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Phelan McDermid syndrome: from genetic discoveries to animal models and treatments

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Abstract

Phelan McDermid syndrome (PMS) or 22q13.3 deletion syndrome is a rare neurodevelopmental disorder characterized by generalized developmental delay, intellectual disability, absent or delayed speech, seizures, autism spectrum disorder, neonatal hypotonia, physical dysmorphic features, and recurrent medical comorbidities. In most cases, individuals with PMS have terminal deletions of the chromosomal region 22q13.3 encompassing *SHANK3*, a gene encoding a structural component of excitatory synapses indispensable for proper synaptogenesis and neuronal physiology.

Here, we review the clinical aspects of the syndrome and the genetic findings shedding light onto the underlying etiology. We also provide an overview on the evidence from genetic studies and mouse models that supports *SHANK3* haploinsufficiency as a major contributor of the neurobehavioral manifestations of PMS. Finally, we discuss how all these discoveries are uncovering the pathophysiology of PMS and are being translated into clinical trials for novel therapeutics ameliorating the core symptoms of the disorder.

Clinical phenotype of Phelan McDermid

Phelan McDermid syndrome (PMS) or 22q13.3 deletion syndrome is a rare neurodevelopmental disorder with over 600 cases documented so far. In 1985 the first case was reported (Watt et al., 1985), but only in 1994 Nesslinger and colleagues proposed that the symptoms described in individuals with 22q13.3 deletions clustered under a common

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phenotype (Nesslinger et al., 1994). Since then, the ~150 cases described in the literature have helped delineating a complex phenotype with neonatal, physical, neurological and behavioral features (Luciani et al., 2003; Nesslinger et al., 1994; Phelan and McDermid, 2012; Sarasua et al., 2014a; Sarasua et al., 2011; Soorya et al., 2013).

PMS presents with neonatal hypotonia, which can contribute to poor feeding and hyporeflexia and often persists in children and adults as gait disturbances. Physical dysmorphic features, including macrocephaly, dolichocephaly, tall stature, dysplastic toenails and a typical *facies* with long thick eyelashes, pointed chin, and bulbous nose, are also common. The neurobehavioral symptoms include generalized developmental delay, with intellectual disability of variable severity, absent or delayed speech, seizures and autism spectrum disorder (ASD). It has been estimated that over 80% of individuals with PMS meet diagnostic criteria for ASD (Soorya et al., 2013), and PMS is emerging as one of the most frequent and penetrant causes of autism (Betancur and Buxbaum, 2013). In many cases, changes in the corpus callosum (thinning or hypoplasia) and white matter anomalies can be detected (Aldinger et al., 2013; Dhar et al., 2011; Philippe et al., 2008; Soorya et al., 2013). Amongst the recurrent medical comorbidities are pain insensitivity, cardiac and renal abnormalities, recurring upper respiratory tract infections, gastroesophageal reflux, lymphedema and strabismus (Soorya et al., 2013). To date, several studies have also implied that subjects with PMS are at a higher risk to develop atypical bipolar disorder (BPP) (Denayer et al., 2012; Verhoeven et al., 2012; Vucurovic et al., 2012) and show progressive loss of skills during their lifetime. Prominent neurological regression during aging and even death in early and mid adulthood has been also reported in PMS (Bonaglia et al., 2011; Durand et al., 2007; Willemsen et al., 2012).

The etiology underlying Phelan McDermid syndrome

PMS is typically caused by the loss of the distal segment of the long arm of chromosome 22, as the consequence of terminal or interstitial (subtelomeric) deletions, balanced or unbalanced translocations, ring chromosomes (the chromosome arms are fused together to form a ring), and mosaicisms of these anomalies (Bonaglia et al., 2009; Bonaglia et al., 2011; Dhar et al., 2010; Jeffries et al., 2005; Luciani et al., 2003; Phelan and McDermid, 2012; Sarasua et al., 2014a; Soorya et al., 2013; Wilson et al., 2003). Microscopically visible rearrangements, including large deletions, translocations and ring chromosomes, can be detected with G-banding karyotyping (Bonaglia et al., 2011; Soorya et al., 2013), while cryptic translocations can be revealed using fluorescence *in situ* hybridization (FISH) (Bonaglia et al., 2001). Microarray technologies, including array-based comparative genomic hybridization (array-CGH) and chromosome microarray analysis (CMA), as well as targeted deletion analysis by FISH or multiplex ligation-dependent probe amplification (MLPA), are efficient in capturing submicroscopic deletions (Bonaglia et al., 2011; Dhar et al., 2010; Luciani et al., 2003; Soorya et al., 2013). As we shall discuss further below, sequencing technologies are expanding the landscape of PMS etiology to intragenic variation, including point mutations. Since PMS can be misdiagnosed with other disorders (Phelan and McDermid, 2012), the technological advances and integration of cytogenetic and molecular genetic techniques are essential to enable earlier and more accurate diagnosis. For example, individuals diagnosed with nonsyndromic ASD can be found with 22q13.3

deletions, as revealed by screenings of ASD cohorts using FISH (Durand et al., 2007), microarrays (Bremer et al., 2011; Guilmatre et al., 2009; Leblond et al., 2014; Marshall et al., 2008; Moessner et al., 2007; Pinto et al., 2014; Pinto et al., 2010; Rosenfeld et al., 2010; Sebat et al., 2007), or analysis of copy number variations from whole-exome sequencing data (De Rubeis et al., 2014; Poultney et al., 2013).

About 75% of PMS subjects have terminal deletions that in most cases are *de novo* and paternally derived (Bonaglia et al., 2011; Luciani et al., 2003; Sarasua et al., 2014a; Wilson et al., 2003). Terminal deletions have typically nonrecurrent breakpoints and can extend up to 9 Mb (Bonaglia et al., 2011; Sarasua et al., 2014a; Soorya et al., 2013). Studies on genotype-phenotype correlations have converged on an association between the size of the deleted region and the number and/or severity of clinical manifestations (Luciani et al., 2003; Sarasua et al., 2014a; Sarasua et al., 2014b; Sarasua et al., 2011; Soorya et al., 2013; Wilson et al., 2003), although the specific findings on phenotypic variables are not completely consistent across studies. A report on 30 individuals carrying ring 22 chromosome found that the deletion size correlated with certain dysmorphic features and with the severity of developmental and speech delay (Jeffries et al., 2005). Another study on 56 individuals with terminal deletions found that the deletion size was associated with developmental delay, hypotonia, head circumference, recurrent ear infection, pointed chin, and dental anomalies (Wilson et al., 2003). Analyses from three non-independent PMS cohorts [the extended cohort comprising 201 participants (Sarasua et al., 2014a)] have shown that developmental delay, abnormal growth, and dysmorphic features are more pronounced in carriers of larger deletions, with macrocephaly and speech ability being the more robust associations (Sarasua et al., 2014a; Sarasua et al., 2014b; Sarasua et al., 2011). Conversely, aggressive behavior and ASD (assessed using parent report) are inversely correlated with deletion size (Sarasua et al., 2014a; Sarasua et al., 2014b; Sarasua et al., 2011). An investigation of 32 subjects has noted a significant correlation between deletion size and number of dysmorphic features or medical comorbidities (Soorya et al., 2013). In contrast with the results from Sarasua and colleagues, no changes were found in speech ability. Also, two standard ASD diagnostic scores related to social interactions and communication were positively correlated with deletion size (Soorya et al., 2013). These results indicate that the ASD behavioral manifestations exacerbate with increasing deletion size.

The evidence that the penetrance and expressivity of the symptoms correlate with the extent of the deleted region suggests that more than one haploinsufficient gene contributes to PMS phenotype. Amongst the 90 genes that can be deleted as a consequence of the 22q13.3 rearrangements, *SHANK3*, one of the three genes lost in all terminal deletions (Luciani et al., 2003), has emerged as the most robust candidate gene for the neurobehavioral correlates of PMS. First of all, the majority of subtelomeric deletions (Bonaglia et al., 2011; Bonaglia et al., 2006; Delahaye et al., 2009) or translocations (Bonaglia et al., 2001) have their breakpoints in *SHANK3*. In the first report, the authors described a *de novo* reciprocal balanced translocation between chromosomes 12 and 22 with the breakpoint in exon 21 of *SHANK3* in a child exhibiting the typical facial dysmorphic features, mild hypotonia, dolichocephaly, mild intellectual disability and severe speech impairment (Bonaglia et al., 2001). Only 12 individuals with interstitial deletions not involving *SHANK3* have been

reported thus far (Disciglio et al., 2014; Wilson et al., 2008). These patients display a phenotype partially overlapping with PMS, including developmental delay, hypotonia, overgrowth and macrocephaly (Disciglio et al., 2014; Wilson et al., 2008). However, the ASD symptomatology has not exhaustively been described in these patients: ASD was assessed only in three patients out of 12, among which two were reported as being affected (Disciglio et al., 2014).

Another line of evidence supporting that *SHANK3* haploinsufficiency is the major determinant of the PMS neurobehavioral symptoms relates to the discovery of *SHANK3* point mutations in subjects with phenotypes overlapping PMS and ASD. Amongst the 32 subjects examined by Soorya and colleagues, two carry *de novo* mutations (a nonsense and a frameshift mutation) and show core features of PMS (Soorya et al., 2013). The subject with a *de novo* frameshift mutation (p.R834RfsX59) displays dolichocephaly and short stature, hypotonia, intellectual disability, repetitive behavior, hyperactivity, aggression, seizures, but no ASD (Soorya et al., 2013). The subject with a *de novo* nonsense mutation (p.W509X) has several dysmorphic features, macrocephaly, intellectual disability, repetitive behavior, hyperactivity and autism (Soorya et al., 2013). Furthermore, large-scale targeted resequencing (Boccutto et al., 2013; Durand et al., 2007; Gauthier et al., 2009; Leblond et al., 2014; Moessner et al., 2007) and whole-exome sequencing (De Rubeis et al., 2014) have identified *de novo SHANK3* point mutations or indels in subjects diagnosed with idiopathic ASD. Some of these patients retrospectively present with manifestations of PMS, although with a certain degree of variability (Leblond et al., 2014). It is also worth mentioning that the contribution of point mutations/indels is likely underestimated by the current studies: the identification of variants in *SHANK3* has been hindered by the poor coverage of whole-exome sequencing due to the high CG content of this gene (Betancur and Buxbaum, 2013; Krumm et al., 2014; Wilson et al., 2003), therefore targeted resequencing has been the most effective approach. Finally, the genetic evidence pinpointing *SHANK3* as a major determinant in PMS phenotype is coalescing with findings from cell and mouse models, as we shall further discuss below. Briefly, mice deficient or ablated for *Shank3* show an array of neuroanatomical, physiological and behavioral phenotypes that are compatible with the clinical manifestations of PMS (Bozdagi et al., 2010; Drapeau et al., 2014; Kouser et al., 2013; Wang et al., 2011; Yang et al., 2012). Also, some of the physiological defects observed in functional neurons differentiated from induced pluripotent stem cells derived from individuals with PMS can be alleviated by reintroducing *SHANK3* (Shcheglovitov et al., 2013).

In addition, there are observations implicating 22q13.3 and/or *SHANK3* perturbations in other disorders. Five subjects have been reported with 22q13.3 duplications and other psychiatric conditions [Asperger syndrome (Durand et al., 2007), attention-deficit/hyperactivity disorder (ADHD) (Moessner et al., 2007), schizophrenia (Failla et al., 2007), and hyperkinetics disturbances (Han et al., 2013)]. Furthermore, *de novo SHANK3* mutations have been detected in individuals diagnosed with atypical schizophrenia (Gauthier et al., 2010) or intellectual disability (Gong et al., 2012; Hamdan et al., 2011a). Finally, mutations or copy number variants in other genes of the *SHANK* family (*SHANK1* and *SHANK2*) have been described in cases of ASD and intellectual disability (Berkel et al., 2010; Leblond et al., 2012; Leblond et al., 2014; Pinto et al., 2010; Sato et al., 2012).

The Shank family

Each of the *SHANK1*, *SHANK2* and *SHANK3* genes is positioned on a different chromosomal location and encode for the Shank1, Shank2 and Shank3 proteins, respectively, all of which are related to the same family of proteins known by different names; the Shank protein family, the Proline-rich synapse-associated proteins (ProSAP) (Boeckers et al., 1999), the Cortactin binding proteins (CortBP) (Du et al., 1998), the Somatostatin receptor interacting proteins (SSTRIP) (Zitzer et al., 1999), or the Synamons (Yao et al., 1999). This family of proteins is highly enriched in a specific compartment of the postsynaptic membrane of excitatory synapses, known as the post-synaptic density (PSD) (Sheng and Kim, 2011). The PSD is an ultrastructural thickening that is composed of a network of scaffolding proteins, among which the Shank molecules represent about 5% of the total protein molecules and total protein mass (Sugiyama et al., 2005). Each of the Shank encoding genes contains numerous splicing sites and alternative promoters thus producing several isoforms (Wang et al., 2011) (Boeckers et al., 1999; Lim et al., 1999; Yao et al., 1999; Zitzer et al., 1999), which contribute to the transcriptional and functional complexity that these genes deliver (Wang et al., 2014). Shank members are expressed in the peripheral organs as well as in the brain. Within the brain they were all shown to be expressed in the cortex and hippocampus, while Shank1 expression was also detected in the amygdala, substantia nigra, and thalamus and Shank2 expression was detected in the olfactory bulb and the central gray. High levels of Shank3 were detected in the striatum (Peca et al., 2011). In the cerebellum, Shank2 and Shank3 are differentially expressed in the Purkinje and the granule cells, respectively (Sheng and Kim, 2000) (Boeckers et al., 1999). This differential expression could be mediated by epigenetic modifications including DNA methylation, which was previously shown to regulate tissue-specific expression of Shank3 (Beri et al., 2007; Ching et al., 2005; Maunakea et al., 2010).

A full length Shank isoform harbors five protein-protein interaction motifs/domains; the ankyrin repeats domain (ANK), a Src homology 3 (SH3) domain, a PDZ domain, a proline rich region, and a sterile α -motif (SAM) domain (Kreienkamp; Sheng and Kim, 2000). Through the SAM domain, the Shank proteins can self-assemble to form the Shank platform at the PSD, which plays a vital role in synaptogenesis and synaptic maintenance and plasticity (Baron et al., 2006; Grubruker et al., 2011; Gundelfinger et al., 2006). Through the other domains, Shank proteins can also form direct and indirect interaction with additional scaffolding proteins within the PSD, with the glutamatergic receptors (AMPA and NMDA receptors) and cell adhesion molecules at the membrane, and with cytoskeletal components within the post-synapse (Boeckers et al., 2002; Naisbitt et al., 1999; Sheng and Kim, 2000).

Since they have an essential role in structuring and organizing the PSD, Shank proteins shape the synaptic morphology and function, thus perturbations of their expression profoundly affect synaptic composition and physiology (Arons et al., 2012; Durand et al., 2012; Grubruker et al., 2011; Hung et al., 2008; Roussignol et al., 2005; Sala et al., 2001; Sala et al., 2005). For example, Shank1 and Shank3 overexpression in hippocampal cultures increased the levels of polymerized actin (F-actin) in dendritic spines and promoted their maturation (Durand et al., 2012; Sala et al., 2001), while Shank1 silencing affected both

spine size and number, and led to reduced PSD thickness (Hung et al., 2008). Moreover, altering Shank2 and Shank3 levels by manipulating the Zn^{2+} extracellular concentration led to changes in the PSD size and affected the assembly of the immature synapse (Grabrucker et al., 2011; Sugiyama et al., 2005). Finally, expression of Shank3 into aspiny cerebellar neurons provoked the maturation of spines with functional excitatory synapses (Roussignol et al., 2005). Changes in synaptogenesis and spine morphology induced by Shank proteins mirror effects on neuronal activity, including AMPAR and NMDAR-mediated synaptic transmission, transsynaptic transmission and synaptic plasticity (Arons et al., 2012; Durand et al., 2012; Hung et al., 2008; Roussignol et al., 2005; Sala et al., 2001). Furthermore, the neuronal effects of 22q13.3 deletions have recently been studied in human neurons differentiated from induced pluripotent stem cells derived from two individuals with PMS (Shcheglovitov et al., 2013). Compared with control neurons, PMS neurons showed deficits in excitatory, but not inhibitory, synaptic transmission, fewer excitatory synapses, and decreased expression of excitatory neurotransmitter receptors. The alterations in synaptic transmission could be reversed by restoring SHANK3 expression (Shcheglovitov et al., 2013).

De novo and inherited *SHANK3* mutations have been found in individuals with ASD (Durand et al., 2007; Moessner et al., 2007). By transfecting wild-type and mutant SHANK3 in primary hippocampal neurons, independent studies have found that three of the reported point mutations (p.E12C, p.R300H, p.Q321R, the latter two in the ANK domain) do not interfere with the postsynaptic targeting, but affect the number and the morphology of dendritic spines, synaptic F-actin levels, and spontaneous neuronal activity (Arons et al., 2012; Durand et al., 2012; Mameza et al., 2013). Furthermore, a prematurely truncated SHANK3 resulting from a frameshift mutation in exon 21 (Durand et al., 2007) and missing the SAM domains and the binding sites for critical partners (Homer and Cortactin) was retained in the soma when overexpressed in hippocampal neurons. The truncated SHANK3 also exerts a dominant-negative effect on spine induction and synaptic transmission (Arons et al., 2012; Durand et al., 2012). Another missense mutation (p.L68P) (Gauthier et al., 2009) located in the N-terminal domain does not compromise postsynaptic targeting or synaptic transmission, but enhances the interaction with cytoskeletal components (Mameza et al., 2013).

To further clarify the function and the role of the Shank proteins in the brain, several groups have developed and characterized genetically modified mice models with deficiency in one of the Shank family members, aiming to mimic some of the aforementioned human mutations.

Animal models for Shank genes deficiency

Animal models are advantageous for understanding the function of genes and their encoded proteins in the context of the entire organism. In addition to their particular value in investigating the complex interplay of molecular and cellular pathways within the brain, they are preferable models for studying neurodevelopmental disorders where behavioral deficits are among the core symptoms. Given the relevance for *SHANK1* and *SHANK2* mutations in ASD, we will briefly report on the findings in animal models mimicking their deficiency. We

will provide a more extended summary of the available *Shank3*-deficient mice models, which are being used as a mean for understanding the pathophysiology of PMS and ASD and for the discovery of new therapeutic targets.

Shank1-deficient mice

Shank1-deficient mice were the first to be generated to study the function of the Shank proteins *in vivo* (Hung et al., 2008). In this model, Shank1 is lacking most of the PDZ domain leading to undetectable levels of the protein. The mice show altered PSD protein composition, reduced dendritic spine size, smaller and thinner PSDs, and reduced basal synaptic transmission with no change in synaptic plasticity. Behaviorally, the mice have anxiety, impaired motor learning and contextual fear memory, and enhanced performance in spatial learning but impaired long-term memory retention in the same task. They do not show any ASD-relevant social interaction deficits (Silverman et al., 2011). However, they display deficits in the emission of ultrasonic vocalizations in the pups and urine-based communicative behaviors in the adult males, indicating a specific deficit in social communicative behavior (Wöhr et al., 2011), and alterations in self-grooming and marble burying behaviors, reflecting changes in their repetitive behavior (Sungur et al., 2014).

Shank2-deficient mice

Several mutations and small deletions in *SHANK2* have been reported in subjects with ASD (Berkel et al., 2010). Functional studies on three mutations found in patients (p.L1008_P1009dup, p.T1127M, p.R462X) have shown that overexpression of mutant SHANK2 proteins lead to smaller spines, fewer synapses and more complex dendritic branches, and that one mutation (p.R462X) causes mislocalization. Furthermore, when newborn mice were injected with viral particles carrying Shank2-R462X; electrophysiological recordings of the hippocampal slices isolated three months after the injection showed reduction in miniature postsynaptic AMPA receptor currents (Berkel et al., 2012). Also, the Shank2-R462X expressing mice were less likely to investigate a novel object and less capable at navigating a maze than control mice (Berkel et al., 2012).

Following these studies, two *Shank2*-deficient mice models were generated by targeting the PDZ domain of the Shank2 gene. The first model harbors a deletion of exons 6 and 7 (*Shank2*^{-/-}, ex6-7) (Won et al., 2012), while the second one has a deletion spanning exon 7 (*Shank2*^{-/-}, ex7) (Schmeisser et al., 2012). Using different social behavior paradigms, both models showed impaired social behaviors, altered social communication assessed as ultrasonic vocalizations, and stereotypies, measured as grooming and digging behavior. The *Shank2*^{-/-}, ex6-7 model was further challenged on the Morris water maze to examine the cognitive functioning; using this task, mice displayed impaired spatial learning, but no deficits in working memory, object recognition memory or motor learning. Despite the similar behavioral phenotype of both models, findings related to synaptic physiology showed some discrepancies. While Schmeisser and colleagues reported an increase in NMDAR-mediated synaptic function in the *Shank2*^{-/-}, ex7 model, Won and colleagues reported an opposite observation.

Shank3-deficient mice

To investigate the pathophysiological consequences of *SHANK3* haploinsufficiency, pinpoint one or more nexus for therapeutic intervention, and establish animal models for drug testing, four independent groups have generated murine lines mimicking Shank3 deficiency or ablation (Bozdagi et al., 2010; Peca et al., 2011; Wang et al., 2011) (Kouser et al., 2013). Also, given the clinical relevance of *SHANK3* duplications (Durand et al., 2007; Failla et al., 2007; Moessner et al., 2007), a *Shank3*-overexpressing mouse model recapitulating hyperkinetic disorder has been generated (Han et al., 2013).

In the first published model of *Shank3* deficiency, the targeted region spans exons 4–9 (encoding the ANK domain), a region of clinical relevance since it can harbor mutations detected in subjects with ASD and intellectual disability (Hamdan et al., 2011b; Moessner et al., 2007). Exons 4–9 excision disrupted the full length Shank3 isoform (Shank3α) (Bozdagi et al., 2010). Mice heterozygous for exons 4–9 deletion have a significant reduction in basal glutamatergic synaptic transmission in the hippocampus, specifically in the component mediated by the AMPA receptors (AMPA), as compared with wild-type littermates (Bozdagi et al., 2010). Such effect is likely due to a decrease in synaptic AMPARs, since neurons from *Shank3* heterozygous animals have an attenuated expression of the AMPAR subunit GluR1. In addition to basal transmission, *Shank3* deficiency caused defects in synaptic plasticity, with robust deficits in the maintenance of long-term potentiation (LTP) but no perturbations in long-term depression (LTD). The lack of stabilization of the synaptic potentiation was accompanied by a transient enlargement of the dendritic spine volume, which was conversely persistent in control animals. The authors hypothesized that the deficits in synaptic consolidation could be due to altered AMPA receptor trafficking. Behavioral analyses also indicated that *Shank3* heterozygotes had deficits in reciprocal social interaction and communication, as documented by reduced social sniffing and ultrasonic vocalizations during interactions between males and estrus females. These analyses and other behavioral tests were performed on two additional experimental cohorts to obtain a comprehensive phenotyping of *Shank3* heterozygous and homozygous mice at infant, juvenile, and adult ages (Yang et al., 2012). The larger study confirmed previous data on impaired AMPA-mediated basal transmission and LTP maintenance, and extended these findings to homozygous animals. The electrophysiology data were also corroborated by a subsequent study on the same model (Bozdagi et al., 2013). However, behavioral testing on the three cohorts revealed variability across animal cohorts (Yang et al., 2012). Overall, *Shank3* mutant mice displayed mild behavioral deficits, including reduction in some measures of juvenile reciprocal social interactions, higher levels of repetitive self-grooming, and impaired motor learning (Yang et al., 2012). The model generated by Bozdagi and colleagues was also screened for strain-specific effects on behavioral performances (Drapeau et al., 2014). Negligible strain effects were observed when comparing the behaviors of heterozygous or homozygous mice across genetic backgrounds (Drapeau et al., 2014), indicating that behavioral phenotypes associated with Shank3 haploinsufficiency are largely strain-independent.

Using the same approach of Bozdagi et al. (2010), an independent group generated a homozygous model targeting exons 4–9 and suppressing Shank3α expression (*Shank3^{ex4-9}*

model) (Wang et al., 2011). *Shank3^{ex4-9}* mice showed abnormal social behavior (reduced preference for social interaction, decreased social initiation, excessive self-grooming) and deficits in social communication (altered ultrasonic vocalizations). Mutant mice also displayed modest learning impairments as measured in the water maze test, obsessive behaviors, and mild motor deficits with tests in vertical placement, climbing down a vertical pole, foot-misplacement tasks, and accelerating rotarod. On the neuroanatomical side, an increase in the density of dendritic spines and significantly longer spines (resembling an immature phenotype) were noted in 4 weeks old but not 10-weeks old *Shank3^{ex4-9}* mice. In line with earlier findings from Bozdagi and colleagues (Bozdagi et al., 2010), the mutant mice displayed reduced hippocampal LTP, and the AMPA receptor subunit GluR1 showed reduced surface expression upon LTP induction. Unlike previous observations (Bozdagi et al., 2010), basal transmission function was intact.

Two more *Shank3* murine models were generated and characterized by Peca and colleagues (Peca et al., 2011). In the first line (*Shank3A^{-/-}*), exons 4–7 (ANK domain) were targeted, causing the ablation of the full-length Shank3 α , with no perturbations of the smaller isoforms Shank3 β and Shank3 γ . In the second line (*Shank3B^{-/-}*), exons 13–16 (PDZ domain) were deleted; as a consequence, Shank3 α and Shank3 β were fully suppressed, while Shank3 γ was significantly downregulated. *Shank3A^{-/-}* mice showed little to no behavioral alterations when compared with controls. Conversely, *Shank3B^{-/-}* mice developed excessive grooming, anxiety-like behavior and substantial deficits in social interaction, including perturbed social interactions and impaired recognition of social novelty. These behavioral anomalies were mirrored by biochemical, anatomical and electrophysiological changes in the striatum. The expression of several scaffolding proteins, including SAPAP3, Homer1, PSD93 and glutamate receptor subunits GluR2, NR2A and NR2B, was reduced in PSD preparations isolated from the striatum of *Shank3B^{-/-}* mice. Also, the striatum of the mutant mice was hypertrophic and the striatal medium spiny neurons had more complex dendritic arborization with reduced dendritic spine density. The dendritic spines had no alterations in morphology, but exhibited thinner and shorter PSDs. Electrophysiological recordings indicated perturbed cortico-striatal circuitry function in these mice, likely due to a loss in the number of functional synapses on the striatal medium spiny neurons and decreased postsynaptic responses from these synapses. In contrast with other observations (Bozdagi et al., 2010; Yang et al., 2012), no changes were detected while recording the hippocampal synaptic transmission.

Another mouse model was generated by deleting exon 21 (*Shank3^{C/C}*), which encodes the Homer binding domain, resulting in the loss of all the major isoforms detectable by Western blot (Kouser et al., 2013). The design was based on two clinical reports implicating exon 21, one subject with PMS and a balanced translocation having a breakpoint within exon 21 (Bonaglia et al., 2001), and another individual diagnosed with ASD carrying a disrupting frameshift in this exon (Durand et al., 2007). *Shank3^{C/C}* mice showed no global changes in the PSD proteins in hippocampal total lysates and purified synapses (synaptosomes), whereas the synaptic expression of the metabotropic glutamate receptor mGluR5 was elevated. Furthermore, *Shank3^{C/C}* animals had impaired spatial learning, poor motor coordination, avoidance towards inanimate objects, and excessive self-grooming in advanced adulthood. No alterations in ultrasonic vocalizations were observed. The

neuronal morphology appeared unaltered in the mutant mice, with no differences in spine density and shape when compared with control littermates. Electrophysiological recordings on hippocampal slices indicated impaired LTP, but no changes in mGluR-dependent LTD, despite the observed increase in mGluR5 (Kouser et al., 2013).

The comparison of these studies highlight discrepancies in many of the domains explored, and isoform specificity might account for some of the differences observed. Alterations in the biochemical composition of the PSD were found in the striatum of the *Shank3B*^{-/-} model (Peca et al., 2011), in the hippocampus of *Shank3*^{e4-9} homozygous mice (Wang et al., 2011), but not in the hippocampus of *Shank3*^{C/C} mice (Kouser et al., 2013). Spine density and morphology were found abnormal in striatal medium spiny neurons of *Shank3B*^{-/-} animals (Peca et al., 2011) and hippocampal neurons of *Shank3*^{e4-9} homozygous mice (Wang et al., 2011), but not *Shank3*^{C/C} mice (Kouser et al., 2013). Basal glutamatergic synaptic transmission in the hippocampus was intact in *Shank3*^{e4-9} homozygous mice (Wang et al., 2011), altered as a result of AMPAR-mediated transmission in the independent model with the same deletion (Bozdagi et al., 2010; Yang et al., 2012), or altered as a consequence of NMDAR-mediated transmission in the *Shank3*^{C/C} animals (Kouser et al., 2013). All the studies converge on a decrease in LTP but not in LTD (Bozdagi et al., 2010; Bozdagi et al., 2013; Kouser et al., 2013; Wang et al., 2011; Yang et al., 2012). Grooming was excessive in *Shank3B*^{-/-} but not in *Shank3A*^{-/-} animals (Peca et al., 2011), in *Shank3*^{C/C}, but only in old animals (Kouser et al., 2013), and in male *Shank3* heterozygous mice a trend was reported (Drapeau et al., 2014; Yang et al., 2012). Motor impairments assessed with different paradigms were reported in some studies (Bozdagi et al., 2013; Kouser et al., 2013; Wang et al., 2011; Yang et al., 2012) but not others (Drapeau et al., 2014). Anxiety levels were found normal (Yang et al., 2012), increased (Peca et al., 2011) or decreased (Drapeau et al., 2014). Variability across studies affects social interactions and social communication, two core features for ASD. Social interactions were found consistently affected in freely interacting animal pairs (Peca et al., 2011; Wang et al., 2011). In three-chamber arenas, mutant models showed higher preference for the interactions with the object rather than the social partner in a study (Peca et al., 2011) but not in other models (Drapeau et al., 2014; Kouser et al., 2013; Yang et al., 2012), along with reduced interest for social novelty in two models (Kouser et al., 2013; Peca et al., 2011) but not others (Drapeau et al., 2014). As for social communication, emission of ultrasonic vocalizations was found affected in some models and cohorts (Bozdagi et al., 2010; Wang et al., 2011; Yang et al., 2012), but not in others (Kouser et al., 2013).

The discrepancies discussed above illustrate how difficult it is to draw a unifying phenotype when dealing with different models. Yet, the convergences across models indicate that *Shank3* knockdown or knockout affect synaptic structure and function, forms of synaptic plasticity and ASD-related behaviors. Also, the results of these studies emphasize the importance of comparing phenotypes at all scales (biochemical, neuroanatomical, electrophysiological and behavioral) across experimental designs. This is of uttermost importance for PMS, given its phenotypic complexity and the relevance for these animal models to help disentangling the contribution of *SHANK3* from other potentially haploinsufficient genes in the 22q13.3 deleted region.

Behavioral and therapeutic interventions in PMS

Given the heterogeneity in the PMS phenotype, there is no generalized intervention that applies to all cases of PMS, and no pharmacological treatments are currently available to target the underlying pathophysiology of the disorder. Treatment strategies are tailored to the patient after a thorough medical history and physical examination to identify the clinical manifestations, so that each symptom and co-morbidity can be addressed specifically, usually with approaches or medications adopted and adapted from those applied in the general population. For example, early intervention programs and intensive physical and occupational therapies are recommended for motor skill deficits, appropriate medication is prescribed for hyperactivity, anxiety and self-stimulatory behaviors, and cardiac, renal, respiratory and other conditions are managed using to standard protocols [see (Phelan and McDermid, 2012) for further discussion]. If ASD is ascertained in PMS, behavioral and psychological treatments are considered as a first line of intervention. The aim for such treatments is to shift the trajectory of the child's atypical development toward a more typical developmental path (Reichow and Wolery, 2009; Seida et al., 2009; Warren et al., 2011). Clearly, none of the aforementioned treatments addresses the core symptoms or targets the core deficits at the molecular, cellular or circuitry level. Therefore, there is still an urgent need to develop pharmacological treatments that address the bases of the disorder. Here is where animal models for PMS are extremely valuable. A thorough analysis of these models allows us to understand the basic neurobiology of *SHANK3*, and to reveal mechanisms or pathways that are affected by *Shank3* deficiency. Once identified, components of these pathways can be targeted pharmacologically to reverse the deficits at the physiological and behavioral level. This translational approach has been recently applied in monogenic disorders associated with ASD including Fragile X syndrome, tuberous sclerosis, Rett syndrome, neurofibromatosis (Bear et al., 2004; Ehninger and Silva, 2009; Tropea et al., 2009), and, based on our work in rodents (Bozdagi et al., 2013), for PMS (Kolevzon et al., 2014). For instance, basic studies on animal models for Fragile X syndrome have pointed to excessive mGluR5 signaling as a major determinant of the pathology (Bear et al., 2004). Therefore, studies in preclinical models have shown the efficacy of mGluR5 antagonists or allosteric modulators in reversing the core deficits associated to the syndrome, and these findings have been brought into clinical trials (Dolen and Bear, 2008; Dolen et al., 2010; Krueger and Bear, 2011; Scharf et al., 2014). A similar approach is being used for therapeutic candidates targeting the GABAergic system, which has also been found altered in Fragile X (Braat and Kooy, 2015). Another example evolves from studies on mice models for tuberous sclerosis, which imply that mTOR signaling is excessively activated, contributing to the neuropsychiatric phenotypes, including cognitive deficits (Auerbach et al., 2011). Therefore, mTOR inhibitors were tested in these models for their ability to reverse the observed phenotypes and were found to have a beneficial effect on the cognitive function and the neurological symptoms, even if treatment was initiated in adult animals (Ehninger and Silva, 2009). As for Fragile X syndrome, these findings are being translated into clinical trials (Curatolo and Moavero, 2012).

In PMS, our study on *Shank3* heterozygous mice showed beneficial effects of IGF-1 treatment in reversing synaptic and motor deficits (Bozdagi et al., 2013), and an independent

study has found that IGF-1 is effective in reversing the synaptic deficits observed in neurons derived from induced pluripotent stem cells of patients with PMS (Shcheglovitov et al., 2013). These preclinical findings have led to a first pilot clinical trial with IGF-1 in PMS which has shown benefits with IGF-1 on core symptoms of ASD, suggesting a potentially disease-modifying effect, and forming the basis for larger studies to inform the efficacy of IGF-1 in PMS (Kolevzon et al., 2014). Notably, independent clinical studies on IGF-1 in Rett syndrome has provided clinical evidence for the efficacy of IGF-1 (Jou et al., 2011), thus increasing the interest in this compound and suggesting a broader application in other monogenic forms of ASD.

Several caveats need to be considered when evaluating the potential of translating preclinical findings into therapeutics. In fact, even clinical trials based on robust preclinical evidence have failed to provide clinical efficacy in humans and have therefore been terminated (Scharf et al., 2014). Yet, rigorous basic studies and drug testing in increasingly sophisticated animal models represent the most promising resource for the discovery of novel therapeutics. In particular, genetically modified rat models are evolving as a new model for ASD and ASD-related syndromes including PMS, and they will provide the research community with a powerful tool not only for neurobiological and behavioral studies, but also for boosting drug discovery and development.

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