GABAergic System Dysfunction in Autism Spectrum Disorders

Haisheng Zhao, Xijing Mao, Cuilin Zhu, Xiaohan Zou, Fanzhen Peng, Wei Yang, Bingjin Li, Guangquan Li, Tongtong Ge* and Ranji Cui*

Jilin Provincial Key Laboratory on Molecular and Chemical Genetic, Second Hospital of Jilin University, Changchun, China

Autism spectrum disorder (ASD) refers to a series of neurodevelopmental diseases characterized by two hallmark symptoms, social communication deficits and repetitive behaviors. Gamma-aminobutyric acid (GABA) is one of the most important inhibitory neurotransmitters in the central nervous system (CNS). GABAergic inhibitory neurotransmission is critical for the regulation of brain rhythm and spontaneous neuronal activities during neurodevelopment. Genetic evidence has identified some variations of genes associated with the GABA system, indicating an abnormal excitatory/inhibitory (E/I) neurotransmission ratio implicated in the pathogenesis of ASD. However, the specific molecular mechanism by which GABA and GABAergic synaptic transmission affect ASD remains unclear. Transgenic technology enables translating genetic variations into rodent models to further investigate the structural and functional synaptic dysregulation related to ASD. In this review, we summarized evidence from human neuroimaging, postmortem, and genetic and pharmacological studies, and put emphasis on the GABAergic synaptic dysregulation and consequent E/I imbalance. We attempt to illuminate the pathophysiological role of structural and functional synaptic dysregulation in ASD and provide insights for future investigation.

Keywords: autism spectrum disorders, GABA, neurodevelopmental disorders, inhibitory neurotransmission, excitatory/inhibitory balance

INTRODUCTION

Autism spectrum disorder (ASD) refers to a series of neurodevelopmental diseases characterized by two hallmark symptoms, social communication deficits and repetitive behaviors (Happe, 1999). Clinical observations suggest that patients with ASD were often comorbid with intellectual disability (ID), mood disorders, epilepsy, and intestinal disorders (Happé and Frith, 1996; Rudolph and Möhler, 2014; Richard et al., 2017). The prevalence of autism has gradually increased over time and with significantly higher frequency in male infants than in female infants (Fatemi et al., 2013). A number of investigations have demonstrated that genetic, epigenetic, environmental perinatal factors and their complicated interaction were implicated in the neurophysiological mechanisms of ASD (Belmonte et al., 2004; Hallmayer et al., 2011; Cattane et al., 2020). However, high clinical heterogeneity challenges both the clinical diagnosis and treatment and further investigations of the cellular and molecular pathogenesis of ASD.

Synaptic neurotransmission, including excitatory and inhibitory neurotransmission, is the neurophysiological basis of various functions of the brain. Aberrant neurotransmission, especially the imbalance of excitation and inhibition in the central nervous system (CNS), might be implicated in the ASD pathogenesis. Based on Genome-Wide Association Study (GWAS) and human genetic evidence, some synaptic genes encoding the synthesis, release, and binding receptor
proteins of neurotransmitters have been reported to contribute to the pathogenesis of ASD (Kumar and Christian, 2009). Gamma-aminobutyric acid (GABA) is a pivotal inhibitory neurotransmitter in the CNS. GABAergic inhibitory neurotransmission is critical for the regulation of brain rhythm and spontaneous neuronal activities during brain development. The imbalance between excitation and GABAergic inhibition might be consequently contributed to the dysfunction of the CNS GABAergic system. Increasing evidence has shown that dysfunction of inhibitory neurotransmission is implicated in neurophysiological mechanisms of neuropsychiatric disorders, including schizophrenia, depression, and anxiety (Amin et al., 2006; Möhler, 2012; Duman et al., 2019). Especially, it was widely studied that abnormal neuronal excitatory firing in specific brain regions caused by the absence of GABAergic inhibition underlies the neurophysiological mechanism of epilepsy (Puts and Edden, 2012; Kaila et al., 2014). GABAergic dysfunction might provide a potential reasonable explanation for high comorbidity with epilepsy or increased susceptibility to epilepsy observed in patients with autism (Richard et al., 2017).

In this review, we summarized evidence from genetic, human neuroimaging, postmortem, and genetic and pharmacological studies focusing on GABAergic dysfunction and investigating the consequent excitatory/inhibitory (E/I) imbalance in ASD. We put emphasis on the convergent and neurophysiological alterations of how GABA is involved in ASD from different lines of evidence. We aimed to illuminate the potentiality of GABA and GABA receptor (GABA R) modulators as potential therapeutic targets for ASD.

EVIDENCE FROM IN VIVO NEUROIMAGING STUDIES

Human evidence from proton magnetic resonance spectroscopy (1H-MRS), neuroimaging, enzyme-linked immunosorbent assay (ELISA), and postmortem brain studies reported altered levels of GABA metabolites and the ratio of GABA to metabolites, such as glutamate and creatine (Rolf et al., 1993; Dhossche et al., 2002; Thatcher et al., 2009; Al-Otaish et al., 2018). A study showed that the electroencephalogram (EEG) phase reset of 54 patients with ASD showed reduced number and/or strength of thalamocortical connections comparing with healthy subjects (Thatcher et al., 2009). Earlier evidence showed increased serotonin levels and decreased levels of amino acids, namely, aspartic acid, glutamine, glutamic acid, and GABA, in patients with autism in comparison with controls (Rolf et al., 1993). However, contradictory results exist that young autism patients exhibited higher plasma GABA levels which decreased with age (Dhossche et al., 2002). Several evidence also reported higher plasma GABA levels and lower glutamate/GABA levels in autistic subjects than in controls (El-Ansary and Al-Ayadhí, 2014; Al-Otaish et al., 2018). Al-Otaish et al. (2018) found a higher glutamate/glutamine ratio and lower levels of plasma glutamine in ASD patients. Another study focusing on alterations of antineuronal antibody levels including anti-glutamic acid decarboxylase antibodies (GAD), anti-glutamate receptor antibodies, and seven types of anti-ganglioside antibodies levels by ELISA found no significant difference between autistic and control subjects (Bayram et al., 2016).

Recently, amounts of evidence using magnetic resonance spectroscopy (MRS) found that the lower ratio of GABA/glutamate (Harada et al., 2011; Kubas et al., 2012; Drenthen et al., 2016; Ajram et al., 2017), N-acetylaspartate/creatinine, GABA/creatinine, and glutamate/creatinine (Kubas et al., 2012) in the frontal lobes, lower GABA/creatinine levels in the anterior cingulate cortex (ACC) of patients with ASD than normal controls (Kubas et al., 2012; Cochran et al., 2015). The binocular rivalry dynamics exhibited in normal controls, which depend on the balance of inhibitory and excitatory cortical dynamics, is absent in participants with autism; these findings indicate reduced GABAergic action in the autistic brain (Robertson et al., 2016). The researchers have drawn a conclusion that children with autism have lower sensorimotor GABA levels via a tactile task detecting amplitude discrimination and frequency discrimination under the presence of an adapting stimulus (Puts et al., 2017). ASD patients exhibited lower GABA concentrations in the sensorimotor cortex, and the GABA concentrations were positively correlated with self-reported tactile hypersensitivity in adults with ASD (Sapey-Triomphe et al., 2019). It has been shown that children with autism showed higher GABA concentration in the bilateral visual cortex (VIS) which was found to be related to more efficient search and social impairments (Edmondson et al., 2020). Intriguingly, these results suggest that GABAergic system dysfunction in ASD patients, especially in children, was associated with higher cognitive function, including somatosensory function, search and social behaviors. A recent investigation found that there was a negative correlation between thalamic GABA and autism-spectrum quotient (AQ) in male participants with autism, while the thalamic GABA levels were positively correlated with AQ in female participants (Fung et al., 2021). These results indicate that GABA levels might correlate with autism symptom severity in a gender-specific way. Considering the high clinical heterogeneity of autism, the altered GABA system might be more pronounced for a specific subtype of clinical ASD. Drenthen et al. found higher Glu/creatinine and lower GABA/glutamate concentrations in the high-functioning autism (HFA) participants, which are consistent with previous research results (Drenthen et al., 2016). Evidence showed that children and adolescents with HFA have reduced total N-acetylaspartate (tNAA) and total creatine, increased glutamate +glutamine (GLx)/tNAA, but unchanged GLx and comparable GABA levels between healthy and autism groups (Carvalho Pereira et al., 2018). Intriguingly, they also observed that smaller absolute and relative GABA levels were associated with poor communication skills of patients with autism (Carvalho Pereira et al., 2018). These results indicate a critical relationship of the altered GABA system with clinical autism, but the levels of GABA metabolites and the ratio of GABA to metabolites are not
sufficient to be a clinical target for diagnosis due to the poor precision and accuracy.

**EVIDENCE FROM IN VITRO POSTMORTEM STUDIES**

Results from postmortem studies have shown that altered GABAergic signaling is involved in the neurophysiological mechanisms of autism. The autistic group had increased GAD 67 mRNA expression in cerebellar interneurons and reduced glutamic acid decarboxylase 65(GAD 65) mRNA in the larger labeled cells in the cerebellar dentate nuclei (Yip et al., 2008, 2009). It suggests that a disturbance of the intrinsic cerebellar circuitry in the autistic brain might affect autism-related cognitive and behaviors by affecting the Purkinje cell (PC) output to other key brain regions. Individuals with autism showed a selective increase in the density of calbindin (CB)+, calretinin (CR)+, and parvalbumin (PV)+ interneurons in the hippocampus than those of normal controls (Lawrence et al., 2010). A recent study using immunohistochemical staining found significantly decreased protein levels of α2 subunit of GABA-A receptor in the axon initial segment of pyramidal cells in supragranular layers of prefrontal cortical areas of autistic participants (Hong et al., 2020). Fatemi et al. reported downregulation of different subunits of GABA-A and GABA-B receptors both in mRNA and protein levels (Fatemi et al., 2009a; Fatemi et al., 2009b; Fatemi et al., 2010; Blatt and Fatemi, 2011; Fatemi and Folsom, 2011). They reported GABBR1, GABBR2, GABRA1, and GABRB3 were significantly decreased in the cerebellum (Fatemi et al., 2009a; Fatemi et al., 2009b), GABRA1, GABRA2, GABRA3, and GABRB3 were significantly reduced in the parietal cortex, and GABRA1 was significantly altered in the superior frontal cortex (Fatemi et al., 2009a).

Evidence from quantitative receptor autoradiographic studies was carried out to determine the density and distribution of excitatory and inhibitory receptors (Blatt et al., 2001; Guptill et al., 2007; Obblak et al., 2009; Lawrence et al., 2010). The binding sites of benzodiazepine, a positive allosteric modulator on GABA-A R, were significantly reduced in the hippocampus and ACC of the autistic brain, while other receptors were not significantly changed (Obblak et al., 2009). Further evidence found that reduced [(3)H]flunitrazepam-labeled benzodiazepines(BDZ) sites in the autistic hippocampus were due to a decrease in the binding site number or number of receptors but not due to the binding affinity (Blatt et al., 2001; Guptill et al., 2007; Lawrence et al., 2010). Besides the hippocampus, the cerebellar region can receive inputs widely from the frontal cortex via the granular layer; a significant decrease of BDZ binding sites in the supragranular layers and infragranular layers of the cerebellar region was found (Obblak et al., 2009). These results indicate that a significant dysregulation of the GABAergic system, from the synthesis to receptors, in autistic brains, might provide potential targets for therapeutics and diagnosis of autism and related neuropsychiatric diseases.

**EVIDENCE FROM GENETIC STUDIES**

ASD and other neurodevelopmental disorders which are characterized by repetitive behaviors and social deficits are highly heritable. Genetic evidence showed that single-gene mutations are rare in families with autism. Each common genetic variant causes a mild genetic effect on ASD risk, while the cumulative effect of common genetic variants contribute up to 15–50% to ASD (Vorstman et al., 2017). Rare genetic variants, including de novo and intrinsic variants, contribute 10–30% of the genetic contribution to the occurrence of ASD (Vorstman et al., 2017). Several evidence have observed cytogenetic abnormalities in chromosome 15q11–q13 region in individuals with autism (Buxbaum et al., 2002; Hogart et al., 2007; Guo et al., 2017). 15q11–q13 site contains coding regions of specific subunits of GABA-AR, including GABRB3, GABRA5, and GABRG3, which are strongly implicated in the pathogenesis of autism (Delahanty et al., 2011; Bonnet-Brilhault et al., 2016; Guo et al., 2017). Duplications of gene E3 ubiquitin ligase UBE3A and three non-imprinted GABA-AR genes which are located in 15q11.2–q13.1 (Dup15q syndrome) can induce autistic phenotype behavioral deficits (Bonnet-Brilhault et al., 2016). It was found that single-nucleotide polymorphisms (SNP)s of GABRB3 rs2081648, GABRA5 rs35586628, and GABRG3 rs208129 polymorphisms are related to symptom-based behavioral deficits in sociability (Noroozi et al., 2018). GABRA4 was also found to increase autism risk through interaction with GABRB1, indicating a complex gene–gene interaction in GABA receptor subunit genes involved in the ethology of autism (Ma et al., 2005). Thus, overall results suggest that GABA-related gene polymorphisms seem to be sufficient to induce the autistic behavioral phenotype; whether rescue of GABA-related gene deficits by genetic approach can improve behaviors is worth to be further discussed.

**GABAERGIC DYSFUNCTION IN ANIMAL MODELS OF ASD**

Although accumulating in vivo and in vitro evidence from human studies suggest dysfunction of the GABA system is associated with ASD and genetic evidence further confirmed it, the molecular and cellular mechanisms are still obscure. Recently, transgenic technology enables translating genetic variations into rodent models to further investigate the structural and functional synaptic dysfunction in ASD. Investigating convergent cellular signalings or GABAergic alterations of current genetic and pharmacological animal models contribute to further understanding the mechanisms of ASD. Current candidate genes induce dysfunction of GABAergic transmission by affecting transcription of GABA-A receptor(GABA-AR), presynaptic GABA release, formation of GABAergic synapse, and synaptic structure-mediated transmission (Figure 1).

**GABA Receptor Mutant Animal Models**

Direct evidence of GABA dysfunction in autism is from that mouse carrying mutant subunit of GABA receptors exhibited...
sociability impairments and repetitive behaviors which belong to core symptoms of ASD. Delahanty et al. (2011) first demonstrated that in vitro mutant β3 subunit-containing a1β3y2 or a3β3y2 GABA-AR showed reduced whole-cell current and functional receptor. In vivo evidence showed that a mouse model carrying β3 subunit GABA-AR in which serine residues 408 and 409 (S408/9) mutated to alanines (S408/9A) and S408/9A homozygotes exhibited core ASD symptoms, including increased repetitive behaviors and decreased social interaction, and altered dendrite spine structure and consequently decreased tonic inhibition and increased phasic inhibition (Vien et al., 2015). These results suggest specific GABA-AR defect exert a risk effect linking 15q11–q13 autism duplication region with autism. Impaired GABAergic signaling during brain development is a key event related to the neurodevelopmental disease. In addition, 15q duplication mice (a model for ASD with human chromosome 15q11–q13 paternal duplication) exhibited facilitated activity-induced long-term potentiation (LTP) of glutamate synapses onto layer 5 pyramidal neurons and reduced number of inhibitory synapses, indicating shifted E/I balance is implicated with autism (Saitow et al., 2020).

### Single-Gene Models of ASD

GABAergic dysfunction could also be a downstream consequence of mutations in genes and not directly be involved in GABA transmission because the proper development and function of GABA synapses rely on numerous signaling and scaffolding proteins. Currently known candidate genes of ASD contain transcription factors, scaffolding proteins, receptors, and signaling pathway proteins (Vorstman et al., 2017) (Table 1). Accumulating evidence has shown some of the current candidate genes induce dysfunction of GABAergic transmission by affecting transcription of GABA-AR subunits, development of GABAergic synapse, synaptic structure–mediated transmission. GABA-related candidate genes and regulatory role are represented by red letters. Pharmacological modulators of GABA-R reported to have alleviation efficacy are represented by blue letters.

![Figure 1](image.png)

**FIGURE 1** | Current known candidate genes of ASD involved in GABAergic synaptic transmission and related mechanisms. Current candidate genes induce dysfunction of GABAergic transmission by affecting transcription of GABA-AR subunits, presynaptic GABA release, formation of GABAergic synapse, and synaptic structure–mediated transmission. GABA-related candidate genes and regulatory role are represented by red letters. Pharmacological modulators of GABA-R reported to have alleviation efficacy are represented by blue letters.
### TABLE 1 | Alterations of GABA system in single-gene models of ASD.

| Candidate gene | Animal models | GABA-related mechanisms | Main findings | Reference |
|----------------|---------------|-------------------------|---------------|-----------|
| FMR1           | Fmr1 KO mice  | GABA-A subunits         | Downregulation of tonic GABA-A-currents. Downregulation of tonic GABA-A receptor subunits α5 and δ | Curia et al. (2009) |
|                | Fmr1 KO mice  | Amygdalar GABAergic neurotransmission | Reduced amygdalar phasic IPSCs and tonic inhibitory currents | Olmos-Serrano et al. (2010) |
|                | Fmr1 KO mice  | GABA-A subunits         | Increased VGAT expression in MNTB Elevated response thresholds to click and tone stimuli | Rotschafer et al. (2015) |
|                | Fmr1 KO mice  |                        | Reduced expression of GABA-A subunits in protein levels | Sabanov et al. (2017) |
| MeCP2          | Mecp2 tm1.1Bird | GABA-A subunits         | Reduced VGAT levels. Reduced levels of postsynaptic GABA-AR subunits α2 and α4 | Medrihan et al. (2008) |
|                | Mecp2-null mice | GABAergic neurotransmission | Unitary responses evoked by minimal stimulation were decreased in the VB but increased in RTN of mutants | Zhang et al. (2010) |
|                | Mecp2-null mice | LC GABAergic neurotransmission | Deficient postsynaptic GABAergic inhibition. Decreased frequency and amplitude of the GABAergic mIPSCs in LC. | Jin et al. (2013) |
|                | Mecp2tm1.1Bird | GABA levels             | Reduced GABA levels in hippocampus, the midbrain, and the cerebellum | El-Khoury et al. (2014) |
|                | Mecp2 with A140V mutant | Hippocampal GABAergic neurotransmission | Increased positive resting membrane potential, and increased AP firing frequency induced by injection of depolarizing current in hippocampal CA1 pyramidal neurons | Ma et al. (2014) |
|                | Mecp2-null mice | NTS GABAergic neurotransmission; GABA-A subunits | Reduced sIPSC amplitude, sIPSC frequency, and mIPSC amplitude in NTS. Increased expression of GABA-AR δ in the NTS. | Chen et al. (2018) |
|                | Mecp2+/− mouse | GABA-AR subunits         | Reduced mIPSCs in DG, no changes in mEPSCs | Oyarzabal et al. (2020) |
| CNTANP         | Cntnap2−/−     | Number of GABAergic neurons | Reduced number of GABAergic interneurons in all laminae | Peñagarikano et al. (2011) |
|                | Cntnap4−/− mice | GABA-A subunits         | Reduced expression of GABA-ARa1 in the hippocampus and thalamus and a reduced trend in the cortex | Heise et al. (2018) |
|                | Cntnap3-null mice | Number of GABAergic neurons | Increased GABAergic interneurons in cortical regions and hippocampus in vivo | Tong et al. (2019) |
|                | Cntnap2-KO mice |                        | Reduced the number of both excitatory and symmetric inhibitory; decreased average amplitude of mEPSCs of pyramidal neurons | Lazaro et al. (2019) |
| Neurolgin      | Nlgn3 R451C knock-in mice | Hippocampal GABAergic neurotransmission | Increased GABAergic drive with enhancement of the frequency of miniature GABA-A- mediated GPSCs on CA3 pyramidal neurons | Pizzarelli and Cherubini, (2013) |
|                | Nlgn4 KO mice  | Hippocampal GABAergic neurotransmission | Altered GABAergic transmission in CA3 | Hammer et al. (2015) |
|                | Nlgn3 R451C knock-in mice | Striatum GABAergic neurotransmission | Impaired LTD at corticostriatal glutamatergic synapses in the dorsal striatum | Martella et al. (2018) |
|                | Nlgn2 R215H KI mice | Hippocampal GABAergic neurotransmission and GABA-A subunits protein levels | Reduced protein levels of subunit GABA-A y2 and gephyrin in hippocampus. Reduced protein level of PV and VGAT in DG and CA1/CA3 pyramidal cells. Reduced mIPSCs in DG, no changes in mEPSCs | Jiang et al. (2018) |
|                | Nlgn 2 Arg215 → His215 mutation knock-in (NL2 R215H KI) mouse | mPFC GABAergic neurotransmission; GABA-A subunits | Reduced mIPSCs and eIPSCs in mPFC. Reduced levels of PV, subunit GABA-A y2, and VGAT in mPFC | Chen et al. (2020) |
| SHANK          | Shank2 knockout mice (e6-7 KO) | GABAergic neurotransmission and GABA-A subunits | Reduced expression of Gabra2 and GABA receptor-mediated inhibitory neurotransmission | Lim et al. (2017) |
|                | Shank1−/− mice | GABAergic neurotransmission | | Mao et al. (2015) |
FMR1
Fragile mental retardation protein (FMRP), encoded by FMR1, is an extensively studied RNA-binding protein that is implicated in the pathogenesis of Fragile X, ASD, and other IDs (De Rubeis and Bagni, 2011). Research confirmed FMR1 and FMRP-targeting gene set associated with ASD and other neuropsychiatry diseases (Jansen et al., 2017). FMRP can affect synaptic phenotype as an mRNA-binding transitional suppressor via modulating the synthesis of synaptic proteins which implicated in synaptic transmission (Bagni and Zukin, 2019). Early studies have widely reported altered GABA-AR subunit expression levels in Fmr1 knockout (KO) mice. Fragile X mice exhibited reduced expression of α1, α3, and α4, β1 and β2, and γ1 and γ2, subunits of GABA-AR both in mRNA and protein levels comparing with wild-type mice. However, GAD65 and GAD67 expression levels were inversely increased in Fragile X mice (D’Hulst et al., 2006; Adusei et al., 2010). It was found that GABA-A β1 and β3, GABAB-R1, two Na⁺-K⁺-2Cl⁻ cotransporter proteins (NKCC1 and NKCC2), gephyrin and ubiquitin, of Fmr1 KO mice were not significantly different from those of wild-type mice at any of the postnatal time points (Adusei et al., 2010). In addition, Fmr1 KO mice exhibited abnormal transmission in cognition and sociability-related brain regions. Centonze et al. found that Fmr1 KO mice have fewer GABAergic synapses whereas higher levels of spontaneous GABA inhibitory transmission in the striatum (Centonze et al., 2008). Phasic GABAergic inhibition was not significantly altered, while the tonic GABA-A currents were downregulated in Fmr1 KO mice, and this might be related to the lack in expression of GABA-AR subunits α5 and δ in the subiculum (Curia et al., 2009). Furthermore, both significant reduction of the phasic inhibitory postsynaptic currents (IPSCs) and tonic inhibitory currents were observed in the Fmr1 KO mice. Fmr1 KO mice showed neuronal hyperexcitability in the amygdala which can be rescued by the administration of GABA agonist gadoxadol (THIP) can rescue the amygdalar neurons via promoting the tonic inhibitory tone (Olmos-Serrano et al., 2010). Lack of RGS4, a regulator of G-protein signaling protein, can inhibit the susceptibility of FMR1 mice to audiogenic seizures. Treatment with GABA-B receptor antagonist is prone to induce seizures in Fmr1/RGS4 double-knockout mice than wild-type mice, whereas agonist of GABA-B baclofen can inhibit seizures in Fmr1/RGS4 double-knockout mice (Pacey et al., 2009). Similarly, baclofen was found to rescue the increased baseline and auditory-evoked high-frequency gamma (30–80 Hz) power in Fmr1 KO mice and improved the impaired working memory (Sinclair et al., 2017). These findings indicate impaired GABAergic transmission, especially in cognition and sociability-related brain regions, appears to be implicated in the contribution of gene FMR1 in neurodevelopmental and neuropsychiatry disorders.

MeCP2
MeCP2 is a structural chromosomal protein encoded by gene MeCP2 which is implicated in Rett syndrome (RTT) pathology (Amir et al., 1999) and subsequently was found to cause nonspecific ASD and neurodevelopmental diseases (Tillotson and Bird, 2019). The loss of MeCP2 from a subset of forebrain GABAergic neurons also recapitulates many features of RTT (Chao et al., 2010). Earlier evidence showed that MeCP2-deficient GABAergic neurons of forebrain showed reduced inhibitory quantal size, which is consistent with a presynaptic reduction in GAD1 and GAD2 expression levels, and GABA immunoreactivity (Chao et al., 2010). It was found that MeCP2 disruption causes the defect of GABA release from presynapse and impaired GABAergic postsynaptic inhibition in locus coeruleus (LC) and NTS (Jin et al., 2013). MeCP2-null mice exhibited increased amplitude of spontaneous miniature and evoked excitatory postsynaptic current (EPSC) without affecting the intrinsic excitability in the nucleus tractus solitarius (NTS), which is associated with decreased brain-derived neurotrophic factor (BDNF) availability in the primary afferent pathway, and these changes can be normalized by exogenous BDNF application (Kline et al., 2010).

Some evidence has demonstrated that the loss of MeCP2 induces reduced expression levels of vesicular GABA transporter (VGAT), GAD1, and GAD2, and altered expression of GABA-AR subunits (Medrihan et al., 2008; Chao et al., 2010; Chen et al., 2018; Oyarzabal et al., 2020). In particular, it was found that GABA-A subunit expression was developmentally decreased especially in the no-symptom stage in MeCP2+/− mouse brain (Oyarzabal et al., 2020). MeCP2 loss decreased the level of spontaneous glutamate receptor-mediated synaptic currents in hippocampal CA3 neurons and induced hyperexcitability in hippocampal neurons (Zhang et al., 2008; Calfa et al., 2011; Ma et al., 2014). Direct evidence was found from

---

TABLE 1 | (Continued) Alterations of GABA system in single-gene models of ASD.

| Candidate gene | Animal models | GABA-related mechanisms | Main findings | Reference |
|----------------|---------------|-------------------------|---------------|-----------|
| EN2            | En2+/− mice   | Number of GABAergic neurons | Reduced PV+ neuron-mediated inhibitory synaptic transmission increased E/I balance | Sgdó et al. (2013) |
| EN2            | En2+/− mice   | Number of GABAergic neurons | Reduced expression of PV, SOM, and NPY-expressing interneurons in hippocampus and cerebral cortex | Provenzano et al. (2020) |

MNTH, medial nucleus of the trapezoid body; eIPSC, equine IPSC; VB, ventrobasal complex; RTN, the reticular thalamic nucleus; SOM, somatostatin; NPY, neuropeptide Y; SST, somatostatin; IPSCs, inhibitory postsynaptic currents; mIPSC, mini-inhibitory postsynaptic currents; mEPSCs, miniature excitatory postsynaptic currents.
studies demonstrating that neurons lacking MeCP2 exhibited the defect of GABAergic transmission whereas not in the neighboring neurons with MeCP2 and forebrain-restricted loss of MeCP2 reduce the number of GABAergic synapses in the cortex (Zhang et al., 2014). These results indicate that MeCP2 loss induces defect in presynaptic GABA release, reduced number of GABAergic synapses, and impaired GABAergic inhibition.

Pharmacological stimulation of the GABAergic system exhibited restoration of abnormal neurotransmission and behavioral phenotype. GABA-A-R agonist THIP (gaboxadol) was reported to alleviate symptoms of RTT, including the motor dysfunction and social abnormalities (Zhong et al., 2017). GABA reuptake inhibitor can improve the life span of MeCP2-deficient mice (El-Khoury et al., 2014). Application of benzodiazepine-like modulator midazolam can transiently abolish the breathing defects of MeCP2-deficient mice (Voituron and Hilaire, 2011). Notably, mirtazapine, an antidepressant known to promote GABA release, was found to inhibit the hopping behaviors of MeCP2-null mice (Bittolo et al., 2016). These results confirmed that GABAergic neurotransmission defect appears to be involved in neurophysiological mechanisms of MeCP2 deficiency–induced abnormal behaviors.

Notably, sex difference of RTT has higher prevalence in girls than in boys and is significantly distinguished with ASD (Beattie, 2004). It was shown that RTT is the second most prevalent cause of intellectual disability in girls (Matagne et al., 2017). The maternal experience transiently enhances GAD67 in the auditory cortex and enhances PV-expressing neurons only in MeCP2 het mice; knockout of MeCP2 specifically in PV neurons is sufficient to impair learning behavior of pups, indicating experience-dependent plasticity might be impaired during development in adult MeCP2 het mice (Krishnan et al., 2017). Only restoration of the MeCP2 expression in GABAergic neurons of male MeCP2 null mice can increase inhibitory neurotransmission and subsequently improve ataxia and social impairments but not anxiety; these physiological and behavioral changes were not observed in female mice (Ure et al., 2016). These results suggest that MeCP2 is critical for the regulation of the GABAergic system, especially during neurodevelopment.

**Nlgn2, 3, and 4**

Neurexins (NRXNs) and neureligins (NLGNs) are critical structural molecules involved in synapse formation and differentiation as cell adhesion molecules. NRXNs localized in presynapse can bind to postsynaptic NLGNs, bridge the synaptic cleft, and contribute to synapse stabilization (Blunk et al., 2014). In vivo and in vitro evidence have demonstrated that neurelin-2 plays an important role in the development and maintenance of GABAergic synapses (Chen et al., 2011; Varley et al., 2011; Fekete et al., 2015; Uchigashima et al., 2016; Panzanelli et al., 2017). NLGN2 can precede KCC2 expression and regulate GABA functional switch in early postnatal development (Panzanelli et al., 2017). Notably, knockdown of Nlgn2 can reverse the inhibitory GABA to excitatory action in mature neurons, indicating NLGN2 involvement in maintenance of GABA function as an inhibitory neurotransmitter in mature neurons (Sun et al., 2013). NLGN2 can induce fast decay of mini inhibitory postsynaptic currents (mIPSC) by regulation of differential expression of postsynaptic GABA-AR subtypes (Fu and Vicini, 2009). Overexpression of NLGN1B and NLGN2A in newborn neurons of the hippocampus can increase the spine density and impaired spatial memory (Krzisch et al., 2017). These data suggest that neureligins might affect the formation of neural connectivity via affecting the early postnatal transition of excitatory GABA.

Several evidence have shown that abnormal NLGN2 expression is associated with neuropsychiatric behaviors. Reduced NLGN2 expression in the prefrontal cortex (PFC) is involved in attention deficits induced by peripubertal stress which can be reversed by virus-mediated NLGN2 rescue (Tzanoulinou et al., 2016). Animal models using the genetic engineering method showed that Nlgn 2 R215H knock-in (KI) mice exhibited schizophrenia-like behaviors and higher anxiety behaviors with significant reduction of mIPSCs in the dentate gyrus (DG), reduced subunit GABA-A y2 expression levels, and reduced presynaptic PV and VGAT levels, while there were no changes of miniature excitatory postsynaptic currents (mEPSCs), vesicular glutamate transporter (VGLUT), and postsynaptic density protein 95 (PSD95) expression levels (Jiang et al., 2018; Van Zandt et al., 2019). NLGN2 of ACC is involved in KCC2-mediated abnormal GABAergic transmission induced aggressive behaviors (Jager et al., 2020).

Social deficits, enhanced spatial learning, increased locomotor activity, and increased aggressive behaviors were observed in Nlgn3 R451C mutant mice (Jaramillo et al., 2014; Burrows et al., 2015). It was found that elevated aggressive behaviors in Nlgn3 R451C mutant mice could be normalized by risperidone, a novel antipsychotic agent that antagonizes serotonin (5-hydroxytryptamine) 5-HT2 and dopamine D2 receptor (Burrows et al., 2015). Earlier findings demonstrated Nlgn3 R451C mutant mice exhibited enhanced GABAergic transmission with increased frequency of IPSCs while having no significant effect on excitatory transmission (Tabuchi et al., 2007). Both Nlgn3 R451C knock-in and Nlgn3 KO mutant impaired tonic endocannabinoid signaling (C et al., 20132020). Nlgn3 R451C knock-in mice appeared to show an increased GABAergic drive with enhancement of the frequency of miniature GABA-A–mediated giant depolarizing potentials (GPDs) on CA3 pyramidal neurons (Pizzarelli and Cherubini, 2013). In addition, mice carrying R451C-Nlgn3 mutation showed impaired long-term depression (LTD) at cortical striatal glutamatergic synapses which could be rescued by exogenous activation of cannabinoid CB1 receptors or enhancement of the endocannabinoid tone (Martella et al., 2018). Mice and rats carrying Nlgn3 mutations showed reduced striatal glutamate indicating glutamate/GABA abnormalities in the corticostratial circuitry (Horder et al., 2018).

Hippocampal Nlgn4 likely localized at excitatory synapses originating from PV-positive interneurons (Marro et al., 2019). ASD-related Nlgn4 mutations can alter the compositions of hippocampal perisomatic inhibitory synapses and impaired the synchronized oscillatory activity which might be implicated in the cognitive dysfunction of ASD (Hammer et al., 2015). Glycine is
another widely acknowledged inhibitory neurotransmitter in the
CNS. It was found that Ngr4 KO mice exhibited impaired
glycinergic transmission in the retina with fewer glycine
receptors mediating fast glycinergic transmission and slower
glycinergic mIPSCs (Hoon et al., 2011). A recent study
confirmed that NLGN4 significantly impaired glycinergic
transmission and reduced the numbers of glycinergic synapses
while having no impact on glutamatergic synapses. Intriguingly,
they found overexpression of NLGN4 causes impairments of
excitatory transmission but not that of inhibitory transmission
(Zhang et al., 2018). These findings confirmed the contribution of
members of NLGN family to neurodevelopmental diseases via
various molecular and cellular mechanisms.

CNTNAP2, 3, and 4
CNTNAP2 encodes contactin-associated protein-like 2 (Caspr2),
a protein of the neurexin superfamily, and interacts with NLGN
proteins, scaffolding proteins PSD95 and gephyrin (Bonnet-
Brilhaut et al., 2016). Human and animal studies have reported
that mutations in CNTNAP2 cause a syndrome form of
ASD with significant behavioral defects in social interaction
and stereotypes (Periagarikano et al., 2011). It was found that
Cntnap2<sup>−/−</sup> mice showed ASD core symptoms, accompanied
with significant reduced GABAergic neurons and reduced cortical
neuronal synchrony; these changes can be rescued by
risperidone treatment (Periagarikano et al., 2011). Cntnap2 KO
mice displayed remarkable reduced excitatory and inhibitory
synaptic inputs onto pyramidal neurons of mPFC with normal
dendritic arborization, and decreased average amplitude of
mEPSCs in pyramidal neurons, while no significant changes in
mIPSCs (Lazar et al., 2019). However, contradictory results from
a genome analysis found that rare heterozygous mutations in any
of the CNT or CNTAP genes, including CNTAP2, were not
significantly involved in the plausible contribution to risk of
autism (Murdoch et al., 2015).

Other members of CNTAP family are reported to be related
to autism (Tong et al., 2019). It was found that knockdown of
CNTAP3 expression led to the decreased dendritic length and
branch. Intriguingly, the formation of excitatory synapse in
CNTAP3-knockdown mice was decreased, while that of the
inhibitory synapse was increased (Tong et al., 2019). ASD-related
mutations in CNTAP3 (R1219X, P614A, and R786C) may be
a loss-of-function mutation (Tong et al., 2019). Earlier evidence
showed that CNTAP4 might be involved in autism (Cottrell
et al., 2011). CNTAP family contained five members from
CNTAP1 to CNTAP5, while how CNTAP5 exert function was obscure. These results indicate that CNTAP
has extensive effect on synapse formation and function; the
specific molecular mechanisms of CNTAP involved in
synapse function and ASD need to be further investigated.

SCN1A
Haploinsufficiency of the SCN1A gene encoding brain voltage-
gated sodium channel NaV1.1. is the genetic etiology of Dravet
syndrome, a developmental disorder characterized by recurrent
intractable seizures, cognitive deficit, and autistic-like behaviors
(Han et al., 2012). Han S et al. reported that haploinsufficiency of
the SCN1A gene impaired GABAergic neurotransmission by
conditional deleting the NaV1.1 channels in forebrain
interneurons which can be fully rescued by low-dose treatment
with clonazepam, a positive allosteric modulator of GABA-AR
(Han et al., 2012). The expression of the SCN1A gene was found
significantly decreased in PFC of offspring of maternal immune
activation (MIA) models, which also indicates impaired
GABAergic transmission involved in pharmacological models of
autism (Yang J. Q. et al., 2021).

SHANK1, 2, and 3
The Shank family of scaffold proteins, mainly containing
members SHANK1 to SHANK3, can bind a variety of
membrane and cytoplasmic proteins to form a
postsynaptic scaffolding protein complex which is related
to the formation and function of excitatory synapses (Sheng
and Kim, 2000). Though de novo deletions, insertion, splicing
mutations, and point mutations of SHANK have been
identified in ASD patients, in vivo mechanisms in which
SHANK relate to ASD remain obscure. Animal evidence
showed absence of shank altered to the expression of
receptor subunits of GABA and GABAergic inhibitory
synaptic transmission. SHANK1 protein is reported to be
highly expressed in PV-positive fast-spiking inhibitory
interneurons in the hippocampus (Mao et al., 2015). Shank1
mutant mice have impaired inhibitory synaptic outputs to
hippocampal CA1 pyramidal neurons and consequently impaired excitatory and inhibitory balance
(Mao et al., 2015). Shank1<sup>−/−</sup> and Shank3<sup>−/−</sup> mice showed reduced PV expression in mPFC and
somatosensory cortex and striatum (Filice et al., 2016).
Shank2 knockout mice (e6-7 KO) showed a reduction in
the expression levels of Gabra2 and GABA R-mediated
inhibitory signaling, which can be reversed by treatment
with an allosteric modulator for the GABA-AR (Lim et al.,
2017). Shank2<sup>−/−</sup> and Shank3<sup>−/−</sup> mice exhibited reduced
levels of several cell surface glutamate receptors, such as
GluN1 and GluA2, in the striatum and cortex (Heise et al.,
2018). Considering that SHANK family proteins are mainly
localized at the postsynaptic densities of excitatory synapses,
there is still lack of evidence showing a direct relationship
between SHANK and GABAergic transmission.

EN2
Several evidence have shown homeobox transcription factor
Engrailed2 (En2) appears to be a susceptibility locus for ASD
(Benayed et al., 2005; Brune et al., 2008; Wang et al., 2008;
Benayed et al., 2009). Mouse carrying mutants in
En2 exhibited similar morphological abnormalities in the
cerebellar with autistic individuals (Gharani et al., 2004).
Protein EN2 is mainly present in postnatal GABAergic
neurons of the mouse hippocampus and cerebral cortex,
and it was found that persistent high expression of EN2
during the prenatal and early postnatal period contributes to
autism by affecting the maturation of Purkinje cell (PCs)
in the cerebellum (James et al., 2013, 2014). EN2 expression
levels alter the number of GABAergic interneurons (Sgadò
et al., 2011).
et al., 2013; Provenzano et al., 2020). Evidence from an in vitro study found that En2 increases the complexity of the dendritic tree of GABAergic neurons and reduced the number of mature synapses as well as the area of postsynaptic densities (Soltani et al., 2017).

### Pharmacological Models

Besides the single-gene models, pharmacological models were widely used to discuss the pathogenesis of ASD and identification of novel drug targets (Table 2). Valproic acid (VPA) as a broad-spectrum antiepileptic drug has a significant facilitative effect on the GABAergic neurotransmission system (Miyazaki et al., 1988; Lattanzi et al., 2019). Intraperitoneal administration of VPA can significantly increase GABA levels and upregulate the seizure thresholds (Nau and Löscher, 1982; Löscher and Vetter, 1984). Amounts of evidence have shown that prenatal exposure with 400–600 mg/kg VPA was found to induce significant autistic behaviors in offspring which was widely used as pharmacological rodent models for the ASD study (Nicolini and Fahnestock, 2018). Earlier evidence has shown that VPA prenatal exposure-induced ASD models have a significant facilitative effect on the GABAergic neurotransmission system (Miyazaki et al., 1988; Richetto et al., 2014). In contrast, contradictory results found that cortical GAD67 expression levels showed an increasing trend from adolescence to adulthood in VPA models (Hou et al., 2018). High-performance liquid chromatography (HPLC) results showed that the levels of glutamate, Gln, and GABA were significantly increased in the hippocampus of male VPA and thalidomide (THAL)-induced autism rats. Nuclear magnetic resonance (NMR) spectroscopy results showed increased levels of Glu and GABA in the VPA models (Zieminska et al., 2018). A recent study reported widely decreased expression of genes encoding different subunits of GABA-AR, including GABRA1, GABRA2, GABRA3, GABBR3, GABBR1, and GABBR2 (Yang et al., 2021), which is consistent with an earlier study reporting reduced GABRA1, GABRA5, and GABBR2 levels in the cortex of VPA-induced autism models (Chau et al., 2017). Yang et al. found that the frequency and amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) were comparable between control and VPA mice models, while the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) was decreased in VPA models (Yang Y. et al., 2021a). It was reported that the normal expression of GABAp3 in the cerebellum was increased linearly throughout the normal development process, while the VPA models exhibited reduced GABAp3 levels in PCs and ependymal glial cells (EGCs) from

### Table 2 | Alterations of the GABA system in pharmacological models of ASD.

| Pharmacological models | Method | GABA-related mechanisms | Main findings | Reference |
|------------------------|--------|-------------------------|---------------|-----------|
| VPA models             | CS7BL/6 mice (E 10.5 500 mg/kg i.p. injection) | Number of GABAergic neurons | PV-positive GABAergic neurons were reduced in the neocortex | Gogolla et al. (2009) |
|                        | BALB/c mice (E 12.5 600 mg/kg i.p. injection) | GABA-related proteins | Excitatory synaptic proteins NR2A, NR2B, NR2C, and inhibitory-related proteins GAD65, GAD67, GABRA1, GABRB1, and GABRB2 were downregulated | Chau et al. (2017) |
|                        | Rats (E12.5 600 mg/kg i.p. injection) | GABA-related proteins | GAD65, GAD67, and GAT1 were evaluated in the amygdala; GAD65 and GAD67 mRNA were decreased in the cerebellum | Olexová et al. (2016) |
|                        | Sprague–Dawley rats (E12.5 600 mg/kg i.p. injection) | GABA-related proteins | GAD67 was decreased in HP and cerebellum, and temporal cortex, and was increased in PFC. GAD65 and GAD67 were decreased in cortical tissue. Increased high-frequency activity during wake and REM sleep | Hou et al. (2018) |
|                        | Sprague–Dawley rats (E12.5 400 mg/kg i.p. injection) | GABA-related proteins | | Cusmano and Mong, (2014) |
|                        | Wistar rats (VPA, 800 mg/kg b.w) | GABA neurotransmitter levels | | Zieminska et al. (2018) |
|                        | CD-1 strain or GFAP-eGFP transgenic mice (E12.5, 500 mg/kg i.p. injection) | GABA receptor subunit expression pattern | GABA\(\times 3\) in PCs and EGCs from lobule X were reduced. GABA\(\times 3\) expression increases linearly throughout normal development of the cerebellum. GABA\(\times 3\) expression is disrupted in the VPA models | Varman et al. (2018) |
| MIA models             | CS7BL/6 mice (E12.5 20 mg/kg of poly (I:C), i.p. injection) | GABAergic neuron phenotype shift | Scn1a mRNA levels were not significantly changed; protein levels of Na\(_{\text{V}}\)1.1 were decreased in the mPFC | Yang et al. (2021a) |
|                        | CS7BL/6 mice (E17 5 mg/kg intravenous (i.v)) | GABAergic neuron phenotype shift; GABA-related protein expression | Increase in the transcription of NKCC1 and decrease in KCNQ2. Reduced mRNA expression levels of GAD65, GAD67, and VGAT | Richetto et al. (2014) |

REM, rapid eye movement; EGCs, ependymal glial cells; PCs, Purkinje cells.
lobule X of the cerebellum (Varman et al., 2018). Administration of both GABA-A and GABA-B receptor agonists can improve the social deficits (Kazlauskas et al., 2016; Yang Y. et al., 2021). Individual GABA-AR agonist, chlorpromazine or GABA-BR baclofen administration can alleviate autistic-like behaviors in the VPA model. Combined chlorpromazine with baclofen can alleviate sociability deficits, anxiety and repetitive behaviors (Yang Y. et al., 2021). This evidence suggests impaired GABAergic inhibitory transmission in prenatally VPA-exposure-induced autism models.

Another widely studied pharmacological model of ASD is the MIA model, which refers to immune activation by immunogen administration during pregnancy, such as lipopolysaccharide (LPS) or polyinosinic-polycytidylic acid (poly (I:C)) (Meyer, 2014; Haddad et al., 2020). The mRNA and protein levels of GAD65, GAD67, and GABA-A subunits were increased in the peripubertal offspring of MIA models (Richetto et al., 2014). The presence of imbalanced KCC2 and NKCC2 were increased in the cortex or cerebellum (2021). Treatment with low-dose benzodiazepines clonazepam can completely reverse behavioral abnormalities of offspring of MIA models by enhancing GABAergic neurotransmission (Yang J. Q. et al., 2021). In addition, the mRNA expression levels of NKCC1 and KCC2 were increased in the peripubertal offspring of MIA models (Richetto et al., 2014). The presence of imbalanced NKCC1 and KCC2 expressions in the adult brain is typically taken as an index of an immature GABAergic phenotype (Ben-Ari et al., 2012). Thus, these results suggest prenatal maternal immune activation might have effect on long-lasting GABAergic changes relevant to autism and other neuropsychiatric disorders with prenatal infectious etiologies.

**POTENTIAL THERAPEUTIC EFFICACY OF GABA R MODULATORS IN ASD**

GABA-A receptors are a series of ion channel–type receptors that are sites of action for benzodiazepines, barbiturates, and anesthetics. GABA-B receptors are metabotropic receptors (Jembrek and Vlainic, 2015). Presynaptic GABA-B receptor act by inhibiting voltage-gated calcium channels (Krystal et al., 2002). As mentioned above, accumulating evidence has shown some of the current candidate genes mutant induce dysfunction of GABAergic transmission by affecting transcription of GABA-A subunits, development of GABAergic synapse, and synaptic structure–mediated transmission. Treatment with GABA agonists can alleviate autism-like behaviors by disinhibition of presynaptic GABAergic neurons. In addition, diuretic NKCC1 chloride importer antagonist bumetanide can reinforce GABAergic inhibition by decreasing Cl−; animal evidence showed that treatment with bumetanide reduces the severity of autism symptoms (Hadjikhani et al., 2018; James et al., 2019). The most widely studied GABA modulator is baclofen, a GABAB receptor agonist, which was reported to reverse social approach deficits and repetitive behaviors of BTBR T+ Ipr3tf/J (BTBR) mice, and also reduced the stereotyped jumping in C58/J (C58), another ASD model (Silverman et al., 2015). In addition, the hyperactivity, anxiety, aggression, and repetitive behaviors of the FMRI KO mouse model were found to be completely rescued by a GABA-AR agonist, gaboxadol, which is also called OV101 or THIP and prefer to activate δ subunit-containing extrasynaptic GABA-AR (Cogram et al., 2019).

Up to now, there are several positive results from clinical trials on the role of GABA modulators on patients with autism. Brondino et al. summarized human clinical trials of pharmacological efficacy of GABA modulator on autism (Brondino et al., 2016). A randomized, controlled, phase 2 trial results showed arbaclofen failed to improve the abnormal behavioral phenotype of children and adults with fragile X syndrome (Berry-Kravis et al., 2012). A randomized, placebo-controlled, phase 2 study of 150 participants with ASD showed there was no difference in the primary outcome measure between the arbaclofen group and control group, while there was improvement on the clinician-rated clinical global impression of severity in a specified secondary analysis (Veenstra-VanderWeele et al., 2017). Baclofen as an adjuvant therapy can enhance the therapeutic effect of antipsychotic drugs, risperidone, in children with ASD, including improvement of irritability, lethargy, stereotypic behavior, hyperactivity, and inappropriate speech (Mahdavinasab et al., 2019). A study with a small sample found that treatment with bumetanide decreased the autistic behaviors in autistic infants (Lemonnier and Ben-Ari, 2010). In addition, an open-label pilot study showed 10 months of bumetanide treatment can improve emotion recognition and stimulate the activation of social and emotional perception-related brain regions during the perception of emotional faces in adolescents and young adults with autism (Hadjikhani et al., 2015). A recent investigation showed bumetanide significantly reduces the severity of ASD core symptoms which relate to a decreased GABA/glutamate ratio in both the insular cortex (IC) and visual cortex (VC) (Zhang et al., 2020). In conclusion, current evidence in clinical trials is limited by small sample sizes and short study follow-ups, complicated clinical heterogeneity, and inconsistent inclusion criteria. Thus, to date, there is insufficient evidence to suggest the use of GABA modulators in autistic patients. Medication safety is also an issue to consider in addition to efficacy.

**CONCLUSION AND FUTURE PERSPECTIVES**

Numerous ASD candidate genes regulate extensive functions of the CNS; thus, it is hard to converge specific molecular mechanisms of ASD according to current genetic and pathological evidence. However, a lot of ASD-related genetic variations from human genetic studies identified the involvement of synaptic genes which consolidate the hypothesis that synaptic structure or/and function dysfunction are involved in ASD. In addition, technology advances propel modeling rodent models of ASD and other neuropsychiatric disorders using manipulation of candidate genes or environmental factors. We summarized primary GABAergic
inhibitory dysfunction of animal models of non-syndromic gene mutations (CNAPNP, neuroligin, SHANK, and EN2) and syndromic gene mutations (FMRI, Mecp2, and SCN1A). Current evidence indicates GABA is related to ASD from two aspects. First, GABA receptor mutants in specific neurons sufficiently recapitulate core ASD symptoms. Duplications of gene E3 ubiquitin ligase UBE3A and three non-imprinted GABA-AR genes located in 15q11.2–q13.1 (Dup15q syndrome) induce autistic phenotype behavioral deficits (Bonnet-Brilhault et al., 2016). Besides, well-acknowledged ASD candidate genes were specifically expressed in GABAergic neurons and implicated in GABA function. Scn1a encodes sodium channel subunits that are upregulated in P+ fast-spiking interneurons. Pharmacological approaches promoting GABA transmission suppress behavioral symptoms of Dravet’s syndrome (Han et al., 2012). The structural chromosomal protein MeCP2, homeobox transcription factor En2, and RNA-binding protein FMRP target metabolism, synthesis and modification of synaptic proteins related with GABA synaptic function. Neuroligins might affect the formation of neural connectivity via affecting the early postnatal transition of excitatory GABA.

Pharmacological intervention with agonist targeting specific GABA receptors was found to partially alleviate ASD-related behavioral abnormalities in animal models and clinical trials. However, the therapeutic efficacy of clinical modulators of GABA receptors needs to be supported by clinical trials with larger sample sizes. These results collectively suggest that GABAergic synaptic transmission dysfunction, especially in specific neurons or circuitry during the developmental stage, might underlie the pathophysiology of ASD. However, increased GABAergic synaptic transmission is also reported in some animal studies, indicating impaired GABAergic transmission might be a critical etiology of imbalance of the E/I ratio involved in the development of ASD. Substantial questions as to the specific etiology of ASD remain unclear and require future investigation. First, researchers need to take the reliability of current behavioral assays of mice into consideration when mimicking the clinical ASD phenotype. Additionally, a complicated reciprocal relation exists between the excitation and inhibition, especially in early brain development. In consideration that GABA can shift from “excitatory” to “inhibitory” and play a critical role in subtle regulation of neuronal network during critical developmental stages, it cannot be easily ruled out that altered excitation might be secondary to reduced inhibition and GABA dysfunction. Thus, further investigations are needed to shed light on the pathogenesis of ASD and provide a comprehensive understanding of synaptopathology in ASD.

**REFERENCES**

Adusei, D. C., Pacey, L. K. K., Chen, D., and Hampson, D. R. (2010). Early Developmental Alterations in GABAergic Protein Expression in Fragile X Knockout Mice. *Neuropharmacology* 59, 167–171. doi:10.1016/j.neuropharm.2010.05.002

Ajram, L. A., Horder, J., Mendez, M. A., Galanopoulos, A., Brennan, L. P., and Wichers, R. H. (2017). Shifting Brain Inhibitory Balance and Connectivity of the Prefrontal Cortex of Adults with Autism Spectrum Disorder. *Transl. Psychiatry* 7, e1137. doi:10.1038/tp.2017.104

Al-Otaish, H., Al-Ayadhi, L., Bjørklund, G., Chirumbolo, S., Urbina, M. A., and El-Ansary, A. (2018). Relationship between Absolute and Relative Ratios of Glutamate, Glutamine and GABA and Severity of Autism Spectrum Disorder. *Metab. Brain Dis.* 33, 843–854. doi:10.1007/s11011-018-0186-6

Amin, Z., Mason, G. F., Cavus, I., Krystal, J. H., Rothman, D. L., and Epperson, C. N. (2006). The Interaction of Neuroactive Steroids and GABA in the Development of Neuropsychiatric Disorders in Women. *Pharmacol. Biochem. Behav.* 84, 635–646. doi:10.1016/j.pbb.2006.06.007

Amir, R. E., Van den Veyver, I. B., Wan, M., Tran, C. Q., Francke, U., and Zoghbi, H. Y. (1999). Rett Syndrome Is Caused by Mutations in X-Linked MECP2. *Science* 284, 635–646. doi:10.1126/science.284.5406.635

Beattie, M. S. (2004). Inflammation and Apoptotic Linked Therapeutic Targets in Spinal Cord Injury. *Trends Mol. Med.* 10, 580–583. doi:10.1016/j.trendsmolmed.2004.10.006

Belmonte, M. K., Allen, G., Beckel-Mitchener, A., Boulanger, L. M., Carper, R. A., and Webb, S. J. (2004). Autism and Abnormal Development of Brain Connectivity. *J. Neurosci.* 24, 9228–9231. doi:10.1523/jneurosci.3340-04.2004

Ben-Ari, Y. (2002). Excitatory Actions of GABA during Development: the Nature of the Nurture. *Nat. Rev. Neurosci.* 3, 728–739. doi:10.1038/nrn920

Ben-Ari, Y., Khalilov, I., Kahle, K. T., and Cherubini, E. (2012). The GABA Excitatory/inhibitory Shift in Brain Maturation and Neurological Disorders. *Neuroscientist* 18, 467–486. doi:10.1177/1073858412438697

Benayed, R., Choi, J., Matteson, P. G., Gharani, N., Kambard, S., Brzustowicz, L. M., et al. (2009). Autism-associated Haplotype Affects the Regulation of the Homeobox Gene, ENGRAILED 2. *Biol. Psychiatry* 66, 911–917. doi:10.1016/j.biopsych.2009.05.027

Benayed, R., Gharani, N., Rossman, I., Mancuso, V., Lazar, G., Kambard, S., et al. (2005). Support for the Homeobox Transcription Factor Gene ENGRAILED 2 as an Autism Spectrum Disorder Susceptibility Locus. *Am. J. Hum. Genet.* 77, 851–868. doi:10.1086/497705

Berry-Kravis, E. M., Hessl, D., Rathmell, B., Zarevics, P., Cherubini, M., Walton-Bowen, K., et al. (2012). Effects of STX209 (Arbaclofen) on Neurobehavioral Function in Children and Adults with Fragile X Syndrome: a Randomized, Controlled, Phase 2 Trial. *Sci. Transl. Med.* 4, 152ra127. doi:10.1126/scitranslmed.3004214

Bittolo, T., Raminelli, C. A., Deiana, C., Baj, G., Vaghi, V., Ferrazzo, S., et al. (2016). Pharmacological Treatment with Mirtazapine Rescues Cortical Atrophy and Respiratory Deficits in MeCP2 Null Mice. *Sci. Rep.* 6, 19796. doi:10.1038/srep19796

**AUTHOR CONTRIBUTIONS**

HZ, XZ, FP and CZ wrote the manuscript. XM and GL made the graphics. WY, BL, TG, and RC provided the final revision.

**FUNDING**

This work was supported by National Key R&D Program of China (#2018YFC1311600), Jilin Science and Technology Agency funds in China (YDZJ202102CJXJD077), Jilin Province Medical and Health Talents (20195SCZT007 and 20195SCZT013), Program of Jilin finance department (20195SRCJ017).
Blatt, G. J., and Fatemi, S. H. (2011). Alterations in GABAergic Biomarkers in the Autism Brain: Research Findings and Clinical Implications. *Anat. Rec.* 294, 1646–1652. doi:10.1002/ar.21252

Blatt, G. J., Fitzgerald, C. M., Guptil, J. T., Booker, A. B., Kemper, T. L., and Bauman, M. L. (2001). Density and Distribution of Hippocampal Neurotransmitter Receptors in Autism: an Autoradiographic Study. *J. Autism Dev. Disord.* 31, 537–543. doi:10.1023/A:1011323880966

Blunk, A. D., Akbergenova, Y., Cho, R. W., Lee, J., Walldorf, U., Xu, K., et al. (2014). Postsynaptic Actin Regulates Active Zone Spacing and GABA Receptor Apposition at the Drosophila Neuromuscular junction. *Mol. Cell Neurosci.* 61, 241–254. doi:10.1016/j.mcn.2014.07.005

Bonnet-Brilhault, F., Alirol, S., Blanc, R., Baulouillet, S., Theipault, R. A., et al. (2016). GABA/Glutamate Synaptic Pathways Targeted by Integrative Genomic and Electrophysiological Experiments Distinguish Autism from Intellectual Disability. *Brain Sci.* 21, 411–418. doi:10.3390/brainsci20150175

Brondino, N., Fusi-Poli, L., Panisi, C., Damiani, S., Barale, F., and Politi, P. (2016). Pharmacological Modulation of GABA Function in Autism Spectrum Disorders: A Systematic Review of Human Studies. *J. Autism Dev. Disord.* 46, 825–839. doi:10.1007/s10803-015-2619-y

Brun, C. W., Korvatska, E., Allen-Brady, K., CookJr, E. H., Dawson, G., Devlin, B., et al. (2015). Maternal Transmission of a Rare GABRB3 Signal Peptide Variant Is Associated with Autism. *Mol. Autism* 6, 62. doi:10.1186/s13229-015-0055-7

Buxbaum, J. D., Silverman, J. M., Smith, C. J., Greenberg, D. A., Kilifarski, M., Reichert, J., et al. (2002). Association between a GABRB3 Polymorphism and Autism. *Mol. Psychiatry* 7, 311–316. doi:10.1038/sj.mp.4001011

Calfa, G., Hablitz, J. J., and Pozzo-Miller, L. (2011). Network Hyperexcitability in Hippocampal Slices from Mecp2 Mutant Mice Revealed by Voltage-Sensitive Dye Imaging. *J. Neurophysiol.* 105, 1768–1784. doi:10.1152/jn.00800.2010

Carvalho Pereira, A., Violante, L. R., Moura, S. Oliveira, G., and Castelo-Branco, M. (2018). Medial Frontal Lobe Neurochemistry in Autism Spectrum Disorder is Marked by Reduced N-Acetylaspartate and Unchanged Gamma-Aminobutyric Acid and Glutamate/Glutamine Levels. *Jautism Dev. Disord.* 48, 1467–1482. doi:10.1016/j.jautism.2017.03.046

Cattane, N., Richetto, J., and Cattaneo, A. (2020). Prenatal Exposure to Valproic Acid Changes Sleep in Juvenile Rats: a Model for Sleep Disturbances in Autism. *Sleep* 37, 1498–1499. doi:10.5665/sleep.3998

D’Hulst, C., De Geest, N., Reeve, S. P., Van Dam, D., De Deyn, P. P., Hassan, B. A., et al. (2006). Decreased Expression of the GABAA Receptor in Fragile X Syndrome. *Brain Res.* 1121, 238–245. doi:10.1016/j.brainres.2006.08.115

De Ruibeis, S., and Bagni, C. (2011). Regulation of Molecular Pathways in the Fragile X Syndrome: Insights into Autism Spectrum Disorders. *J. Neurodev. Disord.* 3, 257–269. doi:10.1186/111689-011-9087-2

Delahanty, R. J., Kang, J. Q., Brun, C. W., Kistner, E. O., Courchesne, E., Cox, N. J., et al. (2011). Maternal Transmission of a Rare GABRB3 Signal Peptide Variant Is Associated with Autism. *Mol. Psychiatry* 16, 86–96. doi:10.1038/mp.2009.118

Dhosche, D., Applegate, H., Abraham, A., Maertens, P., Bland, L., Bencsath, A., et al. (2002). Elevated Plasma Gamma-Aminobutyric Acid (GABA) Levels in Autism. *Autistic Youngsters: Stimulus for a GABA Hypothesis of Autism. Med. Sci. Monit.* 8, PRI–6.

Drenthen, G. S., Barendse, E. M., Aldenkamp, A. P., van Veenedaald, T. M., Puts, N. A., Edden, R. A., et al. (2016). Altered Neurotransmitter Metabolism in Adolescents with High-Functioning Autism. *Psychiatry Res. Neuroimaging* 256, 44–49. doi:10.1016/j.pscychresns.2016.09.007

Duman, R. S., Sanacora, G., and Krystal, J. H. (2019). Altered Connectivity in Depression: GABA and Glutamate Neurotransmitter Deficits and Reversal by Novel Treatments. *Neuron* 102, 75–90. doi:10.1016/j.neuron.2019.03.013

Edmondson, D. A., Xia, P., McNally Keehn, R., Dyudak, U., and Keehn, B. (2020). A Magnetic Resonance Spectroscopy Study of Superior Visual Search Abilities in Children with Autism Spectrum Disorder. *Autism Res.* 13, 550–562. doi:10.1002/aur.2258

El-Ansary, A., and Al-Ayadhi, L. (2014). GABAergic/Glutamatergic Imbalance: A Relative to Excessive Neuron inflammation in Autism Spectrum Disorders. *J. Neuroinflammation* 11, 189. doi:10.1186/s12974-014-0189-0

El-Khoury, R., Panayotis, N., Matagne, V., Ghata, A., Villard, L., and Roux, J. C. (2011). Impairment of Fragile X Mental Retardation Protein-Metabotropic Glutamate Receptor 5 Signaling and its Downstream Cognates Ras-Related C3 Botulinum Toxin Substrate 1, Amyloid Beta A4 Precursor Protein, Striatal-Enriched Protein Tyrosine Phosphatase, and homer 1, in Autism: a Postmortem Study in Cerebellar Vermis and superior Frontal Cortex. *Mol. Autism* 4, 21. doi:10.1186/2040-2392-4-21

Fatemi, S. H., Folsom, T. D., Reutiman, T. J., and Thuras, P. D. (2009a). Expression of GABA(B) Receptors Is Altered in Brains of Subjects with Autism. *Cerebellum* 8, 64–69. doi:10.1007/s12311-008-0075-3

Fatemi, S. H., Folsom, T. D., Reutiman, T. J., and Thuras, P. D. (2009b). Expression of GABA(B) Receptors Is Altered in Brains of Subjects with Autism. *Cerebellum* 8, 64–69. doi:10.1007/s12311-008-0075-3
Fatemi, S. H., Reutiman, T. J., Folsom, T. D., Patel, D. H., and Thuras, P. D. (2010). mRNA and Protein Levels for GABAα1, α2, and β1 Receptors Are Altered in Brains from Subjects with Autism. J. Autism Dev. Disord. 40, 743–750. doi:10.1007/s10803-009-0924-z

Fatemi, S. H., Reutiman, T. J., Folsom, T. D., and Thuras, P. D. (2009b). GABA(A) Receptor Downregulation in Brains of Subjects with Autism. J. Autism Dev. Disord. 39, 223–230. doi:10.1007/s10803-008-0646-7

Fekete, C. D., Chiou, T. T., Miralles, C. P., Harris, R. S., Fiondella, C. G., Loturco, J. J., et al. (2015). In Vivo clonal Overexpression of Neurogenin 3 and Neurogenin 2 in Neurons of the Rat Cerebral Cortex: Differential Effects on GABAergic Synapses and Neuronal Migration. J. Comp. Neurol. 523, 1359–1378. doi:10.1002/jcn.23740

Filice, F., Vörcell, K. J., Sungur, A. Ö., Wöhr, M., and Schwaller, B. (2016). Reduction in Parvalbumin Expression Not Loss of the Parvalbumin-Expressing GABA Interneuron Subpopulation in Genetic Parvalbumin and Shank Mouse Models of Autism. Mol. Brain 9, 10. doi:10.1186/s13041-016-0192-8

Foldy, C., Malenka, R. C., and Südhof, T. C. (2013). Autism-associated Neuroginin 3 Mutations Commonly Disrupt Tonic Endocannabinoid Signaling. Neuron 78, 498–509. doi:10.1016/j.neuron.2013.02.036

Fu, Z., and Vicini, S. (2009). Neurogin-2 Accelerates GABAergic Synapse Maturation in Cerebellar Granule Cells. Mol. Cel. Neurosci. 42, 45–55. doi:10.1016/j.mcn.2009.05.004

Futai, K., Flores, R. E., Go, M., Sun, K. L., James, D., Schuck, R. K., et al. (2021). Thalamic and Prefrontal GABA Concentrations but Not GABA Receptor Densities Are Altered in High-Functioning Adults with Autism Spectrum Disorder. Mol. Psychiatry 26, 1634–1644. doi:10.1038/s41380-020-0756-y

Gharani, N., Benayed, R., Mancuso, V., Brustowicz, I. M., and Millonig, J. H. (2004). Association of the Homeobox Transcription Factor, ENGRAILED 2, 3, in Neurons of the Rat Cerebral Cortex: Differential Effects on GABAergic Synapses and Neuronal Migration. Mol. Brain 11, 212. doi:10.1038/srep44155

Gogolla, N., Leblanc, J. J., et al. (2015). GABA Receptors: Pharmacological Potential and Therapeutic Perspective. Mol. Psychiatry 20, 743–758. doi:10.1038/mp.2014.298

Gonçalves, J., Violante, I. R., Sereno, J., Leitão, R. A., Cai, Y., Abrunhosa, A., et al. (2021). J. Autism Dev. Disord. 40, 743–750. doi:10.1007/s10803-009-0924-z

Hammer, M., Krueger-Burg, D., Tuffy, L. P., Cooper, B. H., Taschenberger, H., Filice, F., Vörckel, K. J., Sungur, A. Ö., Wöhr, M., and Schwaller, B. (2016). Reduction in Parvalbumin Expression Not Loss of the Parvalbumin-Expressing GABA Interneuron Subpopulation in Genetic Parvalbumin and Shank Mouse Models of Autism. Mol. Brain 9, 10. doi:10.1186/s13041-016-0192-8

Heise, C., Preuss, J. M., Schroeder, J. C., Battaglia, C. R., Kolibius, J., Schmid, R., et al. (2018). Heterogeneity of Cell Surface Glutamate and GABA Receptor Expression in Shank and CNNT4 Autism Mouse Models. Front. Mol. Neurosci. 11, 212. doi:10.3389/fnmol.2018.00212

Hogart, A., Nagarajan, R. P., Patzel, K. A., Yasaki, D. H., and Lasalle, J. M. (2007). 15q11-13 GABAA Receptor Genes Are Normally Biallelically Expressed in Brain yet Are Subject to Epigenetic Dysregulation in Autism-Spectrum Disorders. Hum. Mol. Genet. 16, 691–703. doi:10.1093/hmg/ddm014

Höfert, T., Falcone, C., Dufour, B., Amina, S., Castro, R. P., Regalado, J., et al. (2020). GABAARα2 Is Decreased in the Axon Initial Segment of Pyramidal Cells in Specific Areas of the Prefrontal Cortex in Autism. Neuroscience 437, 76–86. doi:10.1016/j.neuroscience.2020.04.025

Hoom, M., Soykan, T., Falkenburger, B., Hammer, M., Patrizi, A., Schmidt, K. F., et al. (2011). Neurogin-4 Is Localized to Glycerinergic Postsynapses and Regulates Inhibition in the Retina. Proc. Natl. Acad. Sci. U.S.A. 108, 3053–3058. doi:10.1073/pnas.1006946108

Horder, J., Petrinovic, M. M., Mendez, M. A., Bruns, A., Takumi, T., Spooeren, W., et al. (2018). Glutamate and GABA in Autism Spectrum Disorder-A Translational Magnetic Resonance Spectroscopy Study in Man and Rodent Models. Transl. Psychiatry 8, 106. doi:10.1038/s41398-018-0155-1

Hou, Q., Wang, Y., Li, Y., Chen, D., Yang, F., and Wang, S. (2018). A Developmental Study of Abnormal Behaviors and Altered GABAergic Signaling in the VPA-Treated Rat Model of Autism. Front. Behav. Neurosci. 12, 182. doi:10.3389/fnbeh.2018.00182

Jager, A., Amir, H., Bieczk, N., van Heukelum, S., Heerschap, A., Aschner, A., et al. (2020). Cortical Control of Aggression: GABA Signalling in the Anterior Cingulate Cortex. Eur. Neuropsychopharmacol. 30, 5–16. doi:10.1016/j.euro.2017.12.007

James, B. J., Gales, M. A., and Gales, B. J. (2019). Bumetanide for Autism Spectrum Disorder in Children: A Review of Randomized Controlled Trials. Ann. Pharmacother. 53, 537–544. doi:10.1177/0003364318817304

James, S. J., Shpyleva, S., Melnyk, S., Pavliv, O., and Pogribny, I. P. (2013). Complex Developmental Study of Abnormal Behaviors and Altered GABAergic Signaling in the VPA-Treated Rat Model of Autism. J. Comp. Neurol. 458, 21958–21972. doi:10.1002/cne.23740

Jembruk, M. J., and Vlainic, J. (2015). GABA Receptors: Pharmacological Potential and Pitfalls. Curr. Pharm. Des. 21, 4943–4959. doi:10.2174/13816128216615914121624

Jiang, D. Y., Wu, Z., Forsyth, C. T., Hu, Y., Yee, S. P., and Chen, G. (2018). GABAergic Deficits and Schizophrenia-like Behaviors in a Mouse Model Carrying Patient-Derived Neurogin-2 R215H Mutation. Mol. Brain 11, 31. doi:10.1186/s13041-018-0375-6
Yang, Y., Wang, B., Zhong, Z., Chen, H., Ding, W., and Hoi, M. (2021b). Clonazepam Attenuates Neurobehavioral Abnormalities in Offspring Exposed to Maternal Immune Activation by Enhancing GABAergic Neurotransmission. Biochem. Pharmacol. 192, 114711. doi:10.1016/j.bcp.2021.114711

Yip, J., Soghomonian, J. J., and Blatt, G. J. (2009). Decreased GAD65 mRNA Levels in Select Subpopulations of Neurons in the Cerebellar Dentate Nuclei in Autism: an In Situ Hybridization Study. Autism Res. 2, 50–59. doi:10.1002/aur.62

Yip, J., Soghomonian, J. J., and Blatt, G. J. (2008). Increased GAD67 mRNA Expression in Cerebellar Interneurons in Autism: Implications for Purkinje Cell Dysfunction. J. Neurosci. Res. 86, 525–530. doi:10.1002/jnr.21520

Zhang, B., Gokce, O., Hale, W. D., Brose, N., and Südhof, T. C. (2018). Autism-associated Neuroligin-4 Mutation Selectively Impairs Glycinergic Synaptic Transmission in Mouse Brainstem Synapses. J. Exp. Med. 215, 1543–1553. doi:10.1084/jem.20172162

Zhang, L., He, J., Jugloff, D. G., and Eubanks, J. H. (2008). The McCP2-Null Mouse hippocampus Displays Altered Basal Inhibitory Rhythms and Is Prone to Hyperexcitability. Hippocampus 18, 294–309. doi:10.1002/hipo.20389

Zhang, L., Huang, C. C., Dai, Y., Luo, Q., Ji, Y., Wang, K., et al. (2020). Symptom Improvement in Children with Autism Spectrum Disorder Following Bumetanide Administration Is Associated with Decreased GABA/glutamate Ratios. Transl. Psychiatry 10, 9. doi:10.1038/s41398-020-0092-2

Zhang, W., Peterson, M., Beyer, F., Frankel, W. N., and Zhang, Z. W. (2014). Loss of McCP2 from Forebrain Excitatory Neurons Leads to Cortical Hyperexcitation and Seizures. J. Neurosci. 34, 2754–2763. doi:10.1523/JNEUROSCI.4909-12.2014

Zhang, X., Ibi, M., Haga, R., Iwata, K., Matsumoto, M., Asaoka, N., et al. (2021). NOX1/NADPH Oxidase Affects the Development of Autism-like Behaviors in a Maternal Immune Activation Model. Biochem. Biophys. Res. Commun. 534, 59–66. doi:10.1016/j.bbrc.2020.11.070

Zhang, Z. W., Zak, J. D., and Liu, H. (2010). McCP2 Is Required for normal Development of GABAergic Circuits in the Thalamus. J. Neurophysiol. 103, 2470–2481. doi:10.1152/jn.00601.2009

Zhong, W., Johnson, C. M., Cui, N., Oginsky, M. F., Wu, Y., and Jiang, C. (2017). Effects of Early-Life Exposure to THIP on Brainstem Neuronal Excitability in the Mecp2-Null Mouse Model of Rett Syndrome before and after Drug withdrawal(Article). Physiol. Rep.

Zieminska, E., Toczyłowska, B., Diamandakis, D., Hilgier, W., Filipkowski, R. K., Polowy, R., et al. (2018). Glutamate, Glutamine and GABA Levels in Rat Brain Measured Using MRS, HPLC and NMR Methods in Study of Two Models of Autism. Front. Mol. Neurosci. 11, 418. doi:10.3389/fnmol.2018.00418

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors, and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Zhao, Mao, Zhu, Zou, Peng, Yang, Li, Li, Ge and Cui. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.