

Synaptic Dysfunction Attributes to Autism Spectrum Disorder

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ABSTRACT

Autism spectrum disorders (ASD) are a range of high prevalent disorders that can be characterized as a syndrome of social communication deficits, repetitive behavior or restrictive interest. Rapid advance beyond environmental influence is made through identifications of associated genetic variations. Emerging evidence from human and animal models demonstrate impairment in synaptic function is an etiology of ASD. This review focuses on several synaptic proteins that are mutated in some patients and their autistic behaviors and cognitive deficits are recapitulated in animal models.

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KEY WORDS: ASD, synapse, mutation, neurexin, neuroligin and SHANK.

INTRODUCTION

Autism, Asperger's syndrome and other related conditions comprise autism spectrum disorder (ASD). ASD is a developmental brain disorder and the disease may slightly differ in individual symptoms, but all share characteristic problems in personal and social communications, stereotyped and repetitive behaviors and narrowed interest. Autism itself is usually associated with language and intelligence deficits. ASD is reported to have a high prevalence rate. A study in the general Korean population showed a prevalence of 3.74% in males and 1.74% in females,¹ whereas Central for Disease Control reported in 2006 the average prevalence for ASD in the USA is 1 in 110 (<http://www.cdc.gov/mmwr/preview/mmwrhtml/ss5810a1.htm>). Monozygotic twin studies have demonstrated greater than 90% of genetic heritability for ASDs.^{2,3} This gives a very strong genetic inheritance over the environmental effect on the disease etiology. ASD is mostly diagnosed between two to three years age, a period that

human brain undergoes active remodeling and synaptogenesis. Recent advance in this field has pointed synaptic dysfunction as one of etiology of ASD. This review will briefly describe these mutations found in synaptic component proteins and the associated dysfunctions in the synapse.

PATHOLOGY IN DEVELOPMENTAL BRAIN

Most brain disorders have their characteristic brain pathology. For instance, Alzheimer's disease is associated with neurofibrillary tangles and amyloid plaques. Muscle eye brain disease and Walker-Warburg syndrome have malformation of brain and neuronal migration defects.^{4,5} The structural abnormalities in ASD brains were revealed by magnetic resonance imaging (MRI) studies, which showed an increased brain volume with a peak around 2-4 years of age,^{6,7} followed by a decreased volume at adolescence. Increased cortical thickness was found throughout the brain at 2-4 years of age.⁸ As we know that some neurons and primitive neural circuitries are normally eliminated during the afterbirth period due to synaptic competitions. This failed elimination in ASD patients was found to form atypical connections and result in abnormal function in functional MRI studies.^{9,10} These atypical neural connections may link to the abnormal social communications and repetitive behavior in ASD. At least, there is evidence showing increased dendritic spines in this area of brain.¹¹ However, some questions remain unknown, for example, what atypical neural circuitries are developed during this period and whether there is any neuronal migration defect.

ETIOLOGY OF ASDS REFLECTS SYNAPTIC DYSFUNCTIONS

Perhaps the most exciting discoveries made in recent years are identifications of genetic mutations and genomic deletion associated with ASDs. Over 100 gene mutations or genomic deletions have been identified.¹² Of them, many synaptic gene mutations have been found. Synapse is the fundamental unit of our brain, where external information is processed, amplified and consolidated. It's conceivable that abnormal synaptic functions are found in diseases such as ASD, Rett syndrome, schizophrenia and many other neurological/psychological disorders and cognitive dysfunctions. Many synaptic proteins are found to have mutations in ASD, which includes neurexin,¹³⁻¹⁵ neuroligins,¹⁶⁻¹⁸ voltage calcium channels,¹⁹ cadherin^{20,21} and SHANK3.^{22,23}

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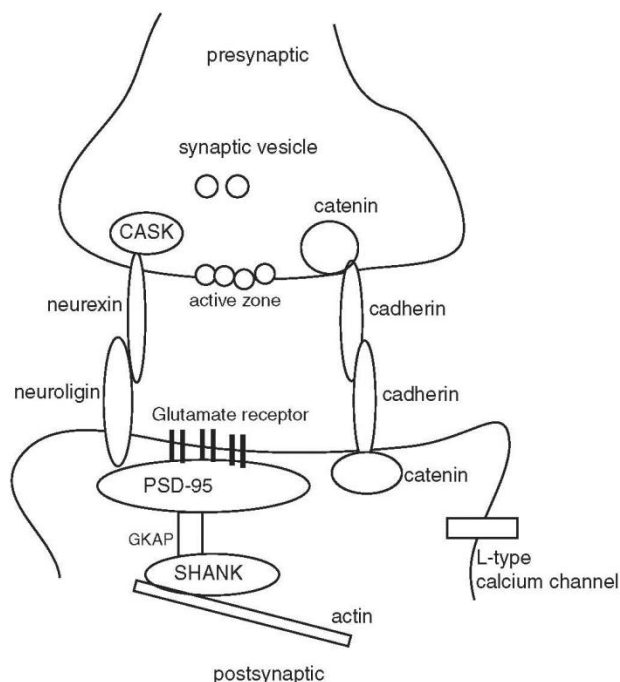


Figure 1. This figure illustrates a synapse within which synaptic components were mutated in some of autistic patients. Neurexin, neuroigin, SHANK, cadherin and L-type calcium channel are all found to bear mutations in some patients. At the synapses, neurexin form trans-synaptic linkage which link CASK to postsynaptic SHANK (including SHANK1, 2, 3) and actin. Cadherin forms homophilic trans-synaptic linkage which connects to intracellular catenin. Glutamate receptors are clustered in the postsynaptic membrane and are inserted in PSD-95 formed postsynaptic scaffolding structures.

NEUREXIN AND NEUROLIGIN

Neurexin is a pre-synaptic transmembrane adhesion molecule, and is connected to postsynaptic membrane through an interaction with neuroigin. Neurexin also connects to CASK intracellularly in the presynaptic terminus whereas neuroigin interacts with PSD95 in the postsynaptic density (**Figure 1**).²⁴ This normal linkage between pre- and post-synapse is crucial to drive normal pre- and post-synaptic differentiation and maturation^{24, 25} in both excitatory and inhibitory synapses. In animal models, neurexin- α null mice show impaired presynaptic voltage-gated calcium channel function, and, thus, suppressed calcium-mediated pre-synaptic vesicle release.²⁶ Analysis in single nucleotide polymorphism (SNP) array in 1200 families identifies two individuals with ASD having deletion in neurexin gene.²⁷ Subsequent studies also identified other neurexin deletions and chromosomal abnormalities in neurexin gene in ASD patients.^{15,28} Most recently, missense mutations in a neurexin family protein CNTNAP2 have also been linked to ASD.²⁹ However, the autistic behavior and cognitive dysfunction

have not been reconstituted in animal models for these neurexin mutations.

Neuroigin is a postsynaptic cell adhesion molecule that promotes both pre- and post-synaptic formations. Five genes encode different neuroigin proteins, and three or more of which are mutated in autism or Asperger syndrome^{16, 18}. Autism mutations of neuroigin decrease its accumulation in postsynaptic membrane and reduce its binding affinity with neurexin.³⁰⁻³² Numerous experiments have demonstrated the critical importance of neurexin-neuroigin linkage. Targeted deletion of either neurexin gene²⁶ or neuroigin gene³³ in mice causes synaptic transmission defects. Especially, in neuroigin knock-out mice, the aggregation and recruitment of glutamatergic, GABAergic and glycinergic receptor to the postsynaptic membrane are altered.³³ Very interestingly, mutations of neuroigin in ASD patients are recapitulated in animal models. R451C knock-in mice, which reconstitute the human ASD neuroigin mutation in mice, have an enhanced inhibitory synaptic transmission and inhibited social communication.³⁴ Moreover, in *Aplysia*, deletion of neuroigin abolishes the long term facilitation, and R451C mutation causes reduced long term synaptic facilitation,³⁵ implicating an impaired memory formation in ASD. Taken together, these studies in human and mice strongly locate ASD to the impaired function of neurexin and neuroigin.

SHANK

SHANK is a postsynaptic scaffolding protein localized in the postsynaptic density (**Figure 1**), which binds directly or indirectly with neuroigin. SHANK forms a huge protein complex with PSD95 and SAPAP between glutamate receptors and actin cytoskeleton (**Figure 1**),³⁶ and serves as a regulator of dendritic spine morphology.³⁷ There are three subtypes of SHANK including SHANK1,2,3. Over-expression of SHANK3 in cultured neurons promotes maturation and enlargement of dendritic spines whereas knock-down of SHANK3 leads to decreased spine formation.^{37,38} The first evidence of involvement of SHANK3 in speech and intellectual deficits comes from chromosomal translocation in 22q13.3 that disrupts SHANK3 gene³⁹. Subsequent analysis demonstrated in some individuals with autistic behaviors that SHANK3 gene is disrupted by micro-insertions within intron 8,³⁹ de novo mutations and two deletions.⁴⁰ In addition, deletions and stop mutation in SHANK2 gene were also found in ASD patients.⁴¹ In a recent mouse model, targeted deletion of SHANK3 gene leads to reduced dendritic spines, abnormal postsynaptic structure and reduced synaptic transmission.⁴² Most importantly, these mice demonstrated autistic behaviors with repetitive grooming and reduced social interactions.⁴² These studies fully establish that disruption in normal SHANK3 function can be the etiology of ASD.

OTHER SYNAPTIC PROTEINS

In synapses, L-type voltage gated calcium channels and cadherin are also associated with ASD. L-type calcium channel mutation G406R is found in cardio-myocytes with

Timothy syndrome, and these patients also show ASD phenotype.¹⁹ Because this channel is also found in neuronal dendritic spines and shafts, studies have shown this type of calcium channel is a regulator of post-synaptic long-term potentiation and synaptic plasticity.⁴³ Cadherins forms homophilic interactions in synaptic clefts to link the pre- and post-synapses (**Figure 1**). They promote synaptic differentiation and maturation, and synaptic plasticity.⁴⁴ Mutations or chromosomal abnormalities in cadherin 9, cadherin 10 and cadherin 15 genes are found in individuals with ASD.^{17,20,45} These mutations destabilize cell-cell connections and impair normal differentiation of synapses, thus, leads to autistic behaviors.

ASD also exists as a comorbid disease with Rett syndrome and tuberous sclerosis. Rett syndrome is caused by mutations in MECP2 gene, a transcriptional regulator. Targeted deletion of MECP2 gene in GABA-releasing neurons leads to reduced inhibitory synaptic vesicle release and demonstrates many characteristics of Rett syndrome and autistic repetitive behavior.⁴⁶ Tuberous sclerosis is caused by mutations in tumor suppressor gene TSC1/TSC2. Data show that, of the 103 patients with tuberous sclerosis, 40% were diagnosed with an ASD.⁴⁷ Tuberous sclerosis forms abnormal tubers in brain, but target deletion of TSC1 and TSC2 shows abnormal dendritic spines and altered shape and size of neurons.⁴⁸ This suggests tubers in brain might not be the cause of autistic behavior but the abnormal morphology of neuron and altered synaptic connections shall be counted as reason for autistic features in tuberous sclerosis.

CONCLUSION

In the past several years, researchers have identified a number of synaptic proteins mutated in autistic individuals. These proteins are functional components of normal synapses. They are either involved in synaptic maturation, synaptic vesicle release, or synaptic stabilization. Most importantly, the typical autistic behavior defects have been recapitulated in animal models that bear these mutations, which confirm the synaptic origin of ASD. The clustering of synaptic genes in ASD may be specific to this disease, but, please keep in mind that over 100 genetic abnormalities were found to give autistic features, only handful are synaptic component proteins or directly involved in synaptic transmission. Moreover, ASD is not merely a single gene disease, multiple gene mutations can be found in one autistic individual.⁴⁹ Further mechanistic studies in ASD will reveal the common pathway for the disease genesis and provide potential therapeutic strategies to the patients.

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