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# Therapeutic Approaches for Shankopathies

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## Abstract

Despite recent advances in understanding the molecular mechanisms of autism spectrum disorders (ASD), the current treatments for these disorders are mostly focused on behavioral and educational approaches. The considerable clinical and molecular heterogeneity of ASD present a significant challenge to the development of an effective treatment targeting underlying molecular defects. Deficiency of *SHANK* family genes causing ASD represent an exciting opportunity for developing molecular therapies because of strong genetic evidence for *SHANKs* as causative genes in ASD and the availability of a panel of *Shank* mutant mouse models. In this article we review the literature suggesting the potential for developing therapies based on molecular characteristics and discuss several exciting themes that are emerging from studying *Shank* mutant mice at the molecular level and in terms of synaptic function.

### Keywords

Autism Spectrum Disorders; Mouse model; SHANK family protein; Synapses; Brain stimulation

## Introduction

Despite the significant progress in recognizing and understanding the etiology of autism spectrums disorders (ASD) in the last decade, we have made few advances in the area of treatments and interventions (Blenner et al., 2011; State, 2010; Volkmar et al., 2009). The current therapeutic options are mostly restricted to programs of behavioral modification such as applied behavioral analysis (ABA), early start Denver model, and Treatment and Education of Autistic and related Communication handicapped Children (TEACCH) (Dawson et al., 2010; Kasari and Lawton, 2010; McPheeters et al., 2011; Myers and Johnson, 2007; Taylor et al., 2012; Warren et al., 2011). These interventions are primarily based on behavioral and educational approaches linked to autistic behaviors but not targeting the underlying biological causes (Vismara and Rogers, 2010). The outcome of these behavioral therapies is quite variable and a vigorous validation for their efficacy is still warranted (Grindle et al., 2012; Hayward et al., 2009; Taylor et al., 2012). Because of the considerable clinical and molecular heterogeneity of ASD that has become apparent from the last decade of research (Betancur, 2011; Devlin and Scherer, 2012; Georgiades et al., 2012), one of the critical questions is whether there is a common pathophysiology at the molecular and circuitry levels underlying ASD that can be targeted for interventions (Dolen

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et al., 2010; Geschwind, 2008; Geschwind and Levitt, 2007; Kelleher and Bear, 2008; Smith and Ehlers, 2012). Currently, there is no medication available to specifically treat the core symptoms of ASD despite the fact that the use of drugs targeting behavioral presentations is common in clinical practice (Carrasco et al., 2012; McPheeters et al., 2011; Myers, 2007; Volkmar, 2001). Two medications, risperidone and aripiprazole, have been approved by the Food and Drug Administration (FDA) to treat the comorbidities commonly seen in ASD (Marcus et al., 2011a; Marcus et al., 2011b)(McPheeters et al, 2011)(McCracken et al., 2002). Similar to behavioral interventions, there is little biological evidence to specifically supports these treatments. Also, the safety profile and efficacy of these treatments in children with ASD remain to be further investigated (Huffman et al., 2011; Panagiotopoulos et al., 2010).

## Genetic defect of SHANKs in ASD

The discovery of genetic defects in a sub-set of ASD patients offers a unique opportunity to explore therapeutic approaches for the core symptoms that is based on the underlying biological mechanism (Devlin and Scherer, 2012; Malhotra and Sebat, 2012; Smith and Ehlers, 2012; Toro et al., 2010). *SHANK* family genes (SHANKs) causing ASD (**Shankopathies**) probably represent one of the best opportunities for this direction of research. *SHANK* family genes include *SHANK1*, *SHANK2*, and *SHANK3* and encode proteins with 5 protein-protein interaction domains including ankrin repeats (ANK), SH3, PDZ, proline-rich region with Homer binding, and SAM (Grabrucker et al., 2011; Kreienkamp, 2008; Sheng and Kim, 2000). SHANK proteins are scaffolding proteins enriched at the post synaptic density (PSD) of excitatory synapses (Naisbitt et al., 1999). Since the first report of a *SHANK3*-specific mutations in ASD in 2007 (Durand et al., 2007), there is now genetic evidence supporting the involvement of all *SHANK* family genes in ASD (Berkel et al., 2010; Berkel et al., 2012; Leblond et al., 2012; Moessner et al., 2007; Sato et al., 2012). Evidence for S*HANK3* causing ASD is particularly strong because it involves different types of genetic defects such as microdeletions and point mutations, and the findings have been independently replicated in different ASD patient cohorts (Durand et al., 2007; Gauthier et al., 2009; Moessner et al., 2007).

## Mutation mechanism underlying the Shankopathies

All types of genetic mutations of *SHANKs* found in ASD are reported in heterozygotes (Moessner et al., 2007). Microdeletion of *SHANK3* in 22q13.2 deletion syndrome (i.e. Phelan-McDermid syndrome) is the most common molecular defect that accounts for more than 95% of cases reported in the literature (Phelan, 2007). Point mutations or small intragenic mutations contribute to a small percentage of *SHANK* causing ASD cases studied (Berkel et al., 2010; Berkel et al., 2012; Bonaglia et al., 2011; Moessner et al., 2007). Chromosome translocation with a breakpoint within the *SHANK3* gene has also been reported (Bonaglia et al., 2005). The fact that microdeletions usually disrupt entire *SHANK* genes generally supports haploinsufficiency as the molecular mechanism underlying the pathogenesis in these patients. For point mutations, particularly missense mutations of *SHANK2* and *SHANK3* found in ASD, the possibility of a gain of function mechanism may also be considered.

One of the interesting features observed in all *SHANK* family genes is complex transcriptional structure and extensive isoforms resulting from multiple intragenic promoters and extensive alternative splicing of coding exons in *SHANK*s (Durand et al., 2011; Leblond et al., 2012; Lim et al., 1999; Wang et al., 2011; Wilson et al., 2003) (Figure 1). For example, 3 promoters and brain-region-specific alternative splicing of several coding exons are reported in *SHANK2* (Leblond et al., 2012). *SHANK3* has 6 promoters and extensive

splicing of several coding exons that result in an array of *SHANK3* isoforms with different combinations of the 5 protein domains (Wang et al., 2011). These data indicate that individual point mutations in different exons of *SHANK* genes may only disrupt selective isoforms of *SHANKs* in ASD. A similar phenomenon is suggested in *SHANK1* but the details remain to be characterized (Lim et al., 1999). Because each isoform has a combination of different protein domains for SHANK proteins, the function of each isoform at synapses is predicted to be different based on the study of domain specific mutations by RNAi in cultured neurons (Roussignol et al., 2005; Sala et al., 2001). The interesting hypothesis to be tested in humans is whether isoform-specific disruption of *SHANK*s contributes to the clinical heterogeneity observed in patients with different types of mutations.

## The pathophysiology of *SHANK* causing ASD and *Shank* mutant mice

The progress to model human *SHANK* mutations in mutant mice has been impressive. Mutant mice for all *Shank* family genes have been produced. The *Shank1* mutant mouse model was first produced well before the discovery of involvement of *SHANK1* in ASD (Hung et al., 2008; Silverman et al., 2011; Wohr et al., 2011). The PDZ domain is disrupted in *Shank1* mutant mice. The phenotypes at both the synaptic and behavioral levels in *Shank1* mutant mice are unexpectedly mild. *Shank1* mutant mice have altered PSD protein composition, reduced size of dendritic spines, smaller and thinner PSDs, weaker basal synaptic transmission, but normal synaptic plasticity. Behaviorally, *Shank1* mutant mice have normal social interaction behavior, increased anxiety, reduced ultrasonic vocalizations, and impaired contextual fear memory. Unexpectedly, *Shank1* mutant mice display enhanced performance in a spatial learning task. Two mutant models for *Shank2* were reported recently (Schmeisser et al., 2012;Won et al., 2012). Schmeisser et al. created *Shank2* exon 7 deletion mutant mice (*Shank2* Δex7). In these animals long-term potentiation (LTP) in hippocampal CA1 synapses was increased but no change in long-term depression (LTD) was observed (Schmeisser et al., 2012). Impairment in social interaction, increased stereotypical behavior, hyperactivity, and altered ultrasonic vocalization patterns were found in *Shank2* $\Delta ex7^{-/-}$ mice. In contrast, Won et al. generated a slightly different *Shank2* mutant mouse where exons 6–7 were deleted (*Shank2* Δex6–7) (Won et al., 2012). *Shank2* $\Delta ex6–7^{-/-}$ mice also display abnormal synaptic function and ASD-like behaviors. Both exon 7 and exon 6–7 deletions resulted in frame shift mutations shortly after exon 7 which suggest that the two mutations should have very similar consequences for the *Shank2* protein. There are similarities and differences between *Shank2* Δex7 *Shank2* Δex6–7 mice. Intriguingly, LTP in the hipppocampal CA1 region was reduced in *Shank2* $\Delta ex6–7^{-/-}$ mice, and this is opposite to *Shank2* $\Delta ex7^{-/-}$ mice. The behavioral profile of *Shank2* $\Delta ex6–7^{-/-}$ mice is similar to *Shank2* $\Delta ex7^{-/-}$. The explanation for the apparent discrepancy in hippocampal LTP in two very similar *Shank2* mutations is not immediately clear and further investigation is warranted.

Recently, four different laboratories have developed five lines of mutant mice carrying different mutations in *Shank3* (Figure 2) (Bozdagi et al., 2010; Peca et al., 2011; Schmeisser et al., 2012; Wang et al., 2011). The mutations include deletions of exons 4–9 by two groups with slightly different design of [($\Delta e4–9^{Buxbaum(B)}$) (Bozdagi et al., 2010) and $\Delta e4–9^{Jiang\ (J)}$ (Wang et al., 2011)], exons 4–7(Δe4–7)(Peca et al., 2011), deletion of exon 11(Schmeisser et al., 2012) and exons 13–16 (Δe13–16) (Peca et al., 2011). Because all of these deletions cause a frame shift for targeted transcripts, they all result in either a truncated Shank3 protein or possible disruption of full length RNA or protein isoforms due to the instability of shortened RNA or protein. Based on our knowledge of alternative *Shank3* promoters and alternative splicing of coding exons, each of these mice is expected to disrupt different *Shank3* isoforms but not completely disrupt the *Shank3* gene (Wang et al., 2011).

*Shank3* mutant mice have been extensively characterized by biochemical, ultrastructural, electrophysiological, and behavioral approaches. Notably, synapse structure phenotypes vary with specific *Shank3* mutations, are different in different brain regions, and display developmental heterogeneity. This could be due to differential spatial and temporal expression of other Shank family members or due to compositional variation across different populations of glutamatergic synapses. Dendritic branching and spine areas were increased in medium spiny neurons (MSNs) in the striatum of $\Delta ex13–16^{-/-}$ mice but PSD thickness and length were decreased at corticostriatal synapses in these mice (Peca et al., 2011). Similar findings, however, were not found in hippocampal CA1 synapses in $\Delta ex4–9^{J-/-}$ (Wang et al., 2011) and $\Delta ex11^{-/-}$ mice (Schmeisser et al., 2012). Spine length was increased in CA1 hippocampus of $\Delta ex4–9^{J-/-}$ mice (Wang et al., 2011), and spine density was decreased in the striatum and CA1 hippocampus of $\Delta ex13–16^{-/-}$ and $\Delta ex4–9^{J-/-}$ mice, respectively. Activity-induced spine growth by theta burst stimulation in cultured brain slices was attenuated at CA1 synapses of $\Delta ex4–9^{B+/-}$ mice (Bozdagi et al., 2010). Similarly, electrophysiological studies of these mutant mice have revealed variable findings. Measurements of miniature excitatory postsynaptic current (mEPSC) frequency and amplitude, paired pulse ratio, input/output (I/O) curves, fiber volley, and population spikes indicated that synaptic transmission was reduced at hippocampal CA1 synapses of $\Delta ex4–9^{B+/-}$ mice (Bozdagi et al., 2010), but not in mice bearing $\Delta e4–9^{J-/-}$ (Wang et al., 2011), or $\Delta e13–16^{-/-}$ (Peca et al., 2011). The explanation for the difference between $\Delta e4–9^{B+/-}$ and $\Delta e4–9^{J-/-}$ is not immediately clear. One possibility is that these mutations induce different cryptic splicing as described in $\Delta e4–9^{J-/-}$ mice (Wang et al., 2011). Another possibility is that heterozygous mutations may produce a dominant gain-of-function phenotype which differs from the phenotype of homozygous deletions. In striatum, the frequency of mEPSCs and amplitude of population spikes were significantly decreased in $\Delta ex13–16^{-/-}$ mice (Peca et al., 2011). Presynaptic responses as measured by paired pulse ratio and input/output curves were not altered at corticostriatal synapses in $\Delta ex13–16^{-/-}$ or $\Delta ex4–7^{-/-}$ mice (Peca et al., 2011). Hippocampal LTP was reduced at CA1 synapses of $\Delta ex4–9^{J-/-}$ and $\Delta ex4–9^{B+/-}$. (Bozdagi et al., 2010; Wang et al., 2011). Alterations in mGluR-dependent LTD was not evident in the $\Delta ex4–9^{B+/-}$ mice as induced by PP-LFS (Bozdagi et al., 2010). However, acute knockdown of Shank3 in cultured neurons decreases mGluR-dependent plasticity (Verpelli et al., 2011), suggesting differences in effects of Shank3 on mGluR1/5 signaling over development and pointing to the need for cautious interpretation regarding the pathogenic versus compensatory roles of synaptic phenotypes observed in *Shank3* mutant mice.

Extensive behavioral analyses were performed in all *Shank3* mutant mice on different genetic backgrounds using different protocols. The most notable and consistent observations are reduced social interaction and affiliation behaviors in all mutant mouse lines (Bozdagi et al., 2010; Peca et al., 2011; Wang et al., 2011). Repetitive behaviors measured by increased self-grooming in the home cage and behavioral inflexibility in the reverse Morris water maze and hole board test were observed in $\Delta e4–9^{J-/-}$ mice (Wang et al., 2011). Significantly increased self-grooming leading to skin lesions are also observed in $\Delta ex11^{-/-}$ and $\Delta ex13–16^{-/-}$ mice (Peca et al., 2011). The number, frequency, and duration of ultrasonic vocalizations were altered in a sex-specific manner in $\Delta e4–9^{J-/-}$ and $\Delta e4–9^{B+/-}$ mice (Bozdagi et al., 2010; Wang et al., 2011).

Deciphering the relationship between the phenotypic diversity and the molecular diversity of *Shank3* mutations remains a significant challenge. It is tempting to speculate that the phenotypic diversity in *Shank3* mutant mice reflects the clinical heterogeneity in *SHANK3* mutations found in human ASD patients. Since each mutation has a different impact on *Shank3* isoform expression, a simple hypothesis is that the diversity of phenotypes in *Shank3* mutant mice reflects the molecular diversity of *Shank3*. However, analysis of

heterozygotes and homozygotes, performing different measurements in different brain regions as well as in animals with different genetic backgrounds and ages could all contribute to the observed phenotypic heterogeneity. Further investigations comparing different lines of *Shank3* mice side by side is necessary to resolve these discrepancies and provide validated data for future pre-clinical trials.

## Therapeutic approaches for Shankopathies

Further investigations in *Shank* mutant mice or in humans are warranted to fully understand the pathophysiology of Shankopathies. However, several exciting themes related to the development of treatments of *SHANK* causing ASD are emerging from the knowledge learned from human genetics and mutant mouse models (Figure 3). Two general questions are worth discussing prior to focusing on SHANK specific treatment strategies. The first question is the critical window of development underlying the pathophysiology of ASD in general. Although ASD are classified as neurodevelopmental disorders clinically and presumably have a developmental origin, the evidence in the human literature as to whether the pathophysiology of ASD is the result of mainly a developmental *versus* functional defect, or a combination of both is very limited (Bale et al., 2010; Rubenstein, 2010; Zoghbi, 2003). Little is known about the developmental window that is critical for the expression of core behavioral features of ASD (LeBlanc and Fagiolini, 2011). Clinically, in the typical course of ASD, particularly for the mild and moderate cases, there may be normal development during the first 12–16 months before significant signs and symptoms of ASD emerge (Lord and Bishop, 2009). One of the best characterized examples is Rett syndrome (Zoghbi, 2003, 2005). However, this traditional view is challenged by recent reports from neuroimaging studies using more sensitive and higher resolution techniques which suggest an early developmental defect may be present before clinically apparent symptoms emerge (Dawson et al., 2004; Wolff et al., 2012; Zwaigenbaum et al., 2009). In the case of *SHANK3* causing ASD, most PMS patients display signs of neurological impairments such as hypotonia at birth or developmental delay during infancy (Phelan, 2007). No reports are in the literature on the neurological deficits or neuroimaging studies for the cases with *SHANK3* only mutations. Only one neuroimaging study of 8 cases of 22q13.3 deletion including *SHANK3* is reported (Philippe et al., 2008). The major findings are thin or morphologically atypical corpus callosum, localized dysfunction of the left temporal polar lobe, and amygdala hypoperfusion. Because other genes in addition to *SHANK3* are also deleted in 22q13.3 deletion syndrome, it could not be determined whether the deficiency of *SHANK3* or other genes, or both are responsible for these imaging finding as well as early neurological impairments. In all *Shank* mutant mice, no apparent structural or histological defects are reported (Bozdagi et al., 2010; Peca et al., 2011; Schmeisser et al., 2012; Wang et al., 2011; Won et al., 2012). These observations do not indicate the presence of an earlier developmental defect associated with the deficiency of Shanks but the possibility of a subtle or ultra-structural defect in specific cell lineages cannot be completely ruled out.

The second important question directly relevant to treatment is the reversibility of neurological impairment in postnatal human brains. There are now several examples in mouse models where neurological impairments can be reversed in postnatal brains (Daily et al., 2011; Guy et al., 2007; Han et al., 2012; Krueger and Bear, 2011). In mutant mice lacking *Mecp2*, genetic restoration of *Mecp2* during late development reverses the neurological impairments including defective synaptic plasticity and abnormal behaviors in adult mice (Giacometti et al., 2007; Guy et al., 2007; Kerr et al., 2012). Similarly, in the Angelman syndrome (AS) mouse model, virus mediated delivery of the AS causing gene *Ube3a* resulted in rescued LTP and amelioration of abnormal behaviors in adult mice (Daily et al., 2011). In the case of fragile X syndrome, pharmacological treatments given postnatally ameliorated major neurological impairments by both mGluR5 and GABA

receptor modulators both in humans and the mouse model (Berry-Kravis et al., 2012; Dolen et al., 2007; Henderson et al., 2012; Michalon et al., 2012). Similar examples are also reported in other ASD mouse models (Bhattacharya et al., 2012; Silverman et al., 2012). These examples, although still limited, are exciting because they support the concept of reversibility in postnatal brains in animal models. It remains untested and an ongoing subject of debate whether the same may be accomplished in humans for these or other CNS related disorders.

## Drugs to enhance glutamatergic synaptic activity

Shank family proteins interact with both ionotropic (NMDA and AMPAR receptors) and metabotropic glutamate receptors (mGluRs) at the PSD through different protein interaction domains (Grabrucker et al., 2011; Gundelfinger et al., 2006; Kreienkamp, 2008). However, how the interactions between *Shanks* and different types of receptors are coordinated is poorly understood. Biochemical analysis of *Shank* mutant mice has led to a general conclusion that there is reduced synaptic function mediated by glutamate receptors (Bozdagi et al., 2010; Hung et al., 2010; Schmeisser et al., 2012; Wang et al., 2011; Won et al., 2012). This result then raises the interesting question as whether pharmacological approaches which enhance glutamate receptor activity may have therapeutic benefit to Shankopathies. This hypothesis has been tested in *Shank2* mutant mice (Won et al., 2012). Treatment with a positive allosteric modulator of the metabotropic glutamate receptor 5 (mGluR5), which enhances NMDA receptor function via mGluR5 activation, normalizes NMDA receptor function and markedly enhances social interaction in *Shank2* mutant mice (Won et al., 2012). These data support a basic premise to test different glutamate receptor agonists such as the NMDA agonist D-cyclosine or other mGluR positive allosteric modulators for mGluR1/5 in *Shank* mouse models (Smith and Ehlers, 2012). The anticipated challenges for using these receptor modulators are their specificity and selectivity. For instance, Shank proteins have different expression patterns in different brain regions or synapses (Bockers et al., 2004; Leblond et al., 2012). Enhancing glutamate receptor activity in neurons that do not express SHANKs may have detrimental effect due to disrupting the balance of circuits mediated by SHANK proteins.

## Molecular restoration of SHANK proteins

Because haploinsufficiency of *SHANKs* is predicted to be the major molecular mechanism for *SHANK* causing ASD in the majority of cases caused by the deletions of entire *SHANK* genes, an interesting possibility for treatment is whether the transcription of *SHANKs* can be up-regulated from the non-mutated allele by a molecular approach. The isoform specific expression of *SHANK3* is epigenetically regulated (Beri et al., 2007; Ching et al., 2005; Maunakea et al., 2010). *SHANK3* has multiple CpG islands in the gene body which harbor multiple intragenic promoters (Wang et al., 2011). These CpG islands display tissue specific DNA methylation and other epigenetic marks both in humans and mice (Beri et al., 2007; Ching et al., 2005). The isoform-specific expression of *SHANK3* could be modified by DNA methylation inhibitors and histone deacetylase (HDAC) inhibitors, as well as methylation promoters in cultured neurons (Beri et al., 2007; Maunakea et al., 2010). Whether these epigenetic modifications may have similar impact *in vivo* has not been investigated. A drug screen to discover compounds that can up regulate *SHANK3* from the non-mutated allele would be an attractive direction for future investigation. It should be noted that the successful use of this approach has been reported in Angelman syndrome using a Ube3a reporter fusion protein approach in mice (Huang et al., 2012). Similarly, multiple CpG islands and brain tissue specific methylation are also found in *SHANK1* and *SHANK2* and an epigenetic mechanism may also be involved in regulating the expression of these genes.

Conceptually, genetic restoration of SHANK proteins by gene delivery is an ideal approach in individuals with *SHANK* mutations. The rationale for this approach is straight forward as in gene therapies proposed in other genetic disorders. The challenge is that SHANK proteins are structural proteins in synapses. The delivery of exogenous SHANK3 protein inside of cells to reach the proper sub-cellular targets in brains is the major obstacle for this experimental design. A decade ago, gene delivery to the brain was limited to stereotaxic injection of viral vectors into the brains of laboratory animals (Mueller et al., 2012). More recently, advancements in vector design and the exploration of alternative routes of administration have made efficient global central nervous system (CNS) gene delivery a possibility despite other remaining significant challenges (Guggenhuber et al., 2010). The most popular CNS gene delivery vector is adeno-associated virus (AAV) (Mueller et al., 2012). Lentivirus-based vectors also play an increasingly significant role in CNS-directed gene therapy because they have the advantage of a larger packaging capacity than AAV (Manfredsson and Mandel, 2011). Multiple groups have now reported in detail the ability of AAV9 vectors to cross the blood brain barrier (BBB) and transduce neurons and astrocytes following intravenous injection in neonatal and adult mice, cats, and nonhuman primates (Duque et al., 2009; Foust et al., 2009). Strategies employing intravenous delivery of AAV9 vectors have successfully treated spinal muscular atrophy (Foust et al., 2010) and mucopolysaccharidosis (MPS IIIB) in mice (Fu et al., 2011). Using a similar design, several studies have treated lysosomal storage diseases in animal models (Li et al., 2012; Sun et al., 2008). Proof of principle studies have also been reported for Rett syndrome and Angelman syndrome, two typical neurological disorders that share a number of similarity with *SHANK* causing ASD (Daily et al., 2011; Gadalla et al., 2012). The major variables influencing the feasibility of any gene therapy approach include 1) whether a secreted factor can be utilized or if the therapeutic gene product is limited to cell autonomous effects, 2) what the range of effective and tolerated gene expression is, and 3) whether the expressed products can pass BBB and what type of delivery efficiency is required for a meaningful therapeutic effect.

## Neuromodulation by transcranial magnetic stimulation as a treatment modality

Repetitive transcranial magnetic stimulation (rTMS) is a technique for non-invasive stimulation of brain via generation of a pulse of high intensity magnetic field by passing a brief electric current through an inductive coil (Pell et al., 2011; Peterchev et al., 2011). Recently, substantial interest in the use of this technique for the treatment of neuropsychiatric disorders such schizophrenia and depression has emerged (Husain and Lisanby, 2011; Rossi et al., 2009; Rossini and Rossi, 2007; Stanford et al., 2011; Wassermann and Lisanby, 2001). One notable development in this regard is that rTMS recently joined electroconvulsive therapy (ECT) as one of FDA approved neuromodulation techniques for treating major depression (Husain and Lisanby, 2011). In terms of therapeutic potential, rTMS paradigms have been shown to reactivate hypoactive structures, inhibit overactive structures, and induce long-term potentiation (LTP)-like effects in human brain and in a few animal studies (Houdayer et al., 2008; Huang et al., 2005). Low frequency (1Hz) stimulation has been shown to suppress excitatory synaptic transmission while high frequency (5–50Hz) or the intermittent form of theta burst stimulation (TBS) may potentiate it (Aydin-Abidin et al., 2008; Pell et al., 2011). The cellular mechanisms underlying these rTMS effects are poorly understood (Funke and Benali, 2011). A factor contributing to this lack of understanding is the considerable variability in the experimental designs employed in prior research (Pell et al., 2011). Nonetheless, several interesting observations in the literature suggest that rTMS may have therapeutic potential by modulating synaptic plasticity in Shankopathies. Chronic treatment with high frequency rTMS in awake animals significantly increases the expression of the AMPAR Glu1A subunit, a key component for

synaptic plasticity in hippocampus (Gersner et al., 2011; Newpher and Ehlers, 2009), and also enhances LTP (Ahmed and Wieraszko, 2006; Esser et al., 2006; Hoogendam et al., 2010; Kim et al., 2006). This suggests that the cellular mechanism underlying rTMS may be mediated by modulating the expression synaptic genes. These observations and the consistent finding of impaired synaptic plasticity in ASD mouse models including impaired hippocampus LTP in *Shank* mouse models (Bozdagi et al., 2010; Shepherd and Katz, 2011; Wang et al., 2011) support that SHANK3 causing ASD would be a good target for investigation into rTMS treatment. In this regard, an advantage of rTMS is that it is non-invasive and relatively limited in risks so that it may be applied to humans immediately without pre-clinical trials. Mouse models of SHANK related ASD then offer an opportunity to dissect the mechanism underlying rTMS treatment.

## Future directions

Studies of Shankopathies in humans and mouse models have provided a framework for future investigations of treatment and intervention of ASD. Numerous questions have also emerged from the analysis of *SHANK* defects in human ASD patients and *Shank* mutant mice. In humans, natural history studies of genotype and phenotype in patients with various *SHANK* mutations are critical. A detailed description and comparison of clinical features in patients with different *SHANK* mutations will provide guidance for modeling human disease in animal models. There is a critical need to directly compare the different *Shank* mutant mice head to head for cellular, synaptic, circuit, and behavioral phenotypes. Such direct comparisons will allow for more definitive identification of common synaptic defects, circuit endophenotypes, and behaviors. Can mutations in *Shanks* open the door to a molecular pathway that provides novel therapeutic targets? Much remains to be learned, but it is tempting to consider Shank3 “restoration” in a loose sense as a therapeutic strategy for Phelan-McDermid syndrome, and perhaps more broadly in ASD. Ultimately, the value of *Shank* mutant mice will depend critically on the ability to use human patients to validate their predictive utility.

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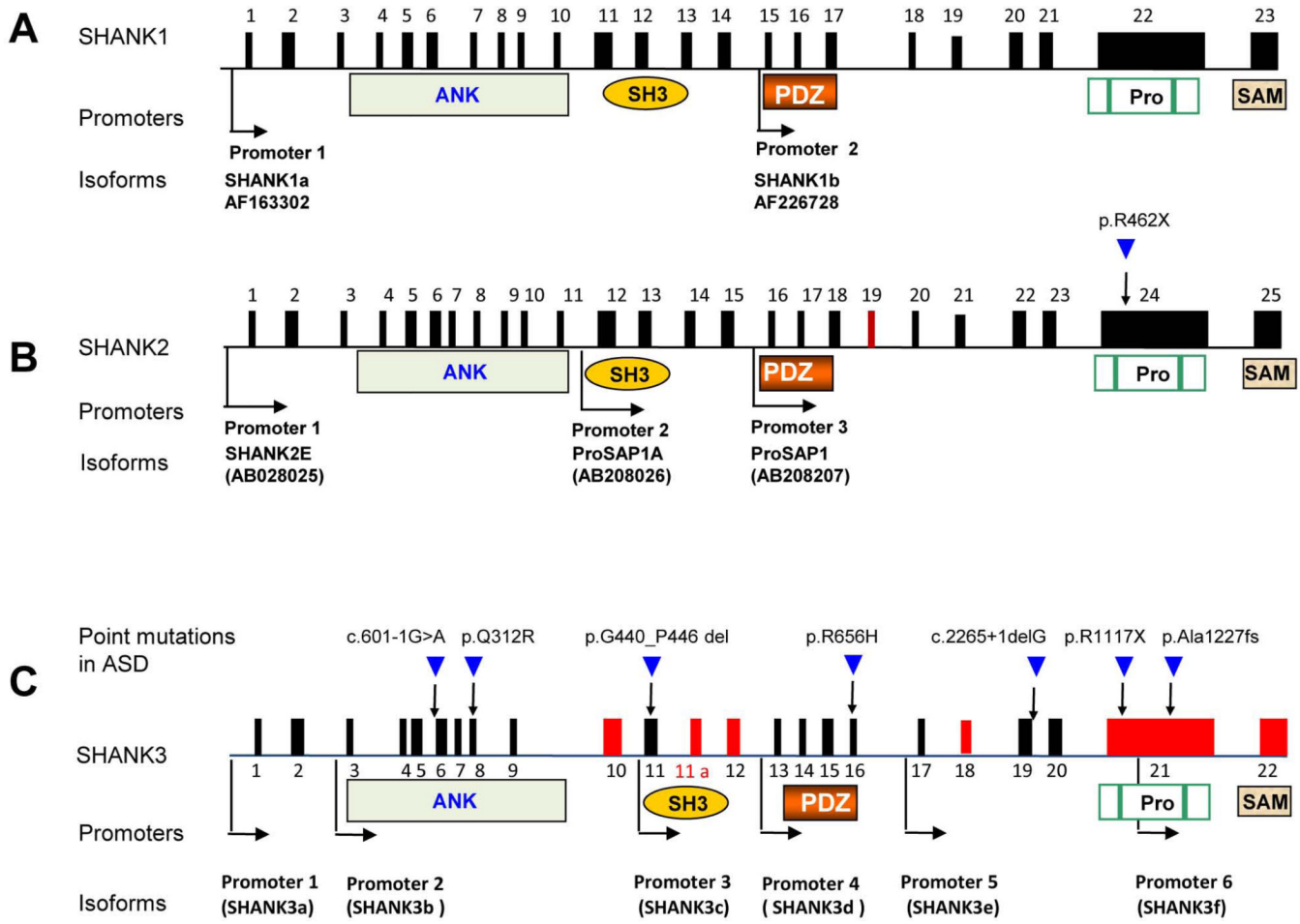


**Figure 1. *SHANK family* gene structure, mutations, and protein domains**

The gene structures are deduced from cDNAs of AF163302 for SHANK1, AB208205 for SHANK2, and AB569469 for SHANK3 deposited in GenBank. The promoters are shown in arrows and the alternatively spliced exons are indicated in red. Microdeletions of SHANK1, SHANK2, and SHANK3 and point mutations of SHANK2 and SHANK3 are reported in ASD. Exon 11a of SHANK3 is a newly identified exon. The positions of six identified promoters are indicated as black arrows. The exons in red are alternatively spliced. The positions of point mutations are indicated as blue arrows and the nature of point mutations are as described above the arrow. c.601-1G>A splicing mutation in intron 5 (Hamdan et al., 2011), p.Q312R in exon 8 (Moessner et al., 2007), p.G440_P446del in exon 11 (Waga et al., 2011), p.R656H in exon 16 (Waga et al., 2011), c.2265+1delG splicing mutation in intron 19 (Gauthier et al., 2009), p.R1117X (Gauthier et al., 2010) and p.Ala1227fs in exon 21 (Durand et al., 2007). Protein domains are shown and aligned to corresponding exons (Pro, proline rich region).

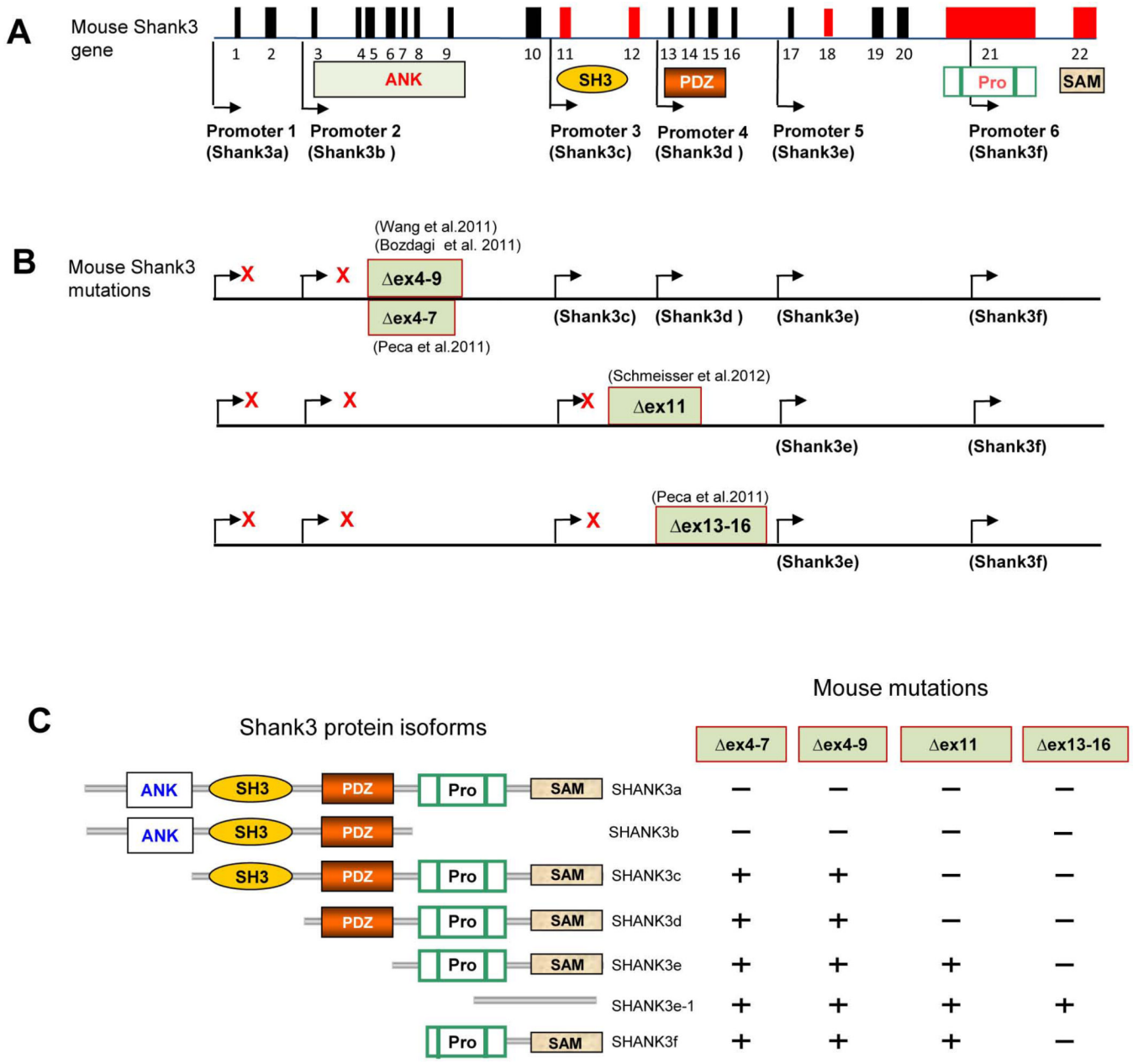


**Figure 2. Targeted Mutations in *Shank3* Mice**

(A) Schematic of mouse gene structure deduced from cDNA AB230103 deposited in GenBank. The promoters are shown in arrows and the alternatively spliced exons are indicated in red. (B) Schematic of *Shank3* mutant mice. The positions of targeted mutations in five different lines of *Shank3* mutant mice are shown. The transcripts that are predicted or confirmed to be disrupted (red X) or intact in each mutant line of mice are indicated. Exon 21 is spliced out in known *Shank3b* and *Shank3e-1* isoforms. Whether the exon 21 is spliced out in transcripts from other promoters has not been determined (Bangash et al., 2011; Bozdagi et al., 2010; Peca et al., 2011; Wang et al., 2011). (C) The predicted isoform-specific expression of Shank3 mRNA and protein in Shank3 mutant mice. The "−" indicates that the isoform is disrupted and "+" indicates the isoform remains intact. The full complement of Shank3 mRNA and protein isoforms that derive from combinations of

alternative promoters and mRNA splicing remains unknown. Therefore, the pattern of isoform-specific expression and disruption by specific mutations is likely more complex than indicated.

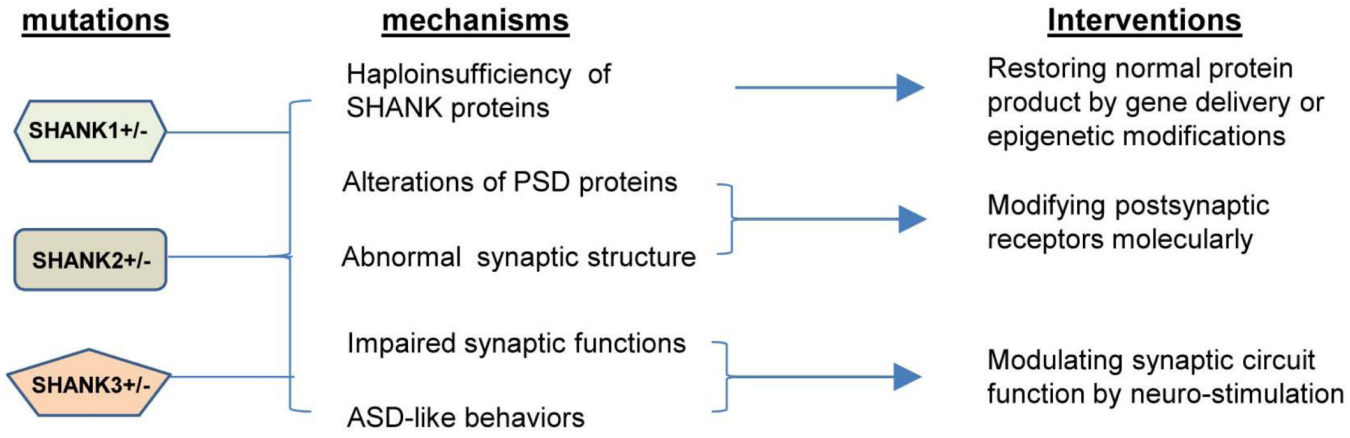


**Figure 3. Therapeutic approach for Shankopathies**

The proposed therapeutic approaches for SHANK family gene causing ASD based on the molecular and circuit mechanism.