SHANK proteins: roles at the synapse and in autism spectrum disorder

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Abstract | Several large-scale genomic studies have supported an association between cases of autism spectrum disorder and mutations in the genes SH3 and multiple ankyrin repeat domains protein 1 (SHANK1), SHANK2 and SHANK3, which encode a family of postsynaptic scaffolding proteins that are present at glutamatergic synapses in the CNS. An evaluation of human genetic data, as well as of in vitro and in vivo animal model data, may allow us to understand how disruption of SHANK scaffolding proteins affects the structure and function of neural circuits and alters behaviour.

During development and throughout life, a number of dynamic processes regulate the number, size, shape and strength of neuronal synapses. These changes occur through alterations in the molecular composition of synapses and through the chemical modification of synaptic proteins\(^1,4\). However, sometimes, the genes encoding these synaptic proteins (such as those that encode SH3 and multiple ankyrin repeat domains protein 3 (SHANK3), neurexin 3, neurexin 4 or neurexin 1) carry particular mutations that result in the production of dysfunctional proteins that cannot fulfil their synaptic role. The consequences of such synaptic mutations often affect both protein structure and function, leading to synaptic and circuitry defects that may have a neurodevelopmental and neuro-psychiatric impact.

Mutations in the SHANK (also known as ProSAP) family genes have been linked to syndromic and idiopathic autism spectrum disorder (ASD), as well as to other neuropsychiatric and neurodevelopmental disorders (schizophrenia and intellectual disability)\(^5,6\). In mice, mutations in the genes encoding SHANK family proteins (SHANK1, SHANK2 and SHANK3) often result in marked behavioural phenotypes. These include an increase in repetitive routines, altered social behaviour and anxiety-like phenotypes, seemingly similar to those described in some human neuropsychiatric disorders\(^7\).

Despite great research progress in establishing links between mutations in SHANK genes and ASD, the physiological role of SHANK proteins has, until recently, been poorly understood. Here, we review the most recent studies that help to clarify the roles of SHANK proteins at the synapse, providing insightful mechanistic links to neuropsychiatric disorders.

The SHANK protein family

SHANK proteins are ‘master’ scaffolding proteins that tether and organize intermediate scaffolding proteins. They are located at excitatory synapses, where they are crucial for proper synaptic development and function\(^1,10\).

In the mouse, SHANK proteins are encoded by Shank1, Shank2 and Shank3 genes. Downstream from their transcription start sites, all three genes have alternative promoter options, resulting in the generation of a wide array of mRNA transcripts and protein isoforms.

SHANK3. SHANK3 (also known as ProSAP2) is the best studied of the three SHANK protein family members. The Shank3 gene is located on mouse chromosome 15E3 (human location: 22q13.3), has 22 exons and spans 60 kilobases of genomic DNA (FIG. 1). Shank3 has multiple intragenic promoters (probably six)\(^11,12\) and several alternative splicing exons (exon 11, exon 12, exon 18, exon 21 and exon 22)\(^15\), resulting in the possible generation of several protein isoforms (see Supplementary information S1 (figure)). Besides alternative promoter usage and mRNA splicing, Shank3 gene expression is regulated by epigenetic mechanisms such as DNA methylation and histone acetylation, causing tissue-specific expression of different SHANK3 isoforms\(^11,15\).

The full length structure of mouse Shank3 contains six domains for protein–protein interactions: according to information contained in the databases UniProt Knowledgebase (UniProtKb) and National Center for Biotechnology Information (NCBI) database of the National Library of Medicine at the US National Institutes of Health (see Further information). These domains comprise protein domain of unknown function 535 (DUF535), ankyrin repeat domain, SRC homology 3 (SH3) domain superfamily, postsynaptic density...
Epigenetic mechanisms
A mechanism of a stable change in gene expression that does not involve changes in DNA sequence.

SHANK2. Shank2 (also known as ProSAP1) is the largest gene among Shank gene family members and is located on mouse chromosome 7F5 (human location: 11q13.2). It has 25 exons and spans about 450 Kb of mouse genomic DNA (FIG. 1). SHANK2 contains five domains: ankyrin repeat domain (located at the N terminus), SH3, PDZ, PRO and SAM (located at the C terminus) (FIG. 1). There are three isoforms of SHANK2 that are produced by alternative promoter usage: SHANK2E (containing all five protein domains), SHANK2A (lacking the ankyrin repeat domain) and SHANK2C (lacking the ankyrin repeat domain and the SH3 domain) (see Supplementary information S2 (figure)). Another isoform, SHANK2B, is transcribed from the same intragenic promoter as SHANK2A but shows alternative splicing. SHANK2E is the longest isoform of SHANK2 and is the only isoform that contains the ankyrin repeat domain (which is thought to coordinate actin-dependent events at the apical membrane of epithelial cells23). SHANK2E owes its name to the fact that it was initially thought to be expressed only in epithelial cells24; however, recent data suggest that SHANK2E is also expressed in human and mouse brain tissue, with high expression levels in the cerebellum25,26. Like SHANK3 and SHANK1, SHANK2 is a scaffolding protein of the PSD that interacts with multiple partners at the synapse20,24.

SHANK1. Shank1 is located on mouse chromosome 7B4 (human location: 19q13.33), has 24 exons and spans about 50 Kb of genomic DNA (FIG. 1). The gene contains two different promoters that can generate two different protein isoforms: SHANK1A (the longest isoform, which contains ankyrin repeat domain, SH3, PDZ, PRO and SAM domains) and SHANK1B (which contains PDZ, PRO and SAM domains) (see Supplementary information S2).
Shank genes and SHANK3 isoforms. The expression pattern of the three members of the SHANK protein family is slightly different in the rodent brain. For example, SHANK3 is the only family member that is highly enriched at corticostriatal glutamatergic synapses. Shank1 mRNA seems to be exclusively expressed in the brain and is particularly enriched in the cortex, thalamus, amygdala, hippocampus (CA1 and CA3), dentate gyrus and in cerebellar Purkinje cells. Shank2 mRNA is enriched in the cortex, thalamus, hippocampus (CA1 and CA3), dentate gyrus and in Purkinje cells. It is also expressed in the kidney and liver, at lower levels. Shank3 mRNA is highly expressed in the heart and moderately expressed in the brain and spleen. In the brain, Shank3 mRNA is enriched in the cortex, thalamus, striatum, hippocampus (more in CA3 than in CA1), dentate gyrus and in cerebellar granule cells. The different SHANK3 protein isoforms are differently expressed according to developmental stage, cell type and brain region (FIG. 2a), suggesting the existence of isoform-specific functions. The different isoforms also present different subcellular localizations. Shank3B (an isoform lacking the PRO and SAM domains) localizes to the nucleus, whereas Shank3A, Shank3C and Shank3E (isoforms containing the PRO and SAM domains) form clusters in the cytoplasm. This data thus suggest that PRO and SAM domains might be important for nuclear targeting of SHANK3 isoforms and that different subcellular localizations might contribute to the existence of isoform-specific functions. An important step for future research would be the generation of isoform-specific antibodies or probes that could enable a systematic analysis of isoform-specific binding partners and isoform-specific brain expression patterns. Understanding SHANK3 protein function from an isoform-specific perspective may also help to explain how different Shank3 gene mutations may result in distinct phenotypic consequences.

**Figure 2** | **Expression patterns of Shank genes and SHANK3 isoforms.**

**a** | The schematics illustrate CNS expression pattern of SH3 and multiple ankyrin repeat domains protein (Shank) genes in the mouse brain. Each Shank gene is indicated by a different colour. Higher colour intensity represents a stronger expression signal (enriched areas). This part is created using data from REFS 9, 11, 31–34. **b** | The table details the expression pattern of different SHANK3 isoforms in the mouse brain. Shank3B is expressed throughout the brain at low levels. Shank3A and Shank3E are enriched in the striatum and are present in the cortex, hippocampus, thalamus and amygdala. Shank3C and Shank3D are mainly enriched in the cerebellum. Shank3F is not represented, as no information on its expression is currently available. Hippocampal neurons strongly express all major isoforms of Shank3, whereas astrocytes express low levels of Shank3A, Shank3C, Shank3D and Shank3E. Isoform expression pattern is extrapolated from REF. 11.
SHANK genes were first implicated in neurodevelopmental disorders by studies of Phelan–McDermid syndrome (PMS), a neurodevelopmental disorder that is caused by a 22q13.3 deletion and is characterized by autistic-like behaviours, hypotonia and delayed or absent speech.\textsuperscript{46–49} Genomic rearrangements in patients with PMS include deletions, ring chromosomes, interstitial deletions and unbalanced translocations\textsuperscript{5,46–49}. \textit{SHANK3} is deleted in nearly all reported cases of PMS\textsuperscript{5,51}, and its disruption is therefore thought to cause the core neurodevelopmental and behavioural deficits that are seen in patients. Supporting the idea that core PMS symptoms are due to \textit{SHANK3} haploinsufficiency\textsuperscript{46}, individuals carrying a ring chromosome 22 in which the \textit{SHANK3} gene is intact are phenotypically normal.

Apart from the syndromic forms of autism (such as PMS, in which \textit{SHANK3} disruption may be responsible for the core phenotype), many \textit{SHANK3} mutations have also been identified in genetic screenings of patients with ASD who have not been diagnosed with PMS\textsuperscript{52–54} (Table 1; see Supplementary information S4 (table)). These mutations include microdeletions, nonsense mutations, breakpoints and missense mutations. In addition, mutations in \textit{SHANK1} and \textit{SHANK2} have also been recently associated with ASD cases\textsuperscript{5,8,12,21,24}. A meta-analysis study found that mutations or disruptions in the \textit{SHANK} gene family account for \textasciitilde1% of all patients with ASD\textsuperscript{5}. There is also a correlation between \textit{SHANK1}–3 mutations and the degree of cognitive impairment: patients with \textit{SHANK3} mutations have more-severe cognitive deficits than those with \textit{SHANK1} or \textit{SHANK2} mutations\textsuperscript{5}. Together these findings indicate that a common neurobiological role, shared by all \textit{SHANK} gene family members, may be of relevance to ASD pathophysiology and that the degree of cognitive impairment in ASD may depend on which \textit{SHANK} family member is mutated. The difference in impairment severity might be explained by the expression pattern of the particular \textit{SHANK} gene that is mutated and by the extent to which the other two remaining \textit{SHANK} members can (or cannot) compensate for its loss. In addition, these findings indicate that mutations in \textit{SHANK} genes are a potential monogenic cause for ASD and indicate that several \textit{SHANK} mutations are of a syndromic nature and should be considered for screening in clinical practice\textsuperscript{5}. It is currently unclear how mutations in the PRO domain of exon 21 might be related to the pathophysiology. One possible explanation is that exon 21 is present in most \textit{SHANK3} isoforms; thus, mutations in this exon are likely to affect the expression and/or function of a large proportion of the \textit{SHANK3} proteins that are produced.

Given the potential importance of these genes in ASD, it is crucial to understand more about their normal roles at the synapse and how they are disrupted by mutations.

### Table 1 | Human \textit{SHANK3} mutations and small deletions

<table>
<thead>
<tr>
<th>Exon and domain affected by mutation</th>
<th>Total number of cases across studies</th>
<th>Diagnoses</th>
<th>Phenotypes and comments</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 21; PRO</td>
<td>1</td>
<td>ASD (7 cases), PDD-NOS (1 case), schizophrenia (3 cases)</td>
<td>• Severe ID (8 cases); mild–moderate ID (3 cases)</td>
<td>5,7, 53,83</td>
</tr>
<tr>
<td>Exon 11; SH3</td>
<td>1</td>
<td>ASD</td>
<td>Patient also carries mutation in \textit{NRXN1} gene</td>
<td>7</td>
</tr>
<tr>
<td>Exon 4; ANK</td>
<td>1</td>
<td>ASD</td>
<td>• Sensitive to touch</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Developmental delay</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Seizures</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Echolalia</td>
<td></td>
</tr>
<tr>
<td>Intron 15–exon 16 boundary</td>
<td>1</td>
<td>Asperger syndrome</td>
<td>Behavioural problems and echolalia at the age of 2 years</td>
<td>7</td>
</tr>
<tr>
<td>Exon 19–intron 19 boundary</td>
<td>1</td>
<td>ASD</td>
<td>Patient ASQ score: 23</td>
<td>6</td>
</tr>
<tr>
<td>Exon 2</td>
<td>1</td>
<td>PDD-NOS</td>
<td>Language deficiency around 18 months of age</td>
<td>6</td>
</tr>
<tr>
<td>Exon 1</td>
<td>1</td>
<td>ASD</td>
<td>• Absence of language and severe ID</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• High pain threshold and low noise threshold</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Impaired non-verbal communication and social interaction</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Motor stereotypes</td>
<td></td>
</tr>
<tr>
<td>Exon 8; ANK</td>
<td>2</td>
<td>ASD</td>
<td>• Repetitive behaviours and circumscribed interests</td>
<td>52,53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Generalized hypotonia and brisk reflexes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Generalized verbal communication and social interaction</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Abnormal EEG</td>
<td></td>
</tr>
</tbody>
</table>

Please note that this table does not cover all known mutations. For other human SH3 and multiple ankyrin repeat domains protein 3 (\textit{SHANK3}) mutations and deletions, see REFS 5, 12. A more detailed table is provided as Supplementary information S3 (table). ANK, ankyrin repeat domain; ASD, autism spectrum disorder; ASQ, Autism Screening Questionnaire (a score higher than 15 indicates ASD); EEG, electroencephalography; ID, intellectual disability; \textit{NRXN1}, neurexin 1; PDD-NOS, pervasive developmental disorder, not otherwise specified; PRO, proline-rich region; SH3, SRC homology 3 domain superfamily.
Role in synaptogenesis and function

Glutamatergic synapses in the CNS are characterized by an electron dense thickening underneath the postsynaptic membrane, the PSD. The PSD contains more than a thousand proteins, including membrane-tethered receptors and channels (such as NMDA receptors (NMDARs), metabotropic GluRs (mGluRs) and AMPARs), adaptor and scaffolding proteins (such as SAPAP and SHANK proteins), cytoskeletal and cell adhesion proteins (such as actin and neurofilins) and signalling molecules (such as calcium/calmodulin-dependent protein kinase type II (CaMKII)). Together, these create a wide macromolecular complex.19,36

At the heart of the PSD lies the membrane-associated guanylate kinase (MAGUK) family, the key organizers of vertebrate CNS synapses. MAGUKs are modular proteins that are composed of multiple domains that enable protein–protein interactions, such as PDZ, SH3 and C-terminal guanylate kinase domains. MAGUK members include PSD95 (also known as SAP90 or DLG4), SAP102 (also known as DLG3), SAP97 (also known as DLG1) and PSD93 (also known as chapsyn 110 or DLG2). Together, these family members build up a dense scaffold that serves as interface between clustered membrane-bound receptors, cell adhesion molecules and the actin cytoskeleton.37 The identification of MAGUK proteins, and the subsequent cloning of their associated proteins, has extended our comprehension of the molecular composition of the PSD.37,58

PSD95 is probably the best characterized MAGUK protein of the PSD. It has a PDZ domain that interacts with NMDARs and a guanylate kinase domain in its C terminus that is linked to members of the SAPAP family of proteins. SAPAP proteins subsequently bind to the PDZ domain of members of the SHANK protein family. SHANK proteins then bind to the actin cytoskeleton and to Homer protein, which in turn interacts with mGluRs. Through these extended links, PSD95, SAPAP, SHANK and Homer proteins form a quaternary complex that brings together mGluR and NMDAR complexes in the PSD (Fig. 3). This functional link between NMDARs and mGluRs is likely to facilitate crosstalk between ionotropic and metabotropic glutamate receptors.39

On a subcellular level, SHANK proteins are located in a distal area of the PSD that extends up to 120 nm away from the postsynaptic membrane.36,60 Recent data suggest that there are two pools of SHANK proteins in the PSD complex: one that is closer to the postsynaptic membrane and that seems to be relatively stable and able to bind to SAPAPs, and another, more dynamic, pool that is present at a distal location and probably cannot interact with SAPAP.41

Consistent with the roles of SHANK proteins as major scaffolding proteins in the postsynaptic site, the deletion of Shank genes in mice alters the PSD levels of several proteins including Homer, SAPAP proteins, NMDARs and AMPARs.8,37 Parallel to these molecular alterations, morphological and functional changes have also been found in several Shank-mutant mice. Depending on which Shank gene is mutated and on the specific nature of the mutation, many other functional and behavioural consequences can be seen. These include impaired social behaviours, self-grooming and synaptic plasticity defects (for a detailed comparison between Shank1-, Shank2- and Shank3-mutant mice, see REFs 8,12). Deletion of Shank genes also affects spine numbers and size,27,64,65 as well as AMPA transmission (with effects on miniature excitatory postsynaptic current (mEPSC) frequency and amplitude being observed).9,36,37,59,61 The different mutant mice thus indicate an important role of SHANK proteins in regulating excitatory neurotransmission.

Owing to the strong genetic association between SHANK3 mutations and ASD (see above), many neurobiology studies have focused on this particular gene. Shank3-knockout (Shank3-KO) mice display reduced corticostriatal connectivity and reduced striatal mEPSC frequency in adults.9,36,37,65 This suggests that loss of Shank3 decreases the number of striatal afferents that are formed during development (resulting in a reduced number of corticostriatal synapses); however, recent electrophysiological data obtained at postnatal day 14...
reveal that Shank3-KO mice actually exhibit increased striatal mEPSC frequency in early development. These results indicate that there is a premature increase in (and subsequent arrest of) the development of excitatory afferents onto striatal neurons in the absence of Shank3 (REF. 71). Given this reduction in basal excitatory synaptic transmission, one could mistakenly assume that there is reduced activity in the striatum of Shank3-KO mice. In fact, Shank3-KO mice have increased in vivo activity in the striatum. This striatal hyperactive profile does not seem to be due to increased intrinsic excitability of striatal neurons but rather to be a downstream consequence of increased cortical activity. The mechanism behind the cortex hyperexcitability profile in the absence of Shank3 is currently not known.

Corroborating these in vivo mouse studies, recent in vitro data also show increased frequency of evoked firing in cultured neurons from Shank3-KO mice, as well as similar changes in cultured human neurons differentiated from induced pluripotent stem cells that are derived from the cells of patients with PMS. Although these studies have focused on excitatory transmission (owing to the location of Shank3 at excitatory synaptic sites), it is important to keep in mind that Shank3 deletion may also affect inhibitory synaptic transmission. Indeed, recent data show reduced numbers of synaptic puncta containing parvalbumin (PV; an interneuron marker in the cortex) and compromised wisteria floribunda agglutinin-containing perineuronal nets that have reduced size and intensity in the insular cortex, as well as reduced PV expression in the striatum. In the cortex, perineuronal nets preferentially enwrap large PV-containing interneurons. Therefore, when perineuronal nets are disrupted, the perisomatic inhibition of the targets of these interneurons is reduced. This indicates an immature or altered inhibitory circuitry in Shank3-KO mice. In line with these data, a different study found that the abnormal network firing pattern that is seen in cultured cortical neurons of Shank3-KO mice can be normalized by application of clonazepam, an enhancer of GABA-mediated inhibitory transmission. Taking a different approach, it has also been shown that mice carrying a deletion in Shank3 exon 9 have an increased excitation/inhibition (E/I) ratio in the medial prefrontal cortex (mPFC). Although these mice have decreased miniature inhibitory postsynaptic currents (mIPSC) frequency in mPFC layer 2/3 pyramidal neurons, they display increased mIPSC frequency in hippocampus CA1 pyramidal neurons. Together, these studies suggest a developmental arrest of neural circuits in the absence of Shank3, with consequent disruption of optimal E/I circuitry balance.

Given the wide array of synaptic roles of Shank3 that are demonstrated by these studies (ranging from scaffolding to regulating spine morphology and neurotransmission), it will be important to understand which role is the most significant with regard to ASD.

**Neurobiology of SHANK proteins in ASD**

As noted above, several human genetic studies have pointed towards a link between mutations in Shank1, Shank2 or Shank3 and ASD. Although ASD neuropathology remains elusive, animal models based on mice carrying relevant genetic mutations may help us to dissect the circuit basis of behaviours that are evolutionary conserved and relevant to ASD. Several behavioural abnormalities have been reported in mice with deletions in Shank genes, some of which are considered ASD-like behaviours. Thirteen different mutant mouse lines carrying Shank3 deletions have been generated so far (TABLE 2; see Supplementary information S5 (table)). Of these, 9 of 13 mutant lines show aberrant social behaviours (1 line has not been tested for these behaviours), and 9 of 13 display repetitive self-grooming behaviour (TABLE 2). Self-injurious grooming is usually observed after complete Shank3 deletion and after Shank3 PDZ-targeted disruption. A key challenge is therefore to understand how the disrupted synaptic function of Shank3 can lead to these behavioural changes.

Social interaction requires the ability to combine sensory information with emotional and cognitive content. Many brain areas have been implicated as part of the ‘social brain’, including the mPFC, the amygdala, the anterior insula, the anterior cingulate cortex, the inferior frontal gyrus and the superior temporal sulcus. A recent study of the mouse insular cortex found multisensory integration deficits in Shank3-mutant mice and suggested that altered GABA circuits in the insular cortex may underlie the asocial urges of these mice.

Other behavioural parameters, such as ultrasonic vocalizations and learning and memory, have been evaluated in some of the different Shank3-mutant lines, revealing distinct results that might be related to isoform-specific disruptions in each line (TABLE 2). Indeed, the idea that isoform-specific disruptions will result in different phenotypic consequences (and even result in different disorders) has recently gained momentum. A recent study generated two novel Shank3-mutant mouse lines harbouring Shank3 mutations that are found in patients with ASD and schizophrenia, respectively. Both mutations are located in exon 21 of the mouse Shank3 gene, and the mutations are located only 325 nucleotides apart. The ASD-linked InsG3680 mutation has a guanine nucleotide insertion at position 3680 of Shank3 cDNA, resulting in a frameshift mutation and the appearance of a stop codon immediately after the mutation. The schizophrenia-linked R1117X mutation, on the other hand, changes arginine 1117 to a stop codon. These two mutant mouse lines exhibit both distinct and shared defects at molecular, synaptic, circuit and behavioural levels. Whereas mice with the ASD-linked mutation display impaired striatal synaptic transmission at postnatal day 14 and abnormal juvenile social interactions (similar to the early-age onset of ASD symptoms), adult mice with the schizophrenia-linked mutation have profound synaptic defects in the PFC and display social dominance behaviour. Given that the ASD-linked mutation results in almost complete loss of Shank3 (similar to a full deletion of the Shank3 gene) and that the schizophrenia-linked mutation results in the generation of a truncated Shank3 protein (which could be either partially functional or act as a dominant negative), this study provides, for the first time, some
Table 2  Characterization of the different Shank3-mutant mice

<table>
<thead>
<tr>
<th>Protein domain/ exon mutated</th>
<th>Altered synaptic proteins</th>
<th>Spine morphology</th>
<th>Social behaviour</th>
<th>USVs</th>
<th>Repetitive behaviours</th>
<th>Learning and memory</th>
<th>Motor coordination</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANK; exons 4–9</td>
<td>GluR1</td>
<td>Defective remodelling</td>
<td>Reduced snuffing</td>
<td>Reduced</td>
<td>Increased self-grooming</td>
<td>MWM test normal</td>
<td>Impaired</td>
<td>NOR test impaired</td>
</tr>
<tr>
<td></td>
<td>SAPAP1, Homer 1b/c, GluR1 and GluN2A</td>
<td>Reduced density</td>
<td>Abnormal interactions</td>
<td>Altered</td>
<td>Increased self-grooming and head pokes in hole-board test</td>
<td>MWM test impaired</td>
<td>Impaired</td>
<td>Reduced distance in OF test; NOR test impaired</td>
</tr>
<tr>
<td></td>
<td>GluR2, GluR3, Homer 1b/c and PSD95</td>
<td>NA</td>
<td>Mild impairment</td>
<td>Increased</td>
<td>Increased self-grooming</td>
<td>MWM test slightly impaired</td>
<td>NS</td>
<td>NOR test impaired</td>
</tr>
<tr>
<td>ANK; exons 4–7</td>
<td>NA</td>
<td>NA</td>
<td>Abnormal social novelty</td>
<td>NA</td>
<td>No increase in self-grooming</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ANK; exon 9</td>
<td>NA</td>
<td>NA</td>
<td>Normal</td>
<td>Normal</td>
<td>Increased rearing in novel environment</td>
<td>Normal</td>
<td>NA</td>
<td>None</td>
</tr>
<tr>
<td>PDZ; exons 13–16</td>
<td>Homer 1, SAPAP3, GluN2A, GluN2B, GluR2 and PSD95</td>
<td>Reduced density</td>
<td>Abnormal</td>
<td>NA</td>
<td>Self-injurious grooming</td>
<td>Normal</td>
<td>NA</td>
<td>Increased anxiety-like behaviour</td>
</tr>
<tr>
<td></td>
<td>Homer 1, SAPAP3, GluN2A, GluN2B and GluR2</td>
<td>Reduced density</td>
<td>Abnormal</td>
<td>NA</td>
<td>Self-injurious grooming</td>
<td>NA</td>
<td>Impaired</td>
<td>Increased anxiety-like behaviour</td>
</tr>
<tr>
<td>SH3; exon 11</td>
<td>GluN2B, SHANK2, Homer 1b/c and mGluR5</td>
<td>Normal</td>
<td>Abnormal</td>
<td>NA</td>
<td>Self-injurious grooming</td>
<td>MWM test impaired</td>
<td>Minor deficits</td>
<td>Hyposensitivity; impaired nesting and marble burying; increased aggression</td>
</tr>
<tr>
<td>PRO; exon 21</td>
<td>mGluR5</td>
<td>Normal</td>
<td>Abnormal social novelty</td>
<td>NA</td>
<td>Increased self-grooming</td>
<td>MWM test impaired</td>
<td>Impaired</td>
<td>Hyposensitivity; impaired nesting and marble burying; anxiety-like behaviour</td>
</tr>
<tr>
<td></td>
<td>p-GluN2B Tyr1472</td>
<td>NA</td>
<td>Inconclusive testing</td>
<td>NA</td>
<td>None</td>
<td>MWM test mildly impaired</td>
<td>Impaired</td>
<td>Impaired nesting and marble burying; anxiety-like behaviour</td>
</tr>
<tr>
<td></td>
<td>Homer proteins, SAPAP3, SYNGAP, GluN1, GluN2A, GluN2B, GluR2 and mGluR5</td>
<td>Reduced number</td>
<td>Abnormal</td>
<td>Normal</td>
<td>Self-injurious grooming</td>
<td>NA</td>
<td>Impaired</td>
<td>Anxiety-like behaviour; PPI test impaired; startle response impaired</td>
</tr>
<tr>
<td>All domains; exons 4–22</td>
<td>Pan–SAPAP, SAPAP3 and Homer 1b/c</td>
<td>Reduced in the striatum</td>
<td>Normal</td>
<td>Altered spectral properties</td>
<td>Self-injurious grooming</td>
<td>MWM test impaired</td>
<td>Impaired</td>
<td>Anxiety-like behaviour; PPI test impaired; impaired instrumental learning</td>
</tr>
</tbody>
</table>

A more detailed table is provided as Supplementary information S5 (Table). ANK, ankyrin repeat domain; GluR1, glutamate receptor 1; mGluR5, metabotropic GluR5; Homer 1b/c, Homer splice variants 1b and 1c; MWM, Morris water maze; NA, not available; NOR, novel-object recognition; NS, nonsignificant; OF, open field; p-GluN2B Tyr1472, phosphorylated Tyr1472 site of GluN2B; PDZ, postsynaptic density protein 95 (PSD95)–discs large homologue 1–zonula occludens 1 domain; PPI, prepulse inhibition; PRO, proline-rich region; SAPAP1, SAP90/PSD95-associated protein 1; SH3, SRC homology 3 domain superfamily; SHANK2, SH3 and multiple ankyrin repeat domains protein 2; USVs, ultrasonic vocalizations.
neurobiological insights into how different mutations in the Shank3 gene may lead to mutation-specific defects and contribute to different disorders.

Besides isoform-specific mutations, SHANK3 gene expression (or perhaps expression of specific isoforms) seems to be crucial. For example, duplications of 22q13 that span SHANK3 have been reported in patients diagnosed with attention deficit hyperactivity disorder (ADHD)\(^2\), and mice in which SHANK3 is overexpressed exhibit synaptic dysfunction and manic-like phenotypes\(^4\).

Mutant mice carrying Shank1 and Shank2 deletions have also been generated and characterized in recent years\(^6,7,8,85,86,88,89,90,91\) (Table 3; see Supplementary information S6 (table)). In line with the human genetic data that showed an association between different SHANK family members and distinct cases of ASD\(^3\), all three lines of mutant mice carrying Shank1 and Shank2 deletions display certain ASD-like phenotypes, such as increased repetitive behaviour and/or decreased social interaction\(^6,7,8,90\). A recent study reveals that, besides regulating excitatory synaptic transmission in neurons\(^6\), SHANK1 also regulates excitatory synaptic transmission in inhibitory interneurons\(^8\). The study further confirms the expression of all three Shank genes in CA1 PV-expressing interneurons and suggests that, in the absence of Shank1, a shift occurs in the E/I balance of the hippocampus. As a consequence, PV-expressing interneurons receive less excitatory input and provide weaker inhibitory output onto CA1 pyramidal neurons. Interestingly, no major changes were found in CA1 inhibitory synapses in Shank2-mutant mice with regard to inhibitory transmission\(^36\) and protein markers\(^8\).

Although the different Shank1-, Shank2- and Shank3-mutant mice have some distinct phenotypes at the molecular and functional level, they all feature a disrupted E/I balance that results from initial changes in excitatory synaptic transmission and composition, as well as ASD-relevant behavioural phenotypes\(^6,7,8,90\). Taken together, the findings from different Shank-mutant mice suggest that each Shank mutation might underlie a defined subset of ASD pathology and contribute to the myriad of phenotypes that are seen in patients.

**Towards the development of treatment**

Given the complex genomic landscape of human mutations that are associated with ASD, animal models pose a starting point to study the neurobiology of specific mutations and establish useful genotype–cellular phenotype correlations. The SHANK3 gene, in particular, has the advantage of having available 13 difference mice models, harbouring different Shank3 gene disruptions. The gene itself is probably also less complicated to study than some other ASD-related genes because of the defined localization of Shank3 protein at glutamatergic synapses. This has advantages over the study of a transcription factor, such as methyl-CpG-binding protein 2 (MeCP2), which may directly regulate the expression of hundreds of genes. Because ASDs are neurodevelopmental disorders (patients typically display symptoms before the age of three)\(^90\), one of the key questions in autism research is whether the pathology is reversible in adults. Recently, this question was tackled in a study that generated a Shank3 conditional knock-in mouse\(^96\). These mice are born as Shank3-KO (carrying a PDZ domain deletion) but have a FLexed PDZ domain inverted, which allows reorientation at any time point in life to restore the Shank3 gene. This strategy design is crucial because it keeps the Shank3 gene under the control of its endogenous genomic locus and avoids Shank3 expression at non-physiological levels that could induce potential confounds. These Shank3-KO mice have deficits in striatal neurotransmission, reduced spine density in the striatum and reduced levels of PSD proteins (SAPAP3, Homer, GluN2A, GluN2B and GluR2) in the striatum. All these alterations can be reverted in the adult by restoring the Shank3 gene. Behaviourally, these Shank3-KO

| Table 3 | Characterization of the different Shank1- and Shank2-mutant mice |
|-------------|---------------|------------------|----------------|-----------------|-------------------|----------------------|-----------------|------------------|
| **Protein domain/ exon mutated** | **Altered synaptic proteins** | **Spine morphology** | **Social behaviour** | **USVs** | **Repetitive behaviours** | **Learning and memory** | **Motor coordination** | **Other** |
| Shank1 PDZ; exons 14–15 | SAPAP and Homer proteins | Reduced density | Inconclusive testing | Reduced | No increase in self-grooming | Enhanced in radial maze | Impaired | Anxiety-like behaviour | 67,85, 87 |
| Shank2 PDZ; exons 16–17 | p-CaMKII α/β, p-ERK1/2, p-p38, p-GluR1 (S831 and S845), GABA\(_A\) R; increased GluN1 | NS | Abnormal | Reduced | Repetitive jumping | MWM test impaired | NA | Anxiety-like behaviour | 68,88 |
| Shank2 PDZ; exon 17 | Increased GluN1, GluN2A, GluN2B, GluR2 and SHANK3 | Reduced density | Abnormal | Abnormal | Increased self-grooming | MWM test impaired | NS | Hyperactivity | 30,88 |

A more detailed table is provided as Supplementary information S6 (table). GABA\(_A\) R, GABA type A2 receptor; MWM, Morris water maze; NA, not available; NS, nonsignificant; p-CaMKII α/β, phosphorylated calcium/calmodulin-dependent protein kinase type II subunit alpha/beta; p-ERK1/2, phosphorylated extracellular signal-regulated kinases 1 and 2; p-GluR1, phosphorylated glutamate receptor 1; PDZ, postsynaptic density protein 95 (PSD95)–discs large homologue 1–zonula occludens 1 domain; SAPAP, SAPAP0/PSD95–associated protein; Shank1, SH3 and multiple ankyrin repeat domains protein 1; USVs, ultrasonic vocalizations.
mice display repetitive self-injurious grooming, anxiety, 
social-interaction deficits (reduced social-interaction fre-
quency and duration) and impaired motor coordination.
After adult restoration of Shank3 expression, repetitive 
self-injurious grooming and social-interaction deficits, 
but not anxiety or motor coordination deficits, can be 
rescued, indicating a selective rescue of certain autism-
like phenotypes. The same study shows that the behav-
ioural deficits that are irreversible in adulthood can be 
improved by early postnatal intervention, highlighting 
the unique behavioural effects of Shank3 expression 
during specific developmental periods and through-
out life. Given the emergence of new genome editing 
approaches (such as CRISPR), these results suggest that 
repair of the Shank3 gene in adulthood could alleviate 
some of the synaptic and behavioural impairments that 
are associated with Shank3 mutations. Although there 
are still technical limitations to the genetic manipulation 
of mature neurons in a fully formed brain, new studies 
have been pushing the boundaries of the use of CRISPR 
in adult brain repair97. More importantly, this study 
suggests the possibility of treatment (whether pharma-
cological or through future genetic repair approaches) 
for patients with Shank3 mutations or deletion, during 
adulthood. Similar encouraging adult-rescue results have 
been reported for mutant mice carrying deletions and 
duplications in other ASD-related genes such as Mecp2 
(REFS 95–95) and ubiquitin-protein ligase E3A (Ube3a)98.

Other studies have taken a different approach towards 
reversing the phenotype resulting from Shank3 deficien-
cy. In one study99, the phosphoproteome of Shank3-deficient 
rat cortical neurons was characterized, revealing an upregulation of CDC-like kinase 2 (CLK2) expression. Inhibition of CLK2 rescued social 
behaviour in mice carrying a mutation in Shank3 exon 21, as well as synaptic deficits in Shank3-deficient 
neurons and neurons derived from patient with PMS. 

Together, these studies suggest that adult restoration of Shank3 levels or restoration of downstream medi-
ators may be a useful approach to alleviate some of the 
synaptic and behavioural impairments that are associ-
ated with Shank3 mutations. Aligned with the idea of 
targeting downstream mediators and proteins associated 
with Shank3 network, two groups have recently looked 
into mGlur5 and Homer as potential therapeutic targets 
in ASD. Using a complete Shank3-KO mouse65, it was 
shown that antagonism of mGlur5 activity ameliorates 
over-grooming behaviour, whereas positive allosteric 
modulation of mGlur5 exacerbates self-grooming. In 
a second study99, it was reported that a pharmacological 
increase in mGlur5 activity ameliorates over-grooming 
behaviour and rescues other behavioural deficits in 
Shank3-KO mouse. Although these studies might, at 
first, seem to be discrepant — the mGlur5 positive ago-
nist CDPPB exacerbates and ameliorates self-grooming 
in the respective studies — it is crucial to note that these 
results were derived from different Shank3-mutant mouse 
lines. Furthermore, the results of the two studies were 
otherwise in agreement. In one study, CDPPB treatment 
slightly improved instrumental learning, normalized 
striatal long-term depression and slightly increased the 
protein level of two splice variants of Homer (Homer 1b 
and Homer 1c) in the striatum of complete Shank3-KO 
mouse65. Similarly, in the other study99, a pharmacol-
ogical increase in mGlur5 activity ameliorated functional 
deficits (NMDA-induced membrane depolarization) 
and behavioural defects (social interaction and Morris 
water maze performance) in mice with deleted exon 11. 
Nevertheless, these studies suggest that the defects in 
downstream signalling, and thus potential therapeutic 
targets (such as mGlur5 and Homer), that result from dif-
ferent mutations of the same gene might be very different.

In an unrelated study99, the authors reported a marked 
loss of cortical actin filaments in mice with deleted 
exon 11, which was associated with reduced RAC1 and 
Pak activity and increased coflin activity (the major 
actin depolymerizing factor). This suggested that actin 
regulators might be another potential molecular target 
for the treatment of ASD. Elevation of RAC1 activity in 
the PFC of these mice rescued their social behaviour defi-
cits and NMDAR hypofunction, whereas inhibition of 
Pak or Rac1 function induced social behaviour deficits 
and NMDAR hypofunction in wild-type mice. 

The aforementioned demonstrations of pharmaco-
logical reversals of phenotypes in Shank3-mutant mice 
highlight potential target pathways that seem to con-
verge with some pharmacological reversal studies car-
ried out in Shank2-mutant mice. In those studies69,100,
NMDA hypofunction was suggested to be a potential 
mechanism underlying ASD-like behaviours. By treating 
Shank2-mutant mice with d-cycloserine (a partial ago-
nist of NMDAR), one study showed an improvement in 
social interaction that could also be attained by treating 
the mice with CDPPB (possibly because of the enhance-
ment of NMDAR function through mGlur5 activation)98. 
Similarly, another study used clioquinol (a zinc chelator 
and ionophore) to rescue social-interaction deficits in 
Shank2-mutant mice. It was suggested that clioquinol 
can be used to mobilize zinc towards postsynaptic sites, 
where it would then increase NMDAR function through tyros-
ine kinase SRC activation100. Alterations in NMDAR func-
tion and excitatory transmission could potentially lead to 
an E/I imbalance, as was also suggested by a recent study 
that showed impaired GABAergic neurotransmission in 
mice with deleted exon 6 and exon 7 (REF. 88). The study 
further demonstrated that administration of a GABA_A 
receptor agonist can slightly improve the spatial-memory 
deficits observed in Shank2-mutant mice, although the 
social-interaction deficits cannot be rescued. Together 
these studies suggest that NMDAR hypofunction con-
tributes to the development of some ASD-like phenotypes 
in Shank-mutant mice and that other related molecular 
targets could potentially be used to modulate NMDAR 
function and improve specific phenotypes.

Conclusions and future perspectives
Many important questions remain. Can we mitigate the 
loss of a missing Shank protein by somehow upregu-
lat ing other Shank protein family members? Which of 
the domains of Shank proteins are essential for their 
roles? Which cell types are more vulnerable to Shank 
protein loss?
Although most of the human SHANK mutations that underlie clinical conditions are heterozygous, the study and characterization of Shank homozygous mutant mice is sometimes essential, as it allows us to gain a clear understanding of the physiological role of a particular gene and the functional consequences of its disruption. Overall, three very important conclusions can be drawn from all the aforementioned studies. ‘The earlier the better’ seems to be the case in terms of therapeutic window; however, adult intervention may still be useful to alleviate some of the impairments that are associated with SHANK3 mutations. It is also clear that efforts need to be taken to understand Shank3 mutations in an isoform-specific manner. Finally, careful genotype–phenotype patient stratification is required before individual testing of specific pharmacological agents. Shank-mutant animal models, together with patient-derived induced pluripotent stem cells, will certainly help us to uncover some of the neurobiological secrets of SHANK proteins.
This study describes the first Shank3-mutant mice (exon 4 and exon 9 deletion). These mice show some ASD-relevant phenotypes.


Zhou, Y. et al. Mice with Shank3 mutations associated with ASD and schizophrenia display both shared and distinct defects. *Neuron 89*, 147–162 (2016).


This study describes the first Shank1/-mutant mouse (exon 14 and exon 15 deletion; PDZ domain deletion).


This study describes one of the first Shank2-mutant mice (exon 6 and exon 7 deletion). These mice show autism-related behavioural phenotypes and NMDAR dysfunction.


This study shows that mice with SHANK3 overexpression exhibit synaptic dysfunction and maniac-like phenotypes, thus reinforcing the idea that proper Shank3 gene dosage is crucial.


