ASD Pathologies and Potential Treatments Involving SHANK3

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Abstract: Autism Spectrum Disorder (ASD) is a pervasive developmental disorder that has effects on social interactions, communication, and creates repetitive patterns of behaviour activities and interests. It is acknowledged that about seventy million people on Earth have Autism. Its importance is therefore very explicit. However, there is still a lot that we don't know about this widely effective disease. Past studies and treatments have mainly focused on the physical features of autism. But it wasn't until recent years that we started to focus on the genes causing this disease. And it turns out that genes are a very important factor in the pathogenesis of this disease, making the studying of genes affecting autism extremely significant. Autism is a strong gene-related neurological disorder with high genetic heterogeneity. SHANK3, a kind of Genetic Association-Syndromic-Genetic Association-Rare single gene variant, is strongly related to autism and its function, relation in autism and treatments related to it will be discussed.

1. Introduction

Autism Spectrum Disorder (ASD) is one of the genetic and mental diseases, which becomes more common to see during recent years. Since the increase of patients, this disorder gets much more attention from the public, but it is still hard to completely understand the causes in molecular and symptoms of this disorder. Over the years, researchers and therapists are trying to treat autism in many ways, but they actually mainly focus on behavioral treatments, instead of genetic treatments due to the limit of technology. Since the emergence and popularization of gene sequencing methods in recent years, we are beginning to understand autism from a genetic perspective and find the methods that can fix the genetic problems. Through the research, people found that more than 1255 genes cause autism Spectrum Disorder (ASD), and the SHANK3 gene is one of them that we mainly focus on. It is typical and common. This scientific paper revolves around Autism Spectrum Disorder (ASD) and talks about the basic information of this disorder, such as the symptoms, gender differences, and the alternations in the brain. Also, the gene analysis includes candidate genes classification, basic connection and mutation are shown. Lastly, the treatments in behavioral and genetic are discussed at the end of the paper.

2. The Profile of ASD

2.1 Basic Introductions involving symptoms

Autism is a pervasive developmental disorder that has effects on social interactions communication and creates repetitive patterns of behavior activities and interests. Symptoms include increased sensitivity to loud noises and bright lights, repetitive behaviors, sleeping issues, and epilepsy, and social interaction and communication problems (decrease in sociability) [1]. Autism is closely related to genetics, which is suggested by family and twin studies done. The possibility of

developing autism is 50 to 100 times greater for siblings of autistic individuals than for the general population. Twin studies have shown a higher concordance for monozygotic than for dizygotic twins, indicating that genetics is an important factor in autism [2].

2.2 Gender Differences in Autism

A tendency for males to be affected by autism has been discovered. The male to female gender ratio for adults was 1.8: 1, and for all children and adolescents (age=0~18years), it was 3.5 male: 1 female [3], the reason is not yet exactly clear, but there have been some theories about this. Theories suggest that differences between genders in genes, brain structure, and hormones can result in a high ratio of males and females with autism disorder. Gene differences, to begin with, contribute a lot to the gender bias in autism. The female protective effect is what has been contributing to the gender bias in autism through genes. Even though the genes related to autism are carried more by females, females do not tend to show autistic behavior. This phenomenon is known as the female protective effect, meaning that, when a boy and a girl have equal amounts of autistic gene mutations, girls tend to be affected less [4]. Research has shown that the female protective effect is not mediated by a single gene locus but by many gene loci, indicating a greater impact on gender bias [5]. Brain structure also plays an important role in the gender bias in autism. Research has shown that the grey matter significantly increases for both genders. However, males seem to have increased grey matter in the left middle occipital gyrus and right superior temporal gyrus, while females tend to have increased grey matter in the bilateral frontal regions [6]. Autistic females tend to have differences in the bilateral Temporo-parietal Area and increase size in the bilateral Cingulate Gyrus, Inferior Longitudinal Fasciculus, Corpus Callosum, right Arcuate Fasciculus are observed. These changes are not very obvious in autistic males [7]. We do not yet know how these differences relate to autism. Another important component for studying the gender bias in autism is hormones. Oxytocin, a hormone mostly secreted by females, is found to be beneficial to the social deficits of autism by increasing the originally diminished brain activity in the medial prefrontal cortex [8]. The fact that females secrete more Oxytocin, therefore, might also explain the gender bias in autism.

2.3 Alternations in the brains of Autistic

Changes in brain structure caused by autism are found by studies done, including asymmetry of brain serotonin synthesis in the left and right cortex, an enlargement of the hippocampus, increases in brain volume and weight, abnormal connectivity, and changes in the cerebellum, and reduced corpus callosum development [9-14]. The brain serotonin synthesis asymmetry in the autistic, to begin with, affects variable territories, including frontal, temporal, parietal cortices, and occipital cortices. Evidence suggests that serotonin acts as a differentiation factor and its role as a neurotransmitter in the brain, and alteration of serotonin levels during brain development alters neuronal differentiation, suggesting that the different patterns of cortical abnormality in serotonin synthesis relate to the dysfunction in hemispheric specialization in autistic children [10, 15]. The Hippocampus, related to memory and learning [16], is enlarged in autism [11, 13]. This enlargement could therefore have an effect on memory in the autistic. It is also shown that hippocampus enlargement might also be linked to sleeping issues [17] which is also one of the symptoms of autism. The reason behind this, however, is not yet clear. The increase in brain volume in the autistic is related to age. Data has shown an increase in total brain volume in children between 8 to 12 years old, but not those who are above 12 years old. A slight decrease in brain volume of adolescents with autism (as compared with younger children with autism) is also found, while adolescents without autism have been found to have a slight increase in brain volume, suggesting a “normalization” in the autistic in adolescent years. The head circumference has, however, been found to increase at all ages [18]. Also, a study has reported that the brain volume increases rapidly in 2- and 3-year-old children with autism——cerebral cortical gray matter was increased by 12%, cerebral white matter by 18%, and cerebellar white matter by 39% [19]. The effects of the brain increase at an early age in the autistic is not yet clear. It is suggested might reflect an increase in brain cells or brain activity. Many factors characterize the abnormal connectivity and changes in the cerebellum in the autistic. Including a

reduction in size and the amount of Purkinje cells [20]. A reduction in Purkinje cell number would release deep cerebellar nuclei from inhibition, producing abnormally strong physical connectivity and potentially abnormally weak computational connectivity along the cerebellothalamocortical circuit. This pattern of cortical excitation may produce abnormal activity-dependent patterning and may be related to abnormal individual variability in cortical maps for motor function, face processing and overgrowth in frontal lobes [9]. Corpus Callosum in the autistic has been found with the decreased overall size, which is localized to posterior regions. With the posterior regions, known to be where parietal lobe fibers are projected, reduction in size, the idea of parietal lobe involvement is a consistent feature in autism is strengthened [14]. On the other hand, the Corpus Callosum is closely related to the information transmission in the two hemispheres, indicating that there might be a transmission deficiency between the two hemispheres in the autistic, causing spatial and timing problems as a result of the inefficiency of information communication.

2.4 The Pathogenesis of ASD

The pathogenesis in autism is not yet clear, but three main factors have been said to contribute to this disease. The first factor refers to genes. As mentioned before, autism is a neurological disease highly related to genes. Many genes have been deduced to be related to autism: SHANK3, NLGN3, NLGN4, NRXN1, DIA1 and many others [21]. The second factor is the immune system. Many links have been found between the immune system and autism, including alterations in many immune system cells such as natural killer cells. Altered cytokine profiles and immune system-related proteins have also been found. All of them are strong evidence that links autism to the immune system [22]. Despite these factors, there are also environmental factors relating to autism and brain damage could cause autism as well.

3. Gene Analysis

3.1 Classification of ASD Candidate Genes

Table.1. Basu SN, Kollu R, Banerjee-Basu S. AutDB: a gene reference resource for autism research. Nucleic Acids Res [23].

Types	Basic Characteristics	Typical Genes
rAut	Genes implicated in rare monogenic forms of ASD. The mutated types include rare polymorphisms and single-gene disruptions as well as sub-microscopic duplications or deletions encompassing single-genes specific for ASD. rAuts mostly have the strongest association with the disorder.	ADNP, ASH1L, NRXN1, SLC6A1, NLGN3, NLGN4X, SHANK2, SHANK3
sAut	Genes implicated in syndromic forms of autism that the patients developed a specific genetic syndrome.	ASXL3, AGO2, CDK8, MAGEL2, TCF4
iAut	Low-relativity candidate genes with common polymorphisms identified from autistic genetic studies.	KCNQ3, AMPD1, ANK3, ATP2B2, NLGN1
fAut	Candidate genes that have not been covered by the upper three genetic categories but have ASD biologically functional relevance.	SMARCC2, ADORA3, APBB1, CELF4

*One gene is possible to be ranked into more than 1 category depending on the specific mutation and its pathological outcome.

*The listed are the representative related genes of each category.

Autism Spectrum Disorder (ASD) is a strong gene-related neurological disorder with high genetic heterogeneity. According to the statistics from AutDB (Jun 2021), more than 1255 genes were officially regarded as potential ASD candidate genes. Those potential genes were identified from associative genetic studies, single-gene mutations and systematically autism-related mutations. The

classification of Autism-related genes can be mainly divided into 4 categories but not limited to the following types: (Table 1) [23].

3.2 Functioning Connection between SHANK3 and ASD

SHANK3 (SH3 and multiple ankyrin repeat domains 3) is a kind of Genetic Association-Syndromic-Genetic Association-Rare single gene variant, which can be ranged into a kind of rAut. It was first identified as having a strong relationship with autism and similar neurological diseases in 2006. SHANK3's function to encode the scaffolding platform in the postsynaptic density (PSD) was discovered serving to construct the PSD complex and bind neuroligins in the glutamatergic synapses in both mice and humans [24, 25]. Clinical evidence revealed its contribution to ASD related disorders. Up to now, 92 reports involving 30 recent reports have shown its relevance with ASD evidently. Y. Zhou, C.Sala, M. Bidinosti, L. Gouder and many other neural-scientists teams have applied mice, non-human primate, and human-induced pluripotent stem cells (hiPSC) and its derived neurons to trace the SHANK3 knock out factors and succeeding autistic behaviour expressed in different development stages. Future methods to ameliorate SHANK3 deficiency and its autistic related symptoms have been tested in models, like the CLK2 inhibition approach from M.Bidinosti's team, pharmacological enhancement of receptor mGlu5 from C.Vicidomini's team and the Oxytocin treatment which improves electrophysiological deficits from H.Harony-Nicolas' team. New-born and cutting-edge treatment methods are keeping emerging, expected to be implemented in clinical occasions [26-44].

SHANK3gene, consisting of 22 exons, is located on a multi-genic region on chromosome 22. SHANK3 has mainly 7 different interacting domains, ANK, SH3, Proline-rich domain, PDZ, homer-binding region, a cortactin-binding region and SAM domain. Two isoforms and six potential isoforms of it have been identified, playing roles as the alternative protein sequences generated to promote alternative splicing, promoter usages, initiation and ribosomal frameshifting. The most common isoforms are SHANK3α which is the canonical sequence carrying all positional information, SHANK3β, and SHANK3γ, produced by alternative promoter usages. The gene plays a unitary role in encoding the multi-domain scaffold protein, which connects neural transmitters, receptors, ion channels, and anchoring proteins on the cell membrane of the PSD. Signaling molecules are abundant in the PSD, which is a multi-protein complex anchoring on the postsynaptic membrane and they have strong reliability with the SHANK protein family's scaffolding ability. In accordance, their availability is under strict control of the PSD central and its local degradation and synthesis and the actin-dependent dynamic rearrangements within the integral dendritic spines [37]. The SHANK family is responsible for the proper organization and functioning of cytoskeletal and signaling complexes at the synapse junctions [38]. The importance of SHANK3's function can be demonstrated by the mental retardation caused by its haploinsufficiency. The SAM region of SHANK3 also contributes to creating positively charged residues and electropositive potential in the post-synapse region to promote RNA binding. It was reported that the heterogeneous nuclear ribonucleoproteins SHANK3 form with the RNA binding complex, controlling the transcription of a number of genes in the nucleus in primary hippocampal neurons [39, 40]. Additionally, the gene promotes the formation of synapse and dendritic spine maturation. The maturation is crucial to the proper functioning of the peripheral nervous system and muscles [43, 44]. Expression deficiency of SHANK3 may lead to the muscle power decline common in Autistic cases, but the mechanism is still not very clear [41, 42].

3.3 SHANK3 Mutations Participating in ASD

The impact of SHANK3 mutation or expression deficiency will be connected to the typical symptoms of ASD, such as deficits in social communication, delaying language development, repetitive behaviour and limited interests and focus, in accordance with the structural and functional changes within the brain [45, 46]. Recent mice models showed that the knockout effect of the SHNAK3 gene results in the elimination of its isoforms SHANK3α and SHANK3β and the reduction of SHANK3γ in the post-synapse density. The level of the scaffolding proteins it encodes,

including the guanylate kinase-associated protein (GKAP) and other glutamate receptors, are obviously reduced because of its high relativity with the glutamatergic synapses. For the study of the mutant, their dendrites show more arborization and spines and have a lower density than the wild-type [47]. The DNA methylation regulates the mutation expressed in the developing rodent brain in intragenic promoters. As a result, the amplitude and frequency of the excitatory postsynaptic currents are weaker, as observed, affecting the central synapse maturation and may contribute to hypotonia [43, 44, 48]. For macro-structure alternations, the overall size of the brain of the mutant does not have observable changes, but the striatum shows slight enlargement. The behavior alternations can be concluded as a reduction of social activities, the loss of social novelty, excessive grooming and self-injurious behavior as the autistic majority. Further genetic studies had identified the SHANK3 gene to be strongly associated with a range of psychiatric disorders as well as neural-developmental disorders that have highly similar conditions to ASD. 22q13.3 deletion, a developmental disorder, also called Phelan-McDermid syndrome, is caused by the deletion, translocation and other structural changes of chromosome 22, which have been identified located on the exon 21 of the SHANK3 proline-rich domain [45, 46]. This syndrome is usually accompanied by typical autistic behaviour like severe expressive language and speech delay as well as retarded body growth. Moreover, not only the 22q deletion but also the duplication, which will cause the presence of 22q partial trisomy correlated to ASD, mainly exist in Asperger Syndrome. Motor deficiencies, including muscle hypotonia, a syndrome of PMDS caused by the deletion of 22q13.3 and the SHANK3 on it, are also common existed ASD. Autistic exhibits motoric development, motor coordination, motor movement, and stereotypic behaviors [49, 50]. Furthermore, eight non-synonymous mutations in the synaptic SHANK3 gene had also been discovered in ASD patients. The alternations include amino acid deletion in the SH3 domain and missense alternation in the PDZ domain, which is guanine changed into adenine. The mutations, including insertion and deletion, cause a frameshift of the encoded SHANK3 protein. The mutations have an impact on the autistic spine morphology and synaptic transmission. For peripheral loss of SHANK3 protein, the loss of some region-specific functions in the CNS and disfunction of presynaptic receptors in motor neurons in the PNS. Patients expressed infantile hypotonia and growth deficiency. According to the MRI, abnormal intensity in the white matter and thinner gray matter in the frontal lobe had been identified in autistic children cases [39].

4. Treatments of ASD

Despite medical technology is very advanced in modern society, and has been in progress, the autism spectrum disorder still cannot be cured. The treatments are only used to minimize and reduce the symptoms, so that help the patients to get the ability of development and learning [51]. The treatments could be separated into two main parts: behavioral and genetic. Behavioral treatments are used most widely.

4.1 Behaviour Treatments

One of the main treatments is treating by behaviors and communications. The autism spectrum disorder would cause the lack of communication or speaking skills, so some of the programs mainly focus on teaching the patients the suitable behaviors in social situations, and how to communicate with others in a better way [51]. Obviously, the behavioral therapies need to spend much time and repeat the behaviors or words to stimulate the patients' brains. For example, Applied Behavior Analysis is a famous behavioral therapy for autism. It is a proficient therapy that has been used for more than 50 years and always teaches the patients different necessary abilities such as play, self-caring, and social skills by providing a high-structured approach. Meanwhile, it could also reduce problematic behaviors. The therapists of Applied Behavior Analysis usually help children with autism to learn skills by repetition, reinforcement, and encouragement. Also, they will observe the patients and determine the best treatment option. However, for the patients with more severe symptoms, this kind of treatment is very basic, and the therapy should be a long period of progress

which spends about 40 hours per week [52]. The other typical therapy is Verbal Behavior Therapy, which is used to teach the children how to communicate with purposes. The therapists treat the patients by providing stimulus. They choose food, activities, or toys frequently due to their attractiveness to the children. After that, the therapists will encourage the children to understand the importance of communication, to achieve therapeutic effects [52]. Through the complete progress of Applied Behavior Analysis, the patients could learn how to communicate with others and attend social activities.

4.2 The Early Start Denver Model (ESDM)

The Early Start Denver Model (ESDM) is the other famous behavioral treatment. Interestingly, the researchers found that one of the therapies can actually change the patients' brains and then normalize their brain activities. In the other words, this therapy is used to activate the brains by practicing for a long time, since the brain with autism usually is less inactive to human face or social activity than objects [53]. The therapies will try to find children's hobbies and abilities by setting different programs to let patients attend to choose available methods to develop patients' skills [54]. For example, if a patient shows a talent for painting, the therapies will try to use painting to communicate with him/her to improve his/her social ability. According to the amount of research, ESDM has great benefits for early treatment and intervention of autism, although it could not cure the disorder totally as usual. The researchers, Rogers and Dawson observed patients' brains by using EEG, which is the electroencephalogram that could illustrate the brain activity. They found that the children treated with ESDM actually have language skills three times higher than those in the community intervention group, which means that the patients' brains activity becomes active as normal by experiencing this therapy [53].

4.3 Gene Fixing of SHANK3

In recent years, people already understood the molecular mechanisms of autism spectrum disorder, but treating the underlying molecular defect is still a huge question that researchers hard to solve.

Correcting the mutation of the SHANK3 gene can reduce some autistic-like behaviors. New research using the fetal mice to do the experiments shows that correcting a mutation in the SHANK3 gene could reduce some of the autistic-like behaviors after birth [54, 55]. The researchers used a protein, cre-activating protein, to correct the SHANK3 gene mutation. However, this method of this research is actually unstable: after correcting the mutation, the autistic-like behaviors were decreased but not disappeared completely. For example, the mice would become more active in social activities, but they still prefer to interact with objects rather than another mouse. Therefore, maybe fixing the SHANK3 gene mutation could certain extent reduce the symptoms of autism spectrum disorder, but we still need to do more research to cure it. ions when writing Figure axis labels to avoid confusing the reader. As an example, write the quantity "Magnetization," or "Magnetization, M," not just "M." If including units in the label, present them within parentheses. Do not label axes only with units. In the example, write "Magnetization (A/m)" or "Magnetization (A (m (1)," not just "A/m." Do not label axes with a ratio of quantities and units. For example, write "Temperature (K)," not "Temperature/K."

5. Conclusion

Autism Spectrum Disorder affects social interactions, communication and creates repetitive behavior. A gender bias is found in autism, also many physical changes. Another feature of autism is that it is strongly related to genes. Many genes were found to be potential ASD candidate genes. SHANK3 is one of them. Many relations were found between SHANK3 and autism, but still, many questions remain. In fact, we still don't know enough about these genes, and more research has to be done. On the other hand, treatments involving the SHANK3 gene are already raising more questions to answer. More research should be done on the SHANK3 gene treatment and other ASD candidate

genes should be researched as well. Obviously, a lot of research has to be done on this disease and its potential candidate genes, and many questions still remain for us to answer.

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