特约稿

Genetic mechanisms underlying synaptic pathology in Autism Spectrum Disorder (ASD)

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【Abstract】 Autism spectrum disorder (ASD) is a neuronal developmental disorder that is characterized by defects both in social interaction and verbal communication, and is often accompanied by restricted interest, and repetitive and stereotyped behaviors. The prevalence of ASD is approximately 0.8% ~ 1.2% in China. Although its etiology remains unclear in most cases, over 1 000 genes or genomic loci have been linked to its pathogenic origin, indicating a strong genetic influence, as well as complicated pathogenic mechanisms. Here, we reviewed recent findings in the possible genetic effects on synaptic pathophysiology of ASD, and a particular focus was put on variants in genes that are related to synaptic morphology or functions, such as *FMR1*, *NRXN, NLGN, SHANK* and *MeCP2*. The synaptic pathology caused by these genetic defects may substantially contribute to the pathogenesis of ASD. This review outlines several lines of evidence that have been recently reported to support the hypothesis that a genetic defect may lead to a synaptic pathology that may underlie the pathogenesis of ASD.

【Key words】 Autism spectrum disorders (ASD) Synapse Synapse elimination Synaptic transmission Synaptic plasticity

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1 Introduction

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders that are characterized by a cluster of clinical core symptoms including impaired social interaction and communication, and repetitive and stereotyped behaviors. The term "autism" is first introduced by Prof Leo Kanner in 1943 at Johns Hopkins University in USA. Dr. Kanner followed up almost 30 years for 11 children who were featured by repetitive, stereotypical attention to objects and considered as ASD at the ages from 2 to 8 [1]. In 1944, Dr. Hans Asperger identified a group of children who showed maladaptation to their social environment and displayed certain repetitive and restricted behaviors [2]. There is a significant clinical heterogeneity in ASD. In addition to the main core symptoms, a large portion of patients may show some mental comorbidities [3-4] such as intellectual disability [5], epilepsy [6], anxiety [7], attention deficit hyperactivity disorder [8] and sleep disorders [9] etc. or others such as gastrointestinal disorders [9]. Except for

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some symptom-based medications, no actually effective medicines are currently available for ASD. Although FDA in USA approved for the clinical uses of Risperidone and Aripiprazole, those two choices are not really effective for any core symptoms of ASD [10-11]. Currently, behavioral interventions, especially at the earlier stage, are the major usefully therapeutic approaches world-widely [12-13].

The global prevalence of ASD is approximately 0.8% ~ 1.2% [14-16]. In China, we still do not have a national-wide epidemiological data basis so far [17]. A recent multi-center study has suggested that the prevalence of ASD in children within the ages between 6 and 12 years in China is about 0.6% ~ 0.8% [18], while a local study in younger kids revealed a prevalence rate of 2.6% [19]. Also, there was a report to claim that the prevalence of ASD in China is very close to that in western countries, that is about 1% [20]. Nevertheless, the prevalence of ASD in most areas in this world is significantly increased during the past 30 years [21-22], due to whatever reasons, such as changes in the environmental exposures, changes in humanity culture, growing public awareness of the disorder, changes in ASD diagnostic criteria, and increasing services for ASD, etc. [23]. However, there is evidence showing that the changes in environments may most dominantly attribute to this increased prevalence [22, 24].

The pathogenic origin of ASD is still not clear. Over the past 50 years, one milestone in ASD studies is the establishment of the notion that the etiology of ASD is at least partially genetic/genomic oriented [14, 25]. The first lines of evidence came from twin studies, in which it was found that the concordance rate for ASD in monozygotic twins (80% ~ 90%) was much higher than that in dizygotic twins (0% ~ 10%) [26-28]. However, due to the small sample size, short follow-up period, and inconsistent diagnostic criteria, those studies did not receive enough attention. Recently, large-scale cohort studies on multiple populations have further supported the findings that the incident rate of ASD in monozygotic twins is much higher than that of dizygotic twins [29-30], and further confirmed the strong influence of genetic components [29, 31]. To date, over 1 000 genes or genomic loci have been associated with ASD. Many of these genes are important players in molecular and cellular functions such as chromatin remodeling [32], mRNA transcription and translation [33], protein modification [34], cellular stress [35] etc, or neuronal function such as neural migration [36], axonal guidance [37], dendritic growth [38], synapse formation [39], neurogenesis [40] and synaptic transmission [41], among which, synaptic dysfunction seems one of the most important mechanisms in the pathogenesis of ASD [42-44].

Synapses in the brain are the neural structure specifically for the functional connections between neurons, via which neural information is transmitted. The pathogenic events for ASD may occur during the late developmental stage, including prenatal and perinatal stages [45], a critical period for the development of synapses. Many genes that are associated with ASD, such as *FMR1*, *NRXN*, *NLGN*, *SHANK* and *MeCP2*, are actually important players in synapse formation, synaptogenesis, synaptic transmission, synaptic plasticity etc. [44, 46-47], implying that synaptic dysfunction may lie on the core of the pathogenesis. In this review, we will provide an overview of the genetic mechanisms that underlie synaptic dysfunctions often observed in ASD, with a hope to strengthen the research on this aspect.

2 Genetic Factors

2.1 Copy number variants (CNVs)

Following the broad uses genome-wide analysis advanced, it has been generally accepted now that CNVs play an essential role in the human genome polymorphism, biodiversity and diseases. In 2007, Sebat *et al*. for the first time systematically reported that de novo CNVs were associated with ASD, in which over 10% ASD kids harbor CNVs, while in control subjects, only less than 1% has a similar genotype [48]. Up to date, numerous studies have revealed that an overall frequency of CNVs in ASD patients range from 8% to 21%, which is significantly higher than that in controls [49-51]. CNVs are generally classified into the recurrent and nonrecur-

rent types. Recurrent CNVs have standard sizes and breakpoints caused by non-allelic homologous recombination (NAHR) between low copy repeats (LCRs), while nonrecurrent CNVs are usually caused by nonhomologous end joining (NHEJ), fork stalling and template switching (FoSTeS), or microhomology-mediated break-induced replication. The most common recurrent CNVs associated with ASD are microdeletions and microduplications of approximately 600 kb in the 16p11.2 region, [52]. In addition, CNVs at 15q11-q13, 7q11.23, 16p11.2, 22q11.2, and 1q21.1 were also linked to ASD [53]. ASD patients with 16p11.2 microdeletions showed a significant deficit in functional connectivity and long-range integration in the prefrontal and parietotemporal region of the brain [54]. The *UBE3A* gene, located at 15q11-q13, encodes a translational ubiquitination protease E3A, and this molecule regulates protein homeostasis and synaptic plasticity. Evidence shows that the duplications of *UBE3A* can cause ASD-like symptoms[55].

2.2 Single nucleotide polymorphisms (SNPs)

In addition to CNVs, SNPs are also obtained a wide attention for their possible implication in ASD etiology. Although results from numerous studies have indicated a significant association of SNPs in over 1 000 genes or loci with ASD [46, 56-57], controversial results are also often reported. Since ASD patients have a high heterogeneity in clinical symptoms, many GWAS studies were seeking to establish a connection between a specific clinical symptom and genetic variants [58-59]. Prof Hu *et al*. screened 18 significant SNPs by dividing all ASD individuals into four subtype groups. When all patients were combined into the same cohort for analysis, however, no definitive association between the SNPs and ASD was found. The recent two studies also suggested that SNPs might make a minor contribution to ASD etiology [59-60]. The cohorts tested so far in autism studies are much smaller than in other common diseases such as epilepsy or schizophrenia. Therefore, it may be necessary to expand the sample size to further look for the association of SNPs with ASD[61-64].

2.3 Chromosomal abnormalities

It has been estimated that the incident rate of chromosomal aberrations, including deletions, duplications, inversions, translocations in ASD patients is approximately 2% ~ 5% [65-66]. Most chromosomal structural abnormalities are rare, and their causative role in ASD is unknown [67]. Among them, the most common chromosomal abnormality is 15q11-q13 duplication of maternal origin [68]. It's variable in size and presents in approximately 1% ~ 3% of children with ASD. Many genes associated with ASD, such as *gabra5*, *GABRB3*, *UBE3A*, *HERC2*, *SNRPN* and *CYFIP1*, are located in this chromosomal region [69-71]. Moreover, sex chromosomal abnormalities were identified in syndrome ASD patients, such as Turner syndrome, Klinefelter syndrome, XXX syndrome and XYY syndrome, which are consistent with the notion that many genes located on the sex chromosomes may play an essential role in brain structure, function and development [72].

3 ASD Risk Genes and Synaptopathology

Synapse formation and maturation are critical for the development of neural circuits in the brain, and synaptic dysfunctions are thought to be underlying the pathogenesis of neurodevelopmental disorders. It has been suggested that ASD-like symptoms may be attributed to deficits in synaptic function, and thus the role of those synaptic function-related genes in the pathogenesis of ASD has received an extensive attention. Mutations in those synaptic function-related genes, such as *FMR1*, *NRXN*, *NLGN*, *SHANK* and *MeCP2*, have been clearly fund to be harmful for synapse formation, maturation, or synaptic plasticity, and many others [43]. Thus, abnormal synaptic development and synaptic dysfunction may be particularly important in triggering pathogenic cascade for ASD.

3.1 FMRP gene

Fragile X Syndrome (FXS) is the most common cause for inherited intelligent deficit (ID), accounting for approximately 1% ~ 2% of all ID patients. Also, FXS is the most common single-gene disorder causing ASD, with approximately 60% ~ 74% of FXS patients

meeting the diagnostic criteria for ASD. The direct cause of FXS mutation is the inactivation of the *FMR1* gene. At the same time, about 2% ~ 5% of all ASD are diagnosed with a test of the *FMR1* gene mutations [73].

The *FMR1* gene cords for the Fragile mental retardation protein (FMRP) that acts as a translational repressor of mRNA binding. In normal cells, FMRP tightly regulates mRNA stability and transport and translation [74]. In recent years, hundreds of possible FMRP target mRNAs have been identified through high-throughput approaches, with a considerable number of mRNAs encoding pre - and post - synaptic proteins associated with ASD, including NMDARs, mGluR5, PSD-95, SHANK1-3, HOMER 1, NEUREXIN and others [75]. The dysfunction of FMRP in promoting translational activities is considered as a critical cause of ASD-associated synaptic and cognitive deficits. An increased local translation of *NLGN* mRNAs and targeting of *nlgn1* and *nlgn3* to the postsynaptic membrane was found in *FMR1* KO mice [76-79]. In FXS mouse models, mGluR5 in the CA1 region of the hippocampus was found to be increased binding to short Homer 1a and reduced binding to the long Homer isoform, resulting in mGluR5 being significantly more mobile post-synaptically [80].

3.2 *SHANK* genes

Shank (also known as pro sap) is a major scaffolding protein localized at PSD of excitatory synapses in the brain. The *Shank* family consists of three major isoforms (*Shank1*, *Shank2* and *Shank3*), and they share the following similar domains: an N-terminal ankyrin repeat, an Src homology 3 (SH3) domain, and a PSD-95/discs large/ZO-1 (PDZ) domain, an extended proline-rich region, and a sterileαMotif (SAM) domain. The function of *Shank* family is linked to cell adhesion molecules, actin remodeling, and they enhance synaptic plasticity, maintain synaptic ecology, induce dendritic spines, induce excitatory synapse formation, and is essential for excitatory glutamatergic synapses.

Shank1 is encoded by the *SHANK1* gene, which is located on chromosome 19q13.33. Sato *et al*. identified deletions in the *Shank1* gene in two multiplex ASD families [81]. Hung *et al*. found that *Shank1* knockout mice (PDZ domain suppression that disrupts all *Shank1* isoforms) showed an reduced locomotor function and impaired memory for fear conditioning, whereas increased spatial memory in the maze test[82]. Wohr *et al*. showed that *Shank1* knockout mice showed reduced ultrasonic articulation levels and odor marker behavior, indicating an inability to learn social experience, in consistent with the phenotype of ASD [83]. These studies all suggest that *Shank1* may play an important role in the pathogenesis of ASD.

Shank2 is encoded by the *SHANK2* gene, which is located on chromosome 11q13.3-11q13.4. *Shank2* variants include deletions, missense mutations, truncations, and changes in the promoter region. Leblond *et al*. found de novo *shank2* gene deletions (truncations) in three individuals with ASD [84]. He also reported a de novo *Shank2* deletion in one ASD patient carrying a paternally inherited deletion of the synaptic translation repressor CYFIP1. Recently, novel *Shank2* knockout models in mice (shank2△7 - / - and shank2 E6-7 - / -) have been generated. Both strains of mice showed a lack of social interaction, increased hyperactivity, self-grooming and anxiety [85]. At the biochemical level, the mutant mice showed a reduced synaptic transmission, reduced frequency of mEPSCs, and an increased NMDA/AMPA ratio etc. All these results suggest that *Shank2* is involved in the pathological process of ASD [86].

Shank3 is encoded by the *SHANK3* gene, which is located on chromosome 22q13.3. *Shank3* was first identified in the 22q13.3 deletion syndrome, also known as Phelan McDermid syndrome (PMS), characterized by global developmental delay. More than 50% of PMS patients have autistic-like behavior [87]. The first ASD case with a de novo deletion mutation in *Shank3* was identified by Durand *et al*. in 2007. Since then, many ASD cases carrying with *Shank3* mutations were reported. *Shank3* Neurons harboring *Shank3* mutation showed reduced PSD proteins, including GKAP, HOMER1b/C, GluA1 subunit of AMPAR, and NR2A subunit of NMDAR [39, 88]. By compared with shank3ab heterozygotes, Yang *et al*. showed that shank3ab heterozygotes have more severe reductions in synaptic transmis-

sion and LTP in hippocampal CA1[89]. Subtle morphological changes in dendritic spines have also been observed upon shank3ab mutations, manifesting with reduced levels of homer1b/C, GKAP, and GluA1 in addition to activity-dependent redistribution of GluA1 (containing AMPA receptors) in primary hippocampal neurons. In summary, studies using *Shank3* mutant murine models have provided with us with evidence in further understanding of the neurobiological basis for ASD.

3.3 NLGNs and NRXNs

NLGNs are a class of cell adhesion factors that are located in the postsynaptic membrane of neurons. This group of protein belongs to the type I transmembrane proteins, which mainly consist of large extracellular domains (containing one acetylcholinesterase homology domain, EF-hand type motif, and O-glycosylation domain), a single-pass transmembrane domain, and intracellular domains. There are 5 NLGNs proteins, namely *NLGN1*, *NLGN2*, *NLGN3*, *NLGN4* and *NLGN4y*. *NLGN1* is exclusively located in excitatory postsynaptic membranes [90], and *NLGN2* is mainly located on the inhibitory postsynaptic membrane [91]. *NLGN3* and *NLGN4* are found in both types of postsynaptic membranes [92-93]. NLGNs bind to axonal proteins (neurexins) of the presynaptic membrane and play an essential role in synapse development and function, regulating the balance between excitatory and inhibitory synapses. Nlgns gene variants were first reported to be associated with ASD by Jamain *et al* [94], who found the r45lc missense mutation in the *Nlgn3* gene and the c.1186inst frameshift mutation in the *Nlgn4* gene on the X chromosome in two Swedish ASD families. By direct sequencing, Xu *et al*. identified four rare missense mutations, namely, p.g426s (*Nlgn3*), p.g84r (*Nlgn4*), p.q162k (*Nlgn4*) and p.a283t (Nlgn4), in 318 Chinese Han ASD patients, and these four missense mutations were all located in the acetylcholinesterase homology domain of Nlgns, a crucial structure for binding to axonal proteins to trigger synaptic activity [95]. Several Nlgns mutant mouse models were used to clarify the role of the Nlgns gene family in the pathogenesis of ASD. Abnormalities in excitatory or inhibitory synaptic neurotransmitter transmission were found in almost all those models, and several studies identified an altered synaptic density and morphology in the hippocampus [96]. The abnormal behavior of Nlgns mutant mice resembles typical symptoms of ASD. for example, Blundell *et al*. found that *Nlgn1* knockout mice exhibited decreased social behavior, impaired spatial learning and memory, and repetitive stereotypical grooming behaviors [96-97]. At the same time, synapse number, synapse length, synaptic connections, dendritic spine density of excitatory synapses were significantly increased in the hippocampus. In consistent, electrophysiological studies revealed a significant increase in excitatory postsynaptic potential (EPSP) and inhibitory postsynaptic potential (IPSP) ratios (E/I) and impaired LTP [97-99]. Similar phenotypes were described in other mouse models of *Nlgn2*, *Nlgn3*, and *Nlgn4* [100].

Genes encoding neurexins include *Nrxn1*, *Nrxn2*, and *Nrxn3*. Axonal proteins are a class of highly polymorphic neuronal surface proteins involved in critical physiological processes such as synapse formation and signaling [101]. In a cohort study of 116 Caucasian children with ASD, J Yan *et al* discovered five missense mutations, including a predicted splice mutation [102]. It has been concluded that mutations and abnormalities of structural genes in the *Nrxn1* may increase susceptibility to ASD [61]. Moreover, such mutations and structural abnormalities occur at different frequencies in different human populations. *Nrxn1* was also associated with ASD in an association study with genome-wide SNPs in a large group of samples. Although without any social impairments or deficits in learning, the *Nrxn1* null animal model exhibits repetitive grooming behavior. The electronic signals transmitted by synapses in the hippocampus are weaker than normal controls [96, 103]. All these results point to a special role of these genes in the pathogenesis of ASD.

3.4 Synaptic elimination

In addition to being associated with abnormalities in synaptogenesis, synaptic neurotransmission, and synaptic plasticity, several ASD risk genes (including *MEF2C*, *FMR1*, *dlg4* and *pcdh10*) are involved in synaptic pruning. Therefore they will also be reviewed in

this article as a whole[104].

Synapse formation initially predominates during prenatal brain development, while excessive synapse formation beyond the required level for normal physiological function may lead to a pathological condition. During subsequent developmental stages, synapses closely related to normal physiological function are retained, while excess synapses are trimmed and eliminated, leading to reduced synapse number. The maintenance of cognition and expected behavior is mainly dependent on the precise formation of mature neuronal circuits. Therefore, proper synaptic pruning is essential to maintain precise neural circuits.

Loss of MEF2C in mice results in a significant increase in the number of excitatory synapses, whereas neuronal expression of the hyper-activated form of MEF2C leads to a decrease number of excitatory postsynaptic sites. MEF2 and FMRP cooperatively regulate the expression of pro-adhesin-10 (pcdh10), and activation of MEF2 leads to PSD95 ubiquitination by the ubiquitin E3 ligase Murine Double Minute-2 (Mdm2), which is then delivered by pcdh10 to the proteasome for degradation, enabling synaptic pruning function [105-107]. All these results indicated that a deficit in synaptic pruning may underlie the cellular and neuronal mechanism of ASD.

4 Conclusions

With the advance of gene sequencing technology, more and more researchers pay much attention to the genetic role in the pathogenesis of ASD. An increasing number of ASD risk genes are being identified, suggesting that the genetic mechanisms are more and more important. However, because of the complexity of ASD inheritance, other than a simple monogenic mode of inheritance, there are multiple genes with complex effects, incomplete penetrance, making it very challenging to define an exact role of a genetic component in the pathogenesis of ASD. Even though, it seems very promising to focus on the genes that are synaptic function related, in our attempts to further understanding the possible genetic and neuronal mechanism. Further elucidation of the underlying synaptic pathology, as well as advances in gene therapy and targeted agents to modulate these processes, may lead to promising new therapies for the treatment of ASD.

参 考 文 献

[1] Kanner L. Follow-up study of eleven autistic children originally reported in 1943[J]. J Autism Child Schizophr, 1971, 1(2): 119-145.

[2] Hippler K, Klicpera C. A retrospective analysis of the clinical case records of ´autistic psychopaths´ diagnosed by Hans Asperger and his team at the University Children´s Hospital, Vienna[J]. Philos Trans R Soc Lond B Biol Sci, 2003, 358(1430): 291-301.

[3] Lai MC, Kassee C, Besney R, *et al*. Prevalence of co-occurring mental health diagnoses in the autism population: a systematic review and meta-analysis[J]. Lancet Psychiatry, 2019, 6(10): 819-829.

[4] Hossain MM, Khan N, Sultana A, *et al*. Prevalence of comorbid psychiatric disorders among people with autism spectrum disorder: an umbrella review of systematic reviews and meta-analyses[J]. Psychiatry Res, 2020, 287: 112922.

[5] Verma V, Paul A, Amrapali Vishwanath A, *et al*. Understanding intellectual disability and autism spectrum disorders from common mouse models: synapses to behaviour [J]. Open Biol, 2019, 9(6): 180265.

[6] Besag FMC, Vasey MJ. Seizures and epilepsy in autism spectrum disorder[J]. Child Adolesc Psychiatr Clin N Am, 2020, 29(3): 483-500.

[7] Wood JJ, Kendall PC, Wood KS, *et al*. Cognitive behavioral treatments for anxiety in children with autism spectrum disorder: a randomized clinical trial[J]. JAMA Psychiatry, 2020, 77(5): 474-483.

[8] Miller M, Musser ED, Young GS, *et al*. Sibling recurrence risk and cross-aggregation of attention-deficit/hyperactivity disorder and autism spectrum disorder[J]. JAMA Pediatr, 2019, 173(2): 147-152.

[9] Devnani PA, Hegde AU. Autism and sleep disorders[J]. J Pediatr Neurosci, 2015, 10(4): 304-307.

[10] McCracken JT, McGough J, Shah B, *et al*. Risperidone in children with autism and serious behavioral problems [J]. N Engl J Med, 2002, 347(5): 314-321.

[11] Gettu N, Saadabadi A. Aripiprazole[M]. Treasure Island (FL): StatPearls Publishing, 2021.

[12] Xu GF, Strathearn L, Liu BY, *et al*. Prevalence and treatment patterns of autism spectrum disorder in the United States, 2016[J]. JAMA Pediatr, 2019, 173(2): 153-159.

[13] Uddin LQ. Brain mechanisms supporting flexible cognition and behavior in adolescents with autism spectrum disorder[J]. Biol Psychiatry, 2021, 89(2): 172-183.

[14] Lai MC, Lombardo MV, Baron-Cohen S. Autism[J]. Lancet, 2014, 383(9920): 896-910.

[15] Kim YS, Leventhal BL, Koh YJ, *et al*. Prevalence of autism spectrum disorders in a total population sample[J]. Am J Psychiatry, 2011, 168(9): 904-912.

[16] Blumberg SJ, Bramlett MD, Kogan MD, *et al*. Changes in prevalence of parent-reported autism spectrum disorder in school-aged U.S. children: 2007 to 2011-2012[J]. Natl Health Stat Report, 2013(65): 1-11, 1 p following 11.

[17] Wang F, Lu L, Wang SB, *et al*. The prevalence of autism spectrum disorders in China: a comprehensive meta-analysis[J]. Int J Biol Sci, 2018, 14(7): 717-725.

[18] Zhou H, Xu X, Yan WL, *et al*. Prevalence of autism spectrum disorder in China: a nationwide multi-center population-based study among children aged 6 to 12 years [J]. Neurosci Bull, 2020, 36(9): 961-971.

[19] Yang WK, Xia H, Wen GM, *et al*. Epidemiological investigation of suspected autism in children and implications for healthcare system: a mainstream kindergarten-based population study in Longhua District, Shenzhen[J]. BMC Pediatr, 2015, 15: 207.

[20] Sun X, Allison C, Wei LP, *et al*. Autism prevalence in China is comparable to Western prevalence[J]. Mol Autism, 2019, 10: 7.

[21] Baxter AJ, Brugha TS, Erskine HE, *et al*. The epidemiology and global burden of autism spectrum disorders[J]. Psychol Med, 2015, 45(3): 601-613.

[22] Nadeem MS, Al-Abbasi FA, Kazmi I, *et al*. Multiple risk factors: a challenge in the management of autism[J]. Curr Pharm Des, 2020, 26(7): 743-754.

[23] Waterhouse L. Autism overflows: increasing prevalence and proliferating theories[J]. Neuropsychol Rev, 2008, 18 (4): 273-286.

[24] Keil KP, Lein PJ. DNA methylation: a mechanism linking environmental chemical exposures to risk of autism spectrum disorders?[J]. Environ Epigenet, 2016, 2(1): dvv012.

[25] Vorstman JAS, Parr JR, Moreno-De-Luca D, *et al*. Autism genetics: opportunities and challenges for clinical translation[J]. Nat Rev Genet, 2017, 18(6): 362-376.

[26] Folstein S, Rutter M. Infantile autism: a genetic study of 21 twin pairs[J]. J Child Psychol Psychiatry, 1977, 18(4): 297-321.

[27] Bailey A, Le Couteur A, Gottesman I, *et al*. Autism as a strongly genetic disorder: evidence from a British twin study[J]. Psychol Med, 1995, 25(1): 63-77.

[28] Steffenburg S, Gillberg C, Hellgren L, *et al*. A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden[J]. J Child Psychol Psychiatry, 1989, 30(3): 405-416.

[29] Sandin S, Lichtenstein P, Kuja-Halkola R, *et al*. The familial risk of autism[J]. JAMA, 2014, 311(17): 1770-1777.

[30] Bai D, Yip BHK, Windham GC, *et al*. Association of genetic and environmental factors with autism in a 5-country cohort[J]. JAMA Psychiatry, 2019, 76(10): 1035-1043.

[31] Mefford HC, Batshaw ML, Hoffman EP. Genomics, intellectual disability, and autism[J]. N Engl J Med, 2012, 366 (8): 733-743.

[32] De Rubeis S, He X, Goldberg AP, *et al*. Synaptic, transcriptional and chromatin genes disrupted in autism[J]. Nature, 2014, 515(7526): 209-215.

[33] Parras A, Anta H, Santos-Galindo M, *et al*. Autism-like phenotype and risk gene mRNA deadenylation by CPEB4 mis-splicing[J]. Nature, 2018, 560(7719): 441-446.

[34] Hörnberg H, Pérez-Garci E, Schreiner D, *et al*. Rescue of oxytocin response and social behaviour in a mouse model of autism[J]. Nature, 2020, 584(7820): 252-256.

[35] Manivasagam T, Arunadevi S, Essa MM, *et al*. Role of oxidative stress and antioxidants in autism[J]. Adv Neurobiol, 2020, 24: 193-206.

[36] Xu Q, Liu YY, Wang XM, *et al*. Autism-associated CHD8 deficiency impairs axon development and migration of cortical neurons[J]. Mol Autism, 2018, 9: 65.

[37] de Anda FC, Rosario AL, Durak O, *et al*. Autism spectrum disorder susceptibility gene *TAOK2* affects basal dendrite formation in the neocortex[J]. Nat Neurosci, 2012, 15(7): 1022-1031.

[38] Guo H, Li Y, Shen L, *et al*. Disruptive variants of *CSDE1* associate with autism and interfere with neuronal development and synaptic transmission[J]. Sci Adv, 2019, 5 (9): eaax2166.

[39] Peça J, Feliciano C, Ting JT, *et al*. Shank*3* mutant mice display autistic-like behaviours and striatal dysfunction[J]. Nature, 2011, 472(7344): 437-442.

[40] Durak O, Gao F, Kaeser-Woo YJ, *et al*. Chd8 mediates cortical neurogenesis via transcriptional regulation of cell cycle and Wnt signaling[J]. Nat Neurosci, 2016, 19(11): 1477-1488.

[41] Tabuchi K, Blundell J, Etherton MR, *et al*. A neuroligin−3 mutation implicated in autism increases inhibitory synaptic transmission in mice[J]. Science, 2007, 318 (5847): 71−76.

[42] Nelson AD, Bender KJ. Dendritic integration dysfunction in neurodevelopmental disorders[J]. Dev Neurosci, 2021: 1−21, DOI: 10.1159/000516657. Epub ahead of print.

[43] Monteiro P, Feng GP. SHANK proteins: roles at the synapse and in autism spectrum disorder[J]. Nat Rev Neurosci, 2017, 18(3): 147−157.

[44] Delorme R, Ey E, Toro R, *et al*. Progress toward treatments for synaptic defects in autism[J]. Nat Med, 2013, 19 (6): 685−694.

[45] Katz J, Reichenberg A, Kolevzon A. Prenatal and perinatal metabolic risk factors for autism: a review and integration of findings from population−based studies[J]. Curr Opin Psychiatry, 2021, 34(2): 94−104.

[46] Stessman HAF, Xiong B, Coe BP, *et al*. Targeted sequencing identifies 91 neurodevelopmental−disorder risk genes with autism and developmental−disability biases[J]. Nat Genet, 2017, 49(4): 515−526.

[47] Iakoucheva LM, Muotri AR, Sebat J. Getting to the cores of autism[J]. Cell, 2019, 178(6): 1287−1298.

[48] Sebat J, Lakshmi B, Malhotra D, *et al*. Strong association of de novo copy number mutations with autism[J]. Science, 2007, 316(5823): 445−449.

[49] Yap CX, Alvares GA, Henders AK, *et al*. Analysis of common genetic variation and rare CNVs in the Australian Autism Biobank[J]. Mol Autism, 2021, 12(1): 12.

[50] Yehia L, Seyfi M, Niestroj LM, *et al*. Copy number variation and clinical outcomes in patients with germline *PTEN* mutations[J]. JAMA Netw Open, 2020, 3(1): e1920415.

[51] Bourgeron T. Current knowledge on the genetics of autism and propositions for future research[J]. C R Biol, 2016, 339(7/8): 300−307.

[52] Jang W, Kim Y, Han E, *et al*. Chromosomal microarray analysis as a first−tier clinical diagnostic test in patients with developmental delay/intellectual disability, autism spectrum disorders, and multiple congenital anomalies: a prospective multicenter study in Korea[J]. Ann Lab Med, 2019, 39(3): 299−310.

[53] Carter MT, Scherer SW. Autism spectrum disorder in the genetics clinic: a review[J]. Clin Genet, 2013, 83(5): 399−407.

[54] Bertero A, Liska A, Pagani M, *et al*. Autism−associated 16p11.2 microdeletion impairs prefrontal functional connectivity in mouse and human[J]. Brain, 2018, 141(7): 2055−2065.

[55] Vatsa N, Jana NR. UBE3A and its link with autism[J]. Front Mol Neurosci, 2018, 11: 448.

[56] Gaugler T, Klei L, Sanders SJ, *et al*. Most genetic risk for autism resides with common variation[J]. Nat Genet, 2014, 46(8): 881−885.

[57] Werling DM, Brand H, An JY, *et al*. An analytical framework for whole−genome sequence association studies and its implications for autism spectrum disorder[J]. Nat Genet, 2018, 50(5): 727−736.

[58] Hu VW, Addington A, Hyman A. Novel autism subtype−dependent genetic variants are revealed by quantitative trait and subphenotype association analyses of published GWAS data[J]. PLoS One, 2011, 6(4): e19067.

[59] An JY, Claudianos C. Genetic heterogeneity in autism: from single gene to a pathway perspective[J]. Neurosci Biobehav Rev, 2016, 68: 442−453.

[60] Ronald A, Butcher LM, Docherty S, *et al*. A genome−wide association study of social and non−social autistic−like traits in the general population using pooled DNA, 500 K SNP microarrays and both community and diagnosed autism replication samples[J]. Behav Genet, 2010, 40(1): 31−45.

[61] Wang K, Zhang HT, Ma DQ, *et al*. Common genetic variants on 5p14.1 associate with autism spectrum disorders [J]. Nature, 2009, 459(7246): 528−533.

[62] Weiss LA, Arking DE, Gene Discovery Project of Johns Hopkins & the Autism Consortium, *et al*. A genome−wide linkage and association scan reveals novel loci for autism[J]. Nature, 2009, 461(7265): 802−808.

[63] Anney R, Klei L, Pinto D, *et al*. A genome−wide scan for common alleles affecting risk for autism[J]. Hum Mol Genet, 2010, 19(20): 4072−4082.

[64] Anney R, Klei L, Pinto D, *et al*. Individual common variants exert weak effects on the risk for autism spectrum disorders[J]. Hum Mol Genet, 2012, 21(21): 4781−4792.

[65] Devlin B, Scherer SW. Genetic architecture in autism spectrum disorder[J]. Curr Opin Genet Dev, 2012, 22(3): 229−237.

[66] Liu XX, Takumi T. Genomic and genetic aspects of autism spectrum disorder[J]. Biochem Biophys Res Commun, 2014, 452(2): 244−253.

[67] Castermans D, Wilquet V, Steyaert J, *et al*. Chromosomal anomalies in individuals with autism: a strategy towards the identification of genes involved in autism[J]. Autism, 2004, 8(2): 141−161.

[68] Hogart A, Wu D, LaSalle JM, *et al*. The comorbidity of autism with the genomic disorders of chromosome 15q11.2-q13[J]. Neurobiol Dis, 2010, 38(2): 181-191.

[69] Puffenberger EG, Jinks RN, Wang H, *et al*. A homozygous missense mutation in *HERC2* associated with global developmental delay and autism spectrum disorder[J]. Hum Mutat, 2012, 33(12): 1639-1646.

[70] Nishimura Y, Martin CL, Vazquez-Lopez A, *et al*. Genome-wide expression profiling of lymphoblastoid cell lines distinguishes different forms of autism and reveals shared pathways[J]. Hum Mol Genet, 2007, 16(14): 1682-1698.

[71] Menold MM, Shao Y, Wolpert CM, *et al*. Association analysis of chromosome 15 gabaa receptor subunit genes in autistic disorder[J]. J Neurogenet, 2001, 15(3-4): 245-259.

[72] Gillberg C. Chromosomal disorders and autism[J]. J Autism Dev Disord, 1998, 28(5): 415-425.

[73] Schaefer GB, Mendelsohn NJ. Genetics evaluation for the etiologic diagnosis of autism spectrum disorders[J]. Genet Med, 2008, 10(1): 4-12.

[74] Darnell JC, Richter JD. Cytoplasmic RNA-binding proteins and the control of complex brain function[J]. Cold Spring Harb Perspect Biol, 2012, 4(8): a012344.

[75] Pasciuto E, Bagni C. SnapShot: FMRP mRNA targets and diseases[J]. Cell, 2014, 158(6): 1446-1446.e1.

[76] Gkogkas CG, Khoutorsky A, Ran I, *et al*. Autism-related deficits via dysregulated eIF4E-dependent translational control[J]. Nature, 2013, 493(7432): 371-377.

[77] Wang HS, Doering LC. Reversing autism by targeting downstream mTOR signaling[J]. Front Cell Neurosci, 2013, 7: 28.

[78] Darnell JC, Van Driesche SJ, Zhang CL, *et al*. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism[J]. Cell, 2011, 146(2): 247-261.

[79] Chmielewska JJ, Kuzniewska B, Milek J, *et al*. Neuroligin 1, 2, and 3 regulation at the synapse: FMRP-dependent translation and activity-induced proteolytic cleavage[J]. Mol Neurobiol, 2019, 56(4): 2741-2759.

[80] Lipton JO, Sahin M. The neurology of mTOR[J]. Neuron, 2014, 84(2): 275-291.

[81] Sato D, Lionel AC, Leblond CS, *et al*. *SHANK1* deletions in males with autism spectrum disorder[J]. Am J Hum Genet, 2012, 90(5): 879-887.

[82] Hung AY, Futai K, Sala C, *et al*. Smaller dendritic spines, weaker synaptic transmission, but enhanced spatial learning in mice lacking Shank1[J]. J Neurosci, 2008, 28(7): 1697-1708.

[83] Hambidge GJ. Therapeutic play techniques; symposium, 1954. 4. Structured play therapy[J]. Am J Orthopsychiatry, 1955, 25(3): 601-617.

[84] Leblond CS, Heinrich J, Delorme R, *et al*. Genetic and functional analyses of *SHANK2* mutations suggest a multiple hit model of autism spectrum disorders[J]. PLoS Genet, 2012, 8(2): e1002521.

[85] Guerney BJ. Filial therapy: description and rationale[J]. J Consult Psychol, 1964, 28: 304-310.

[86] Smith CS, Hung LC. The influence of eastern philosophy on elder care by Chinese Americans: attitudes toward long-term care[J]. J Transcult Nurs, 2012, 23(1): 100-105.

[87] Dp Kiran S, Vithalani A, J Sharma D, *et al*. Evaluation of the efficacy of play therapy among children undergoing dental procedure through drawings assessed by graphological method: a clinical study[J]. Int J Clin Pediatr Dent, 2018, 11(5): 412-416.

[88] Wang XM, McCoy PA, Rodriguiz RM, *et al*. Synaptic dysfunction and abnormal behaviors in mice lacking major isoforms of Shank*3*[J]. Hum Mol Genet, 2011, 20(15): 3093-3108.

[89] Kleijer KTE, Schmeisser MJ, Krueger DD, *et al*. Neurobiology of autism gene products: towards pathogenesis and drug targets[J]. Psychopharmacology (Berl), 2014, 231(6): 1037-1062.

[90] Song JY, Ichtchenko K, Südhof TC, *et al*. Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses[J]. Proc Natl Acad Sci U S A, 1999, 96(3): 1100-1105.

[91] Varoqueaux F, Jamain S, Brose N. Neuroligin 2 is exclusively localized to inhibitory synapses[J]. Eur J Cell Biol, 2004, 83(9): 449-456.

[92] Makowiak M, Mordalska P, Wędzony K. Neuroligins, synapse balance and neuropsychiatric disorders[J]. Pharmacol Rep, 2014, 66(5): 830-835.

[93] Budreck EC, Scheiffele P. Neuroligin-3 is a neuronal adhesion protein at GABAergic and glutamatergic synapses [J]. Eur J Neurosci, 2007, 26(7): 1738-1748.

[94] Jamain S, Quach H, Betancur C, *et al*. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism[J]. Nat Genet, 2003, 34(1): 27-29.

[95] Xu XJ, Xiong ZM, Zhang LS, *et al*. Variations analysis of *NLGN3* and *NLGN4X* gene in Chinese autism patients [J]. Mol Biol Rep, 2014, 41(6): 4133-4140.

[96] Blundell J, Blaiss CA, Etherton MR, *et al*. Neuroligin-1 deletion results in impaired spatial memory and increased repetitive behavior[J]. J Neurosci, 2010, 30(6): 2115-2129.

[97] Dahlhaus R, Hines RM, Eadie BD, *et al*. Overexpression of the cell adhesion protein neuroligin-1 induces learning deficits and impairs synaptic plasticity by altering the ratio of excitation to inhibition in the hippocampus[J]. Hippocampus, 2010, 20(2): 305-322.

[98] Chih B, Afridi SK, Clark L, *et al*. Disorder-associated mutations lead to functional inactivation of neuroligins[J]. Hum Mol Genet, 2004, 13(14): 1471-1477.

[99] Chubykin AA, Atasoy D, Etherton MR, *et al*. Activity-dependent validation of excitatory versus inhibitory synapses by neuroligin-1 versus neuroligin-2[J]. Neuron, 2007, 54(6): 919-931.

[100] Gatto CL, Pereira D, Broadie K. GABAergic circuit dysfunction in the Drosophila Fragile X syndrome model[J]. Neurobiol Dis, 2014, 65: 142-159.

[101] Bishop DVM, Scerif G. Klinefelter syndrome as a window on the aetiology of language and communication impairments in children: the neuroligin-neurexin hypothesis [J]. Acta Paediatr, 2011, 100(6): 903-907.

[102] Yan J, Noltner K, Feng JN, *et al*. Neurexin 1alpha structural variants associated with autism[J]. Neurosci Lett, 2008, 438(3): 368-370.

[103] Etherton MR, Blaiss CA, Powell CM, *et al*. Mouse neurexin-1alpha deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments[J]. Proc Natl Acad Sci U S A, 2009, 106(42): 17998-18003.

[104] Venkataraman GR, O´Connell C, Egawa F, *et al*. DE novo mutations in autism implicate the synaptic elimination network[J]. Pac Symp Biocomput, 2017, 22: 521-532.

[105] Barbosa AC, Kim MS, Ertunc M, *et al*. MEF2C, a transcription factor that facilitates learning and memory by negative regulation of synapse numbers and function[J]. Proc Natl Acad Sci U S A, 2008, 105(27): 9391-9396.

[106] Pfeiffer BE, Zang T, Wilkerson JR, *et al*. Fragile X mental retardation protein is required for synapse elimination by the activity-dependent transcription factor MEF2[J]. Neuron, 2010, 66(2): 191-197.

[107]Cole CJ, Mercaldo V, Restivo L, *et al*. MEF2 negatively regulates learning-induced structural plasticity and memory formation[J]. Nat Neurosci, 2012, 15(9): 1255-1264.

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专 家 简 介



唐亚平，1982年毕业于西南医科大学（原泸州医学院）临床医学系，获医学学士学位；1989年毕业于上海交通大学（原上海第二医科大学），获医学硕士学位。于1992年获得日本文部省奖学金后赴日留学，并于1998年获日本国立名古屋大学医学博士学位。先后在美国普林斯顿大学（博士后），芝加哥大学（助理教授/博导），LSUHSC 医学中心（副教授/博导），美国退伍军人事务局（主任研究员/博导）任职。于2015年回国加入广州市妇女儿童医疗中心，负责组建广州儿科研究所。长期从事神经生物学，行为遗传学，和干细胞生物学的研究工作，在鼠类行为遗传学和学习记忆的神经生物学领域属世界知名学者 。获美国“老年性痴呆病基金会”青年学者奖，美国“神经精神病学基金会”青年学者奖，中国科学院王宽诚教育基金会科研奖等，担任 *PLoS ONE* 和中国现代神经疾病杂志编委；*Science*、*Nat Neurosci*、*Neuron*、*PNAS*、*Mol Psychiatry* 等期刊的审稿人；和 *Alzheimer's Disease Foundation*（美国）、*NARSAD*（美国）、*Philip Morris External Research Foundation*（美国）、*BBSRC*（英国）等基金组织（会）的评审人。已获美国和中国各种科研基金20余项，总金额近10,000万元人民币。已发表SCI论文六十余篇，其中包括世界顶尖级刊物 *Nature, Science, Cell, PNAS, Neuron, Nat Neurosci*，Nat Med 等，其总影响因子已近380分，总引用次数已近7,000次。以第一作者发表在英国《自然》杂志（1999年）的科研成果被美国《时代》周刊评 为1999年度全世界10个最重大科学成果的第四位。目前担任中国神经学会儿童认知与障碍分会副主任委员，广东省医学会精准医学与分子诊断分会副主任委员，中国医师协会儿童健康专业委员会第一届全国儿童肥胖防治学组副组长 和中国妇幼保健协会妇幼健康和精准医学专业委员会副主任委员。