

The Progress of Different Pathways for Shank3 to Cause Autism Spectrum Disorder

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Abstract. Autism spectrum disorder (ASD) is a broad definition of autism based on the core symptoms of typical autism. Statistics show that in every 40 to 59 children, there would be a case of ASD. This severely affects the kid's social acceptability and further development. Although the pathogenesis of ASD is still unclear, it is confirmed that several gene mutations contribute to its symptoms. The Shank3 gene is one of the related genes, and it encodes the multi-domain Shank3 protein, a scaffold protein in the excitatory postsynaptic dense region. The Shank3 gene is widely distributed in the nervous system and plays an important role in maintaining synaptic plasticity. In recent years, researchers have done a large number of experiments as well as investigations about the mechanism of how Shank 3 causes ASD. This paper reviewed some hypothesis of the Shank3 gene mechanisms which leads to ASD, contributes to further understanding and research on the relationship between Shank3 gene and ASD.

Keywords: Shank3, Autism spectrum disorder (ASD), ASD treatment, Homer protein, Haploinsufficiency, mGluR.

1. Introduction

Autism spectrum disorder (ASD) is a complex neuron developmental disorder with a high incidence, which seriously affects adolescents' social, emotional and intellectual development and the quality of life of their families. Its symptoms include abnormal language skills, abnormal communication skills, narrow interests, and stubborn behavior patterns. The cause of autism spectrum disorder has been investigated since 1943. Although the specific genes that cause it are still unsure, family and twin studies have shown that ASD is one of the most inherited neuropsychiatric disorders. According to recent research, there are over 1000 genes that contribute to ASD risk.

The Shank3 gene is one of the members of *the Shank* family. It encodes Shank3 protein that is found in most of the body tissue but mainly in the brain. Shank3 protein is located at the receptor side and roles as a scaffold that supports the synapse functioning correctly. Shank protein is used to connect the neurotransmitter receptors, ion channels, and other membrane proteins to the actin cytoskeleton and G-protein-coupled signaling pathways, so if *the Shank3 gene* mutated, it will impair the function of synapse and signal transportation, that is the potential reason to explain why it related with ASD.

In this paper, we proposed a number of popular hypotheses about the connection between *Shank3* gene mutation and ASD and possible corresponding treatment. Each of the hypotheses is followed by the independent logical chain.

2. Shank3 and Homer protein

The association of Shank3 and Homer protein is one of the hypotheses in the regulation of ASD [1]. Shank3 and Homer are scaffold proteins that are abundant in the postsynaptic density (PSD) region. Together this complex can form a supportive scaffold where other proteins can bind to [2]. It

has been speculated that the ratio of Homer and Shank is essential for the equilibrium in PSD, which stabilizes glutamate receptors [2,3]. Furthermore, Homer and SHANK may assist in regulating synaptic plasticity, which is a cellular learning process in which sensory stimuli strengthen or weaken synapses.

In addition, another study was able to come up with a more specific pathway on the issue. It was found that the lack of Shank 3 caused Homer 1b/c to decrease in the PSD. However, it increased in the soma. Vice versa, mGluR5 increased in the synapse but decreased in the soma. The altered levels of Homer and mGluR5 were distinguished in the basal ganglia nuclei embedded in the striatum, which is a region responsible for learning and memory [4,5]. Regions such as the Neocortex or Hypothalamus, on the contrary, remained normal. In Fig 1 the interaction at synapse level is demonstrated in the mutant mice, but it has remained unknown about the interaction pathway between the cortical-striatal-thalamic axis. The decrease in Homer is associated with instrumental learning, while the increase in mGluR5 is associated with self-grooming behavior. This links those alterations to the phenotypes of autism.

It was confirmed by another study where several PSD proteins such as SAPAP3, Homer, GluR2 were shown to be reduced in striatal synaptic extracts from animals lacking Shank3. Not only were their metabolic changes, but also PSDs thickness and length were reduced [6]. Overall, this alteration shows how disruption of Shank3 can affect many other crucial proteins in the PSD and even cause possible disruption in glutamatergic signalling.

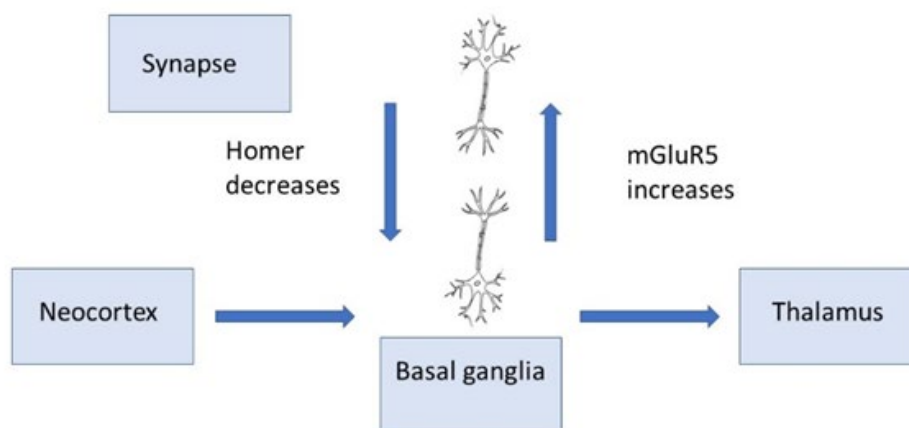


Figure 1. Homer1b/c and mGluR5 redistribution in synapses is aberrant. Increased mGluR5 is associated with the increase of self-grooming. The complicated interactions between molecular alterations are still not understood [1,7].

3. Shank 3 deficiency and indirect pathway

One of the common phenotypes of ASD is repetitive behaviour and involuntary movement. These can be seen in the disruptions of motor processing. The neural circuitry can be divided into direct and indirect pathways. (As shown in Fig 2). Many studies have found differences in the pathway between WT and mutant. Thus, it is important to understand the alteration mechanism to discover new treatments for ASD.

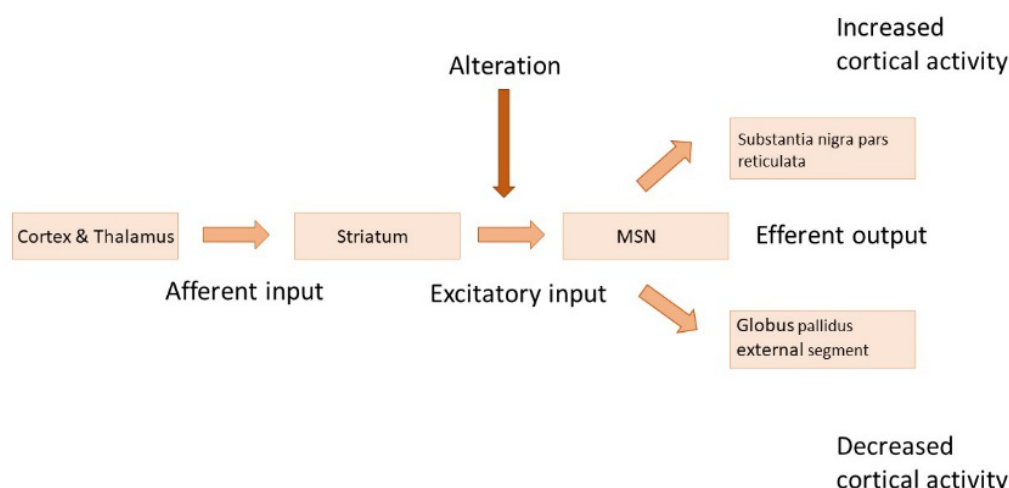


Figure 2. The direct and indirect pathway of neural circuitry. The pathway begins from the cortex and thalamus that will signal afferent input to the striatum region, which will be responsible for facilitating voluntary movements. As the region is activated, excitatory signals will be continued to another inhibitory cell, the medium spiny neurons (MSN). In the MSN, the different output is continuously given to two different regions. Substantia nigra pars reticulata (SNpr), direct pathway and Globus pallidus external segment (Gpe), indirect pathway. The indirect pathway shows decreased cortical activity [8].

The glutamatergic synapse transmissions were decreased in the indirect pathway of the Shank3 mutants by fluorescent proteins signal. This could be due to insufficient afferent stimulation as long-term depression (LTD) was not produced [8]. LTD indicates a decrease in postsynaptic strength and is responsible for motor memory execution [9]. This alteration can be accompanied by a series of other consequences such as decreased number of dendrite spines, a decrease in the number of glutamatergic synapses and inactivity to cortical activity. With these impairments, fewer indirect pathways are activated, and there is less striatal output resulting in more involuntary movements [8].

A similar study arrived at the same conclusion by measuring AMPAR-mediated miniature excitatory postsynaptic currents (mEPSCs). In the Shank3 mutant D1 MSNs in the direct pathway and D2 MSNs in the indirect pathway were both reduced, but only in the indirect pathway, the frequency was reduced. This finding supports the theory that deletion of Shank3 may contribute to a decrease in the number of synapses [10].

Another past research investigated the corticostriate projections to the dorsal striatum as it conveys input to the other basal ganglia structures [11]. It was shown that Substantia nigra pars reticularis (SNpr) is rich in D1 receptors, while the globus pallidus internus (GPi) is rich in D2 receptors. The experiments showed that in ASD, the regulation of striatal neurons by dopamine is disrupted, specifically the *Drd2* mRNA that is expressed in the indirect route. However, the dopamine D2 receptors were not altered, suggesting that changes in mRNA level may not correspond to change in receptors. Changes in mRNA level can only indicate that the GABAergic indirect pathway is altered in ASD [11]. Although this research was not done on Shank 3 deficit subject, this shows an association between ASD and indirect pathway using a dopamine model.

According to the pathway, the treatments can be further designed to be more effective. For example, it has been proposed to selectively enhance the activity of the striatopallidal MSN, which can be activated by the DREADD type of drugs [10]. This would target to reduce the repetitive grooming behaviour. On the other hand, drugs available on the market, such as Risperidone, helps to rebalance serotonin and dopamine levels, which were also disrupted in the GABAergic indirect pathway. Aripiprazole similarly reduces dopamine activity by targeting the D2R receptor to reduce anxiolytic symptoms [11, 12].

4. HCN channel impairment and SHANK3 Haploinsufficiency

Research has suggested that the potential explanation of SHANK3 gene mutation caused by ASD phenotypes is HCN channel impairment.

Previous Reseaches have noted the importance of SHANK3 about ASD shows an estimate of the frequency of haploinsufficiency at the SHANK3 deletions and mutations in ASD through the review of recent studies in ASD that made use of either chromosome microarray or targeted resequencing of SHANK3. A survey of all relevant studies, including negative studies, indicates that at least 0.5% of subjects with ASD have haploinsufficiency at the SHANK3 locus. SHANK3 haploinsufficiency is one of the more prevalent monogenic causes of ASD, explaining at least 0.5% of cases [13].

Other experiments point out the relationship between mutation on SHNAK3 and the basic neuronal electrical property. Then the relation between Shank3-deficient and HCN channelopathy and alterations in intrinsic electrical properties. It is possible that both InsG and complete SHANK3 deletion have a severe impact on the expression of the HCN channel, similar to the detrimental effects on I_h current by exon 13–16 deletion. Since the I_h current plays important physiological roles in the heart and the brain and shows unique biophysical properties, HCN channels are appealing targets for drug development. The drugs that specifically interfere with the function of HCN channels could be effective treatments for certain patients carrying Shank3 [14].

A further experiment on the mice shows Shank3 haploinsufficiency can caused the disorder in synaptic function, social interaction, and social communication. The close correlation between I_h reduction and changes in the AP firing suggests that HCN channels might be an important player in affecting the electrical properties of the Shank3-deficient neurons. Furthermore, the mechanism remains to be determined for the increase in the excitation/inhibition ratio of the Shank3-deficient VB neuron but the close to the normal behaviour of RTN neuron, which should provide new insights at ci3rcuitry level into the Shank3 mutation-related diseases [15].

Following up experiment shows reduced reciprocal social interactions in Shank3 heterozygous mice. Results are consistent with altered synaptic development and function in Shank3 haploinsufficiency, highlighting the importance of Shank3 in synaptic function and supporting a link between deficits in synapse function and neurodevelopmental disorders. The reduced glutamatergic transmission observed in the Shank3 heterozygous mice represents an interesting therapeutic target in Shank3-haploinsufficiency syndromes [15].

The research about the relationship between SHANK3 haploinsufficiency and I_h current point out that mutant SHANK has caused the impairment of HCN channel and that lead to the genotype and phenotype of ASD [16] SHANK3 protein interacted with hyperpolarization-activated cyclic nucleotide-gated channel proteins (HCN proteins) forming I_h-channels, indicating that SHANK3 functions to organize HCN-channels. Data suggest SHANK3 mutations predispose to autism, at least partially, by inducing an I_h-channelopathy that may be amenable to pharmacological intervention [16].

According to the Chinese researchers' experiment from people, they analysed 22q13 deletions and SHANK3 small mutations. They performed genotype-phenotype analysis to determine whether neurological features and other important clinical features are responsible for haploinsufficiency of SHANK3 [17].

Further treatment experiments proposed active compounds were evaluated for efficacy in correcting dysfunctional networks of neurons differentiated from individuals with deleterious point mutations of SHANK3. Among 202 compounds tested, lithium and valproic acid showed the best efficacy at corrected SHANK3 haploinsufficiency associated phenotypes in cellulo. Lithium pharmacotherapy was subsequently provided to one patient and, after one year, an encouraging decrease in autism severity was observed. This demonstrated that pluripotent stem cell-derived neurons provide a novel cellular paradigm exploitable in the search for specific disease-modifying treatments [18].

5. One gene dosage-sensitive synaptic pathway of *Shank3* gene

For this hypothesis, we would talk about how the mutation of a single copy of the *Shank3* gene shed light on one gene dosage-sensitive synaptic pathway, which causes autism spectrum disorder.

Previous research has done the standard karyotype analyses, which suggests that there are chromosomal rearrangements in 3%–6% of the cases. The most common deletions and duplications are shown to be on chromosomes 15q, 22q and 7q. Among these sites, 22q13.3 micro-deletion syndrome is one of the most common rearrangements associated with cognitive deficits. Its characters include neonatal hypotonia, overall developmental delay, language loss to severe delay, autistic behavior, and mild malformation features. However, the loss of terminal 22q13.3 cannot be detected by routine chromosome analysis, which requires FISH (Fluorescence in situ hybridization) to confirm the presence of this deletion [20].

SHANK3 gene is considered to be the main organizer of postsynaptic density (PSD) due to its ability to form multi-molecular complexes with dendritic spines and postsynaptic receptors, signalling molecules, and cytoskeletal proteins in the PSD. There is research investigated chromosome 22q13 and *Shank3* in patients with ASD by FISH. It gave positive results with removing the intron 8 of *SHANK3*, which supports a structural substrate for the formation of the inappropriate telomere. In addition to that, insertion of a guanine nucleotide in exon 21 is shown in two brothers with autism who were heterozygous. The guanine insertion conducts a frameshift at nucleotide 3680, which modifies the C-terminal sequence of the protein. This region is crucial for the binding of mGluR to actin and synaptic targeting and postsynaptic assembly of *Shank3* polymers. This affected the polymerization and depolymerization of actin filaments, the bunching and cross-linking of filaments, and further affected the maintenance of cell morphology, cell movement and many other biological functions [21, 22].

Another research provides further explanation about haploid insufficiency for the *Shak3* gene, which causes autism spectrum disorder. A deletion on chromosome 22q, which starts from the third intron of *Shank3*, was found in a patient. And by comparison with other individuals, it is pointed out that the local dendrite translation of neurons and synaptic proteins plays an important role in the formation and function of synapses, which is often found to be disrupted in ASD patients. In addition, in ASD-associated fragile X syndrome, abnormal dendritic mRNA translation leads to changes in synaptic function and plasticity. This has been clarified to impact synaptic number and function, neuronal morphology and intrinsic neuronal properties, which are associated with language and learning disorders in ASD patients [23].

Multiple research done in another survey suggests that patients have the mutation on chromosome 20q13.33, which has impacts on several kinds of functioning proteins: proteins response for potassium channels, which play an important role in neuronal excitability, and has associations with delayed psychomotor development or mental retardation; *CHRNA4*, which encodes a nicotine-acetylcholine receptor that mediates rapid synaptic signalling—the mutation appears to cause epilepsy at nocturnal frontal lobe; *MYT1*— a myelin transcription factor that is highly expressed in the developing brain; and *OPRL1*, codes for opiate-like G-protein coupled receptors, which is thought to be involved in the regulation of instinct and emotional behavior.

In summary, *Shank3* promotes the formation, maturation, and enlargement of dendritic spines, which requires further clinical experiments and surveys to complete its causes with an autism spectrum disorder.

6. Conclusion

ASD is one of the complex neuron development disorders, it has a wide range of patients worldwide, and it is getting more popular in the view public. Not just because patients will face a significant challenge on social interaction and conversation, but the data shows ASD is more likely caused by genetic inheritance and the harmful chemicals ingesting during pregnancy. ASD include many types of disorder like autism, Asperger's Syndrome etc.

As it is unsure what gene causes ASD, there is an urgent need to research candidate genes and their pathways. The Shank3 gene encodes for the Shank3 scaffold protein, secures different receptors and ensures that the synapse functions normally. Indeed, many ASD phenotypes show deficits in signalling transportation that has been associated with repetitive and antisocial behaviors. Although we cannot conclude whether Shank3 mutation causes ASD as one of the main candidate genes, this gene shows great potential in the field of ASD research.

There are many potential pathways proposed on how Shank3 is related to ASD. As autism has several phenotypes, theoretically, different pathways could contribute to those different phenotypes. However, signalling imbalance in PSD seems to be a prominent theory in most studies. Near all of the hypotheses shown that the Shank 3 mutation alters other protein sequences such as Homer scaffold protein, HCN integral membrane protein, mGluR receptor protein and other synaptic proteins. On the other hand, the theory of the indirect route suggests that the weakening of the glutamatergic synaptic transmission in the indirect route contributes to decreased number of glutamatergic synapses, leading to ASD symptoms. This is linked to the alteration in the signalling pathway rather than a functional deficiency in a specific protein. Treatment wise, dopamine and glutamate receptors are often targeted to rebalance the disrupted signalling. New drugs such as DREADD are still in the process of development as the long-term effect remains unknown. Other genetic research showed Haploinsufficiency of Shank3 caused Ih current impairs in the neuron, which leads to the further damage of the synapse and those impairments have caused ASD. When the Ih current is inhibited, neurons have shown similar phenotypes as those in the ASD patient. This haploinsufficiency has been shown to cause further damage in dendrite translation, interfering with the whole synaptic functioning, including changes in the synapse number.

To better understand the relationship between Shank3 and ASD, many questions are still not answered. It is unknown what kind of alteration Shank3 mutation does on the MSN and how can this be prevented clinically? Or what are the synapse interactions in the cortical-striatal-thalamic axis in the mutant? To reach a solid conclusion for finding new drugs for treatment, an enormous sample would have been required along with highly controlled variables. This remains to be one of the issues in the ASD research as it lacks definiteness in diagnosing the disorder due to its overlap with other disorders such as epilepsy or fragile x syndrome. Furthermore, Shank3 has been shown to alter several different synaptic processes, which means all patients with Shank3 impairment likely can not be treated with one standard treatment. Some of the research has proposed the general direction for the medicine certain Shank3 mutant like medicine aims to repair HCN channel impairment from the mutant of Shank3 haploinsufficiency. Further investigation and research should be carried out on more possible relationships between different types of mutant Shanks gene and ASD and the improvement of treatments for the patients.

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