SHANK3 AND HYPERSENSITIVITY:
THE KNOWN AND THE UNKNOWN

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Abstract

Autism spectrum disorder (ASD) is a very heterogeneous neurodevelopmental disorder on both the genetic- as well as- clinical level. Hyper- and hypoactivity are clinical symptoms commonly seen in ASD patients; affected individuals can respond extremely, either too much or too little, to sensory stimuli. Genetically, ASD has been associated with numerous risk genes, however, one gene has been found to be frequently correlated with hypersensitivity in ASD patients: *SHANK3*. This gene on chromosome 22 codes for the SHANK3 protein that is part of the postsynaptic density (PSD) in which it regulates proper synaptic transmission and thus proper signalling between neurons. It is thought that SHANK3 is mechanistically associated with altered sensory perception. In this review we outline the current knowledge on the gene-to-function correlation between *SHANK3* and the clinical symptom of hypersensitivity.

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by persistent deficits in social communication and interaction, as well as repetitive patterns of behaviour, activities, and interests, present from childhood [1,2]. The prevalence of ASD is estimated to be around 1% although in the Netherlands no quantitative research based on registered diagnoses has been performed [3,4]. Within the diagnosis of ASD there is a wide range of manifestations and severeness of the disorder. This is due to the heterogeneity of the disorder, on both the phenotypic level as well as on the level of the underlying cause. There is consensus on the pathogenesis of ASD, which states that the deficits found in ASD seem to be mainly caused by genetic mutations, leading to developmental alterations in neural connectivity [3,5]. It is found that these genetic mutations can be both inheritable and de novo mutations, and are mostly heterozygous [6,7].

Atypical behavioural responses to sensory information, defined as "hyper- or hypo reactivity to sensory input or unusual interest in sensory aspects of the environment", is one of the impactful and commonly seen symptoms of ASD 1. Abnormalities in sensory sensitivity can possibly lead to abnormal social behaviour and difficulties in daily life. Also, sensory hypersensitivity might play a role in the presence of other ASD-related symptoms, such as learning disabilities, anxiety, attention- and sleep deficits, and hyperarousal [8,9].

Incorrect synaptic transmission is one of the factors that might play a role in sensory hypersensitivity and can be caused by genetic mutations. One of the heterozygous mutations found in ASD leads to a malfunctioning of the SH3 and multiple ankyrin repeat domains 3 (SHANK3) protein. SHANK3 is a postsynaptic scaffolding protein that – together with other proteins – facilitates correct synaptic transmission and development [10,11]. Current literature implies SHANK3 as a risk gene that has been associated with approximately 1% of the non-syndromic ASD patients [12]. Different types of mutations in SHANK3 can be found, relating to different phenotypic outcomes. For instance, some mutations lead to irregular dendritic spine formation of neurons while other mutations lead to a reduction in mGluR5 receptors, which play a role in neurotransmission [13].

SHANK3 contributes to synaptogenesis and proper synaptic transmission. Therefore, it has an impact on the stimulus transmission between neurons, and has a potential correlation to hypersensitivity in which proper synaptic transmission is affected 3. Whether and how the SHANK3 protein and hypersensitivity in ASD are correlated to each other has yet to be determined. Therefore, in this mini-review, we investigate what role SHANK3 plays in hypersensitivity in individuals with ASD.

SHANK3 and the postsynaptic density

The synapse of the postsynaptic excitatory neuron contains several structures to regulate proper neurotransmission (Figure 1). The postsynaptic membrane can contain voltage- and ligand-gated ion channels, and metabotropic receptors to facilitate neurotransmission. Another structure that is part of the synapse is called the postsynaptic density (PSD), which is a dense structure beneath the postsynaptic membrane. Scaffolding proteins in the PSD connect the ion channels and receptors in the postsynaptic membrane to each other, to other membrane components such as adhesion molecules, and to the actin cytoskeleton [14]. A major role for the PSD proteins is to anchor glutamate receptors such as AMPA and NMDA receptors to the postsynaptic membrane [15]. Proper functioning of the hierarchical cascade of scaffold proteins is needed to facilitate neurotransmission, as well as formation, maturation, and maintenance of the synapse [14].

SHANK3, encoded by *SHANK3*, is a synaptic scaffolding protein that can be found in the core of the PSD of excitatory neurons, which thus plays a role in connecting neurotransmitter receptors, ion channels, and other membrane proteins to the actin cytoskeleton or signalling cascades. Each part of SHANK3 has its own protein-protein connection and therefore contributes differently to the regulation of neurotransmission and synapse development. The six ANK repeats make interactions with the actin cytoskeleton, while the SH3 domain interacts with tyrosine kinases, which are part of signalling cascades in the postsynaptic neuron [10,17]. SHANK3 also anchors AMPA- and NMDA- glutamate receptors and metabotropic

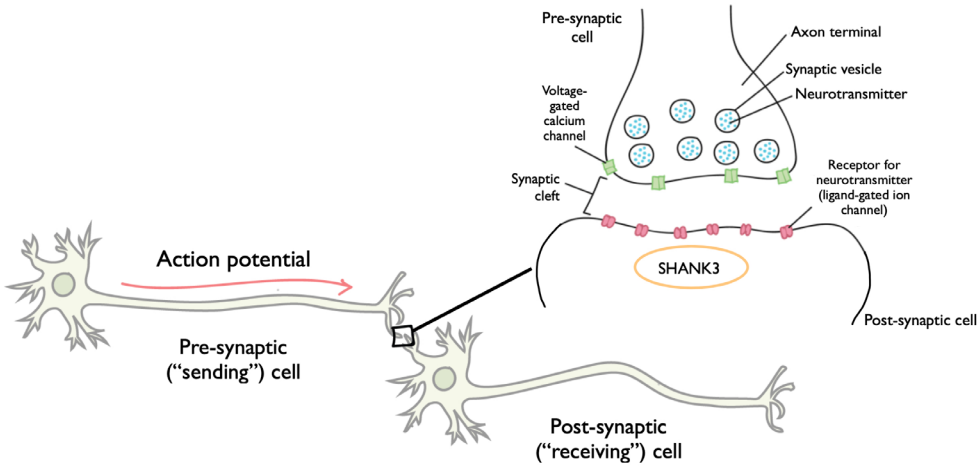

Figure 1: Neuronal cell showing factors contributing to chemical synaptic transmission between two neurons. Location of SHANK3 in the postsynaptic neuron is shown. Figure modified from Khan Academy, 2016 [16]

receptors to the postsynaptic membrane, which is important for chemical synaptic transmission and synapse development (Figure 2) [17,18]. Furthermore, SHANK3 also makes connections with other scaffolding proteins to interact indirectly with receptors, signalling molecules, and the actin cytoskeleton [17].

As SHANK3 has so many different and important functions in the PSD, the protein is often described as the "master regulator" of glutamatergic synapses [15].
Since SHANK3 plays such a fundamental role in coordinating the function and development of excitatory synapses, it seems logical that mutations in SHANK3 impair glutamatergic synaptic structure and function [19].

The history of *SHANK3*

At the tip of chromosome 22 lies the locus for *SHANK3*, more specifically in the 22q13 region. *SHANK3* and its association with ASD go back as far as 1985. However, at that time, scientists did not know it was specifically *SHANK3* that played a part in this. It all started with chromosome 22 instead, as Watt et al.[20] reported the first case of monosomy (absence of one member of a pair of chromosomes)

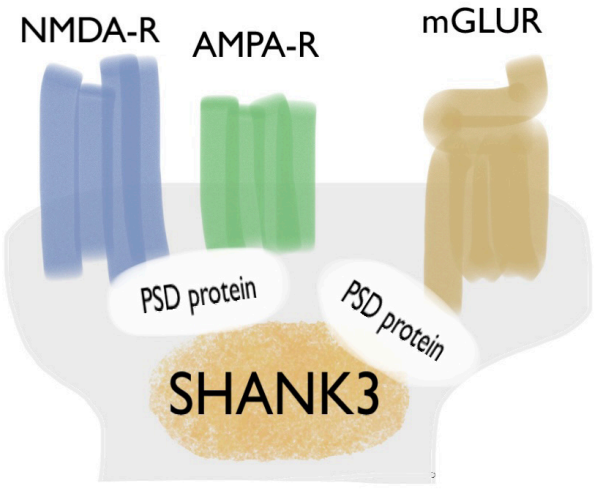

Figure 2: *SHANK3 function in the postsynaptic neuron where it, together with other PSD proteins, binds to the postsynaptic receptors to regulate chemical synaptic transmission.*

for the distal long arm of chromosome 22, currently known as the location for *SHANK3*. The 14-year-old male had developmental delays and absence of speech, features that are currently part of the clinical phenotype of ASD.
In 2001, the discoveries continued when Phelan et al.[21] described features of multiple individuals with a deletion in a specific region of chromosome 22, namely 22q13. The research group compared the reported clinical phenotypes to the features of individuals previously described in the literature. Symptoms such as developmental delay, delayed speech, and hypotonia were commonly present in both groups. Later that year, Bonaglia et al.[22] also described similar features for a 4.5-year-old male with a de novo translocation between 12q24.1 and 22q12.
Scientists unravelled the correlation between *SHANK3* and chromosome 22, more specifically the 22q13 region. Throughout the years, this location has been associated with several similar clinical phenotypes; therefore, it was suggested that a disruption of *SHANK3* could be responsible for several phenotypic features of 22q13.3 deletion syndrome.

Two years later, in 2003, *SHANK3* was identified as the most likely candidate gene for the neurodevelopmental and behavioural impairments in individuals with 22q13.3 deletion syndrome. To date, 22q13 deletion syndrome is known as Phelan-McDermid syndrome (PMDS). It is characterised by developmental delay, hypotonia, delayed development of speech, and autistic behaviours [23]. *SHANK3* mutations have also been identified in non-syndromic ASD, also known as classic ASD [24-26].
As mentioned before, SHANK3 has been associated with approximately 1% of non-syndromic ASD patients [12]. This includes mostly de novo *SHANK3* deletions or mutations, together referred to as SHANK3-deficiency. The loss of one copy (haploinsufficiency) of *SHANK3* is sufficient to cause neurobehavioral symptoms as seen in ASD [27]. Typical behavioural phenotypes like abnormal social behaviour and elevated anxiety, are found in SHANK3-deficient animal models. These characteristics resemble clinical features seen in human ASD patients. Furthermore, altered PSD levels of NMDA and AMPA receptors are also present in SHANK3-deficient animals, which is in line with the previously mentioned function(s) of SHANK3 [11,28-30].

SHANK3 and hypersensitivity

Hyposensitivity and hypersensitivity are common features of both PMDS patients as well as non-syndromic ASD patients [31]. These

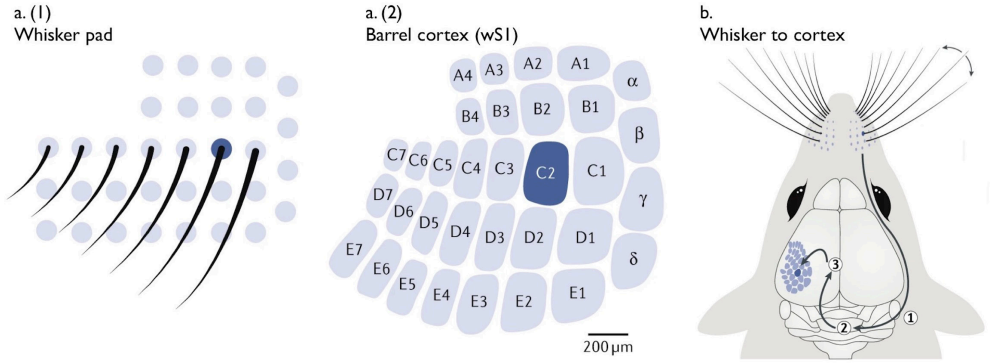

Figure 3: *a) Barrel cortex in S1, every whisker is represented by a specific region in the barrel cortex. Dark blue area around the whisker correlates to the dark blue area in the barrel cortex.*
b) Trajectory from an individual whisker to the thalamus to the barrel cortex in S1.
Figure modified from Petersen., 2019 [38].

individuals, with *SHANK3* mutations, often show an increased pain tolerance and aberrant tactile sensitivity [27]. This led to the suggestion that SHANK3-deficiency may also be correlated with abnormal sensory function in individuals diagnosed with ASD.
To investigate this hypothesis, rodent studies were used as it is to some extent ethically approved to alter the gene expression of rodents. Most studies on SHANK3-deficient mice have been focused on nociception – pain perception. Multiple research groups have reported hyposensitivity to painful stimuli in SHANK3-deficient conditions [32-35].
On the other hand, somatosensory function abnormalities, such as physical touch, have been little investigated in SHANK3-deficient mouse models. Chen et al. [36] were the first research group to investigate this by performance of a vibrissae (whiskers) motion detection task on SHANK3-deficient mice.
The researchers focused on the vibrissae since the neural trajectory from the vibrissae to the brain is well-defined. Fibres from a specific nucleus (ventral posteromedial nucleus) in the thalamus project specifically to the barrel region (representative sensory location of the whiskers) of the primary somatosensory cortex (S1) [37]. The S1 has a somatotopic map where each whisker is represented as a barrel which allows for precise activation of each whisker (Figure 3) [38].
During the task, an individual whisker was stimulated with different electrical intensities. The mouse was taught to lick his paws when he could detect, thus feel, the electrical shock. SHANK3-deficient mice licked their paws more often to weaker electrical stimulation than wild type mice without a SHANK3-deficiency. This suggests that a SHANK3-deficiency is associated with increased sensitivity to physical touch, also referred to as hypersensitivity.Chen et al. [36] also investigated the underlying neural mechanism of hypersensitivity in the SHANK3-deficient mice by performing calcium imaging on the neurons in the barrel region of S1. Calcium imaging relies on the calcium concentration inside the presynaptic terminal after an action potential (AP) has depolarized the synapse. Calcium in the presynaptic terminal controls the release of neurotransmitters, and therefore the firing activity between two neurons [39].
After the whisker was stimulated with a set electrical intensity, the calcium concentration inside the neurons of the corresponding barrel region was measured. Glutamatergic excitatory neurons of the SHANK3-deficient mice showed an increased firing pattern compared to the wild type mice. While the GABAergic interneurons of the SHANK3-deficient mice showed a decreased firing pattern compared to the wild type mice.
The altered excitatory and inhibitory neural activity in the SHANK3-deficient mice comes as no surprise. Many neurodevelopmental disorders such as ASD, epilepsy, and intellectual disability have been associated with an excitatory versus inhibitory imbalance, as it is thought to contribute to the pathophysiology of these types of disorders. Normally, excitatory and inhibitory neurons work in harmony where excitatory neurons activate the inhibitory neurons, and inhibitory neurons keep the excitatory neurons from firing too strongly. In neurodevelopmental disorders, however, a disturbed excitatory versus inhibitory balance usually means reduced activity of inhibitory neurons which leads to reduced inhibition of excitatory neurons, hence overexcitation [40,41].
The current theory states that SHANK3 expression is probably reduced in excitatory neurons of individuals with SHANK3 mutation(s). Since decreased expression of SHANK3 leads to reduced activity of the excitatory neurons, it cannot properly activate inhibitory neurons as it would normally do. As previously mentioned, inhibitory neurons attenuate the firing activity of excitatory neurons. However, as the inhibitory neurons are hypoactive, the excitatory neurons are overly active leading to overexcitation, which is thought to underlie the hypersensitivity in SHANK3-deficient mice [36].
Overall, these findings provide evidence that SHANK3-deficiency results in hypoactive inhibitory neurons causing decreased inhibitory control over excitatory neurons, hence hyperactivity.

Conclusion

Hypersensitivity is one of the most impactful symptoms commonly seen in ASD. Recently, *SHANK3* has been found to be of importance in the research on somatosensory hypersensitivity in ASD patients. According to the conducted research, a SHANK3-deficiency likely leads to decreased inhibitory control over the excitatory neurons, leading to an overexcitatory network in the brain, which is thought to underlie hypersensitivity. However, the research on *SHANK3* and its correlation to hypersensitivity is still in its infancy. Therefore, the pathway involved in the overexcitation contributing to hypersensitivity remains unclear. Future research could focus on the pathway from the thalamus to S1 as somatosensory stimuli are processed following this trajectory. Given the high prevalence of hypersensitivity in individuals with ASD and the urgency of unravelling the underlying cause of this phenomenon, it is of high importance to study this concept further.

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