice on the C57Bl/6	Antisense transcript of *UBE3A*	Rescued the anatomical and behavioural phenotypes in a mouse model of Angelman syndrome
[86]	HEK293FT cells	*FMR1*	Fragile X syndrome improved by knocking out the CGG
[89]	Mef2c L35P knock-in mouse	*MEF2C*	Reversal of autistic-like behaviour

ASD: Autism spectrum disorder; CGG: Cytosine-guanine-guanine; *FMR1*/*Fmr1*: Fragile X messenger ribonucleoprotein 1; iPSC: Induced pluripotent stem cell; *MECP2*: Methyl CpG binding protein 2; *MEF2C*: Myocyte-specific enhancer factor 2C; *mGluR5*: Metabotropic glutamate receptor subtype 5; *SHANK2*: SH3- and multiple ankyrin repeats protein 2.

exaggerated repetitive behaviours in mice caused by fragile X syndrome[83]. The study demonstrated minimal off-target effects, and the editing target used was the metabotropic glutamate receptor subtype 5 gene, one of the overexpressed targets in ASD-associated syndromes[83-85].

In another study, the CRISPR-Cas9 tool was used to correct the *MECP2* mutations responsible for ASD-associated Rett syndrome *via* homology directed repair in hiPSCs[86]. Loss-of-function mutations in the *SHANK2* gene has been associated with monogenic ASD. CRISPR-Cas9-mediated correction of a nonsense mutation on *SHANK2* was demonstrated in iPSCs, and the positive impacts on nerve cells were reported, including an increase in synapse number and dendritic complexity and length[87].

In Angelman syndrome (monogenic form of ASD) caused by deletion of the maternally inherited *UBE3A* allele, the CRISPR-Cas9 approach was used to knock out the antisense transcript of *UBE3A* in cultured human neurons and a mouse model. The antisense transcript of *UBE3A* is a long non-coding RNA that silences the paternal copy of the *UBE3A* allele and leads to the neurodevelopmental syndrome. The CRISPR-Cas9 approach was used to terminate the long non-coding RNA termed as antisense transcript of *UBE3A*, which led to the copy of the *UBE3A* allele available for transcription (activation of *UBE3A*) and hence rescued the anatomical and behavioural phenotypes in the mouse model of Angelman syndrome[88].

In another study, the CRISPR-Cas9 approach was used to improve fragile X syndrome by knocking out the cytosine-guanine-guanine (CGG) repeats expansion, recovering *FMR1* expression *in vitro*. *FMR1* encodes fragile X mental retardation protein, which undergoes epigenetic silencing because of the addition of CGG repeats and excessive DNA methylation, thus the CRISPR-Cas9 approach was used to excise the CGG expansion in the iPSCs[89]. A recent study used the CRISPR-Cas9 tool to activate the extracellular matrix receptor b3 integrin. The study also validated the involvement of b3 integrin haploinsufficiency in the pathophysiology of ASD and ASD-associated fragile X syndrome[90].

All CRISPR-Cas9-based therapeutic strategies established so far mainly comprise the proof of principle studies and have used the conventional homology-directed repair pathway to correct the mutations in the monogenic form of ASD. However, with the advancements in CRISPR-Cas9 genome editing tools, the most recently introduced concept of the base editing technique for more specific genome editing has been explored in fewer studies. One such study used CRISPR-Cas9-based cytidine base editors and the fourth generation base editor system to selectively modify the disco-interacting protein 2a and 2c genes in cell culture. Both of these genes are highly expressed in the central neuron system and known to be associated with ASD[91].

In another study, the CRISPR-mediated cytidine base editor system was used to restore the impairments in social interactions and repetitive behaviours in a knock-in mice model of autism. The *de novo* mutation in the gene myocyte-specific enhancer factor 2C was introduced in the mice brain, which displayed autistic-like behaviour. With the help of the base editing system the myocyte-specific enhancer factor 2C mutation was eradicated, and the reversal of symptoms was reported in mice[92]. A study demonstrated the use of CRISPR-Cas9 for correcting the mutation in *CNTNAP2* in an organoid model derived from patients with syndromic ASD by rescuing the phenotype of organoid overgrowth. This *CNTNAP2*-organoid model provided an opportunity for further mechanistic inquiry and development of new therapeutic strategies for ASD[93]. Another finding has shown the use of a CRISPR activation-based approach for rescuing abnormalities in *SCN2A* haploinsufficiency-associated ASD[94].

The CRISPR-Cas9 mediated base editing system is just the beginning of an era of targeted gene modification, which can bring a breakthrough in the treatment of ASD.

A plethora of studies is being conducted worldwide using several targets in cultured cells or in animal models. However, the extrapolation to patients has not been achieved yet. The advances in the techniques leading to improved specificity, targeted delivery and personalized therapeutics will definitely help in the bench-to-bedside conversion of these CRISPR-Cas9 based therapies and help in reducing the disease burden.

CONCLUSION

Understanding brain function and its complexities have only been made possible by emerging genomic engineering tools like transcription activator like effector nucleases, zinc finger nucleases and CRISPR-Cas9. Opportunities for manipulating the genome have created the possibility to generate models for understanding a complex neurological disorder like ASD. Among these genomic editing tools, CRISPR-Cas9 is being considered the most extensive and effective, with the advantages of low mutation rate, high target efficiency and cost-efficient. CRISPR has enabled the creation of models that reproduce exactly the same causal mutations identified in patients, which has made it possible to determine an appropriate and disease-specific drug therapy.

Owing to the heterogeneous nature of ASD, it is difficult to identify the exact cause of ASD in patients as it could be genetic or environmental. No standard medication has been developed for treating ASD, except for aripiprazole and risperidone for irritability and aggressiveness. Thus, creating a reliable model, establishing a causal factor and representing all the characteristics of the disease is difficult. *In vitro* modelling of ASD has been a great benefit for understanding the underlying mechanism involved in the pathogenesis of ASD. However, it does come with limitations like high heterogeneity among hiPSCs lines. Therefore, reprogramming strategies need to be optimized. CRISPR-Cas9 potentially overcome such limitations by generating isogenic cell lines and increasing the reproducibility of experiments.

To further investigate the pathogenesis of ASD, the genome of animals can be successfully edited to construct a validated KO and knock-in models using CRISPR. These animal models have been reported to present phenotypes, including neuroanatomical, behavioural and morphological characteristics, caused by ASD-associated genes. In that regard, such models are helpful in determining the aetiology of the condition as well as screening appropriate drugs to restore the altered phenotype. Advancement in genomic editing systems is an encouraging indication that could restore the wild-type sequence and potentially be effective in human treatment trials. Utilization of the CRISPR-Cas9 tool is not only limited to the modelling of ASD but also has been helpful in targeting the mutated genes and correcting them.

Based on the available genetic information, ASD-associated genes have been widely explored, but their therapeutic potential is limited to monogenic forms of ASD and remains unexplored in polygenic form of ASD. Also, due to lack of target specificity, genetic therapy using CRISPR-Cas9 is unable to target every ASD- associated gene. Other approaches, such as CRISPR-mediated activation of a gene in which nuclease-deficient Cas9 was fused with a transcriptional activator or the CRISPR-mediated base editor system in gene therapy, have been helpful in restoring and normalizing gene dosage in ASD. However, this method has not been explored well, and optimization of this procedure is necessary before utilization.

Despite advancements in CRISPR-Cas9 tools, there are certain numbers of limitations like off-targeting, delivery method and immunogenicity and associated risks that make it challenging to use in clinical trials. A high frequency of off-targets is a prime concern while using CRISPR for gene therapy because it can lead to further mutations in undesired genomic locations. However, emergence of bioinformatic tools have been helpful in reducing the off-target effects while predicting the off-target modifications. Another major concern is immunogenicity caused by the introduction of Cas9 and delivery methods using viral vectors. Cas9 is derived from *Streptococcus pyogenes*, which is responsible for various human infections. Therefore, many patients would already harbour pre-existing anti-Cas9 antibodies. Therefore, when it is introduced for therapy purposes in humans, it will be recognised as a foreign antigen. An immune response may develop and cause degradation of Cas9, which would prevent it from gene editing. Another safety concern is the DSBs induced by CRISPR, which often trigger apoptosis. In addition to this, induced DSBs have also resulted in unnecessary massive deletions and rearrangements of sequences, suggesting a significant safety concern for the clinical use of DSB-inducing CRISPR therapy.

Given the challenges involved in using these gene editing techniques, gene therapy is still a distant therapeutic approach. Considering all limitations and the need for improvising CRISPR technology, studies using genomic editing tools is limited to cultured cells or animal models. Extrapolation of such experiments in patients has not been yet achieved. Therefore, the application of results from preclinical studies to the clinical treatment of ASD will require extreme care.

FOOTNOTES

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REFERENCES

1 **Hsu PD**, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. *Cell* 2014; **157**: 1262-1278 [PMID: 24906146 DOI: 10.1016/j.cell.2014.05.010]
2 **Akram F**, Sahreen S, Aamir F, Haq IU, Malik K, Imtiaz M, Naseem W, Nasir N, Waheed HM. An Insight into Modern Targeted Genome-Editing Technologies with a Special Focus on CRISPR/Cas9 and its Applications. *Mol Biotechnol* 2023; **65**: 227-242 [PMID: 35474409 DOI: 10.1007/s12033-022-00501-4]
3 **Domadenik A**. Overview of current mouse models of autism and strategies for their development using CRISPR/Cas9 technology. *Acta Agric Slov* 2018; **112**: 19 [DOI: 10.14720/aas.2018.112.1.3]
4 **Singh V**, Gohil N, Ramírez García R, Braddick D, Fofié CK. Recent Advances in CRISPR-Cas9 Genome Editing Technology for Biological and Biomedical Investigations. *J Cell Biochem* 2018; **119**: 81-94 [PMID: 28544016 DOI: 10.1002/jcb.26165]
5 **Havdahl A**, Niarchou M, Starnawska A, Uddin M, van der Merwe C, Warrier V. Genetic contributions to autism spectrum disorder. *Psychol Med* 2021; **51**: 2260-2273 [PMID: 33634770 DOI: 10.1017/S0033291721000192]
6 **Yin J**, Schaaf CP. Autism genetics – an overview. *Prenat Diagn* 2017; **37**: 14-30 [PMID: 27743394 DOI: 10.1002/pd.4942]
7 **Peters SU**, Beaudet AL, Madduri N, Bacino CA. Autism in Angelman syndrome: implications for autism research. *Clin Genet* 2004; **66**: 530-536 [PMID: 15521981 DOI: 10.1111/j.1399-0004.2004.00362.x]
8 **Bey AL**, Jiang YH. Overview of mouse models of autism spectrum disorders. *Curr Protoc Pharmacol* 2014; **66**: 5.66.1-5.66.26 [PMID: 25181011 DOI: 10.1002/0471141755.ph0566s66]
9 **Schaefer GB**, Mendelsohn NJ; Professional Practice and Guidelines Committee. Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions. *Genet Med* 2013; **15**: 399-407 [PMID: 23519317 DOI: 10.1038/gim.2013.32]
10 **Mitchell KJ (ed)**. The genetics of neurodevelopmental disorders. Hoboken, New Jersey: Wiley-Blackwell
11 **Bailey A**, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M. Autism as a strongly genetic disorder: evidence from a British twin study. *Psychol Med* 1995; **25**: 63-77 [PMID: 7792363 DOI: 10.1017/s0033291700028099]
12 **Steffenburg S**, Gillberg C, Hellgren L, Andersson L, Gillberg IC, Jakobsson G, Bohman M. A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden. *J Child Psychol Psychiatry* 1989; **30**: 405-416 [PMID: 2745591 DOI: 10.1111/j.1469-7610.1989.tb00254.x]
13 **Tick B**, Bolton P, Happé F, Rutter M, Rijsdijk F. Heritability of autism spectrum disorders: a meta-analysis of twin studies. *J Child Psychol Psychiatry* 2016; **57**: 585-595 [PMID: 26709141 DOI: 10.1111/jcpp.12499]
14 **Folstein SE**, Rosen-Sheidley B. Genetics of autism: complex aetiology for a heterogeneous disorder. *Nat Rev Genet* 2001; **2**: 943-955 [PMID: 11733747 DOI: 10.1038/35103559]
15 **Rosenberg RE**, Law JK, Yenokyan G, McGready J, Kaufmann WE, Law PA. Characteristics and concordance of autism spectrum disorders among 277 twin pairs. *Arch Pediatr Adolesc Med* 2009; **163**: 907-914 [PMID: 19805709 DOI: 10.1001/archpediatrics.2009.98]
16 **Gordon A**, Geschwind DH. Human in vitro models for understanding mechanisms of autism spectrum disorder. *Mol Autism* 2020; **11**: 26 [PMID: 32299488 DOI: 10.1186/s13229-020-00332-7]
17 **Maeder ML**, Gersbach CA. Genome-editing Technologies for Gene and Cell Therapy. *Mol Ther* 2016; **24**: 430-446 [PMID: 26755333 DOI: 10.1038/mt.2016.10]
18 **Ramaswami G**, Geschwind DH. Genetics of autism spectrum disorder. *Handb Clin Neurol* 2018; **147**: 321-329 [PMID: 29325621 DOI: 10.1016/B978-0-444-63233-3.00021-X]
19 **Lupski JR**, Stankiewicz P. Genomic disorders: molecular mechanisms for rearrangements and conveyed phenotypes. *PloS Genet* 2005; **1**: e49 [PMID: 16444292 DOI: 10.1371/journal.pgen.0010049]

20 **Cook EH Jr**, Scherer SW. Copy-number variations associated with neuropsychiatric conditions. *Nature* 2008; **455**: 919-923 [PMID: 18923514 DOI: 10.1038/nature07458]

21 **Canitano R**, Bozzi Y. Editorial: Autism Spectrum Disorders: Developmental Trajectories, Neurobiological Basis, Treatment Update. *Front Psychiatry* 2017; **8**: 125 [PMID: 28751868 DOI: 10.3389/fpsyt.2017.00125]

22 **Giovedí S**, Corradi A, Fassio A, Benfenati F. Involvement of synaptic genes in the pathogenesis of autism spectrum disorders: the case of synapsins. *Front Pediatr* 2014; **2**: 94 [PMID: 25237665 DOI: 10.3389/fped.2014.00094]

23 **Perenthaler E**, Yousefi S, Niggl E, Barakat TS. Beyond the Exome: The Non-coding Genome and Enhancers in Neurodevelopmental Disorders and Malformations of Cortical Development. *Front Cell Neurosci* 2019; **13**: 352 [PMID: 31417368 DOI: 10.3389/fncel.2019.00352]

24 **Yu TW**, Chahrour MH, Coulter ME, Jiralerspong S, Okamura-Ikeda K, Ataman B, Schmitz-Abe K, Harmin DA, Adli M, Malik AN, D'Gama AM, Lim ET, Sanders SJ, Mochida GH, Partlow JN, Sunu CM, Felie JM, Rodriguez J, Nasir RH, Ware J, Joseph RM, Hill RS, Kwan BY, Al-Saffar M, Mukaddes NM, Hashmi A, Balkhy S, Gascon GG, Hisama FM, LeClair E, Poduri A, Oner O, Al-Saad S, Al-Awadi SA, Bastaki L, Ben-Omran T, Teebi AS, Al-Gazali L, Eapen V, Stevens CR, Rappaport L, Gabriel SB, Markianos K, State MW, Greenberg ME, Taniguchi H, Braverman NE, Morrow EM, Walsh CA. Using whole-exome sequencing to identify inherited causes of autism. *Neuron* 2013; **77**: 259-273 [PMID: 23352163 DOI: 10.1016/j.neuron.2012.11.002]

25 **Turner TN**, Coe BP, Dickel DE, Hoekzema K, Nelson BJ, Zody MC, Kronenberg ZN, Hormozdiari F, Raja A, Pennacchio LA, Darnell RB, Eichler EE. Genomic Patterns of De Novo Mutation in Simplex Autism. *Cell* 2017; **171**: 710-722.e12 [PMID: 28965761 DOI: 10.1016/j.cell.2017.08.047]

26 **Weiss LA**, Arking DE; Gene Discovery Project of Johns Hopkins & the Autism Consortium, Daly MJ, Chakravarti A. A genome-wide linkage and association scan reveals novel loci for autism. *Nature* 2009; **461**: 802-808 [PMID: 19812673 DOI: 10.1038/nature08490]

27 **Ishino Y**, Shinagawa H, Makino K, Amemura M, Nakata A. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. *J Bacteriol* 1987; **169**: 5429-5433 [PMID: 3316184 DOI: 10.1128/jb.169.12.5429-5433.1987]

28 **Jansen R**, Embden JD, Gaastra W, Schouls LM. Identification of genes that are associated with DNA repeats in prokaryotes. *Mol Microbiol* 2002; **43**: 1565-1575 [PMID: 11952905 DOI: 10.1046/j.1365-2958.2002.02839.x]

29 **Bolotin A**, Quinquis B, Sorokin A, Ehrlich SD. Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. *Microbiology (Reading)* 2005; **151**: 2551-2561 [PMID: 16079334 DOI: 10.1099/mic.0.28048-0]

30 **Mojica FJ**, Díez-Villaseñor C, García-Martínez J, Soria E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J Mol Evol* 2005; **60**: 174-182 [PMID: 15791728 DOI: 10.1007/s00239-004-0046-3]

31 **Gasiunas G**, Barrangou R, Horvath P, Siksnys V. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proc Natl Acad Sci U S A* 2012; **109**: E2579-E2586 [PMID: 22949671 DOI: 10.1073/pnas.1208507109]

32 **Jinek M**, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 2012; **337**: 816-821 [PMID: 22745249 DOI: 10.1126/science.1225829]

33 **Liu Z**, Dong H, Cui Y, Cong L, Zhang D. Application of different types of CRISPR/Cas-based systems in bacteria. *Microb Cell Fact* 2020; **19**: 172 [PMID: 32883277 DOI: 10.1186/s12934-020-01431-z]

34 **Mei Y**, Wang Y, Chen H, Sun ZS, Ju XD. Recent Progress in CRISPR/Cas9 Technology. *J Genet Genomics* 2016; **43**: 63-75 [PMID: 26924689 DOI: 10.1016/j.jgg.2016.01.001]

35 **Shao M**, Xu TR, Chen CS. The big bang of genome editing technology: development and application of the CRISPR/Cas9 system in disease animal models. *Dongwuxue Yanjiu* 2016; **37**: 191-204 [PMID: 27469250 DOI: 10.13918/j.issn.2095-8137.2016.4.191]

36 **Ceasar SA**, Rajan V, Prykhozhij SV, Berman JN, Ignacimuthu S. Insert, remove or replace: A highly advanced genome editing system using CRISPR/Cas9. *Biochim Biophys Acta* 2016; **1863**: 2333-2344 [PMID: 27350235 DOI: 10.1016/j.bbamcr.2016.06.009]

37 **Liu M**, Rehman S, Tang X, Gu K, Fan Q, Chen D, Ma W. Methodologies for Improving HDR Efficiency. *Front Genet* 2018; **9**: 691 [PMID: 30687381 DOI: 10.3389/fgene.2018.00691]

38 **Jiang F**, Doudna JA. CRISPR-Cas9 Structures and Mechanisms. *Annu Rev Biophys* 2017; **46**: 505-529 [PMID: 28375731 DOI: 10.1146/annurev-biophys-062215-010822]

39 **Yang H**, Ren S, Yu S, Pan H, Li T, Ge S, Zhang J, Xia N. Methods Favoring Homology-Directed Repair Choice in Response to CRISPR/Cas9 Induced-Double Strand Breaks. *Int J Mol Sci* 2020; **21** [PMID: 32899704 DOI: 10.3390/ijms21186461]

40 **Russo FB**, Freitas BC, Pignatari GC, Fernandes IR, Sebat J, Muotri AR, Beltrão-Braga PCB. Modelling the Interplay Between Neurons and Astrocytes in Autism Using Human Induced Pluripotent Stem Cells. *Biol Psychiatry* 2018; **83**: 569-578 [PMID: 29129319 DOI: 10.1016/j.biopsych.2017.09.021]

41 **Marchetto MC**, Belinson H, Tian Y, Freitas BC, Fu C, Vadodaria K, Beltrao-Braga P, Trujillo CA, Mendes APD, Padmanabhan K, Nunez Y, Ou J, Ghosh H, Wright R, Brennand K, Pierce K, Eichenfield L, Pramparo T, Eyler L, Barnes CC, Courchesne E, Geschwind DH, Gage FH, Wynshaw-Boris A, Muotri AR. Altered proliferation and networks in neural cells derived from idiopathic autistic individuals. *Mol Psychiatry* 2017; **22**: 820-835 [PMID: 27378147 DOI: 10.1038/mp.2016.95]

42 **Engle SJ**, Blaha L, Kleiman RJ. Best Practices for Translational Disease Modeling Using Human iPSC-Derived Neurons. *Neuron* 2018; **100**: 783-797 [PMID: 30465765 DOI: 10.1016/j.neuron.2018.10.033]

43 **Deneault E**, White SH, Rodrigues DC, Ross PJ, Faheem M, Zaslavsky K, Wang Z, Alexandrova R, Pellecchia G, Wei W, Piekna A, Kaur G, Howe JL, Kwan V, Thiruvahindrapuram B, Walker S, Lionel AC, Pasceri P, Merico D, Yuen RKC, Singh KK, Ellis J, Scherer SW. Complete Disruption of Autism-Susceptibility Genes by Gene Editing Predominantly Reduces Functional Connectivity of Isogenic Human Neurons. *Stem Cell Reports* 2018; **11**: 1211-1225 [PMID: 30392976

DOI: 10.1016/j.stemcr.2018.10.003]

44 **Yi F**, Danko T, Botelho SC, Patzke C, Pak C, Wernig M, Südhof TC. Autism-associated SHANK3 haploinsufficiency causes Ih channelopathy in human neurons. *Science* 2016; **352**: aaf2669 [PMID: 26966193 DOI: 10.1126/science.aaf2669]

45 **Deneault E**, Faheem M, White SH, Rodrigues DC, Sun S, Wei W, Piekna A, Thompson T, Howe JL, Chalil L, Kwan V, Walker S, Pasceri P, Roth FP, Yuen RK, Singh KK, Ellis J, Scherer SW. CNTN5(-)(/+)or EHMT2(-)(/+)human iPSC-derived neurons from individuals with autism develop hyperactive neuronal networks. *Elife* 2019; **8** [PMID: 30747104 DOI: 10.7554/eLife.40092]

46 **Frega M**, Linda K, Keller JM, Gümüş-Akay G, Mossink B, van Rhijn JR, Negwer M, Klein Gunnewiek T, Foreman K, Kompier N, Schoenmaker C, van den Akker W, van der Werf I, Oudakker A, Zhou H, Kleefstra T, Schubert D, van Bokhoven H, Nadif Kasri N. Neuronal network dysfunction in a model for Kleefstra syndrome mediated by enhanced NMDAR signaling. *Nat Commun* 2019; **10**: 4928 [PMID: 31666522 DOI: 10.1038/s41467-019-12947-3]

47 **Rao SR**, Kostic A, Baillargeon P, Fernandez-Vega V, de Anda MR, Fletcher K, Shumate J, Scampavia L, Buxbaum JD, Spicer TP. Screening for modulators of autism spectrum disorder using induced human neurons. *SLAS Discov* 2022; **27**: 128-139 [PMID: 35123134 DOI: 10.1016/j.slasd.2022.01.004]

48 **Wang P**, Lin M, Pedrosa E, Hrabovsky A, Zhang Z, Guo W, Lachman HM, Zheng D. CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in neurodevelopment. *Mol Autism* 2015; **6**: 55 [PMID: 26491539 DOI: 10.1186/s13229-015-0048-6]

49 **Ross PJ**, Zhang WB, Mok RSF, Zaslavsky K, Deneault E, D'Abate L, Rodrigues DC, Yuen RKC, Faheem M, Mufteev M, Piekna A, Wei W, Pasceri P, Landa RJ, Nagy A, Varga B, Salter MW, Scherer SW, Ellis J. Synaptic Dysfunction in Human Neurons With Autism-Associated Deletions in PTCHD1-AS. *Biol Psychiatry* 2020; **87**: 139-149 [PMID: 31540669 DOI: 10.1016/j.biopsych.2019.07.014]

50 **Rontani P**, Perche O, Greetham L, Jullien N, Gepner B, Féron F, Nivet E, Erard-Garcia M. Impaired expression of the COSMOC/MOCOS gene unit in ASD patient stem cells. *Mol Psychiatry* 2021; **26**: 1606-1618 [PMID: 32327736 DOI: 10.1038/s41380-020-0728-2]

51 **Rabeling A**, Goolam M. Cerebral organoids as an in vitro model to study autism spectrum disorders. *Gene Ther* 2022 [PMID: 35790793 DOI: 10.1038/s41434-022-00356-z]

52 **Englund C**, Fink A, Lau C, Pham D, Daza RA, Bulfone A, Kowalczyk T, Hevner RF. Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. *J Neurosci* 2005; **25**: 247-251 [PMID: 15634788 DOI: 10.1523/JNEUROSCI.2899-04.2005]

53 **Paulsen B**, Velasco S, Kedaigle AJ, Pigoni M, Quadrato G, Deo A, Adiconis X, Uzquiano A, Kim K, Simmons SK, Tsafou K, Albanese A, Sartore R, Abbate C, Tucewicz A, Smith S, Chung K, Lage K, Regev A, Levin JZ, Arlotta P. Human brain organoids reveal accelerated development of cortical neuron classes as a shared feature of autism risk genes. *Developmental Biology* [DOI: 10.1101/2020.11.10.376509]

54 **Zhang W**, Ma L, Yang M, Shao Q, Xu J, Lu Z, Zhao Z, Chen R, Chai Y, Chen JF. Cerebral organoid and mouse models reveal a RAB39b-PI3K-mTOR pathway-dependent dysregulation of cortical development leading to macrocephaly/autism phenotypes. *Genes Dev* 2020; **34**: 580-597 [PMID: 32115408 DOI: 10.1101/gad.332494.119]

55 **Amir RE**, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet* 1999; **23**: 185-188 [PMID: 10508514 DOI: 10.1038/13810]

56 **Trujillo CA**, Adams JW, Negraes PD, Carromeu C, Tejwani L, Acab A, Tsuda B, Thomas CA, Sodhi N, Fichter KM, Romero S, Zanella F, Sejnowski TJ, Ulrich H, Muotri AR. Pharmacological reversal of synaptic and network pathology in human MECP2-KO neurons and cortical organoids. *EMBO Mol Med* 2021; **13**: e12523 [PMID: 33501759 DOI: 10.15252/emmm.202012523]

57 **Xiang Y**, Tanaka Y, Patterson B, Hwang SM, Hysolli E, Cakir B, Kim KY, Wang W, Kang YJ, Clement EM, Zhong M, Lee SH, Cho YS, Patra P, Sullivan GJ, Weissman SM, Park IH. Dysregulation of BRD4 Function Underlies the Functional Abnormalities of MeCP2 Mutant Neurons. *Mol Cell* 2020; **79**: 84-98.e9 [PMID: 32526163 DOI: 10.1016/j.molcel.2020.05.016]

58 **Sun AX**, Yuan Q, Fukuda M, Yu W, Yan H, Lim GGY, Nai MH, D'Agostino GA, Tran HD, Itahana Y, Wang D, Lokman H, Itahana K, Lim SWL, Tang J, Chang YY, Zhang M, Cook SA, Rackham OJL, Lim CT, Tan EK, Ng HH, Lim KL, Jiang YH, Je HS. Potassium channel dysfunction in human neuronal models of Angelman syndrome. *Science* 2019; **366**: 1486-1492 [PMID: 31857479 DOI: 10.1126/science.aav5386]

59 **Blair JD**, Hockemeyer D, Bateup HS. Genetically engineered human cortical spheroid models of tuberous sclerosis. *Nat Med* 2018; **24**: 1568-1578 [PMID: 30127391 DOI: 10.1038/s41591-018-0139-y]

60 **Dooves S**, van Velthoven AJH, Suciati LG, Heine VM. Neuron-Glia Interactions in Tuberous Sclerosis Complex Affect the Synaptic Balance in 2D and Organoid Cultures. *Cells* 2021; **10** [PMID: 33445520 DOI: 10.3390/cells10010134]

61 **Wang Y**, Chiola S, Yang G, Russell C, Armstrong CJ, Wu Y, Spampanato J, Tarboton P, Ullah HMA, Edgar NU, Chang AN, Harmin DA, Bocchi VD, Vezzoli E, Besusso D, Cui J, Cattaneo E, Kubanek J, Shcheglovitov A. Modeling human telencephalic development and autism-associated SHANK3 deficiency using organoids generated from single neural rosettes. *Nat Commun* 2022; **13**: 5688 [PMID: 36202854 DOI: 10.1038/s41467-022-33364-z]

62 **Brighi C**, Salaris F, Soloperto A, Cordella F, Ghirga S, de Turris V, Rosito M, Porceddu PF, D'Antoni C, Reggiani A, Rosa A, Di Angelantonio S. Novel fragile X syndrome 2D and 3D brain models based on human isogenic FMRP-KO iPSCs. *Cell Death Dis* 2021; **12**: 498 [PMID: 33993189 DOI: 10.1038/s41419-021-03776-8]

63 **Doi M**, Li M, Usui N, Shimada S. Genomic Strategies for Understanding the Pathophysiology of Autism Spectrum Disorder. *Front Mol Neurosci* 2022; **15**: 930941 [PMID: 35813066 DOI: 10.3389/fnmol.2022.930941]

64 **Zhou Y**, Sharma J, Ke Q, Landman R, Yuan J, Chen H, Hayden DS, Fisher JW 3rd, Jiang M, Menegas W, Aida T, Yan T, Zou Y, Xu D, Parmar S, Hyman JB, Fanucci-Kiss A, Meisner O, Wang D, Huang Y, Li Y, Bai Y, Ji W, Lai X, Li W, Huang L, Lu Z, Wang L, Anteraper SA, Sur M, Zhou H, Xiang AP, Desimone R, Feng G, Yang S. Atypical behaviour and connectivity in SHANK3-mutant macaques. *Nature* 2019; **570**: 326-331 [PMID: 31189958 DOI: 10.1038/s41586-019-1278-0]

65 **Celen C**, Chuang JC, Luo X, Nijem N, Walker AK, Chen F, Zhang S, Chung AS, Nguyen LH, Nassour I, Budhipramono A, Sun X, Bok LA, McEntagart M, Gevers EF, Birnbaum SG, Eisch AJ, Powell CM, Ge WP, Santen GW, Chahrour M, Zhu H. Arid1b haploinsufficient mice reveal neuropsychiatric phenotypes and reversible causes of growth impairment. *Elife* 2017; **6** [PMID: 28695822 DOI: 10.7554/eLife.25730]

66 **Gompers AL**, Su-Feher L, Ellegood J, Copping NA, Riyadh MA, Stradleigh TW, Pride MC, Schaffler MD, Wade AA, Catta-Preta R, Zdilar I, Louis S, Kaushik G, Mannion BJ, Plajzer-Frick I, Afzal V, Visel A, Pennacchio LA, Dickel DE, Lerch JP, Crawley JN, Zarbalis KS, Silverman JL, Nord AS. Germline Chd8 haploinsufficiency alters brain development in mouse. *Nat Neurosci* 2017; **20**: 1062-1073 [PMID: 28671691 DOI: 10.1038/nn.4592]

67 **Zhu T**, Liang C, Li D, Tian M, Liu S, Gao G, Guan JS. Histone methyltransferase Ash1L mediates activity-dependent repression of neurexin-1α. *Sci Rep* 2016; **6**: 26597 [PMID: 27229316 DOI: 10.1038/srep26597]

68 **Silva AI**, Haddon JE, Ahmed Syed Y, Trent S, Lin TE, Patel Y, Carter J, Haan N, Honey RC, Humby T, Assaf Y, Owen MJ, Linden DEJ, Hall J, Wilkinson LS. Cyfip1 haploinsufficient rats show white matter changes, myelin thinning, abnormal oligodendrocytes and behavioural inflexibility. *Nat Commun* 2019; **10**: 3455 [PMID: 31371763 DOI: 10.1038/s41467-019-11119-7]

69 **Rannals MD**, Page SC, Campbell MN, Gallo RA, Mayfield B, Maher BJ. Neurodevelopmental models of transcription factor 4 deficiency converge on a common ion channel as a potential therapeutic target for Pitt Hopkins syndrome. *Rare Dis* 2016; **4**: e1220468 [PMID: 28032012 DOI: 10.1080/21675511.2016.1220468]

70 **Dodge A**, Peters MM, Greene HE, Dietrick C, Botelho R, Chung D, Willman J, Nenninger AW, Ciarlone S, Kamath SG, Houdek P, Sumová A, Anderson AE, Dindot SV, Berg EL, O'Geen H, Segal DJ, Silverman JL, Weeber EJ, Nash KR. Generation of a Novel Rat Model of Angelman Syndrome with a Complete Ube3a Gene Deletion. *Autism Res* 2020; **13**: 397-409 [PMID: 31961493 DOI: 10.1002/aur.2267]

71 **Bernier R**, Golzio C, Xiong B, Stessman HA, Coe BP, Penn O, Witherspoon K, Gerdts J, Baker C, Vulto-van Silfhout AT, Schuurs-Hoeijmakers JH, Fichera M, Bosco P, Buono S, Alberti A, Failla P, Peeters H, Steyaert J, Vissers LELM, Francescatto L, Mefford HC, Rosenfeld JA, Bakken T, O'Roak BJ, Pawlus M, Moon R, Shendure J, Amaral DG, Lein E, Rankin J, Romano C, de Vries BBA, Katsanis N, Eichler EE. Disruptive CHD8 mutations define a subtype of autism early in development. *Cell* 2014; **158**: 263-276 [PMID: 24998929 DOI: 10.1016/j.cell.2014.06.017]

72 **Hu J**, Chen L, Yin J, Yin H, Huang Y, Tian J. Hyperactivity, Memory Defects, and Craniofacial Abnormalities in Zebrafish fmr1 Mutant Larvae. *Behav Genet* 2020; **50**: 152-160 [PMID: 32048109 DOI: 10.1007/s10519-020-09995-7]

73 **Ruzzo EK**, Pérez-Cano L, Jung JY, Wang LK, Kashef-Haghighi D, Hartl C, Singh C, Xu J, Hoekstra JN, Leventhal O, Leppä VM, Gandal MJ, Paskov K, Stockham N, Polioudakis D, Lowe JK, Prober DA, Geschwind DH, Wall DP. Inherited and De Novo Genetic Risk for Autism Impacts Shared Networks. *Cell* 2019; **178**: 850-866.e26 [PMID: 31398340 DOI: 10.1016/j.cell.2019.07.015]

74 **Liu CX**, Li CY, Hu CC, Wang Y, Lin J, Jiang YH, Li Q, Xu X. CRISPR/Cas9-induced shank3b mutant zebrafish display autism-like behaviors. *Mol Autism* 2018; **9**: 23 [PMID: 29619162 DOI: 10.1186/s13229-018-0204-x]

75 **Staahl BT**, Benekareddy M, Coulon-Bainier C, Banfal AA, Floor SN, Sabo JK, Urnes C, Munares GA, Ghosh A, Doudna JA. Efficient genome editing in the mouse brain by local delivery of engineered Cas9 ribonucleoprotein complexes. *Nat Biotechnol* 2017; **35**: 431-434 [PMID: 28191903 DOI: 10.1038/nbt.3806]

76 **Warrier V**, Chee V, Smith P, Chakrabarti B, Baron-Cohen S. A comprehensive meta-analysis of common genetic variants in autism spectrum conditions. *Mol Autism* 2015; **6**: 49 [PMID: 26322220 DOI: 10.1186/s13229-015-0041-0]

77 **Wiśniowiecka-Kowalnik B**, Nowakowska BA. Genetics and epigenetics of autism spectrum disorder-current evidence in the field. *J Appl Genet* 2019; **60**: 37-47 [PMID: 30627967 DOI: 10.1007/s13353-018-00480-w]

78 **Guo H**, Zhang Q, Dai R, Yu B, Hoekzema K, Tan J, Tan S, Jia X, Chung WK, Hernan R, Alkuraya FS, Alsulaiman A, Al-Muhaizea MA, Lesca G, Pons L, Labalme A, Laux L, Bryant E, Brown NJ, Savva E, Ayres S, Eratne D, Peeters H, Bilan F, Letienne-Cejudo L, Gilbert-Dussardier B, Ruiz-Arana IL, Merlini JM, Boizot A, Bartoloni L, Santoni F, Karlowicz D, McDonald M, Wu H, Hu Z, Chen G, Ou J, Brasch-Andersen C, Fagerberg CR, Dreyer I, Chun-Hui Tsai A, Slegesky V, McGee RB, Daniels B, Sellars EA, Carpenter LA, Schaefer B, Sacoto MJG, Begtrup A, Schnur RE, Punj S, Wentzensen IM, Rhodes L, Pan Q, Bernier RA, Chen C, Eichler EE, Xia K. NCKAP1 Disruptive Variants Lead to a Neurodevelopmental Disorder with Core Features of Autism. *Am J Hum Genet* 2020; **107**: 963-976 [PMID: 33157009 DOI: 10.1016/j.ajhg.2020.10.002]

79 **Levitt P**, Campbell DB. The genetic and neurobiologic compass points toward common signaling dysfunctions in autism spectrum disorders. *J Clin Invest* 2009; **119**: 747-754 [PMID: 19339766 DOI: 10.1172/JCI37934]

80 **Muhle R**, Trentacoste SV, Rapin I. The genetics of autism. *Pediatrics* 2004; **113**: e472-e486 [PMID: 15121991 DOI: 10.1542/peds.113.5.e472]

81 **Shailesh H**, Gupta I, Sif S, Ouhtit A. Towards understanding the genetics of Autism. *Front Biosci (Elite Ed)* 2016; **8**: 412-426 [PMID: 27100348 DOI: 10.2741/e776]

82 **Huang K**, Wu Y, Shin J, Zheng Y, Siahpirani AF, Lin Y, Ni Z, Chen J, You J, Keles S, Wang D, Roy S, Lu Q. Transcriptome-wide transmission disequilibrium analysis identifies novel risk genes for autism spectrum disorder. *PloS Genet* 2021; **17**: e1009309 [PMID: 33539344 DOI: 10.1371/journal.pgen.1009309]

83 **Lee B**, Lee K, Panda S, Gonzales-Rojas R, Chong A, Bugay V, Park HM, Brenner R, Murthy N, Lee HY. Nanoparticle delivery of CRISPR into the brain rescues a mouse model of fragile X syndrome from exaggerated repetitive behaviours. *Nat Biomed Eng* 2018; **2**: 497-507 [PMID: 30948824 DOI: 10.1038/s41551-018-0252-8]

84 **Silverman JL**, Smith DG, Rizzo SJ, Karras MN, Turner SM, Tolu SS, Bryce DK, Smith DL, Fonseca K, Ring RH, Crawley JN. Negative allosteric modulation of the mGluR5 receptor reduces repetitive behaviors and rescues social deficits in mouse models of autism. *Sci Transl Med* 2012; **4**: 131ra51 [PMID: 22539775 DOI: 10.1126/scitranslmed.3003501]

85 **Tao J**, Wu H, Coronado AA, de Laittre E, Osterweil EK, Zhang Y, Bear MF. Negative Allosteric Modulation of mGluR5 Partially Corrects Pathophysiology in a Mouse Model of Rett Syndrome. *J Neurosci* 2016; **36**: 11946-11958 [PMID: 27881780 DOI: 10.1523/JNEUROSCI.0672-16.2016]

86 **Le TTH**, Tran NT, Dao TML, Nguyen DD, Do HD, Ha TL, Kühn R, Nguyen TL, Rajewsky K, Chu VT. Efficient and

Precise CRISPR/Cas9-Mediated MECP2 Modifications in Human-Induced Pluripotent Stem Cells. *Front Genet* 2019; **10**: 625 [PMID: 31333716 DOI: 10.3389/fgene.2019.00625]

87 **Zaslavsky K**, Zhang WB, McCready FP, Rodrigues DC, Deneault E, Loo C, Zhao M, Ross PJ, El Hajjar J, oom A, Thompson T, Piekna A, Wei W, Wang Z, Khattak S, Mufteev M, Pasceri P, Scherer SW, Salter MW, Ellis J. SHANK2 mutations associated with autism spectrum disorder cause hyperconnectivity of human neurons. *Nat Neurosci* 2019; **22**: 556-564 [PMID: 30911184 DOI: 10.1038/s41593-019-0365-8]

88 **Wolter JM**, Mao H, Fragola G, Simon JM, Krantz JL, Bazick HO, Oztemiz B, Stein JL, Zylka MJ. Cas9 gene therapy for Angelman syndrome traps Ube3a-ATS long non-coding RNA. *Nature* 2020; **587**: 281-284 [PMID: 33087932 DOI: 10.1038/s41586-020-2835-2]

89 **Xie N**, Gong H, Suhl JA, Chopra P, Wang T, Warren ST. Reactivation of FMR1 by CRISPR/Cas9-Mediated Deletion of the Expanded CGG-Repeat of the Fragile X Chromosome. *PloS One* 2016; **11**: e0165499 [PMID: 27768763 DOI: 10.1371/journal.pone.0165499]

90 **Jaudon F**, Thalhammer A, Zentilin L, Cingolani LA. CRISPR-mediated activation of autism gene Itgb3 restores cortical network excitability *via* mGluR5 signaling. *Mol Ther Nucleic Acids* 2022; **29**: 462-480 [PMID: 36035754 DOI: 10.1016/j.omtn.2022.07.013]

91 **Adlat S**, Hayel F, Yang P, Chen Y, Oo ZM, Myint MZZ, Sah RK, Bahadar N, Al-Azab M, Bah FB, Zheng Y, Feng X. CRISPR-mediated base editing in mice using cytosine deaminase base editor 4. *Electron J Biotechn* 2021; **52**: 59-66 [DOI: 10.1016/j.ejbt.2021.04.010]

92 **Li W**, Chen J, Peng W, Yuan B, Han W, Yuan Y, Xue Z, Wang J, Chen Z, Shan S, Zhu S, Xu M, Cheng T, Qiu Z. Whole-brain in vivo base editing reverses autistic-like behaviors in mice. *Neuroscience* [DOI: 10.1101/2022.01.25.477781]

93 **de Jong JO**, Llapashtica C, Genestine M, Strauss K, Provenzano F, Sun Y, Zhu H, Cortese GP, Brundu F, Brigatti KW, Corneo B, Migliori B, Tomer R, Kushner SA, Kellendonk C, Javitch JA, Xu B, Markx S. Cortical overgrowth in a preclinical forebrain organoid model of CNTNAP2-associated autism spectrum disorder. *Nat Commun* 2021; **12**: 4087 [PMID: 34471112 DOI: 10.1038/s41467-021-24358-4]

94 **Tamura S**, Nelson AD, Spratt PWE, Kyoung H, Zhou X, Li Z, Zhao J, Holden SS, Sahagun A, Keeshen CM, Lu C, Hamada EC, Ben-Shalom R, Pan JQ, Paz JT, Sanders SJ, Matharu N, Ahituv N, Bender KJ. CRISPR activation rescues abnormalities in SCN2A haploinsufficiency-associated autism spectrum disorder. *Neuroscience* [DOI: 10.1101/2022.03.30.486483]

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