Signalling pathways in autism spectrum disorder: mechanisms and therapeutic implications

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Autism spectrum disorder (ASD) is a prevalent and complex neurodevelopmental disorder which has strong genetic basis. Despite the rapidly rising incidence of autism, little is known about its aetiology, risk factors, and disease progression. There are currently neither validated biomarkers for diagnostic screening nor specific medication for autism. Over the last two decades, there have been remarkable advances in genetics, with hundreds of genes identified and validated as being associated with a high risk for autism. The convergence of neuroscience methods is becoming more widely recognized for its significance in elucidating the pathological mechanisms of autism. Efforts have been devoted to exploring the behavioural functions, key pathological mechanisms and potential treatments of autism. Here, as we highlight in this review, emerging evidence shows that signal transduction molecular events are involved in pathological processes such as transcription, translation, synaptic transmission, epigenetics and immunoinflammatory responses. This involvement has important implications for the discovery of precise molecular targets for autism. Moreover, we review recent insights into the mechanisms and clinical implications of signal transduction in autism from molecular, cellular, neural circuit, and neurobehavioural aspects. Finally, the challenges and future perspectives are discussed with regard to novel strategies predicated on the biological features of autism.

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INTRODUCTION

Autism spectrum disorder (ASD), a group of early developmental disorders, is characterized by deficits in social communication and repetitive stereotyped behaviours. Over the past 80 years, risk factors, diagnostic criteria, clinical treatment options, and societal implications of ASD have attracted the concerns of neuroscientists and clinicians (Fig. 1).

In 1943, Leo Kanner of Johns Hopkins University published “Autistic disturbances of affect contact” in the special issue of the journal The Nervous Child, which systemically examined 11 cases of autism and named it “early infantile autism”.1 Kanner used the term “infantile autism” to describe the children with symptoms of social isolation and linguistic disorders. However, some aspects of Kanner’s views also called the origin of early confusion in the field, such as the vague definition between schizophrenia and autism.2 In 1944, Hans Asperger identified a group of children who have severe social abnormalities and motor disorders but with very high intellectual functioning.3 This led to the diagnosis of high-functioning autism, that has been incorporated into the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) and the 10th edition of the World Health Organization’s International Statistical Classification of Diseases and Related Health Problems (ICD-10) and named “Asperger’s Syndrome”.4-6

In the 1960s and 1970s, early pioneering works on the diagnosis and treatment of autism were initiated. In 1964, Bernard Rimland first began to investigate new approaches to the objective diagnosis of autism.7 In 1972, based on studies of clinical phenomenology, Rutter made clear that autism has significant differences from schizophrenia in terms of onset, clinical symptoms, and family history.8 Rutter’s research also suggested that it would be more attributable to autistic behaviours to developmental disorders from birth to early childhood. By the late 1970s, a consensus emerged about the importance of studying autism independently of schizophrenia, which promoted the updating of diagnostic criteria.9,10 In 1978, Rutter proposed new diagnostic criteria for autism emphasizing social skill dysfunction, language and communication impairment, and repetitive behaviours as three aspects of the basic criteria, abandoning the “special skills and attractive appearance” of Kanner’s criteria.9 The diagnostic approach provided by Rutter directly influenced the revision of DSM-III. In 1980, DSM-III first regarded “infantile autism” as a pervasive developmental disorder (PDD) and focused on early development. Over the same period, studies on intervention and treatment also greatly improved. In 1973, Bartak and Rutter recommended the importance of a structured, behaviour improvement-focused treatment plan.11 Subsequently, an increasing number of behavioural intervention studies have supported the notion that behavioural psychology and special education can be applied to inform autism therapy.

In the 1980s, autism research entered a new era, especially in terms of mechanisms. Autism gradually began to be viewed as a
somatic developmental disorder unrelated to parenting styles. Researchers began exploring the aetiology of autism from a biological perspective and completely distinguished autism from schizophrenia on account of clinical symptom recognition and clinical diagnosis. In 1977, Folstein and Rutter’s first study on twins revealed the high heritability of autism. Subsequently, with the in-depth understanding of autism, people gradually realized that autism is a developmental disorder under the influence of certain genetic factors. On this foundation, substantial research into the genesis of autism has been conducted, including molecular genetics, neuroimmunity, functional imaging, neuroanatomy, and neurochemistry research.

ASD is considered to be the result of complex interactions among genetic, environmental, and immunological factors. There have been incredible improvements in the investigation of genetic correlations with autism over the past two decades, ranging from monoclonal gene studies to contemporary large-scale studies using whole-genome sequencing (WGS). A number of highly reliable and repetitive risk genes have been discovered. Based on studies of genetically modified mice, considerable progress has been made in illustrating the functions of genes such as MeCP2 (Rett syndrome), Tsc1/2 (tuberous sclerosis), Fmr1 (fragile X syndrome), Pten and Shank3 (Phelan–McDermid syndrome) in several monogenic diseases. These advances in disease mechanism research provide the basis for the design of drugs such as rapamycin (mTOR) inhibitors (tuberous sclerosis), and fragile X syndrome, metabolic glutamate receptor (mGluR) antagonists (fragile X syndrome and 16p11.2 deletion), and insulin growth factor (IGF-1) (Rett syndrome and Phelan–McDermid syndrome).

In addition to the downregulation of synapse-related genes, microglia and immune-related genes were increased in the brains of autistic patients. The correlations among astrocytes, microglial activation, neuroinflammation caused by gut microbiota and immune dysregulation in ASD patients are also involved in the pathological mechanism. In particular, infection during pregnancy has been established to induce maternal immune activation that affects the offspring nervous system.

Another pathological mechanism of ASD that has garnered much attention is the functional impairment of brain regions and neural circuits. Autopsies of patients with ASD have revealed significant structural changes in their brains, including altered grey/white matter ratios, increased neuronal numbers, decreased neuronal body volume, increased numbers of glia, and changes in dendritic spines and cerebral blood vessels. Additionally, there is established evidence of alterations in glutamate circuits and GABAergic circuits in ASD patients, as manifested by increased numbers of excitatory synapses and spine densities, significantly reduced levels of glutamic acid decarboxylase, and GABAA and GABAB receptor alterations in the postmortem brains of patients with autism.

In this review, we integrate recent advances from genetic, neuropathological, and neurobiochemical studies on ASD to further elucidate the pathogenetic mechanism at the molecular, cellular, and neural circuit levels.

**CLINICAL OVERVIEW AND GENETIC FEATURES**

**Definition and diagnosis of ASD**

Since autism was discovered 80 years ago, its clinical definition and diagnostic criteria have undergone several iterations. In 1980, the DSM-III classified “infantile autism” as one of the generic “PDDs.” In 1994, five PDDs were included in the DSM-IV: autism disorder, Asperger’s syndrome, PDD-not otherwise specified (PDD-NOS), Rett syndrome and childhood disintegrative disorder. Given the large variability in symptom severity across disease groups, it is difficult to effectively distinguish diseases. To remove this uncertainty, the DSM-5 classifies autism, Asperger’s syndrome, and PDD-NOS as ASD. With this revision, the diagnostic criteria have changed as well. ASD is characterized by two main symptoms: deficits in social interaction/communication, as well as repetitive stereotyped behaviours that first occur in early developmental stages and cause clinically substantial impairment. Aside from the core features above, individuals with ASD are frequently associated with co-occurring symptoms, including dyskinesia (hypotonia, bradykinesia), speech delay, sleep disorder,
gastrointestinal problems, anxiety and epilepsy, which are the most common symptoms in preschool children, while in adolescents and adults, the proportion of depressive symptoms is higher. These comorbidities also pose challenges to disease modelling of ASD, as they may complicate the evaluation of ASD core behaviours in animal models.

The diagnosis of autism is based on thorough consideration of medical history, physical and neurological examination, psychiatric examination, and auxiliary examinations. A comprehensive review of the family history of ASD or other neurological disorders should also be included. Autism diagnoses from preschool to mid-childhood are highly stable. Due to the complexity, severity, and overlap of ASD features, the correct diagnosis of ASD with instruments and scales is essential for improving the clinical management of patients. Several scales have been suggested that can be helpful for identifying ASD.

Epidemiology of ASD
Over the past two decades, the prevalence of ASD reported worldwide has been steadily increasing. In 2000, according to the Autism and Developmental Disabilities Monitoring (ADDm), the incidence of ASD was estimated to be 1 in 150 children. In 2006, the incidence was 1 in 110 children, and by 2008, the incidence had increased to 1 in 88 children. According to recent estimates, more than 70 million people worldwide have suffered from autism, and the overall estimated prevalence is between 1.5% and 2%. Modifications in diagnostic criteria and increased awareness of autism in people might be responsible for the surge in autism. Estimates of autism prevalence in different populations and settings vary by definition, sampling, and assessment of independent population cases among studies.

Notably, there is a prominent sex difference in the prevalence of ASD, with prevalences of 2.8% in males and 0.65% in females and a male-to-female ratio of 4.3:1. This suggests that unknown biological factors may play a role. Moreover, a recent study showed an increased female-to-male odds ratio for ASD comorbidities and showed that comorbidity occurrence was associated with the age at first autism diagnosis. There may be differences in gene expression induced by gonadal hormones or sex chromosomes in mammals. In the brain, more genes are expressed from the X chromosome than from the Y chromosome. The mutations in the X chromosome are generally associated with intellectual disability syndrome, which is more prevalent in males than in females. The earliest studies on the rare variant of ASD have also tended to focus on the contributions of chromosomal abnormalities in girls. A rare LGD mutation has been found in the NLGN4 and NLGN3 genes, both of which are located on the X chromosome. As an X-linked neurodevelopment disorder, Rett syndrome almost exclusively influences females. One possibility is that mutations in Rett syndrome occur almost exclusively on the paternally derived X chromosome and are lethal in male embryos. In general, the contribution of gender aetiology to autism remains largely unexplained. Human studies have only identified minor sex variations in cerebral cortical gene expression. Resolving sex differences is a significant aspect in the process of ASD and shows great potential for the development of widely applicable therapies. Many psychiatric disorders, including ASD, will probably be better understood if key sex differences in cellular and molecular events during brain differentiation can be identified.

Genetic architecture of ASD
Twin and family studies have consistently suggested that autism have a strong heritability. Recent advances in genetic technology, microarrays, WGS, and whole-exome sequencing (WES) have revealed patterns of genetic variation that result in ASD. Here, we highlight the contributions of inheritance patterns, variation types and epidemic rates to ASD (Fig. 2). Heritability measurements have been derived from investigations on identical twins, fraternal twins and sibling concordance, including a survey of more than 2 million Swedish households in 2014, which is the largest human-based ASD study to date, eventually estimating the heritability of ASD as ranging from 52% to 90%. Moreover, the epidemiological and molecular data suggest that the genetic contribution of ASD results from the combination of rare deleterious variants and a large number of low-risk alleles. Therefore, different phenotypes can arise because prevalent low-risk alleles buffer the effects of detrimental variation.

The genetic structure of ASD is extremely complex. Approximately 600–1200 genes and genomes have been identified that associated with autism. At least 5% of ASD cases are caused by single-nucleotide polymorphisms (SNPs) in genes such as NLGN3, NLGN4, NRXN1, MECP2, SHANK3, FMR1, TSC1/2 and UBE3A. In addition, rare de novo mutations of CHD8, SCN1A, SCN2A, SYNGAP1, ARID1B, GRIN2B, DSCAM, TBR1, KATNAL2, LAMC3 and NTNG1 have been identified, with strong evidence for their association with ASD. Approximately 10% of them are copy number variations (CNVs) that disrupt protein coding, including chromosomal duplications, large deletions, inversions, and translocations, such as 1q21.1 duplications or deletions, 3q29 deletions, 7q11.23 duplications, 15q11-q13 deletions, 15q13.3 microdeletions, 15q11-13 duplications, 17q12 deletions, 22q11.2 deletions and 22q13.33 duplications or deletions. Mutations located in intronic and intergenic regions are the third variation type of ASD.

ASD is thought to contain two subtypes: syndromic and non-syndromic forms. Syndromic generally refers to mutations in a specific gene or genome, manifesting as neurological syndromes (such as fragile X syndrome, tuberous sclerosis, Rett syndrome, Phelan–McDermid syndrome and Angelman syndrome). Non-syndromic, also regarded as idiopathic, which accounts for the vast majority, is not associated with other neurological disorders (or syndromes) but is related to some genes associated with autism. In heterogeneous genetic structures, syndromic ASD caused by high-penetration single-gene mutations represent only a minority of ASD cases, the majority of cases are idiopathic. In fact, due to the overlap of phenotypes and growing understanding of intersecting biology, it remains controversial that the definition and boundary between syndromic and idiopathic ASD. With the advance of genetics, more efforts have been invested in identifying individuals with rare mutations of same gene and the convergence among them. Some retrospective analysis of gene fragments (for example, CDH8 and ADNP) from individuals with typical idiopathic ASD has revealed different clinical phenotypic features. This suggests significant variability in the symptoms, as well as the persistence of previously overlooked syndromes in idiopathic ASD. Therefore, continuous and holistic analysis rather than isolated studies may help us better comprehend ASD.

NEUROBIOLOGICAL MECHANISMS OF ASD

Due to the above unknown factors and challenges, many genetic variations associated with ASD have been suggested to be possibly concentrated on common molecular or cellular pathways. Key literature from recent years has suggested that ASD-associated genes enriched in aspect of transcription and translation, synapse, epigenetics, immunity and inflammation. These are closely related to the occurrence, development and outcome of autism. The first category is the dysregulation of important transcripts and translational signalling pathways. The second category involves synaptic proteins, including cell adhesion, scaffolding, and signalling molecules, which can affect synapse structure and function during different processes of synapse formation, elimination, transmission, and plasticity. The third category is the overtranscription of certain transcripts,
which can lead to widespread epigenetic dysregulation, creating a positive feedback loop between translation and transcription processes that exacerbates neuronal dysfunction in ASD.\textsuperscript{93} The immunoinflammatory response caused by the activation of reactive glial cell proliferation and intestinal flora dysbiosis can be classified into the fourth type of abnormal signal transduction.\textsuperscript{94,95} These types of signalling pathways can interact or participate in the pathophysiology of ASD in a cascading manner rather than acting independently. For example, alterations in Wnt signalling, alterations in neuronal translation and defects in synaptogenesis or synaptic function during brain development can all affect the formation and activity of neural circuits.\textsuperscript{96,97} In turn, altered neural activity can further influence transcription factors or chromatin remodelling by transmitting action potential cascades that trigger signals and initiate specific transcriptional programmes.\textsuperscript{89,98}

Numerous animal genetic models of autism have been developed and characterised as a result of genetic advances, allowing relevant phenotypes and mechanisms to be discovered and further studied (Table 1). Mouse models have provided a mountain of evidence for molecular pathways in autism, especially in translation and synaptic function.\textsuperscript{15} Manipulation of individual risk genes in model systems may lead to identification of important phenotypes. Although they cannot completely simulate the pathological process of human beings, these techniques still help us to understand the occurrence and development of autism.
| Target | Mice | Behaviour phenotypes | Molecular, cellular and circuit phenotypes | Mechanism | Ref. |
|--------|------|----------------------|------------------------------------------|-----------|-----|
| Nlgn   | Nlgn-3 KO | Reduced ultrasound vocalization | Selective synapse impairment | Nlgn-3 mutations specifically impede synaptic inhibition on D1-dopamine receptor-expressing neurons | 370,557 |
|        | Nlgn-3 R451C | Impaired social interactions | Altered inhibitory synaptic transmission | Neurologin dysfunction altered the E/I balance and synaptic transmission | 193,195 |
|        | Nlgn-4 KO | Impaired social interactions and social memory | Reduced brain volume | Loss of Nlgn-4 selectively impaired glycineric synaptic transmission | 558,559 |
| Nrxn   | Nrxn-1a KO | Increased repetitive grooming | Deficient excitatory synaptic strength | Nrxn-1α deficiency reduced excitatory synaptic transmission and resulted in an E/I imbalance | 560,561 |
|        | Nrxn-2a KO | Deficient social interaction | Reduced spontaneous transmitter release at excitatory synapses in the neocortex | E/I imbalance | 562 |
|        | MeCP2-/- | Impaired motor coordination | Reduced brain volume | Absence of MeCP2 | 563 |
|        | MeCP2-TG1 | Motor defects | Increased Crh and Oprm1 in the amygdala | Social approach deficits may be due to increased Oprm1 levels | 564 |
|        | Shank3 e4−9 KO | Repetitive grooming | Decreased levels of Homer1b/c, GKAP and GluA1 at the PSD | Homozygous deletion of exons 4-9 induce loss of isoforms of Shank3 | 204,565 |
|        | Shank3B-/- | Repetitive grooming | Altered PSD composition in the striatum | Dysfunction of Nrxn/Nlgn/PSD95/PSAPa-P/Shank complex | 202 |
|        | Shank3 HET | Impaired social interaction | Reduced basal neurotransmission | Shank3 deficiency influence AMPA receptor recruitment and synaptic development | 205 |
|        | Shank3ΔC | Social deficits | Diminished NMDAR synaptic function and synaptic distribution | Shank3 deficiency leads to the reduced expression of 1PIX (GEF for Rac1), and Rac1/PAK/LIMK signalling | 566 |
|        | InsG3680 | Impaired social interaction | Severe striatal synaptic defects | Impaired synaptic transmission induced long-lasting alterations in striatal connectivity | 206 |
|        | Shank2 HET | Impaired motor coordination | Reduced dendritic spines basal synaptic transmission | Altered glutamatergic neurotransmission can lead to the core symptoms of ASD | 203,207 |

**References:**
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| Target       | Mice                          | Behaviour phenotypes            | Molecular, cellular and circuit phenotypes                                                                 | Mechanism                                                                                       | Ref.  |
|--------------|-------------------------------|---------------------------------|----------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|-------|
| L7-Shank2    | −/−                           | Impaired motor learning         | Decreased AMPAR in cerebellar synaptosomes                                                               | Shank2 deficiency impairs PC intrinsic plasticity and induction of LTP at the parallel fibre to PC synapse | 208   |
|              |                               | Abnormal social and repetitive  | Increased sIPSCs and spiking irregularity                                                                 |                                                                                                  |       |
|              |                               | behaviour                       | Impaired synaptic and intrinsic plasticity in PC                                                        |                                                                                                  |       |
|              |                               |                                 | elevated basal protein synthesis                                                                       |                                                                                                  |       |
| Fmr1         | Fmr1 KO                       | Deficient social behaviour       | Elevated basal protein synthesis                                                                         | The absence of FMRP leads to enhanced activity of mGlur5 signal transduction pathways            | 567, 568 |
| Tsc2         | Tsc1<sup>−/−</sup>, Tsc2<sup>−/−</sup> | Deficient social interaction     | Hyperactivation of mTOR                                                                                    | Uninhibited mTOR signalling pathways                                                            | 569   |
|              | L7Cre; Tsc1<sup>−/−</sup>      | Abnormal social interaction and  | Decreased PC excitability                                                                               | Overactivity of the mTOR signalling pathway                                                       | 146, 376 |
|              |                               | vocalizations                    |                                                                                                           |                                                                                                  |       |
|              | Tsc2<sup>−/−</sup>             | Deficient social interaction     | Deficient spine pruning and cortical projection neurons                                                  | Tsc2 mutations caused unregulated mTOR activity                                                   | 567   |
| Ube3a        | Ube3a 1× and 2× transgenic     | Defective social interaction     | Suppressed glutamatergic synaptic transmission                                                            | Increased E3A ubiquitin ligase gene dosage results in reduced excitatory synaptic transmission     | 570   |
|              |                               | Impaired communication           |                                                                                                           |                                                                                                  |       |
|              |                               | Increased repetitive stereotypic  |                                                                                                           |                                                                                                  |       |
|              |                               | behaviour                       |                                                                                                           |                                                                                                  |       |
| Chd8         | Chd8<sup>−/−</sup>             | Deficient social behaviour       | Synaptic dysfunction within MSNs in the NAc                                                             | Reduced expression of CHD8 is associated with abnormal activation of REST                        | 131, 368 |
|              |                               | Communication difficulties       | Delayed neurodevelopment                                                                                   |                                                                                                  |       |
|              |                               | Repetitive behaviour             |                                                                                                           |                                                                                                  |       |
| Snc1         | Snc1α<sup>−/−</sup>            | Stereotyped behavior            | Decreased NMDAR synaptic function and synaptic distribution                                              | Snc1α haploinsufficiency impaired GABAergic neurotransmission and NaV1.1 dysfunction induce      | 181   |
|              |                               | Deficient social interaction     | Decreased cortical actin filaments                                                                        | behavioural and cognitive impairments                                                            |       |
|              |                               | Impaired context-dependent       | Insufficient NMDAR                                                                                       |                                                                                                  |       |
|              |                               | spatial memory                  |                                                                                                           |                                                                                                  |       |
| SynGAP1      | SynGAP1 HET                    | Deficient social memory         | Dendritic spine synapses develop prematurely                                                             | SYNGAP1 deficiency impaired NMDAR-CAMKII-SynGAP-GluR1 pathway                                    | 571, 572 |
|              |                               | Tendency to social isolation    | Premature spine maturation enhanced excitability                                                         | SYNGAP1 haploinsufficiency altered E/I balance                                                   |       |
|              |                               |                                 |                                                                                                           | And1b haploinsufficiency suppressed H3K9Ac overall, and reduced H3K9Ac of the Pvalb promoter, resulting in decreased transcription |       |
| And1b        | And1b<sup>−/−</sup>            | Abnormal cognitive and social    | Decreased number of cortical GABAergic interneurons                                                      | And1b haploinsufficiency altered E/I balance                                                   | 573   |
|              |                               | behaviour                       | Reduced proliferation of interneuron progenitors in the ganglionic eminence                              |                                                                                                  |       |
|              |                               |                                 | Imbalance between excitatory and inhibitory synapses                                                    |                                                                                                  |       |
| Tbr1         | Tbr1<sup>−/−</sup>             | Impairment of social interaction,| Defective axonal projections of amygdala neurons                                                        | Tbr1 gene altered the expression of Ntrng1, Cntn2 and Cdh8 and reduced both inter- and intra-amygdala connections | 110   |
|              |                               | ultrasound vocalization,         |                                                                                                           |                                                                                                  |       |
|              |                               | associative memory and cognitive |                                                                                                           |                                                                                                  |       |
|              |                               | flexibility                      |                                                                                                           |                                                                                                  |       |
| Pten         | Pten<sup>−/−</sup>             | Deficient social behaviour       | Brain overgrowth                                                                                        | Desynchronized growth in key cell types                                                          | 574, 575 |
|              |                               | Repetitive behaviour             | Abnormal immune system                                                                                   |                                                                                                  |       |
|              |                               | Lower circadian activity         | Altered cytoarchitecture and synaptic                                                                     |                                                                                                  |       |
|              |                               | Impaired emotional learning      |                                                                                                           |                                                                                                  |       |
|              |                               |                                 |                                                                                                           |                                                                                                  |       |
| Nse-cre; Pten<sup>−/−</sup> | Abnormal social interaction     | Macrocephaly                      | Abnormal activation of the PI3K/AKT pathway in specific neuronal populations                             | Hypervalent expression of fragile X mental retardation protein                                   | 147, 576, 577 |
|              |                               | Heightened anxiety               | Neuronal hypertrophy                                                                                    |                                                                                                  |       |
|              |                               | Decreased motor activity         | Loss of neuronal polarity                                                                                 |                                                                                                  |       |
| NS-Pten KO   | Repetitive behaviour           | Decreased mGluR                  | Hyperactivation of the PI3K/AKT/mTOR pathway                                                            |                                                                                                  | 578   |
|              |                               | Deficient social behaviour       | Decreased phosphorylated fragile X mental retardation protein                                           |                                                                                                  |       |
|              |                               |                                 | Decreased dendritic potassium channel Kv4.2                                                          |                                                                                                  |       |
|              |                               |                                 | Decreased PSD-95 and SAP102                                                                             |                                                                                                  |       |
Table 1. continued

| Target Mice | Behaviour phenotypes | Molecular, cellular and circuit phenotypes | Mechanism | Ref. |
|-------------|----------------------|-------------------------------------------|-----------|-----|
| Nestin-cre; Pten<sup>fl/fl</sup> | Impaired social interactions Increased seizure activity | Increased differentiation to the astrocytic lineage Stem/progenitor cells develop into hypertrophied neurons with abnormal polarity Deficient PPI | Altered AKT/mTOR/GSK3β signalling pathway | 579 |
| En2 En2<sup>−/−</sup> | Deficient social behaviour Deficient novel object recognition memory and spatial learning Increased depression-like behaviour | Neuronal migration abnormalities Reduced number of interneurons Abnormal neuronal network activity Reduced cortical neuronal synchrony | En2 deficiency influence Syn1 mRNA and protein levels | 580,581 |
| Cntnap2 Cntnap2<sup>−/−</sup> | Abnormal vocal communication Repetitive and restrictive behaviours Abnormal social interactions | Increased differentiation to the astrocytic lineage Stem/progenitor cells develop into hypertrophied neurons with abnormal polarity | Cntnap2 deficiency may induce overactivation of direct pathway which promotes motor behaviour | 421 |
| 15q11-13 patDp<sup>−/+</sup> | Deficient social interaction Behavioural inflexibility Abnormal ultrasonic vocalizations Correlates of anxiety | Increased [Ca<sup>2+</sup>] response to 5-HT<sub>2</sub>Cr signalling | Increased MBlI52 snoRNA within the duplicated region, affecting 5-HT<sub>2</sub>Cr | 582 |
| 15q13.3 Df (h15q13)<sup>−/−</sup> | Impairment in social interactions Restricted-repetitive behaviours Deficient communication | Enlarged brains and lateral ventricles Altered gamma-band EEG and ERPs | 15q13.3 microdeletion impair expression of Fan1, Mmnr10, Chrna7, Trpm1, Klf13, or Otud7a | 583,584 |
| 16p11.2 df/+ dp/+ | Stereotypic motor behaviour | Increased numbers of Drd2 MSNs in the striatum Downregulation of DA signalling | 16p11.2 deletion induce ENK dysregulation | 585,586 |
| 22q11 DF (16)1/+ | Deficient hippocampus-dependent spatial memory | Enhanced short- and long-term synaptic plasticity at hippocampal CA3–CA1 synapses Altered calcium kinetics in CA3 presynaptic terminals upregulated SERCA2 | Presynaptic SERCA2 upregulation | 587 |
| (COX)-2<sup>−/−</sup> | Decreased motor activity Increased anxiety-linked behaviours Decreased social behaviour | Altered expression of Wnt2, Gli1, Gm5 and Mmp9 Decreased glyoxalase 1 expression | Altered COX2/PGE2 pathway change neuronal cell behaviour and differential expression of genes and proteins related to ASD | 588 |
| mice treated with VPA | Decreased social interaction | Chronic activation of glial in the hippocampus and the cerebellum Increased expression of TNF-α and IL-6 in the cerebellum Increased microglia density in the hippocampus | VPA-treatment led to decreased expression of PTEN and increased levels of p-AKT protein | 297,589 |
| BTBR <sup>T</sup><sup>−/−</sup>lpr3<sup>−/−</sup>| Increased self-grooming Impaired social behaviour | Increased IgG and IgE in serum and IgG anti-brain antibodies Increased expression of cytokines in the brain Increased proportion of MHC-II-expressing microglia | Different autoimmune profile of BTBR mice is implicated in their aberrant behaviours | 298,590,591 |
| MIA | Decreased sociability Increased repetitive/stereotyped behaviour | Deficits in dendritic spine density, levels of synaptic proteins, synaptic transmission, LTP, and cortical malformations | Immune activation within the maternal compartment likely influences the developing fetal CNS through inflammatory mediators found in the blood and amniotic fluid of mothers | 37,286 |

Nlg1 neuroligin, Nrxn neurexin, PPI prepulse inhibition, E/I excitatory/inhibitory, NMDAR N-methyl-D-aspartate receptor, PSD postsynaptic density, HET heterozygous, LTP long-term potentiation, PAK p21-activated kinase, LIMK LIM-domain containing protein kinase, spSC spontaneous inhibitory postsynaptic currents, AMPA α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid, PC Purkinje cell, LTD long-term synaptic depression, REST RE-1 silencing transcription factor, mGluR5 metabotropic glutamate receptor 5, ERPs event-related potentials, MGNs medium spiny neurons, SERCA2 sarco (endo)plasmic reticulum calcium-ATPase type 2, COX cyclooxygenase-1, PGE2 prostaglandin E2, VPA valproic acid, MIA maternal immune activation.
Stem cell models have also demonstrated that abnormalities in specific molecular processes contribute to the pathogenesis of ASD (Table 2), including chromatin remodelling, Ca²⁺ and Wnt signalling. In recent years, accumulated evidence from modelling studies has identified many specific types of viable mutations, which may paint a bright picture for elucidation of the underlying pathogenesis of ASD.

Activity-dependent gene transcription and mRNA translation
Neuronal activity regulates gene transcription and mRNA translation in a dynamic manner. Many transcription factors and de novo mutations associated with ASD are thought to regulate or engage in cross-talk with canonical Wnt signalling, such as CDH8 and CTNNB1. Disorders in several upstream signalling pathways of translation, including mTOR, Ras and MAPK pathways, contribute to increased protein synthesis and therefore to altered synaptic plasticity (Fig. 3).

Activity-dependent gene transcription. Neuronal activity regulates programmes of gene expression in the nucleus, and disruption of activity-dependent transcriptional regulators or their targets is associated with ASD. Such disruption includes mutations in methyl-CpG-binding protein 2 (MeCP2), activity-dependent neuroprotective protein (ADNP), engraved 2 (ENG), voltage-dependent calcium channel subunit α1C (CACNA1C), T-box brain 1 (TBR1), myocyte enhancer factor 2C (MEF2C) and de novo deletions or duplications in 15q11-q13 (which cover the signalling. Notably, many key proteins in both signalling pathways are localized at synapses and play key roles in synaptic growth and maturation. Canonical Wnt signalling acts indirectly on β-catenin to enhance its stability, allowing it to translocate from the cell surface to the nucleus, thereby linking extracellular signalling to nuclear gene expression regulation through downstream transcriptional machinery (Fig. 3). On the one hand, ASD-associated MET tyrosine kinases (such as CDH8) release β-catenin to bind to surface calcium. On the other hand, free cytoplasmic β-catenin is phosphorylated by GSK3β to reflect the level of proteosomal degradation. Multiple Wnt molecules, including Wnt2, transmit signals at the surface membrane by interacting with frizzled receptors and LRPS/6 coreceptors.

It is noteworthy that the gene CTNNB1, which encodes β-catenin, has been identified among ASD risk variation. CDH8 is one of the best examples of an autism-related chromatin modifier that regulates the expression of other autism risk genes. As a negative regulator, CDH8 participates in the canonical Wnt signalling pathway by directly binding to β-catenin or being recruited to the promoter regions of β-catenin-responsive genes. This is consistent with the hypothesis that elevated canonical Wnt signalling contributes to the hyperproliferation of embryonic neural progenitor cells (NPCs) in the brain, which may partially explain the macrocephaly observed in individuals with autism. However, some studies have also found that CHD8 is a positive regulator of the Wnt/β-catenin signalling pathway in NPCs and negatively regulates this pathway in nonneuronal cell lines, suggesting that CHD8 may regulate Wnt signalling in a cell-specific manner.

In addition, PTEN participates in Wnt signalling by working with β-catenin to regulate normal brain growth. A dynamic trajectory of brain overgrowth and elevated β-catenin signalling has been reported in the developing cerebral cortex in Tuberous sclerosis is an autosomal dominant disorder arising from heterozygous mutations in the TSC1 and TSC2 genes that is commonly associated with deficits in long-term and working memory, intellectual disability, and ASD. TSC1 acts as a regulator of the stability of TSC2, preventing the degradation of TSC2, while TSC2 is a GTPase activating protein (GAP) that inactivates Rheb, a GTPase of the Ras family, and other small G proteins. Activated AKT can phosphorylate and inhibit TSC2, which regulates translation, transcription, and other cellular processes by removing the inhibition of mTORC1 by the TSC1/2 complex and promoting mTORC1 activity. In the absence of a functioning TSC1/2 complex, overactive mTORC1 leads to unregulated protein synthesis and subsequent cell growth.
Table 2. iPSC models of ASD

| Target | Cell type               | Molecular, cellular and circuit phenotypes                                                                 | Mechanism                                                                 | Targeting strategy                          | Ref.                  |
|--------|-------------------------|-------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|----------------------------------------------|-----------------------|
| NLGN4  | Neurons                 | Fails to enhance synapse formation                                                                         | ΔE4 mutation in NLGN4 compromises the ability of NLGN4 to induce synaptic differentiation | -                                            | 592                   |
| NRXN1α | Neurons                 | Increased sodium currents, higher AP amplitude and accelerated depolarization time<br>Altered neuronal excitability and non-synaptic function<br>Depressed calcium-signalling activity<br>Impaired maturation of excitatory neurons<br>NRXN1α deletions can lead to neuronal hyper-excitability<br>Deletion of NRXN1α lead to skewed differentiation of NES cells into immature and inhibitory neurons | NRXN1α deletions can lead to neuronal hyper-excitability<br>Deletion of NRXN1α lead to skewed differentiation of NES cells into immature and inhibitory neurons | -                                            | 593,594               |
| MECP2  | Neurons                 | Reduced synapses and spine density, smaller soma size<br>Altered calcium signalling and deficient electrophysiological | Altered excitatory synaptic strength may underlie global network changes in RTT<br>IGF1<br>Gentamicin | -                                            | 595,596,597            |
| NPCs   | Increased miR-199 and miR-214<br>Delayed GABA functional switch | miR-199 and miR-214 regulate extracellular signal-regulated kinase (ERK/MAPK) and protein kinase B (PKB/AKT) signalling<br>Delayed GABA functional switch due to deficit in neuron-specific KCC2 expression<br>Overexpression mi-199 and miR-214<br>Restoring KCC2 level | -                                            | -                                            |                       |
| Astrocytes | Shorter total neurite length<br>Decreased terminal ends | Loss of MeCP2 in astrocytes contributes to neuronal abnormalities<br>MECP2 deficiency in neurons induces cell-autonomous dysfunctions<br>IGF-1<br>GPE | -                                            | -                                            |                       |
| MECP2dup| Neurons                 | Increased synaptogenesis and dendritic complexity<br>Altered neuronal network synchronization | MECP2 overexpression promotes early postnatal dendritic and synaptic growth<br>NCH-51<br>histone deacetylase inhibitor | -                                            | 599                   |
| SHANK3 | Neurons                 | Altered morphologies of dendritic spines from pyramidal neurons<br>Impaired both early stage of neuronal development and mature neuronal function<br>Smaller cell bodies, more extensively branched neurites, reduced motility | Deficient excitatory synaptic transmission<br>Lack of SHANK3 during early neuronal development may impair the structural integrity of neurons and lead to synaptic defects in later mature neurons | -                                            | 600–602               |
| SHANK2 | Neurons                 | Increased dendrite length, dendrite complexity, synapse number, and frequency of sEPSC<br>Abnormal proliferation<br>Increased protein synthesis | SHANK2 haploinsufficiency disrupts the complex interaction between synaptic formation and dendritic formation<br>Rescued by gene correction of an ASD SHANK2 mutation<br>Repairing the genetic mutation in the FMR1 gene | -                                            | 603                   |
| FMR1   | Neurons                 | Decreased expression of PSD95<br>Decreased synaptic puncta density, neurite length<br>Higher amplitude and increased frequency of calcium transients<br>Abolished homoeostatic synaptic plasticity | FMR1 mutation induce functional differences in vGlut responses<br>FMRI inactivation impaired homoeostatic plasticity by blocking retinoic acid-mediated regulation of synaptic strength | -                                            | 604,605               |
| iPSCs  | Increased cell fate commitment and cell cycle<br>Cell-type-specific translational dysregulation<br>Abnormal proliferation | Hyperactive PI3K activity due to lack of FMRP may be associated with deficient protein synthesis and proliferation<br>Inhibition of PI3K signalling | -                                            | -                                            |                       |
| TSC2   | NPCs                    | Increased proliferative activity and Pax6 expression<br>Neurons differentiated showed abnormal morphology<br>Increased saturation density and higher proliferative activity of astrocytes<br>Slow differentiated into neurons | Enhanced mTOR pathway<br>Reduced PI3B/PIK/ST expression and IRS1 expression | -                                            | 607,608               |
| Neurons | Increased cell body size and process outgrowth | mTORC1 hyperactivation<br>Changes in RMP may be directly related to UBE3A loss and AP and synaptic changes may be secondary effects<br>Pharmacologically unsilencing paternal UBE3A expression | -                                            | -                                            |                       |
| UBE3A  | Neurons                 | Impaired maturation of RMP and AP firing<br>Decreased synaptic activity and synaptic plasticity | CHD8 affects GABAergic interneuron development, by modulating DLX gene expression | -                                            | 611                   |
| CHD8*−/−| Cortical organoids      | Increased expression of TCF4, DLX6-AS1 and DLX1 | -                                            | -                                            |                       |
| Target  | Cell type   | Molecular, cellular and circuit phenotypes                                                                 | Mechanism                                                                                                                                                                                                 | Targeting strategy                          | Ref. |
|---------|-------------|-----------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|------|
| SYNGAP1 | Neurons     | Enhanced dendritic morphogenesis Stronger excitatory synapses and expressed synaptic activity earlier in development | SYNGAP1 regulates the postmitotic maturation of human neurons made from hiPSCs, which influences how activity develops within nascent neural networks                                                        |                                              | 612  |
| CDKL5   | Neurons     | Abnormal dendritic spines                                                                                  | CDKL5 contributes to correct dendritic spine structure and synaptic activity CDKL5-dependent phosphorylation on S631 controls the association of NGL-1 with the postsynaptic molecular hub PSD95                                                                 |                                              | 613  |
| NTNG1   | Neurons     |                                                                                                           |                                                                                                                                                                                                          |                                              |      |
| RELN    | NPCs        | Decreased Reelin secretion Impaired Reelin–DAB1 signal transduction                                         | Overactivation of the mTORC1 pathway contributes to the downregulation of the Reelin–DAB1 cascade                                                                                                                                                       | Rapamycin                                    | 614  |
| CNTNAP2 | Cortical organoids | Increase in volume and total cell number | Homozygous c.3709DelG mutation in CNTNAP2 leads to abnormal brain development                                                                                             | Site-specific repair of c.3709DelG mutation using CRISPR-Cas | 615  |
| FOXL1   | Neurons     | Accelerated cell cycle Overproduction of GABAergic inhibitory neurons                                       | Changed fate of GABAergic neurons induced by FOXL1                                                                                                                                                  |                                              | 616  |
| TRPC6   | Neurons     | Shortening of neurites Reduced dendritic spine density                                                     | MeCP2 levels affect TRPC6 expression                                                                                                                                                               | TRPC6 complementation                       | 617  |
| CACNA1C | Neurons     | Deficient Ca\(^{2+}\) signalling Abnormal differentiation Abnormal expression of tyrosine hydroxylase Increased synthesis of norepinephrine and dopamine Activity-dependent dendrite retraction Abnormal migratory of interneurons | Ca(v)1.2 regulates the differentiation of cortical neurons in humans Ectopic activation of RhoA and inhibition by overexpressed channel-associated GTPase Gem | Roscovitine Pharmacologically manipulate LTCC | 108,618,619 |
| CNTN5   | Neurons     | Enhanced excitatory neuron synaptic activity                                                              | EHMT2 impacts the synaptic function of glutamatergic neurons through H3K9me1/2 catalyzing ability                                                                                                  |                                              | 620  |
| 15q11-q13 | Neurons       | Increased excitatory synaptic event frequency amplitude, density of dendritic protrusions, AP firing Decreased inhibitory synaptic transmission Impaired activity-dependent synaptic plasticity and homeostatic synaptic scaling | Altered expression of UBE3A and other several genes in this region                                                                                                                                 Restoring normal UBE3A expression levels |                                              | 621,622 |
| 15q13.3  | Neurons     | Increased endoplasmic reticulum stress Dyregulated neuronal gene expression Increased AP firing and elevated cholinergic activity Increased homomeric CHRNA7 channel activity | Common functional anomalies may be conferred by CHRNA7 duplication                                                                                                                                 Ryonadine receptor antagonist JTV-519 Wnt signalling agonist |                                              | 623  |
| 16p11.2  | Neurons     | Increased soma size and dendrite length in 16pdel neurons Decreased neuronal size and dendrite length in 16pdup neurons Decreased synaptic density | Changes of the 16p11.2 region may influence genes encoding proteins that interact with the PI3K/AKT or Ras/MAPK pathway                                                                                   |                                              | 624  |
| 22q11.2  | Cortical organoids | Deficient spontaneous neuronal activity and calcium signalling Downregulated expression of miR-1290 | Changed expression of DGC8                                                                                                                                                                           | Raclopride, Sulpiride, Olanzapine DGC8 overexpression Overexpression miR-1290 | 625,626 |
| 22q13.3  | Neurons     | Reduced SHANK3 expression Deficient excitatory synaptic transmission                                       | Loss of SHANK3                                                                                                                                                                                          Restoring SHANK3 expression IGF-1            |                                              | 28   |
It is worth mentioning that a major activator of TSC1/2 signalling is BDNF, a secreted protein that binds to the receptor tyrosine factor TrkB and is thereby involved in the PI3K/mTOR pathway. PTEN is an ASD risk gene located on chromosome 10q23 that encodes a lipid specific for phosphatidylinositol (3,4,5)-trisphosphate (PIP3), which is a negative regulator of PI3K/AKT/mTORC1 signalling upstream of TSC1/TSC2, resulting in symptoms of ASD. Mutations that inactivate PTEN lead to a constitutively active PI3K/AKT/mTOR signalling pathway and ultimately may result in abnormal protein synthesis. FMRP loss of function causes fragile X syndrome and autistic features, which is the most commonly known single-gene cause of ASD. FMRP is an RNA-binding protein whose target mRNAs encode transcription factors, and chromatin modifiers have been identified by high-throughput sequencing of RNA isolated with cross-linking immunoprecipitation (HITS-CLIP). The target genes of the mRNAs include several well-studied autism candidate genes, such as ARK, NLGN3, NRXN1, SHANK3, PTEN, TSC2 and NF1. Notably, the proteins encoded by FMRP target mRNAs regulate the balance of activity-dependent translation in synaptic plasticity. The proteins include mGlur5 and the NMDAR subunits, consistent with findings of altered mGlur5 and NMDAR-dependent synaptic plasticity in fragile X syndrome mouse models. Moreover, mGlur activation regulates FMRP-mediated translational repression, whereas FMRP regulates AMPAR trafficking and mGlur-mediated LTD. In summary, current evidence suggests that there is a complex level of dynamic regulation between translation and transcription that likely contributes to ASD pathophysiology. Interestingly, most mutations in translation pathways such as mTOR, ERK, and FMRP-eliF4E-CYFIP lead to abnormally high levels of synaptic translation and synaptic proteins. This is one of the few convergences seen in the heterogeneous context of autism and provides a good foundation for pharmacological target development. Moreover, determining the dynamics of spatio-temporal relationship between transcription and translation will help us to link the molecular dysfunction to the complex behavioural characteristics of ASD patients.

Synaptic function
A growing number of genes that have been associated with ASD seem to play roles in synaptic structure and function by directly encoding synaptic scaffold proteins, neurotransmitter receptors, cell adhesion molecules, and actin cytoskeletal dynamics-related proteins (Fig. 4). Therefore, abnormalities in synaptic proteins might be some of the mechanisms that increase the risk of developing ASD. Among the synaptic proteins, cell adhesion molecules (neuroligins (NLGNs) and neurexins (NRXNs)), postsynaptic scaffolding proteins (SH3 and multiple ankyrin repeat domains protein (SHANK)), glutamate receptors (NMDAR subunit, GluN2B), inhibitory GABA receptors subunits α3 and β3 (GABRA3 and GABRB3, respectively), and permeable ion channels (voltage-dependent calcium channel subunit α1C (CACNA1C) and sodium channel protein type 1 subunit-α (SCN1A)) are reported to be important signal transduction molecules associated with ASD. Signalling changes in these proteins can modulate the strength or number of synapses and ultimately alter the structure and functional connectivity of neuronal networks in the brain.
**Synaptic structure and homoeostasis.** Intact synaptic structure and homoeostasis are fundamental for the normal function of the brain. Neuropathological studies have provided evidence of increased dendritic spine density and aberrant dendritic spine morphology in individuals with ASD. Moreover, reduced developmental synaptic pruning in layer V pyramidal neurons in the postmortem ASD temporal lobe has been shown to hyperactive mTOR and defective autophagy. At excitatory synapses, the molecular diversity of surface receptors impacts proper synapse formation, maturation and transmission by organizing clustering of interaction partners at postsynaptic regions. For example, the intracellular carboxy-terminal portions of cell adhesion molecules (NLGNs) can bind to several scaffolding proteins of the postsynaptic density, such as postsynaptic density protein 95 (PSD95) and SHANKs. SHANK3 can interact with PSD95, AMPA receptor and glutamate receptor 1 (GluR1), which is critical for dendritic spine formation and synaptic transmission.

NRXNs and NLGNs are presynaptic and postsynaptic binding partners that cooperate to form transsynaptic complexes that directly mediate synapse formation and stabilization but are abnormally manifested during autism pathology. Whereas NLGN-1, NLGN-3 and NLGN-4 localize to the glutamate postsynaptic membrane, NLGN-2 localizes primarily to GABA synapses. NLGNs can participate in the formation of glutamatergic and GABAergic synapses in an activity-dependent manner. Specifically, inhibition of NMDARs or the downstream protein CaMKII suppresses the formation of glutamatergic synapses through the activity of NLGN1, whereas inhibition of NLGN2 activity suppresses the formation of GABAergic synapses.
Various combinations of these cell adhesion molecules have been linked to the differentiation of glutamatergic or GABAergic synapses in Nlgn-3 and Nlgn-4 mutant mice. In addition to alterations in NLGNs, mutations in NRXNs result in extensive changes in synaptic structure and plasticity. Moreover, NRXNs are critical for Ca2+–triggered neurotransmitter release but are not required for synapse formation, which has also been demonstrated in knockout mice.

SHANK genes, including SHANK1, SHANK2 and SHANK3, directly encode the proteins in the postsynaptic scaffolding protein family, which are located in the PSDs of excitatory synapses. SHANKs were first implicated in ASD by studies on Phelan–McDermid syndrome, a neurodevelopmental disorder caused by 22q13.3 deletion, and are deleted in almost all reported Phelan–McDermid syndrome cases. Consistent with studies in humans, different studies on Shank mutation sites in mice have also confirmed the strong genetic associations between Shank genes and ASD, especially Shank3. Individuals with ASD with Shank3 mutation exhibit defects in dendrite development and morphology and axonal growth cone motility. Shank3-knockout mice showed a decrease in the number of corticostriatal connections, whereas defects in NMDAR-dependent excitatory neurotransmission and synaptic plasticity have been observed in Shank2-knockout mice.

In addition, recent genome-wide association studies have linked polymorphisms and rare variations in ion channels and their subunits to ASD susceptibility. Haploinsufficiency of SCN1A encoding the voltage-gated sodium channel Na(V)1.1 causes Dravet’s syndrome, which has been proven to result in the display of autism-like behaviour. The Na(V)1.1 channel is the major Na+ channel expressed in the somata and axon initiation segments of excitatory and inhibitory neurons in the brain. In GABAergic interneurons, Na currents and action potential firing are harmed when Na(V)1.1 is deleted. Calcium channels act as sensors electrical activity sensors, converting membrane potential changes into protein conformational changes and transmitting information about neuronal activity to downstream effector systems.

There is clear evidence to illuminate that defective Ca2+ channel function can lead to ASD with penetrance as high as 60-80%. Mutations relevant to ASD typically sensitize voltage-dependent Ca2+ channel gating, shifting their activation to more hyperpolarized potentials of ~10 mV. CACNA1C and CACNA1D encode the Ca(V)1.2 and Ca(V)1.3 proteins, respectively, which localize to the postsynaptic membrane and signal to the nucleus. In excitatory neurons, CaMKII functions as a shuttle molecule to collect Ca2+/Calmodulin from the cytoplasm and transport it to the nucleus, where Ca2+/Calmodulin release activates CaMKK and its substrate CaMKIV to further phosphorylate CREB, thereby participating in the regulation of transcription and translation.

Synaptic signalling pathways

Neuronal activity-dependent synaptic mRNA translation pathways can directly influence the levels of synaptic proteins, thereby controlling synaptic strength and number. The extracellular mTOR and FMRP-elf4E-CYFIP1 signalling pathways are the two primary regulators of mRNA translation. Interestingly, the majority of ASD-related gene mutations (such as MEF2C, FMRI, PTEN, TSC1, TSC2 mutations) result in enhanced gene transcription and mRNA translation, ultimately leading to an aberrant increase in the strength or number of synapses within certain neural networks. In fact, glutamate and BDNF can also induce a cascade of mTOR and FMRP pathways, resulting in an increase in mRNA expression.

**Fig. 4 Molecular pathways implicated in synaptic function for ASD.** At the excitatory synapse, encoded proteins including synaptic scaffold proteins (for example, SHANKs), neurotransmitter receptors (for example, NMDARs, AMPARs and mGluRs) and cell adhesion molecules (NRXNs and NLGNs) associated with autism risk genes. Activation of cell surface receptors is closely linked to activation of the Ras/ERK and PI3K/AKT/mTOR pathways. In addition, mutations in ion channels, such as L-VSCCs and sodium channel protein type 1 subunit-α (SCN1A), both of which have been illustrated result in synaptic dysfunction and autism-like behaviour.

**Signalling pathways in autism spectrum disorder: mechanisms and...**

Jiang et al.
transcriptional activation or inactivation, and chromatin packaging. Post-transcription by blocking protein synthesis or inducing mRNA degradation.

16p11.2-knockout models,26 have shown dysregulation of mGluRs and increased ARC expression and subsequently decreases the number of AMPARs, ultimately impairing synaptic plasticity at excitatory synapses.

Epigenetic factors
Increasing evidence indicates that ASD is the result of a complicated interaction between genes and the environment.234 Epigenetic factors are ideally positioned at the genome-environment interface, allowing for steady gene expression regulation without alterations to the underlying DNA sequence.29,33,39 Epigenetic mechanisms, including DNA methylation, histone modification, chromatin remodelling, and non-coding RNA activity, are involved in the regulation of social behaviour in autism.93,237 Together, these mechanisms form an epigenetic network that integrates transient social experiences into the genome to regulate social-emotional dispositions in mammals (Fig. 5).

DNA methylation. Many epigenetic researches have focused on DNA methylation with consideration of the contact between genes and environmental factors.240–242 Early studies on ASD-associated DNA methylation focused on several candidate genes, such as Mecp2, glutamate decarboxylase 65 (GAD65), reelin (RELN), oxytocin receptor (OXTR), Shank3 and UBE3A.

Mecp2 is a chromatin architectural regulator and a reader of epigenetic information contained in methylation (or hydroxymethylation) DNA that has been well studied.243 Decreased MeCP2 expression in the PFC in ASD patients is associated with aberrant hypermethylation of its promoter.244,245 MeCP2 binds to methylated CpG sites in gene promoters and associates with chromatin silencing complexes, thereby suppressing gene expression.246–248 GAD1 and RELN are downregulated in postmortem ASD and are selectively expressed in GABAergic neurons.249 Enhanced binding of MeCP2 to GAD1 and GAD2 promoters, which leads to reduced expression of RELN and mRNA, has been found in the cerebellum and frontal cortex in ASD patients.249,250 While the methylation
rate of CpG islands is elevated during mouse brain development, SHANK3 is upregulated two weeks postnatal, suggesting that methylation of CpG islands is a strong regulator of SHANK3 expression.\textsuperscript{251} The neuropeptide oxytocin (peptide: OT, gene: OXT) sends signals via its receptor OXTR, which is a highly conserved G protein-coupled receptor. Both genetic and epigenetic changes in OXTR have been identified to be related to ASD.\textsuperscript{252–255} OXTR mRNA expression is affected by methylation of promoter, and high levels of methylation have been associated with ASD.\textsuperscript{252,256} Consistent with this, a study on siblings and adults with ASD found increased OXTR promoter methylation.\textsuperscript{257,258}

Taken together, the findings indicate that DNA methylation status may serve as a potential biomarker for risk prediction, diagnosis, and targeting, as well as provide information for the study of ASD pathological mechanisms. Highly specific DNA methylation has been identified that may help predict transcriptional regulation in autism.\textsuperscript{93}

### Histone modification and chromatin remodelling

Recent studies have revealed a characteristic histone acetylation signature in the brains of ASD patients, providing strong evidence that histone modifications, especially acetylation, lead to ASD-like behaviours.\textsuperscript{259} A cross-generational study has confirmed that children exposed to prenatal anticonvulsants and the mood stabilizer valproate, a well-known histone deacetylase (HDAC) inhibitor, are at increased risk of being diagnosed with autism, providing insights into the involvement of histone modifications in ASD.\textsuperscript{256–258} Furthermore, treatment with a histone deacetylase inhibitor in Shank3-knockout mice significantly improves the behavioural phenotype of the mice, suggesting that abnormal histone modification is a potential mechanism of ASD.\textsuperscript{262} Trimethylation of the fourth lysine residue of histone H3 (H3K4me3) is essential for chromatin formation and gene activation, regulating hippocampal plasticity by recruiting chromatin remodellers to gene transcription initiation sites.\textsuperscript{263,264} H3K4me3-ChIP deep sequencing of the prefrontal cortex in postmortem tissue from patients aged 6 months to 70 years has revealed that alterations of H3K4me3 levels in neurons are associated with autism.\textsuperscript{265} Mutations in the lysine-specific demethylase 5 C (KDM5C) gene damage its function of transcriptional regulation, resulting in reduced H3K4me3 methyl group removal and suppressed gene expression in ASD patients.\textsuperscript{266–268} Chromatin remodelling is mediated via ATP-dependent enzymes or chromatin remodelling complexes.\textsuperscript{269} The chromatin structure or proteins that bind to DNA are altered when nucleosomes positioned differently, causing gene expression to shift. Chromatin remodelling genes (including CHD8, ARID1B, BCL11A and ADNP) have been identified to be linked to autism.\textsuperscript{106} De novo mutations in the autism-related chromatin modifi er CHD8 are well studied\textsuperscript{88,270} with multiple de novo, truncating, or missense mutations observed in ASD patients\textsuperscript{81,82,88,130} CHD8 is located at active transcription sites with the histone modification H3K4me3 or H3K27ac and recruits histone H1 to target genes by remodelling the chromatin structure.\textsuperscript{141,270} ARID1B is a component of SWI/SNF (or BAF), an ATP-dependent human chromatin remodelling complex that is frequently mutated in ASD.\textsuperscript{269,271} Proteins encoded by BCL11A and ADNP can also interact directly with members of the SWI/SNF complex, which is related to alternative splicing of tau and prediction of tauopathy.\textsuperscript{106,272}

### Non-coding RNAs

The majority of genome-wide association studies have concentrated on protein-coding regions, disregarding non-coding RNA. Because non-coding RNAs primarily target transcripts and rarely interact directly with DNA, they are considered nonclassical epigenetic pathways.\textsuperscript{13,273} Posttranscriptional regulation by non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), is associated with ASD. miRNAs are short non-coding RNA molecules that regulate the expression of most genes by blocking protein synthesis or increasing mRNA degradation at the posttranscriptional level. A preliminary assessment suggested that autism does not induce global dysfunction of miRNA expression, as only 28 of 466 miRNAs were significantly altered in postmortem cerebellar cortex tissue of ASD patients.\textsuperscript{274} Interestingly, the predicted targets of the differentially expressed miRNAs were enriched with genes related to neurobiology, the cell cycle, and cell signalling and largely overlapped with genes previously identified via differential miRNA expression analysis of ASD patients.\textsuperscript{30,275} Considering that miRNAs can be delivered into cells without being integrated into the host genome, miRNA-based therapy is a prospect strategy for the treatment of ASD.\textsuperscript{237} Highly expressed miRNAs in ASD patients can be downregulated by miRNA antagonist treatment (i.e., miRNA-inhibitory therapy), while miRNA mimic replacement therapy can compensate for weakly expressed miRNAs.\textsuperscript{276} Compared with miRNAs, lncRNAs exhibited higher tissue-specific expression, and a considerable number of lncRNAs were confined to the brain. The evolution of lncRNA-specific and synaptic function-enriched gene expression in primates suggests that this category of RNAs may have a broad range of roles in the brain and may help to elucidate the aetiology of ASD.\textsuperscript{31,276,279}

In animal studies, mice with heterozygous knockout of miR-137 show repetitive behaviours and social behavioural deficits.\textsuperscript{280} Another example of the use of miRNA profile screens in a genetic model of ASD comes from a study on MeCP2-knockout mice. Expression profiling of miRNAs in the cerebellum of MeCP2-knockout mice revealed the downregulation of a subset of miRNAs.\textsuperscript{281} Moreover, some of these miRNAs targeted BDNF, which is consistent with the finding that miR-132 targets MeCP2 and BDNF in vitro and is downregulated in the cortices of MeCP2-knockout mice.\textsuperscript{281,282} Therefore, the regulatory loop including BDNF, miR-132 and MeCP2 may be involved in ASD.\textsuperscript{237,282} The deletions in regions of differentially expressed IncRNAs are similar to those reported for miRNAs and mRNAs.\textsuperscript{30} BC1 is an IncRNA whose deletion in the mouse cortex can cause social dysfunction. The underlying mechanism is that BC1 tends to increase the affinity of FMRP and CYFIP1, both of which are ASD risk genes.\textsuperscript{168,283,284}

In general, many differentially expressed and functionally significant non-coding RNA genes and overall epigenetic disorders have been identified in ASD patients and animal models. Preliminary evidence for a relationship between epigenetic regulation and social behaviour has been obtained at the animal level. Nevertheless, the epigenetic network is intricate, and the recently discovered genes with differential expression may be just the tip of the iceberg in the context of ASD. The important topic is how social stress induces temporary changes in the epigenetic network and whether gene expression might contribute to long-term social–behavioural adaptations. Future studies need to further identify more brain-specific epigenetic regulatory genes and clarify their practical functional significance.

### Immunology and neuroinflammation

Immunology dysfunction is another factor attributed to gene–environment interactions in the context of ASD. Persistent immune dysregulation has been identified in ASD patients and animal models.\textsuperscript{277,285,286} An earlier study identified 150 differentially expressed genes in ASD patients compared to controls, 85% of which were upregulated and involved in immune response pathways.\textsuperscript{275} Infl ammatory molecular signalling pathways in both the central nervous system and the periphery can affect brain connections and synaptic function by affecting components including microglia, complement factors, cytokines and their receptors, MET receptors, and major histocompatibility complex class I molecules (MHC-I) (Fig. 6).\textsuperscript{36}

Alterations of immune mediators in the central and periphery. In the brains of ASD patients, the numbers and activation of reactive
microglia and astrocytes are increased in multiple brain regions. A cascade of cytokines and chemokines can be released by reactive microglia and astrocytes, which can signal across cells. Dysregulation of cytokines in ASD has also been associated with symptom severity and presentation on diagnostic tests for ASD. Therefore, abnormal cytokine profiles may be sensitive biomarkers indicative of immune system disturbances and abnormal neuroinflammation in autism. Some studies have found increases in GM-CSF, IL-6, IL-8, TNF-α, TGF-β, CCL2 and IFN-γ levels in the brains of individuals with ASD, which supports this theory. Paralleling findings in humans, findings from several established animal models of ASD, including offspring with maternal immune activation (MIA) (IL2, IL6 and IL17) and offspring of VPA-treated rodents (TNF-α and IL-6) have shown alterations in the secretion of cytokines and chemokines. Due to the secretion of signalling molecules and cytokines, the cross-talk between microglia and astrocytes is enhanced, which can lead to vascular-endothelial dysfunction and damage to blood–brain barrier (BBB) permeability. Some cytokines, such as IL-1α, IL-1β, IL-6 and TNF-α, can migrate from the periphery into the brain via the BBB transport systems.

Moreover, multiple studies have indicated different expressions of cytokine and chemokine in the periphery in autism patients. The results of cerebrospinal fluid and blood tests of ASD samples are similar, and cytokine changes in the blood can potentially provide information on inflammation and alterations in synapse connectivity in the brain. The levels of proinflammatory cytokines (such as IL-1β, IL-6, IL-8, IL-12p40, IFN-γ, TNF-α and GM-CSF) are increased, while those of anti-inflammatory cytokines (such as IL-10 and TGF-β) are decreased, in the blood of ASD patients. However, some alterations in cytokines are different between the central and peripheral regions, including IL-1β and TGF-β. In the CNS, IL-1β levels appear to be unchanged, but they have increased in the periphery. TGF-β1 levels have been reported to be rising in one study, while the vast majority of data point to a
diarrhoea, and also experience gastrointestinal symptoms, including constipation, flatulence. This affects brain function through neural, hormonal, and immune mechanisms.296,310 Cytokines such as IL-2, IL-6 and IL-10 levels, which may explain the results of recent animal studies at the macroscopic scale. 36 MET is an immune gene encoding hepatocyte growth factor (HGF), mutations in which induce disruption of multiple downstream targets in signalling cascades, resulting in critical functional deficits in brain development.346,347 Decreases in MET expression have been observed in ASD postmortem tissues.348,349 MET can indirectly lead to changes in neural circuits and functions by negatively regulating immune responses and gastrointestinal homeostasis, which is a putative hallmark of ASD pathophysiology.350,351 In addition to mediating the adaptive and innate immune responses, MHCI molecules contribute to controlling axonal and synaptic growth and participate in the regulation of synaptic plasticity and synaptic homeostasis in the presynaptic and postsynaptic regions associated with glial cells.352–356 Cortical neurons from offspring of MIA exhibit increased expression of MHCI molecules and its downstream effect factors MIF2. Remarkably, normalizing the MHCI-MEF2 signalling pathway in cultured MIA neurons prevents the MIA-induced decrease in synapse density.353 Notably, despite recent advances, most of the details of when, where and how immune molecules function in the brain remain unknown.

In summary, dysregulation of immunoregulatory signalling molecules, including cytokines, microglial complement, MET, and MHCI, is an important link in the pathological process of ASD that possibly regulates synaptic morphology and plasticity in the CNS through common downstream pathways. Among them, mTOR serves as a focal point for integrating immunological signalling in the brain, cytokine signalling, perinatal environmental exposures, and chronic immune disorders. Determining whether and how immune contributions concentrate on the common mTOR pathway in future studies will be critical for our understanding of the importance of mTOR in different aspects, not just from an immune perspective, as well as for future targeted drug development.

**BRAIN FUNCTIONAL CONNECTIVITY AND THE NEUROTRANSMITTER SYSTEM**

Early brain development in people with ASD is accelerated, which leads to changes in brain connectivity, including physical and functional connectivity between different regions and concomitant neurotransmitter changes. Different types of genetic variants may disrupt the circuits of social interactions and repetitive behaviours, resulting in a complex matrix of genes, synapses, circuits, and behaviours. Here, we summarize and review these topics on three levels. We first describe abnormal functional connectivity in the brains of ASD patients at a macroscopic scale. We then summarize the results of recent animal studies at the level of neural circuits, providing insights into the mechanisms of multiple types of specific neuronal and molecular regulation of circuit networks (Fig. 7). Finally, we summarize the relevant signal transduction pathways that regulate neurotransmitters in ASD patients.
Brain regions and neural circuits

According to human neuroimaging and neuropathological investigations, global brain developmental anomalies in children with ASD emerge in the cerebral cortex, striatum, cerebellum, brainstem, and other subcortical structures.357–363 Recent studies have identified that the medial prefrontal cortex (mPFC) integrates social and spatial information through neuronal coding. The mPFC is one of the best-studied brain regions related to social behaviour.364,365 In both mice and humans, several pieces of evidence imply that striatal dysfunction is a neurological substrate for repetitive behaviours.366–368 For example, Nlgn1-knockout mice exhibit ASD-like repetitive behaviours and corticostriatal synaptic abnormalities,369 whereas mice lacking Nlgn3 exhibit similar behavioural changes caused by neuronal inhibitory transmission from D1-MSN in the nucleus accumbens (NAc).370 Mice lacking Shank3 exhibit striatal hypertrophy and decreased corticostriatal excitatory synaptic transmission, as well as repetitive behaviours.202 In early assessments of autism, the amygdala exhibits reduced volume and increased neuronal density in the medial, central and lateral nuclei, which play critical roles in modulating fear conditioning, anxiety and social behaviour.357,367,371–373 Consistently, amygdalar axonal projections and neuronal activation are defective in Tbr1(+/-) mice, but these defects are ameliorated by infusion of an NMDA receptor agonist (D-cycloserine).10 The cerebellum is best known for its role in controlling motor behaviours, and most individuals with ASD have comorbidities associated with movement disorders such as ADHD. Histopathological changes in cerebellar neuronal structure, such as loss of Purkinje cells (PCs), have been discovered in the postmortem brains of many ASD patients.357,374,375 Validation data on key signalling molecules suggest that cerebellar PC-specific knockout of Tsc1, Tsc2 and Bmal1 is sufficient to induce core ASD-like behaviour.376–378 Notably, a growing number of studies have found that the cerebellum is involved in the pathophysiology of autism in the form of nonmotor regulation.

Rodents and humans share similar brain regions and neural circuits, facilitating our investigation of social behaviour and related signalling mechanisms.382 Currently, rodents and nonhuman primates, such as chimpanzees, are accepted models for identifying social behavioural changes in autism. Numerous studies have shown that mice exhibit unique social behaviours, such as territorial aggression and mating, interpret olfactory traits as social information, and transmit and interpret emotional contagion and empathic responses.383–385 Novel approaches in optogenetics, chemical genetics, electrophysiology and behavioural neuroscience have helped to construct the links between various social behaviours and brain circuit activity (Fig. 7).386–389 In the huge and complex neural network involving social behaviour, the PFC and its massive reciprocal loop connections constitute a

Fig. 7 Social behaviour-related neural circuits, neurotransmitter system and E/I balance in the rodent brain associated with ASD. a A sagittal view of the rodent brain used to illustrate the local and distal circuits implicated in social behaviours. Recent studies use behavioural neuroscience, optogenetics, chemical genetics and electrophysiology have illuminated the relationships between various social behaviour and the activity of specific neural circuits. Alterations in brain connectivity usually accompany changes of neurotransmitter, including glutamate, GABA, oxytocin, serotonin and dopamine. b In addition, the hypothesis of disruption of cortical “E/I imbalance” in autism is widely accepted, which has also been highlighted in the figure. AMY amygdala, AOB olfactory bulb, BNST bed nucleus of the stria terminalis, DRN dorsal raphe nucleus, LS lateral septum, MOB main olfactory bulb, MOE main olfactory epithelium, NAc nucleus accumbens, PFC prefrontal cortex, PVN paraventricular nucleus, RCrus right Crus I, VNO vomeronasal organ, VTA ventral tegmental area.
top-down social behaviour regulation system. Various subcortical networks communicate with the mPFC, including the amygdala (responsible for social incentive), and the hypothalamus (responsible for stress regulation). Recently, the right crus I (R Crus I) of the cerebellum was identified as a key brain region for social interaction in mice that can project to the cortex to modulate social interaction and repetitive behaviours in mice. In addition, oxytocinergic, serotonergic and dopaminergic-related circuits also play critical roles in social regulation, which will be discussed below.

Neurotransmitter system

From a neurobiochemical perspective, the activity of brain structures and neural circuits is coordinated by multiple neurotransmitters and neuromodulators. Therefore, dynamic changes in neurotransmitter concentration, release, and receptor density may directly affect neural circuit function and thus behavioural performance. Increasing evidence shows that disturbances in neurotransmitter systems, including the glutamate, GABA, serotonin (5-HT), melatonin, dopamine (DA), and arginine vasopressin (AVP) systems, are associated with autism (Fig. 7).

Classic neurotransmitters. Glutamate and GABA: An appropriate balance between excitation and inhibition (E/I) in synaptic transmission and neural circuits is essential for the proper brain functioning. In 2011, Yizhar et al. used optogenetics to study excitatory projection neurons and inhibitory PV neurons of the mPFC and subsequently found that an increase in the cellular E/I ratio leads to severe impairments in information processing and behaviour. Currently, the hypothesis of cortical “E/I imbalance” in autism is widely accepted (Fig. 7).

E/I balance is controlled by the ratio of excitatory to inhibitory cells, as well as their activity. Plasma levels of GABA and glutamate are changed in autistic children, who exhibit significantly increased GABA levels and decreased glutamate/GABA ratios. Previous findings have highlighted the importance of glutamate dysfunction in contributing to the aetiology of autism. In addition to the above-mentioned changes in glutamatergic neurons in ASD, the functional role of GABAergic inhibitory neurons is becoming increasingly clear. Neurrophysiological studies have provided evidence of reduced GABAR levels in the cortex and hippocampus, aberrant GAD1 and GAD2 mRNA expression in the postmortem cortex and cerebellum, and the interneuron markers parvalbumin (PV) and somatostatin (SST) are down-regulated. Loss of inhibitory neurons and impairment of inhibitory neurotransmission are also observed in ASD mouse models as a result of mutations in genes such as Pten, Mecpr2, Cnnap2, Shank3 and BTBR mice, which may directly lead to alterations in the balance of excitation and inhibition. It is worth noting, however, that investigations on E/I imbalance have primarily been carried out using animal models, therefore a detailed assessment of the pathophysiology of E/I imbalance contributing to human ASD is warranted.

Biogenic amines. 5-HT and DA: 5-HT has long been suggested to be related to social behaviour. Early research suggested increased 5-HT levels in the blood of children with autism. According to data from neuroimaging and neurobiochemical analyses, up to 45% of individuals with autism have hyperserotonemia. Abnormal 5-HT neurotransmission and social behavioural deficits have been reported in SERT and MAOA mutant animal models. Serotonergic neurons are mainly located in the dorsal raphe nuclei (DRNs), which can project to the PVN of the hypothalamus and modulate OT release. Moreover, other brain areas, such as the NAc, can also receive projections from the DRNs and display OXTR. A study in mice has elucidated that the coordinated activity of OT and 5-HT inside the NAc is essential for social reward. The majority of DA-producing neurons are located in two primary regions, the substantia nigra (SN) and VTA, in the brain. VTA dopaminergic neurons project to various brain structures, such as the NAc, involved in the control of social cognition. Although DA release has long been linked to reward, there is growing evidence that DA is released in response to aversive behaviour. The NAc has been well studied for its role in reward processing behaviour, which is predominantly composed of inhibitory MSNs that differ in the type of DA receptor they express, D1R or D2R. Notably, the two subtypes of neurons may play different roles in social and repetitive behaviours.

Neuropeptides. OT and AVP: The neuropeptide hormones OT and AVP belong to the same superfamily, and genetic variants in OTX, OXTR, arginine vasopressin receptor 1a (AVPR1a) and CD38 (lately demonstrated as essential for social behaviour because it mediates oxytocin secretion) have been verified to be associated with autism. Compared to neurotransmitters (approximately 5 ms), neuropeptides (approximately 20 min) display a substantially longer half-life and are stored in dense core vesicles, which are much larger in size and scope than synaptic vesicles. Hence, OT and AVP have much broader neuromodulatory roles and less spatial/temporal specificity than classical neurotransmitters. The changes in OT and AVP levels in autistic patients’ plasma are often associated with abnormal functional connectivity. For example, OT administration increases the connectivity of brain regions critical for processing socioemotional information, such as the NAc, amygdala and PFC. Studies in animals have implicated OT and AVP in mammalian sexual, territorial, attachment and social behaviours. Moreover, OT also plays a recognized role in anxiety, which is common in a comorbid symptom of ASD.

OT is mainly produced by neurons located in the paraven tricular nucleus (PVN) and supraventricular nucleus (SON) of the hypothalamic–neurohypophyseal system. Social cues induce OT release from the PVN; the OT acts on downstream structures such as the LS, amygdala, VTA and NAc, eliciting OT release from oxytocinergic neuron axon terminals in the VTA drives the excitability of dopaminergic neurons in the NAc, and eventual activation of the PVN–VTA circuit enhances social behaviour. For nearly two decades, an increasing number of studies on the modulation of circuits and neurotransmitter systems have gained insight into different brain areas and circuits involved in particular behavioural states. Nevertheless, it is unclear to what extent the mouse phenotypes recapitulate the relationships among neural circuits in autism. It should be noted that the human brain with its multimodal structure has undergone dramatic changes in brain regions such as the frontal and temporal lobes during evolution. Therefore, more comparative studies between primate and mouse models are required to precisely correlate neuroanatomical features with candidate brain circuits involved in ASD pathogenesis. Moreover importantly, identification of molecular mechanisms that are specific to social behaviours and circuits is needed. Such information will be essential for developing targeted treatments aimed at ASD.

THERAPEUTIC STRATEGIES

The current treatment strategies for autism are divided into nonpharmacological treatment and pharmacological treatment.
approaches. Combining pharmacotherapy with behavioural psychosocial learning interventions may have significant impacts on long-term outcomes for people with autism. However, based on the complex mechanism of the superposition of multiple aetiologies of autism, there is still a lack of clinical cures for core symptoms. In any case, the lack of molecular targets is the rate-limiting barrier for new drug research for autism. Innovative drug development for autism is currently the most challenging work in the field. The development of strategies to intervene in or block the transcription of key signalling molecules involved in the pathogenesis of autism is a primary research direction. In this section, we mainly review and discuss pharmacotherapies based on pathological features and signal transduction mechanisms (Fig. 8).

Nonpharmacological therapies
Nonpharmacological treatment mainly refers to educational interventions and behaviour modification but also includes adjunctive treatments such as music and art therapy. The main purpose of nonpharmacological treatment is to develop children’s self-care and social skills, thus improving their quality of life. With advances in neuroscience, brain stimulation has also gradually attracted clinicians’ attention and has shown potential to improve the symptoms of ASD patients.450,451

**Behavioural and psychological intervention.** Physical intervention is usually considered a priority because many young autistic children have difficulty communicating and interacting with others. Music therapy, cognitive behavioural therapy (CBT) and social behavioural therapy (SBT) have all showed promise in helping autistic patients improve their social interaction and verbal communication.452 One potential pathway by which music therapy affects ASD is by changing the structural and functional connectivity of the cortex to achieve a greater degree of multisensory integration across cortical and subcortical regions during early development.453 CBT is a commonly used psychotherapeutic intervention and can both target core symptoms and treat comorbid anxiety and depression symptoms of ASD.454,455 SBT targets emotional regulation, social skills and functional communication, with an emphasis on independence and quality of life. Considering that the behavioural symptoms of ASD appear at a fairly early stage of development, intervening before symptoms appear may lead to better outcomes. Although treatments vary widely around the world, they generally follow a typical developmental psychology sequence that emphasizes play, social interaction, and communication with children. It is worth noting that clinical services should not be solely diagnosis oriented but should provide step-by-step specific interventions.175

**Brain stimulation.** Non-invasive brain stimulation is a relatively recent treatment option that has shown hope in the treatment of ASD. The molecular mechanisms underlying brain stimulation-dependent neuronal excitability and synaptic plasticity have been well elucidated with extensive preclinical animal models.466,467 Neuroimaging studies have demonstrated structural and functional imaging abnormalities in several brain regions of ASD patients. There have been more than a dozen trials of brain stimulation techniques, including transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS), in the ASD population. tDCS is primarily conducted in the brain via a constant current through scalp electrodes. In contrast, in TMS, intracranial currents are induced in the cortex by fluctuating extracranial magnetic fields. Both techniques modulate regional cortical excitability and are well tolerated in children and adults.459,460 Neural stimulation has been reported to modify cortical excitability by affecting GABAergic function and causing LTP or LTD-like excitatory synaptic strength.461–463 tDCS has been shown to improve autism symptoms and language in several small clinical trials.467,468 Recent studies examining executive function in the dorsolateral prefrontal cortex (DLPFC) after TMS and improvements in social behaviour and social cognition in the posterior superior temporal sulcus and DLPFC in autistic patients after tDCS have shown preliminary therapeutic effects.469–472

Together, nonpharmacological therapies can partially alleviate autism symptoms. Although sufficient evidence is still lacking, the therapeutic effects of behavioural and psychological interventions and brain stimulation on autistic patients must have a theoretical basis related to neurobiochemistry and signal transduction.
Drug targets and pharmacological therapies
Because the pathogenetic and pathological mechanisms are still unclear, there is no effective treatment drug for the eradication of autism that has been officially approved. Several drugs targeting autism are under study (Table 3) and clinical trials (Table 4). At present, clinical drug treatment of autism generally involves appropriate amounts of atypical antipsychotics, antidepressants, and sleep disorder-improving drugs according to the core symptoms of children.

Atypical antipsychotics, including risperidone (a dopamine antagonist) and aripiprazole (a dopamine agonist), are FDA-approved drugs that have been shown to relieve irritability symptoms such as aggression and self-mutilation in adolescent autistic patients in several large clinical trials. α-Adrenergic drugs such as guanfacine are used for ADHD and disruptive behaviour. Antidepressants such as SSRIs improve the symptoms of emotional instability, anxiety, and stereotyped repetitive behaviours in patients with ASD by blocking the reuptake of 5-HT and increasing the concentration of 5-HT in the synaptic cleft. Clinical drugs that can treat ASD by improving sleep include melatonin, ramelteon, niperazine, and clonidine. It is worth mentioning that many investigations have reported aberrant melatonin secretion in autistic patients, particularly decreased melatonin and metabolite secretion at night, and altered circadian rhythms of melatonin.

Several clinical trials have shown that melatonin reduces sleep latency and improves sleep duration and nighttime arousal, suggesting that it is an effective treatment for sleep disturbances in children with ASD. In addition, a meta-analysis and some placebo-controlled studies have suggested that melatonin supplementation may also have positive effects on autistic behavioural disorders. One study on VPA-treated rats has proven that melatonin treatment significantly improves social behavioural deficits through CaMKII/PKA/PKC signalling. Therefore, melatonin or novel analogues may be promising drug therapies for improving disorders of autism in the future, it will be necessary to study the regulatory mechanism of melatonin-related signal transduction and to verify the dose–response relationship in the improvement of behavioural disorders in clinical trials to test the therapeutic benefits of melatonin.

In addition, the development of other ASD-targeted drugs has been promoted due to in-depth basic scientific research on the pathogenesis of ASD in the past decade. Clinical trials targeting E/I balance, transcriptional and epigenetic regulation, immune regulation, biological peptides and intestinal flora are advancing in an orderly manner (Table 3).

Targeting E/I balance. The cortical E/I imbalance hypothesis in ASD patients highlights the potential of glutamate and GABAergic receptor modulators as therapeutic agents. Different pharmacological methods have been applied to restore E/I imbalance, such as mGluR5 antagonist treatment, NMDAR agonist treatment and GABAR agonist treatment. Extensive preclinical data demonstrate that overactivity of mGluR5 is central to the pathogenesis of fragile X syndrome. In addition to targeting fragile X syndrome, mGluR5 inhibition has been shown to salvage many phenotypes, including learning and memory deficits, social deficits, repetitive behaviours, hyperactivity, and dendritic spine dysmorphogenesis, in 16p11.2 deletion mice, BTBR mice and Shank3-knockout mice. Unfortunately, mGluR5 inhibitors developed by two companies have exhibited negative effects in large-scale patient trials targeting fragile X syndrome. Further reasons should be sought for the discrepancies in preclinical and clinical outcomes. In addition to expanding and refining the preclinical analyses of new drugs, it will also be necessary to scientifically stratify patients enrolled in clinical trials in order to increase the expected efficacy in patients.

NMDA receptors and mGluRs show positive reciprocal regulation. NMDA receptor agonist (d-cycloserine) intervention attenuates impaired sociability in Shank2-transgenic mice, highlighting the need for accurate signalling at excitatory synapses. The spatial and temporal selectivity offered by subtype-selective positive allosteric modulators of the NR2 receptor make these agents promising candidates for the treatment of ASD. Drugs targeting the NMDA receptor, such as memantine, have been demonstrated to alleviate core symptoms of ASD in early open-label trials. Although subsequent RCTs have shown no differences in primary and secondary indicators, memantine improves symptoms of ASD such as stereotyped behaviours, and social communication/interaction impairment as an adjuvant therapy.

The results from the memantine trial have been mixed, suggesting that further research is needed, and a large randomized controlled trial is currently being conducted on the therapy of social impairment in adolescents. Several trials on other NMDA-modulating drugs, including ketamine, d-cycloserine, and d-cycloserine, have been negative for the primary endpoint, indicating that further studies with increased sample sizes are required.

Evidence from fragile X syndrome mice has indicated that alterations in GABA-mediated synaptic transmission are present in the mice, suggesting that there is potential therapeutic benefit of GABA receptor agonism. Arbaclofen, a GABA-B agonist, regulates glutamatergic activity through presynaptic action to reduce glutamate release. Fmr1-knockout mice, arbaclofen reverses protein synthesis, synaptic abnormalities and dendritic spine density phenotypes. Consistently, two clinical studies have suggested that arbaclofen has the potential to improve symptoms of ASD, Bumetanide, an NKCC1 (Na–K–2Cl cotransporter) chloride-importer inhibitor that reduces (Cl–) levels, enhances GABAergic inhibition, which improves the behavioural symptoms of individuals with ASD. Data from three follow-up studies have been obtained: two studies showed improvement in the primary endpoint (the Childhood Autism Rating Scale), while the other study showed no difference in the primary endpoint (the Social Responsiveness Rating Scale).

Targeting translation and epigenetic regulation. Transcriptional and translational studies have provided a scientific foundation for the discovery of drug targets for underlying mechanisms, such as PI3K/mTOR pathways. mTOR inhibitors, such as rapamycin and everolimus, have been utilized to cure behavioural and molecular abnormalities in TSC-deficient mice. Unfortunately, chemotherapeutic agents acting on the mTOR pathway have not been discovered to improve social interaction of children with tuberous sclerosis. Preliminary data have shown that the pharmacological effects of IGF-1 affect synaptic development primarily by modulating the MAPK and mTOR pathways, as validated in Phelan–McDermid syndrome and Rett syndrome. Specifically, IGF-1 treatment results in increases in synaptic protein levels and activation of signalling pathway proteins and enhances cortical excitatory synaptic transmission and dendritic spine density. Trials of the effects of IGF-1 on social interactions in individuals with ASD have shown positive results, but larger trials will provide more definitive information on efficacy.

In terms of epigenetic regulation, many autism risk genes are involved in histone modification and chromatin remodelling, and disruption of this process has been observed in individuals with autism. Treatment strategies with epigenetic enzymes, primarily targeting histone modifiers (such as histone deacetylase, histone demethylase and histone methyltransferase) show...
| Drug               | Pharmacological target                        | Improvement of symptoms                                      | Clinical therapeutic effects                                                                 | Adverse effects                                      | Ref. |
|--------------------|-----------------------------------------------|---------------------------------------------------------------|-----------------------------------------------------------------------------------------------|------------------------------------------------------|------|
| Guanfacine         | Selective α2A adrenergic receptor agonist     | Oppositional behaviour, Anxiety, Repetitive behaviour, Sleep disturbance | Improved oppositional behaviour, Significantly improved repetitive behaviour on the CYBOCS, Effective in reducing oppositional behaviour, Slightly improved repetitive behaviour | Drowsiness, fatigue, irritability, decreased appetite | 478  |
| Melatonin          | MT1R agonist                                  | Sleep disorders                                               | Effective in reducing insomnia symptoms, No serious AEs reported                             |                                                      | 629  |
| Clonidine          | α2-adrenergic receptor agonist               | ASD relevant behaviour                                         | Reducing sleep initiation latency and night awakening, slightly improve attention deficits hyperactivity, mood instability and aggressiveness | Sedation, dizziness or mild depression                | 630  |
| Memantine          | Non-competitive NMDAR antagonist              | Social impairment                                             | Significant improvement on the CGI-I and CGI-S                                               | Increased seizures, irritability, emesis and sedation | 631  |
| D-cycloserine      | Partial agonist of NMDA glutamate receptor   | ASD relevant behaviours, Self-stimulatory behaviours, Cognitive, behavioural, and memory dysfunction | Significant improvement on CMSDLS and ABC subscales including hyperactivity, lethargy, and irritability, Minimal improvement on CGI-I | No serious AEs reported                             | 498  |
| Baclofen           | Selective GABA-B agonist                     | Irritability                                                  | Significant improvement for all the ABC subscales, Greater effect on improvement of hyperactivity symptoms | No serious AEs reported                             | 632  |
| Arbaclofen         | Selective GABA-B agonist                     | ASD relevant behaviours                                        | Improvement on ABC-I, LSW, SRS, CY-BOCS-PDD, and CGI                                         | Agitation and irritability                           | 509  |
| Bumetanide         | Selective NKCC1 antagonist                   | Neurophysiological, cognitive, and behavioural measures       | Significant improvement in irritable behaviour, social behaviour and hyperactive behaviour     | No serious AEs reported                             | 512  |
| IGF-1              | IGF-1R receptor agonist                      | Core deficits of ASD                                          | Significant improvement in social impairment and restrictive behaviours                         | No serious AEs reported                             | 517  |
| Folate             | Vitamin B                                    | Language impairment                                           | Improvements in subscales of the VABS, the ABC, the ASQ and the BASC for Children            | No serious AEs reported                             | 526  |
| Oxytocin           | Biological peptides                           | Repetitive behaviour, Social deficits                         | Significantly reduce repetitive behaviours, Improvements in affective speech comprehension from pre- to post-infusion | Mild side effects                                   | 634,635 |
| Balovaptan         | Vasopressin V1a receptor antagonist           | Social behaviours                                             | Improvements on the V-II ABC composite score, No serious AEs reported                         |                                                      | 538  |
| Pioglitazone       | PPAR-γ agonist                                | Core symptoms of ASD                                          | Significant improvement in social withdrawal, repetitive behaviours, and externalizing behaviours | No serious AEs reported                             | 545  |
| PS128              | Lactobacillus plantarum                      | ASD associated symptoms                                       | Improved opposition/defiance behaviours, Significantly improved in SNAP-IV                    | No serious AEs reported                             | 553  |
| MTT                | Microbiota                                   | Gut microbiota composition, GI and ASD symptoms                | Significant improvement in the GSRS, reduction of GI symptoms and significantly improved behavioural symptoms | No serious AEs reported                             | 546  |
| Paliperidone       | Dopamine and serotonin receptors antagonist  | Irritability                                                  | Improvement on the ABC-I, Mild-to-moderate extrapyramidal symptoms                            | Weight gain                                         | 636  |
| Drug         | Pharmacological target                  | Improvement of symptoms | Clinical therapeutic effects                                                                 | Adverse effects                          | Ref. |
|--------------|-----------------------------------------|-------------------------|-----------------------------------------------------------------------------------------------|------------------------------------------|------|
| Donepezil    | Cholinesterase inhibitor                | ASD relevant behaviours  | Significant improvement in ABC and the CGI-I Improvement in the Irritability and Hyperactivity subscales | Gastrointestinal disturbances            | 637  |
| Mecamylamine | Nicotinic acetylcholine receptor        | ASD relevant behaviours  | Improvement in OACIS Decreased hyperactivity and irritability Improved verbalization            | Constipation                             | 638  |
| Acamprosate  | Modulate GABA transmission              | Social impairment       | Much improved on the CGI-I and improvement on both the ABC Social Withdrawal subscale and the total raw score of the SRS Improved hyperactivity as measured by the ABC Hyperactivity subscale | Reduced appetite                         | 639  |
| Amantadine   | Noncompetitive NMDA antagonist          | Hyperactivity Irritability | Significant improvements on ABC-CVs for hyperactivity and inappropriate speech Improvement on CGI | Insomnia                                 | 640  |
| N-Acetylcysteine | Glutamatergic modulator                | Behavioural disturbance | Significant improvements on ABC-Irritability subscale No serious AEs reported                    | No serious AEs reported                  | 641  |
| Olanzapine   | 5-HT2, DA receptor antagonist           | ASD relevant behaviours  | Significant improvement on three subscales of the ABC (Irritability, Hyperactivity, and Excessive Speech) and the TARGET | Weight gain, increased appetite, and loss of strength, extrapyramidal symptoms | 642  |
| Lurasidone   | D2, 5-HT2A antagonist and SHT1A partial agonist | Irritability           | Significantly improvement in CGI-I Vomiting and somnolence                                       | Vomiting and somnolence                  | 643  |
| Galantamine  | Acetylcholinesterase inhibitor          | Irritability            | Improvement in ABC No serious AEs reported                                                      | No serious AEs reported                  | 644  |

**Table 3. continued**

*ABC* Aberrant Behaviour Checklist, *AE* adverse effect, *CGI* Clinical Global Impressions (-I = Improvement, -S = Severity), *RFLRS* Ritvo-Freeman Real Life Rating Scale, *ABC-CV* Aberrant Behaviour Checklist-Community Version, *PDD* pervasive developmental disorders, *CY-BOCS* Children's Yale-Brown Obsessive Compulsive Scale, *CMSDLS* Children's Memory Scale Dot Learning Subtest, *VABS* Vineland Adaptive Behaviour Scale, *ASQ* Autism Symptom Questionnaire, *BASC* Behavioural Assessment System for Children, *V-II* ABC Vineland-II Adaptive Behaviour Scales, *SNAP-IV* The Swanson, Nolan, and Pelham-IV-Taiwan version, *MTT* Microbiota Transfer Therapy, *GSRS* Gastrointestinal Symptom Rating Scale, *GI* gastrointestinal, *OACIS* Ohio Autism Clinical Impressions Scale, *SRS* Social Responsiveness Scale, *TARGET* a checklist of five target symptoms, Lethargy/Social Withdrawal subscales.
therapeutic potential in animal models. The Shank3-mutant mouse model is one of the most commonly used models to study epigenetic enzymes, and it was found that using histone methyltransferase inhibitors and histone acetylase inhibitors alone or in combination can both significantly improve NMDA dysfunction and social interactions in Shank3-mutant mice. In a recent small randomized controlled trial, dietary supplementation with methylation-modifying leucovorin/folate improved core symptoms of ASD. Folate is crucial to normal neurodevelopment. Abnormal folate metabolism has been identified in patients with ASD. Three randomized double-blind placebo-controlled trials evaluated the effect of folic acid on verbal communication in patients with ASD. Encouragingly, compared to placebo, folic acid improved scores in communication and social interaction, providing promising preliminary evidence for language impairment in children with autism.

**Other biological targets: biological peptides, neuroinflammation and the intestinal flora.** The neuropeptide theory of autism is backed up by evidence from animal research. OT has been discovered to play an important role in relationship formation and social functioning. Dozens of clinical trials have studied the effects of intranasal oxytocin on ASD. Although there is no substantial treatment-specific improvement in core social symptoms, recent findings on the long-term beneficial effects on repeated behaviours and feelings of avoidance are encouraging and suggest that OT may have therapeutic promise in the treatment of ASD. Given the difficulty of exogenous drug interventions in penetrating the blood–brain barrier, several trials on strategies to promote endogenous OT production are underway. AVP is a neuropeptide primarily used to regulate renal water reabsorption and increase perivascular resistance that has been detected at lower levels in the cerebrospinal fluid of ASD children than in controls and has also been studied as a target for ASD drug therapy. A randomized double-blind controlled trial of intranasal AVP in children showed a beneficial effect on sociability deficits. Combined with evidence from preclinical studies, this evidence indicates that V1a receptor antagonists may exert prosocial, antidepressant, and anxiolytic effects in disorders of social and emotional dysfunction. In a large trial conducted in adult men, balovaptan, an orally administered selective vasopressin V1a receptor antagonist, showed promise in terms of improving social interaction and communication among people with ASD.

| Drug candidates | Pharmacological target | Improvement of symptoms | Registration number | Phase | Status | Ref. |
|-----------------|------------------------|-------------------------|---------------------|-------|--------|------|
| Lorazepam       | D2 and 5-HT-2A receptor antagonist | Irritability | NCT01911442 | Phase 3 | Completed | – |
| Flunitrazepam   | selective adrenergic uptake inhibitor | ADHD symptoms | NCT00498173 | Phase 3 | Completed | – |
| Paliperidone    | D2 partial agonist and 5-HT-2A receptor antagonist | Aggression, self-injury, irritability | NCT00549562 | Phase 3 | Completed | – |
| Melatonin       | MT1R agonist | Sleep disorders | NCT01906866 | Phase 3 | Completed | – |
| Oxytocin        | Biological peptides | Social difficulties | NCT01944046 | Phase 2 | Completed | 647 |
| Guanfacine      | Selective α2A adrenergic receptor antagonist | PDD | NCT01238575 | Phase 4 | Completed | – |
| Acamprosate     | GABA agonist and partial glutamate antagonist | Social skills deficits | NCT01813318 | Phase 1 | Completed | – |
| Memantine       | Non-competitive NMDAR antagonist | Core symptoms of autism | NCT00872898 | Phase 2 | Completed | – |
| Nuedexta        | NMDA receptor antagonist | Irritability | NCT01630811 | Phase 2 | Completed | – |
| D-cycloserine   | Partial agonist of NMDA glutamate receptor | Symptoms of autism | NCT00198120 | Phase 3 | Completed | – |
| Arbaclofen      | Selective GABA-B agonist | Social withdrawal | NCT01288716 | Phase 2 | Completed | – |
| Bumetanide      | Selective NKCC1 antagonist | ASD | NCT03156153 | Phase 2 | Completed | – |
| Donepezil       | Cholinesterase inhibitor | Communication skills, social interaction | NCT01887132 | Phase 2 | Completed | – |
| Mecamylamine    | Nicotinic acetylcholine receptor | Core symptoms of autism | NCT00773812 | Phase 1 | Completed | – |
| Olanzapine      | 5-HT2, DA receptor antagonist | Disruptive behaviours | NCT00057408 | Phase 2 | Completed | – |
| Galantamine     | Acetylcholinesterase inhibitor | ASD related | NCT00252603 | Phase 3 | Completed | – |
| N-Acetylcysteine| Glutamatergic modulator | Behavioural disturbance | NCT00627705 | Phase 2 | Completed | 641 |
| Pioglitazone    | PPAR-γ agonist | Core symptoms of ASD | NCT01205282 | Phase 2 | Completed | 545 |
| Balovaptan      | Vaspressin V1a receptor antagonist | Social behaviours | NCT01418963 | Phase 1 | Completed | 649 |
|                |                        | Socialisation and communication difficulties | NCT03504917 | Phase 3 | Completed | 650 |
| Amitriptyline   | inhibition of serotonin and norepinephrine reuptake | Repetitive Behaviours | NCT0472583 | Phase 3 | Not yet recruiting | – |
| Mirtazapine     | 5-HT2 and 5-HT3 receptors antagonist | Anxiety | NCT01302964 | Phase 3 | Completed | 651 |
| Tasmelteon      | Melatonin receptor agonist | Sleep disturbances | NCT05361707 | Phase 3 | Recruiting | – |
| IGF-1           | IGF-1R receptor agonist | Social withdrawal | NCT01970345 | Phase 2 | Recruiting | – |
| JNJ-42165279    | Fatty acid amide hydrolase | Symptoms of autism | NCT03664232 | Phase 2 | Recruiting | – |
| N-Acetylcysteine| Glutamatergic modulator | Behavioural disturbance | NCT00627705 | Phase 2 | Completed | 641 |
| Nuedexta        | NMDA receptor antagonist | Irritability | NCT01630811 | Phase 2 | Completed | – |
| D-cycloserine   | Partial agonist of NMDA glutamate receptor | Symptoms of autism | NCT00198120 | Phase 3 | Completed | – |
| Arbaclofen      | Selective GABA-B agonist | Social withdrawal | NCT01288716 | Phase 2 | Completed | – |
| Bumetanide      | Selective NKCC1 antagonist | ASD | NCT03156153 | Phase 2 | Completed | – |
| Donepezil       | Cholinesterase inhibitor | Communication skills, social interaction | NCT01887132 | Phase 2 | Completed | – |
| Mecamylamine    | Nicotinic acetylcholine receptor | Core symptoms of autism | NCT00773812 | Phase 1 | Completed | – |
| Olanzapine      | 5-HT2, DA receptor antagonist | Disruptive behaviours | NCT00057408 | Phase 2 | Completed | – |
| Galantamine     | Acetylcholinesterase inhibitor | ASD related | NCT00252603 | Phase 3 | Completed | – |
| N-Acetylcysteine| Glutamatergic modulator | Behavioural disturbance | NCT00627705 | Phase 2 | Completed | 641 |
| Pioglitazone    | PPAR-γ agonist | Core symptoms of ASD | NCT01205282 | Phase 2 | Completed | 545 |
| Balovaptan      | Vaspressin V1a receptor antagonist | Social behaviours | NCT01418963 | Phase 1 | Completed | 649 |
|                |                        | Socialisation and communication difficulties | NCT03504917 | Phase 3 | Completed | 650 |
| Amitriptyline   | inhibition of serotonin and norepinephrine reuptake | Repetitive Behaviours | NCT0472583 | Phase 3 | Not yet recruiting | – |
| Mirtazapine     | 5-HT2 and 5-HT3 receptors antagonist | Anxiety | NCT01302964 | Phase 3 | Completed | 651 |
| Tasmelteon      | Melatonin receptor agonist | Sleep disturbances | NCT05361707 | Phase 3 | Recruiting | – |
| IGF-1           | IGF-1R receptor agonist | Social withdrawal | NCT01970345 | Phase 2 | Recruiting | – |
| JNJ-42165279    | Fatty acid amide hydrolase | Symptoms of autism | NCT03664232 | Phase 2 | Recruiting | – |
| N-Acetylcysteine| Glutamatergic modulator | Behavioural disturbance | NCT00627705 | Phase 2 | Completed | 641 |
Conclusions and Perspectives

In conclusion, ASD is a complex disease caused by a series of combinations of different aetiological factors, including genetic factors, environmental and immune activation, etc., and ultimately manifests as abnormal changes in molecular signalling pathways, neuronal synapses, immune environment and brain functional connections. Animal models provide an opportunity to identify potential changes in circuit levels and their relation to behaviour regulation. Frustratingly, present medication only target comorbid symptoms rather than the core symptoms of autism, and the development of key molecular targets for signal transduction pathways is still in the basic research. To date, few trials have reached their primary endpoints, and little evidence has promoted the approval of drug administration agencies or the use of the tested treatments in clinical practice. For example, the efficacy of several molecular targets has been well demonstrated in animal models, such as mGluRs inhibitors, OT, Memantine, and mTOR inhibitors, but is still unsatisfactory in clinical trials. A serious challenge is how ASD can bridge the vast gap between molecular, cellular, and circuit convergence mechanisms to the heterogeneity of clinical manifestations. Therefore, basic research to clinical transformation remains the rate-limiting step in the development of treatment strategies for ASD, and the degree of heterogeneity may be considered, which may obscure the effect of experimental treatments. Conducting in-depth mechanistic studies using models such as nonhuman primates that can truly simulate human pathological processes would be crucial. The development of methods for manipulating nonhuman primate genomes may provide key insights for translation from model system experiments to human studies.

Despite these challenges, new therapies based on elucidated genes have been developed in recent years, such as gene replacement, gene editing and translating oligonucleotides. Relatively modest manipulation of gene expression using normal alleles may be sufficient to mitigate the effects of deleterious mutations. The development of technologies such as CRISPR-Cas9, which is based on targeted DNA editing, has facilitated rapid progress in gene therapy, and these technologies have also shown therapeutic effects in mice with fragile X syndrome. Thus, gene editing provides a new personalized medicine approach for the treatment of autism.

To optimize and change the treatment strategy for autism, it is necessary to bridge biochemical molecular events, electrical oscillations and information processing and to explore the pathological mechanism of autism from a new systemic perspective. The coexistence of many clinical disorders in autism is quite common, but this autism comorbidity has not received enough attention thus far. Studies exploring potential biomarkers should design laboratory tests related to specific clinical syndromes based on the presence or absence of some specific comorbidities. Such research will require large-scale clinical cohort studies involving the same population, as well as focusing on spatiotemporal dynamics such as behaviour, development, and types of comorbidities. In conclusion, research on ASD is still challenging. ‘Bench to bedside’ progress will depend on integrative multidisciplinary approaches between basic scientists and clinical investigators to reveal the pathological mechanism of autism.

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Additional Information

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