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Rodent models of genetic and chromosomal variations in psychiatric disorders

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Elucidating the molecular basis of complex human psychiatric disorders is challenging due to the multitude of factors that underpin these disorders. Genetic and chromosomal changes are two factors that have been suggested to be involved in psychiatric disorders. Indeed, numerous risk loci have been identified in autism spectrum disorders, schizophrenia, and related psychiatric disorders. Here, we introduce genetic animal models that disturb excitatory-inhibitory balance in the brain and animal models mirroring human chromosomal abnormalities, both of which may be implicated in autism spectrum disorder pathophysiology. In addition, we discuss recent unique translational research using rodent models, such as *Cntnap2* knockout mouse, *Mecp2* mutant mouse, *Pick1* knockout mouse, and neonatal ventral hippocampal lesion rat. By using these models, several types of drugs are administered during the developmental period to see the effect on psychotic symptoms and neural activities in adults. The accumulating evidence from recent animal studies provides an informative intervention strategy as a translational research.

Key words: animal models, autism, copy number variation, early intervention, excitatory inhibitory balance.

THE PATHOPHYSIOLOGY OF psychiatric disorders is complex. Factors that may contribute to the development of psychiatric disease include imbalance in excitatory and inhibitory (E/I) transmission and chromosomal changes. The E/I imbalance can be caused by modulation of receptors that in turn lead to abnormal activity of glutamatergic neurons and γ-aminobutyric acid (GABA)ergic interneurons. Indeed, genes involved in E/I transmission have been reported to be dysregulated in postmortem brains of patients with schizophrenia and Rett syndrome (RTT).[1,2] Chromosomal abnormalities are another factor associated with psychiatric disorders. Copy number variations (CNV) are duplications or deletions of genomic loci, ranging from kilobase (kb) to megabase (Mb), and can pertain to either a single gene or multiple genes.[3] Autism spectrum disorder (ASD), schizophrenia, and RTT are diseases attributed to CNV.

Uncovering the mechanism whereby specific genetic abnormalities lead to psychiatric disease is difficult to do in clinical studies. However, rodent models can be a useful tool in understanding the potential role of E/I imbalance and chromosomal abnormalities on the development of psychiatric disorders. In addition, rodent models permit the systematic testing of therapeutics during different stages of disease onset to determine the efficacy of the timing of treatment interventions. This review

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will focus on rodent models of E/I imbalance and CNV relevant to psychiatric disorders. Rodent models suggesting the usefulness of treatment early in disease progression are also discussed.

Rodent models of E/I imbalance

Patients with ASD and other psychiatric disorders are thought to have E/I imbalance in the brain.[4,5] ASD patients frequently present with epileptic seizures, with a prevalence rate estimated to range between 8% and 25%.[6] Epileptic seizures may be due to excess excitatory neuronal inputs of the cortical circuit, suggesting E/I imbalance in the brains of ASD patients.[7] Indeed, a recent study showed that 3-D cultures (cortical organoids) derived from individuals with severe idiopathic ASD have normal levels of glutamatergic excitatory neurons and increased numbers of GABAergic inhibitory neurons.[8] Also, a functional genetic analysis on patients with schizophrenia has uncovered genes involved in glutamatergic and GABAergic neurotransmission, suggesting that these patients also have E/I imbalance.[9] In addition, patients with the X-linked neurodevelopmental disorder, RTT, have been shown to have hypoexcitability in the forebrain, possibly indicating E/I imbalance.[3] Postmortem brain studies of RTT patients have shown a decrease in glutamatergic α-amino-3-hydroxy-5-methylisoxazol-4-propionic acid receptor (AMPAR) and *N*-methyl-*D*-aspartate receptor (NMDAR) densities in the putamen and a decrease in GABA receptors in the caudate.[10] These studies demonstrate the heterogeneity of E/I imbalance associated with mental illness. However, the mechanism of E/I-imbalance-induced psychiatric symptoms is difficult to identify in human studies. Therefore, rodent models are useful tools in elucidating the behavioral, molecular, cellular, and electrophysiological outcomes of E/I imbalance associated with psychiatric diseases (Table 1).[11–17]

Rodent models with E/I imbalance exhibit phenotypes reminiscent of patients with ASD and schizophrenia. For example, Yizhar *et al.*[11] utilized optogenetics to elevate excitatory neurons in the medial prefrontal cortex (mPFC) of mice. These mice displayed social deficits and an increase in electroencephalogram frequency as compared to controls, reminiscent of deficits observed in patients with ASD and schizophrenia.[11] Another mouse model is the *insulin receptor substrate protein 53 kDa* (*IRSp53*) knockout (KO) mouse. IRSp53 is an excitatory synaptic scaffolding molecule detected throughout the brain.[18] *IRSp53* KO mice showed enhanced hippocampal NMDAR function and impaired social interaction, a behavior observed in patients with ASD as well as the optogenetics mouse model of elevated mPFC excitatory neurons. Interestingly, administration of the NMDAR antagonist memantine or the selective metabotropic glutamate receptor5 (mGluR5) antagonist 2-methyl-6-(phenylethynyl) pyridine (MPEP) was able to recover the social deficits observed in *IRSp53* KO mice.[14] Testing these drugs for the ablation of social deficits in other mouse models would be an interesting step towards efficacy in treating humans. While the optogenetics and *IRSp53* KO rodent models increase the E/I ratio in the brain by modifying excitatory neurons, other rodent models increase the E/I ratio by modulating inhibitory neurons. Brown *et al.*[12] increased the E/I ratio in the neocortex and dentate gyrus of mice by knocking down *Gad1* in these brain regions. *Gad1* encodes the 67-kDa isoform of glutamic acid decarboxylase (Gad67) and is important for inhibitory neurons. *Gad1* knockdown mice exhibited a decrease in inhibitory parvalbumin (PV)-expressing interneurons and displayed sensorimotor gating deficits, increased novelty-seeking, and reduced fear extinction.[12] Another group ablated *metabotropic glutamate receptor 5* (*mGluR5*) on PV-expressing interneurons, which resulted in reduced numbers of PV neurons, decreased inhibitory currents, and behavioral abnormalities in mice. These mice exhibited increased repetitive behavior, impaired recognition memory, and sensory motor gating abnormalities.[13] Treatment of conditional *mGluR5* KO (in forebrain glutamatergic neurons) mice with MPEP decreased mPFC firing of GABAergic neurons and increased firing of glutamatergic neurons.[15] These results elucidate a possible antipsychotic action of MPEP and suggest that mGluR5 directly regulates E/I balance by dominantly regulating inhibitory neuronal input onto glutamatergic neurons. While memantine recovers deficits in a genetic KO, another NMDAR antagonist, ketamine, has also been assessed by Patrizi *et al.*[17] They reported that chronic administration of ketamine from either postnatal day (P)15 or P30–P55 increased neuronal activity, lifespan, respiratory function, and visual acuity of *Mecp2*-null (KO) mice. The authors confirmed that low-dose chronic ketamine administration (8 mg/kg) is rapidly detected

Table 1. Representative animal models of E/I imbalance

Animal model	E/I ratio	Phenotypes	Rescue	Reference
Optogenetic control	Increase in mPFC	Social deficits, high frequency electroencephalogram (30–80 Hz)	Optogenetic	Yizhar *et al.*[11]
Gad1 knockdown	Increase	Sensorimotor gating deficits, increased novelty-seeking, and reduced fear extinction	—	Brown *et al.*[12]
Postnatal ablation of *mGluR5* from PV-expressing interneuron	Increase	Impaired recognition memory, increased repetitive behaviors, and sensory motor gating abnormalities	—	Barnes *et al.*[13]
IRSp53 KO	Increase	Social deficits	Memantin (NMDAR antagonist) MPEP (selective antagonist for the metabotropic glutamate receptor subtype mGluR5)	Chung *et al.*[14]
mGluR5 KO in GABAergic neurons	Increase	Antidepressant-like behavior	—	Lee *et al.*[15]
mGluR5 KO in glutamatergic neurons	Decrease	Depression-like behavior	MPEP	Lee *et al.*[15]
Mecp2 KO	Decrease	Several neurological and psychological phenotypes[16]	Ketamine (NMDAR antagonist)	Patrizi *et al.*[17]

E/I, excitatory-inhibitory; KO, knockout; MPEP, 2-methyl-6-(phenylethynyl) pyridine; mPFC, medial prefrontal cortex; NMDAR, *N*-methyl-*D*-aspartate receptor; PV, parvalbumin.

in the brain without any adverse side-effects. Intriguingly, the time-points chosen for the start of ketamine administration correlate with when PV cortical circuits are altered (P15) and the onset of RTT phenotypes (P30). Since the spiking activities of cortical excitatory neurons are tightly regulated by PV-positive inhibitory neurons,[19] it is possible that ketamine preferentially modulates NMDAR on PV-positive cells, leading to disinhibition of pyramidal cells and thus renormalizing E/I balance in the cortex of the *Mecp2* KO mouse model of RTT. The pharmacological studies discussed in this section support previous findings that E/I imbalance in the mPFC affects sociability,[11] and show that pharmacological intervention of receptors, such as mGluR5 or NMDAR, may be effective against psychosocial deficits.

Rodent models of copy number variations

CNV is defined as deletion or duplication of a genomic locus larger than 1 kb in size. Some CNV encompass dozens of genes that may include coding gene(s) or genes in regulatory regions,[20] such as the promoter, enhancer, and silencer, while others pertain to only a single gene. Available mouse models of CNV include 7q11.23 (deletion),[21] 15q11-13 (duplication),[22] 15q13.3 (deletion),[23,24] 16p11.2 (deletion and duplication),[25] 17p11.2 (deletion and duplication),[26] 22q11.2 (deletion),[27] and MECP2 (deletion and duplication). These mice are known to display behavioral and brain abnormalities paralleling what has frequently been reported in patients.[28] In this review, we focus on CNV mouse models of 15q11-13, 22q11.2, and MECP2.

15q11-13 duplication

A duplication in 15q11-13 is one of the most prevalent chromosomal abnormalities in ASD. This region is syntenic to mouse chromosome 7qB5-qC. The 15q11-13 chromosomal duplication confers >85% risk of developing ASD, and is found in 1–3% of individuals with ASD.[29] Mice with

paternally inherited 6-Mb 15q11-13 duplication (6-Mb patDp/+) displayed social deficits, behavioral inflexibility, and abnormal ultrasonic vocalization, which are typical autistic behavioral phenotypes in rodents. These behaviors were not present in mice with a maternally inherited duplication (matDp/+).[22] In addition to behavioral abnormalities, 6-Mb patDp/+ mice had decreased serotonin levels in the developing brain[30] and displayed late-onset obesity,[31] both of which resemble what is observed in patients with paternally inherited 15q11-13 duplication.[32] Recently, in order to identify a causal gene of obesity, Kishimoto *et al.* generated a new mouse model with a 3-Mb 15q11-13 paternal duplication (3-Mb patDp/+).[31] Transcriptome analysis on these mice revealed an upregulation in the gene *secreted frizzled-related protein 5* (*Sfrp5*). Sfrp5 induces adipocyte differentiation and increases adipocytes during obesity. This gene may therefore be a possible target for combating or preventing obesity accompanied by autistic symptoms, but it may not be effective against cognitive dysfunction or social deficits.

Another hallmark of ASD and some behavioral changes, such as deregulation of motor coordination and learning,[33] is cerebellar dysfunction. Six-megabase patDp/+ mice exhibited impaired cerebellum-dependent motor learning and coordination, but normal cerebellar morphology.[22,34,35] This is consistent with a magnetic resonance imaging (MRI) study in patDp/+ mice that revealed regional difference existing in the dentate gyrus, medial striatum, and dorsal raphe nucleus, but not in the cerebellar cortex.[36–38] In addition, patDp/+ mice showed impaired long-term depression at cerebellar parallel fiber-Purkinje cell synapses, which may reflect a cellular mechanism underlying motor learning impairment.[39] Since the cerebellum is likely involved in motor and non-motor aspects of ASD phenotypes,[33] cerebellar abnormalities may be involved in a portion of the ASD pathophysiology.

22q11.2 deletion

The 22q11.2 deletion, a region syntenic to mouse chromosome 16qA3, is also known as DiGeorge syndrome and velocardiofacial syndrome. It is one of the most common chromosomal abnormalities, occurring in 1 out of every 4000 live births.[40] Most cases (90%) are of a 3-Mb microdeletion on the long arm of chromosome 22, but a 1.5-Mb microdeletion is also common and has been reported in 8% of cases.[41] The 22q11.2 microdeletion has been associated with congenital heart defects (CHD), palatal anomalies, hypoparathyroidism, hypocalcemia, facial dysmorphisms, and learning disabilities. Additionally, this chromosomal deletion has been strongly associated with schizophrenia and other mental illnesses, such as anxiety disorders, mood disorders, ASD, and attention-deficit hyperactivity disorder.[42]

In recent years, through genetically modified mutant mice that carry multigene and single gene mutations, we have learned that a mutation in *Tbx1*, a member of the T-box family transcription factor gene, is responsible for most of the congenital defects seen in patients and mouse models.[43–46] Rodent models suggest that 22q11.2 deletion leads to behavioral changes and deficits in RNA processing. Mice carrying a hemizygous 1.3-Mb chromosomal deficiency (Df(16)A+/–)[27] showed deficits in pre-pulse inhibition (PPI), a test of sensory information-processing of external stimuli. PPI deficits are frequently observed in several psychiatric and neurological diseases, including schizophrenia, obsessive–compulsive disorder, attention-deficit hyperactivity disorder, and Huntington's disease.[47] In addition to PPI deficits, Df (16)A+/– mice showed dysregulation in miRNA biogenesis.[27] In these mice, a subset of pri-miRNA was upregulated and a smaller subset of the mature form of the miRNA was downregulated due to haploinsufficiency of the *DiGeorge syndrome critical region 8* gene (*Dgcr8*: also known as *Pasha*). Because *Dgcr8* is an essential component of pri-miRNA processing,[48] haploinsufficiency of this gene may affect brain cognitive functions[27,49] due to dysregulation of critical downstream genes involved in neural development. For example, *insulin growth factor* (*IGF*) is one gene downstream of *Dgcr8*. Administration of IGF to *Dgcr8* KO mice rescued hippocampal functions.[50] Recently, Mukai *et al.* reported that deletion of the 22q11 locus *zinc finger and Asp-His-His-Cys (DHHC) domain-containing protein 8* (*Zdhhc8*) led to changes in axonal growth, dendrite arborization, synaptic connectivity, spatial working memory, and prefrontal-hippocampal synchrony.[51] Behavioral and neuronal abnormalities were consistent between Df (16)A+/– and *Zdhhc* KO mice. This interesting consequence gives rise to new insights into post-translational modification of proteins related to

cognitive function and abnormal brain circuit formation.

MECP2

Mutations in the *MECP2* gene cause the neurodevelopmental disorder RTT. This syndrome is characterized by small head size (microcephaly), gait abnormalities, loss of language, breathing disturbances, and poor motor movements with repetitive stereotypies. *MECP2* is located on region Xq28 of the X chromosome where deletions, mutations, and duplications of this gene have been reported.[52] Classically, RTT has been recognized as a girl's disorder, with symptomatic women having either a *MECP2* point mutation or deletion. Women with a *MECP2* duplication remain healthy, likely due to skewed X inactivation.[53] In contrast, boys with an *MECP2* duplication present with clinical features of RTT. For instance, Meins *et al.* reported on an *MECP2* duplication of approximately 430 kb in a boy with hypotonia, mental retardation, lack of speech, and loss of motor movements.[53] Other male patients with *MECP2* duplications have presented with infantile hypotonia, mental retardation, poor speech development, recurrent infections, epilepsy, progressive spasticity, developmental regression, and ASD.[52]

Mecp2 mutant mice are available to elucidate the role of *Mecp2* on neurological and psychological functions.[16] Sztainberg *et al.* generated a *Mecp2* duplication mouse model through conditional *Mecp2*-overexpression.[54] These mice displayed hypoactivity, anxiety-like behavior, motor abnormalities, and social deficits, which are behaviors seen in patients with a *MECP2* duplication. Observed deficits were recovered by: (i) deleting the conditional *Mecp2*-overexpression allele by using tamoxifen-inducible Cre recombination; and (ii) stereotactic injection of antisense oligonucleotides into the right ventricles of the brain, which then hybridized with target mRNA to silence *Mecp2*. These rescue experiments successfully ameliorated the abnormal cortical electroencephalography, molecular, and electrophysiological deficits displayed by *Mecp2* duplication mice. As antisense oligonucleotides efficiently suppressed *Mecp2* gene expression in mouse brains as well as *MECP2* duplication patient-derived lymphoblastoids, it may be a suitable drug candidate for *MECP2* duplication patients. However, possible off-target effects still need to be determined.

Intervention during brain development may treat psychiatric symptoms

Abnormalities arising from a genetic mutation may effectively be recovered by intervention at a young age (Table 2).[17,55–57] One study suggesting this was performed on *contactin-associated protein-like 2* (*Cntnap2*) KO mice.[58] *Cntnap2* is a member of the *NRXN* gene superfamily and encodes a neuronal transmembrane protein that acts as a cell adhesion molecule, implicating the protein in neural plasticity. *Cntnap2* KO mice showed abnormalities similar to patients with a CNTNAP2 mutation,[55] such as epileptic seizures and an abnormal electroencephalography pattern.[58] Indeed, *Cntnap2* is one of the best-replicated risk genes for ASD.[59] It is therefore not surprising that *Cntnap2* KO mice display core features of ASD, including repetitive behaviors, behavioral inflexibility, and social deficits. Social deficits in neurodevelopmental disorders, such as schizophrenia and ASD, have also been suggested to be caused in part by an imbalance in the neurotransmitter oxytocin (OXT).[60–62] OXT treatment of *Cntnap2* KO improved social deficits observed in the reciprocal social interaction and three-chamber social interaction tests. Interestingly, the recovery of behavioral deficits was dependent upon the age at which OXT was administered. Adult mice (6–-8 weeks of age) acutely treated with OXT showed improvement in social interactions. Similarly, early postnatal mice (7–21 days of age) chronically treated with OXT showed improved social interaction up to 1 week after OXT cessation.[55] It is therefore possible that OXT can be used to treat social deficits, particularly when administered early in life.

Another study suggesting intervention prior to adulthood may better recover deficits was performed on *protein interacting with C kinase-1* (*Pick1*) KO mice. *Pick1* is a postsynaptic protein involved in synaptic plasticity and affects AMPAR trafficking. Indeed, hippocampal and cerebellar long-term depression are significantly impaired in *Pick1* KO mice, likely due to impaired internalization, recycling, or intracellular retention of AMPAR.[63] During development, neonatal *Pick1* KO mice have decreased levels of the NMDAR co-agonist D-serine in the prefrontal cortex and hippocampus.[64] As D-serine levels recover later in life, one may be able to use *Pick1* KO mice as a conditional D-serine knockdown model. Interestingly, adult *Pick1* KO mice, who have levels of D-serine comparable to wild-type

Table 2. Phenotypic rescue by early intervention

Animal model	Drug/main effect	Time period	Rescued behavioral phenotypes	Reference
Cntnap2 KO mouse	Oxytocin (hormone: modulate social behavior)	P7–P21	Social behaviors (three-chamber test, reciprocal social interaction test)	Peñagarikano *et al.*[55]
Pick1 KO mouse	D-serine (endogenous co-agonist for NMDAR)	P3–P14	Sensory motor gating (PPI)	Nomura *et al.*[56]
Mecp2 KO mouse	Ketamine (non-competitive NMDAR antagonist)	P15–P55 P30–P55	RTT-like phenotypes (hindlimb-clasping, apnea episode, visual acuity)	Patrizi *et al.*[17]
NVHL rat	*N*-acetyl cysteine (antioxidant)	P5–P50	Sensory motor gating (PPI)	Cabungcal *et al.*[57]

KO, knockout; NMDAR, *N*-methyl-*D*-aspartate receptor; NVHL, neonatal ventral hippocampal lesion; P, postnatal day; PPI, pre-pulse inhibition; RTT, Rett syndrome.

mice, exhibited impaired synaptic plasticity and behavioral abnormalities, including learning and memory, PPI, and cognitive deficits.[56,65] This suggests that decreased levels of D-serine during development adversely affect the brain during adulthood. To test whether rescuing D-serine levels during development would prevent behavioral deficits during adulthood, *Pick1* KO mice were treated with D-serine during the neonatal development period (P3–P17). In the treated mice, abnormal NMDA-elicited firing in prefrontal pyramidal neurons and PPI deficits were rescued in adulthood.[56] Interestingly, these electrophysiological and behavioral deficits were rescued only with D-serine treatment of neonates (P3–P17), and not with D-serine treatment of adults (P56–P70).[56] These results indicate that neonatal NMDAR activation seems indispensable for adult psychopathology.

Behavioral abnormalities of *Mecp2* KO mice can also be recovered by intervention during brain development with the chronic NMDAR antagonist ketamine.[17] In this study, the authors treated mice at two different time windows in order to identify the critical time period to recover physiological abnormalities. Ketamine was administered from either: (i) before the onset of RTT symptoms (P15) to P55 (40 days total administration); or (ii) at the onset of RTT symptoms (P30) until P55 (25 days total administration). Interestingly, both ketamine treatment starting before the onset of RTT symptoms (P15) and at the onset of RTT symptoms (P30) rescued respiratory function, visual activity, neuronal evoked and spontaneous activities, and PV immunofluorescence intensity in the visual cortex of *Mecp2* KO mice. Pharmacokinetic analysis revealed that the low dose of ketamine (8 mg/kg) administered was promptly absorbed in the brain without any detrimental side-effects. Therefore, ketamine administration might be a suitable pharmacotherapy for RTT patients. However, whether ketamine can recover existing symptoms of RTT adult patients remains to be elucidated.

Intervention prior to adulthood has also been shown to recover deficits induced by oxidative stress. Oxidative stress affects neuronal synapses, myelin, and interneuron integrity. It is one of the known causal factors of neurodegenerative disorders,[66] and may also be involved in the development of psychiatric disorders.[67] Accumulating clinical and epidemiological studies suggest an imbalance in genes involved in oxidative and redox signaling in patients with psychiatric disorders. Specifically, patients show changes in *glutathione* (*GSH*), *microsomal glutathione S-transferase 1* (*MGST1*), *superoxide dismutase* (*SOD*), and *catalase* (*CAT*). One rodent model of oxidative stress is the neonatal ventral hippocampal lesion (NVHL) rat model. This rat model develops behavioral, neurochemical, and electrophysiological deficits relevant to schizophrenia during adolescence.[57,68] Using NVHL rats, Cabungcal *et al.*[57] assessed whether pre-symptomatic oxidative stress can lead to schizophrenic-like symptoms in adults. Indeed, they identified increased oxidative stress in the prefrontal cortex of both P21 (juvenile) and P61 (adult) NVHL rats. In addition, there was decreased PV immune-positive neuron in the

prefrontal cortex of P61 rats, which may reflect cortical disinhibition that is frequently seen in patients with schizophrenia. Treatment of NVHL rats from P5 to P50 with the antioxidant *N*-acetyl-cysteine, a glutathione precursor, prevented the reduction of cortical PV interneurons, electrophysiological changes, and behavioral deficits. These findings suggest that the redox pathway may be a potential drug target for patients with schizophrenia, particularly when manipulated during brain development.

DISCUSSION

The best way to ultimately elucidate psychopathological conditions in the brain is to use samples from human patients. Yet, acquiring brain tissues from living patients is impossible, post-mortem brain samples are limited, and non-invasive methods to analyze brain structure and activities, such as functional magnetic resonance imaging (fMRI) and MRI, have limited outcomes. To overcome these hurdles, some scientists have turned to induced pluripotent stem cells (iPSC) and induced neurons (iN), which are derived from patient samples and therefore helpful in understanding the cellular dysfunction of patients. Several types of iPSC have been generated from patients with various diseases to be used in disease modeling and drug screening of therapeutics.[69] For example, iPSC from three independent patients with *MECP2* duplication have recently been established.[70] Expression analysis revealed changes in markers for neural progenitors, migration, and development of different brain regions. The cellular morphology of neurons derived from iPSC with MECP2 duplication showed that synaptogenesis and dendritic complexity were increased compared with controls, suggesting hyperconnected neural networks, which are observed in children with ASD.[71,72] Furthermore, patient-derived neurons displayed higher numbers of action potentials than controls, which may underlie abnormal glutamatergic neurotransmission. Deficits observed in *MECP2* iPSC were ameliorated by the administration of the histone deacetylase (HDAC) inhibitor, NCH-51. This study reveals the usefulness of iPSC as a disease model, as well as a tool in drug screening at the cellular level. In addition to iPSC, fibroblasts can now be directly converted into serotonergic, glutamatergic, GABAergic, or dopaminergic neurons,[73] aiding in unraveling how specific cellular systems are affected in certain diseases. The recent success of culturing organoids will accelerate human disease modeling. While iPSC, iN, and organoids are useful tools because they are derived from patients, they are unable to provide a complete picture of deficits throughout the brain.

Rodent models of genetic mutations found in humans with mental illness have been useful in understanding complex brain abnormalities. However, it is obvious that rodents are vastly different from humans, and therefore rodent disease models may not always reflect human pathophysiological conditions. A good example is the mouse model of RTT. Although some exceptions have been reported in clinical studies, most MECP2 mutations are lethal in males. However, *Mecp2* null mutation in male mice is not lethal although they die around 10 weeks of age. Similarly, female *Mecp2* heterozygous mice appear normal at a young age although RTT is characterized as early neurological regression.[74] In a mouse model of Fragile X syndrome, it was found that an mGluR5 antagonist potently ameliorates phenotypic abnormalities.[75,76] When the pharmaceutical companies Roche and Novartis went into Phase II trials of the mGluR5 antagonist, they found no beneficial effect. This is a common theme for many drugs that show promise in rodent models, but not in humans. In addition, as psychiatric disorders are multifactorial disorders and may be due to changes in many systems throughout the body (e.g., brain vs microbiome and immune system), analysis of the whole body seems to be essential to understanding the pathophysiology of psychiatric disorders. Despite differences between rodents and humans, animal models are still valuable tools to study human diseases because we can study rodents at the system level, within a living organism, and assess drugs in different ways in real time, which are all difficult and more costly to do in human subjects.

As we have discussed, human, rodent, and cell culture studies all have their strengths and weaknesses in understanding the mechanism and treatment of genetic disorders as they pertain to the brain. Studies combining all tools may therefore result in the most promising avenues to follow through to clinical drug trials. One step in this direction is exemplified by a study reported by Hao *et al.*[77] This group applied deep brain stimulation, which is commonly used in the clinical treatment of humans with movement disorders, such as Parkinson's and dystonia,[78] to work on an RTT mouse

model. Using deep brain stimulation, the authors successfully rescued neurological and cognitive deficits in the RTT mice.[77] As suggested by this work, combining human studies (post-mortem samples, iPSC or iN, brain imaging) with animal models of human disease may be necessary to truly understand multifactorial psychiatric disorders, such as ASD and schizophrenia.

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DISCLOSURE STATEMENT

All authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

J.N. drafted the manuscript. All authors edited it.

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