Cellular and synaptic network defects in autism

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Abstract

Many candidate genes are now thought to confer susceptibility to autism spectrum disorder (ASD). Here we review four interrelated complexes, each composed of multiple families of genes that functionally coalesce on common cellular pathways. We illustrate a common thread in the organization of glutamatergic synapses and suggest a link between genes involved in Tuberous Sclerosis Complex, Fragile X syndrome, Angelman syndrome and several synaptic ASD candidate genes. When viewed in this context, progress in deciphering the molecular architecture of cellular protein-protein interactions together with the unraveling of synaptic dysfunction in neural networks may prove pivotal to advancing our understanding of ASDs.

Introduction

Autistic behaviors were independently identified as recognizable syndromes in the early 20th century by Kaner [1], Asperger [2] and Heller [3]. In the DSM-IV, the autism diagnosis spans a broad continuum of what are collectively known as ASDs or Pervasive Developmental Disorders. These encompass Rett syndrome, Asperger syndrome, Childhood Disintegrative Disorder (Heller syndrome), idiopathic autism and atypical autism (Pervasive Developmental Disorder Not Otherwise Specified) [4]. Although heterogeneous, ASDs are united by a combination of three core behavioral symptoms: a) impairments in language and communication; b) abnormal sociability or deficits in social interaction; and c) restricted interested and repetitive or stereotyped behaviors. In addition to these, other common pathological disturbances include gait and motor disturbances, anxiety, epilepsy, sensorial abnormalities, sleep disturbances and comorbidity with psychiatric disorders such as attention deficit hyperactivity disorder, obsessive-compulsive disorder (OCD) and mood disorders [5]•. ASDs are considered to be highly heritable and a genetic predisposition is thought to play an important role in the etiology of these disorders [5] •. For Rett syndrome in particular, mutations in the MECP2 gene are known to be responsible for the majority of cases and only a small percentage remain idiopathic. Moreover, autism is also highly prevalent in patients afflicted with other genetic syndromes such as Tuberous Sclerosis, Cowden, Fragile-X, Angelman, 16p11.2 deletion, Cohen, Smith-Magenis, and Phelan-McDermid syndromes among others [6]. Therefore, the prominence of ASD symptomatology associated with genetic conditions again further suggests that genetic factors may play a key role in the etiological origins of the idiopathic autism phenotype.

As our understanding and sophistication for genetic diagnosis grows, so do the number of discrete genes and chromosomal regions that may afford susceptibility to ASDs. Currently, more than 100 genes are susceptibility candidates, indicating ASDs represent a collection of conditions with heterogeneous causation [7]. Nevertheless, recent evidences have unveiled a remarkable convergence of several of these genes on common cellular pathways that intersect at neuronal glutamatergic synapses. Functional overlap such as this may provide a framework that reconciles the presence of a wide array of genes triggering a set of conditions with a high degree of phenotypic similarity in the core behavioral manifestations. Therefore, it is expected that the identification of common pathways, the better understating of the underlying neurobiological disease substrates, and the analysis of neural circuitry dysfunctional in ASD may open the door to new and broadly effective therapies. Here, we highlight some of the intersecting pathways overlapping at glutamatergic synapses and explore how these pathways interconnect. Our focus is directed to the Shank3 and synaptic protein network, and their relations with the genes involved in Tuberous Sclerosis Complex, Fragile X syndrome and Angelman syndrome.

Shank3 and Postsynaptic Density Proteins in ASD

The interdependence among proteins working as a biochemical complex, suggests that even single gene disorders reflect the perturbation of complex intracellular pathways and cellular networks. Therefore, an emerging notion is that if a particular gene or protein plays a role in pathogenesis, its direct and close network interactors are associated with an increased likelihood of precipitating similar endophenotypical manifestations when they are dysfunctional [8,9]. Thus, the analysis of protein networks and their association at the cellular level may shed light on the etiological landscape of interrelated conditions. For ASDs, the transsynaptic complex composed of Neurexin/Neuroligin/PSD-95/SAPAP/Shank pathway is a good example of a set of genes converging on both function, location and associated disorders (Figure 1). From these, Neurexin-1 [10–13], Neuroligin-3 and -4 [14], PSD-95 [15], SAP97 [16], SAPAP2 [17], Shank2 and Shank3 [17–19] have all been implicated in ASDs and validated across multiple studies. Shank3 in particular is present in the terminal q13 region of chromosome 22 that is deleted/rearranged in patients afflicted with Phelan-McDermid syndrome [20–22]. Interestingly, while even minimal deletions in Shank3 have been shown to induce the complete symptomatology of PMS, a ring chromosome aberration that leaves the Shank3 gene intact does not [23,24]. Moreover, point mutations in the Shank3 gene have been identified in patients diagnosed with ASDs [18,25]. Together, evidence from mutations and chromosomal abnormalities affecting Shank3 in ASDs and PMS patients suggest that deficits in this gene can trigger major neurobehavioral complications and autism-spectrum related behaviors.

At the cellular level, all three genes in the Shank family (Shank1-3) give rise to proteins containing multiple protein-protein interaction domains. Shank proteins prominently concentrate in the postsynaptic density (PSD) of dendritic spines [26]. The PSD is key for organizing and maintaining proper synaptic communication, and within this structure Shanks have been hypothesized to function as master scaffolds. Among other roles, Shank can act by bridging together multiple synaptic pathways and provide a link between the PSD and the actin cytoskeleton. Together, Shank and Homer multimers [27,28] form a common platform (with the SAPAP and PSD-95 family of proteins) for the anchoring of ionotropic (AMPAR and NMDAR) and metabotropic glutamate receptors (mGluR) at synapses (Figure 1) [29]. To understand the function of the Shank proteins, overexpression and knockdown strategies have been used to show a clear role in the genesis, maturation and stabilization of neuronal spines [30,31]. Shank3 overexpression in particular has been demonstrated to induce the formation of functional spines in cultured aspiny cerebellar granule cells [31]. More recently, targeted manipulations in the mouse genome have provided further insight

into the *in vivo* functions of Shank1 and Shank3. An analysis of these mutant animals showed that spine density was reduced in hippocampal neurons of Shank1 knockout mice and in medium spiny neurons of Shank3 knockout mice [32,33]••. Moreover, the four different lines of Shank3 mutant mice characterized so far have unveiled neuronal deficits concentrating in decreases of glutamatergic signalizing, loss of synaptic strength, and at the behavioral level, in deficiencies pertaining to social interaction and other ASD-relevant behaviors [33–36]••. Interestingly, the expression of Shank3 is enriched in the striatum [37] and Shank3 mutant mice have cortico-striatal circuitry defects [33]••.

Tuberous Sclerosis Complex

Tuberous Sclerosis Complex (TSC) originates from two genetic disorders caused by mutation in either the TSC1/hamartin or TSC2/tuberin genes [38,39]. TSC is commonly recognized by the appearance of hamartomas (tumor-like nodules) in the skin and the central nervous system. Common neurological and behavioral symptoms include seizures, developmental delays, intellectual disability [40] and up to 45% of patients also fulfill the diagnostic criteria for ASD [41]. Hamartin and tuberin form a complex that participates in the inhibition of the mammalian target of rapamycin (mTOR) [42]. mTOR is a biochemical energy sensor and a regulator of cell growth that plays an important role in stimulating protein synthesis at the synapse [43]. In line with these roles, Ehninger and colleagues observed a basal increase in activity of the mTOR signaling-pathway in Tsc2 heterozygous mutant mice [44]•. This in turn leads to abnormal neuronal plasticity and memory defects that are rescued by treating the Tsc2 mutant mice with rapamycin—an inhibitor for mTOR activity [44]•. Additionally, post-mitotic perturbation of Tsc1 levels leads to neuronal morphology alterations, such as an increase in soma and spine size, but a decrease in dendritic spine density [45]. Interestingly, recent work from Zoghbi and colleagues has provided evidence for the existence of shared binding partners (e.g. Homer3 and Actinin) between the Tsc1/Tsc2 protein complex and Shank3, indicating an intermolecular overlap between these two ASD relevant pathways [46]••. Furthermore, the Tsc1/Tsc2 complex also overlaps with the Fragile X mental retardation 1 (FMR1) gene network at synaptic sites. Specifically, FMR1 was shown to interact with TSC2 transcripts and other autism associated genes [47]•. Nevertheless, recent results alert to the notion that the precise molecular changes occurring from the disruption of these pathways may not be trivial to predict. Specifically, Tsc2 mutant mice display defects that oppose those caused by FMR1 mutants in regards to mGluR-dependent synaptic plasticity [48]•. Moreover, the genetic cross of both mutant mice for Tsc2+/ and Fmr1/y rescued synaptic and behavioral impairments, further corroborating an apposition in molecular roles [48]•. This could suggest that Tsc2 and FMRP proteins/transcripts may be regulated through complex feedback and compensatory mechanism, or alternatively, that Tsc2/mTOR and FMR1 prime the regulation of distinct pools of synaptic proteins necessary for mGluR-dependent synaptic plasticity.

Fragile X Syndrome

Trinucleotide repeats and mutations leading to the silencing of fragile X mental retardation protein (FMRP) production gives rise to the Fragile X syndrome (FXS) [49,50]. This disorder is the single most common cause for heritable intellectual disability, and due to the high prevalence in the general population; it is also the most widely diagnosed cause for ASD [51]. The fragile X mental retardation protein (FMRP) binds and transports mRNA transcripts to spines where it associates with polyribosomes [52] to exert control over protein translation and regulate several families of synaptic and other autism-relevant proteins [53,54]. Recent evidence suggests that the neuronal deficits in FXS may occur in part due to the loss of translational constraint imposed by FMRP on transcripts for Neuroligin-3, Neurexin-1, Shank3, PTEN (Cowden syndrome), TSC2 and NF1

(Neurofibromatosis type 1) [47]•. In hippocampal synapses, long-term depression (LTD) induced by group 1 mGluR requires translation of synaptic transcripts [55]. In FMR1 mutant mice, mGluR-dependent LTD is enhanced [56]; and FMRP mutant mice crossed to harbor a mGluR5 (a group 1 mGluR) heterozygous deletion display an amelioration of several abnormalities present in the FMRP mutants [57]. This has led to the hypothesis that the manipulation of mGluR signaling is a plausible new target for treating symptoms in FXS patients [58]. Furthermore, the FMRP/mGluR pathway also intersects with that of Shank proteins. Besides associating with FMRP [47]•, Shank3 mRNA is one of rare transcripts strongly enriched in dendritic spines [37], and protein levels of Shank1 and Shank3 are increased in the PSD of FMR1 knockout mice [59]. Therefore, the interaction between mGluRs and Shank3 through Homer, the interrelatedness in FMRP/mGluR synaptic pathways and the regulation of Shank3 transcripts by FMRP again points to a molecular and functional convergence at the PSD of glutamatergic synapses.

Ubiquitin-pathway Genes

While inducing or inhibiting protein synthesis can accomplish control over synaptic protein function, another prominent cellular mechanism for the rapid regulation of protein abundance is the ubiquitination and proteasomal system. Perhaps the correlation between the ubiquitin system and autism is best illustrated by UBE3A (Ubiquitin protein ligase 3A), a gene which when silenced or mutated can cause Angelman syndrome, a disorder with high comorbidity with ASDs [60,61]. In addition to UBE3A, information collected from ASD patients revealed copy number variations (CNV) in other ubiquitin genes, including – PARK2, RFWD2 and FBXO40 [62]••. *In vivo,* Ube3a localizes to synaptic sites and plays a role in dendritic spine development [63]. Ube3a deficient mice display abnormal spine morphology and decreases in spine density [63]. However, even while the full gamut of Ube3a targets may not be entirely understood, the *in vivo* importance of this gene is clear for synaptic plasticity in and in the experience-dependent maturation of neocortical circuits in Ube3a mutant mice [64,65]. Additionally an Ube3a mouse model engineered to carry an increased gene dosage was also reported to display the core symptomatology of ASD [66]. Furthermore at the synaptic level the increase in Ube3a level promoted the suppression of excitatory glutamatergic signaling [66], suggesting a critical role for this protein at excitatory synapses [66]. Interestingly, changing neuronal activity levels produces a rapid bidirectional change in the composition of PSD proteins, an effect that is mediated by the proteasome system [67]•. Additionally, from a set of PSD proteins investigated, Shanks were among the few found to display obvious variations in levels of ubiquitination in response to changes in neuronal activity [67]•. More recently, a mutant mouse line that produces a truncated Shank3 protein was found increased ubiquitination and abnormal protein turnover of other Shank3 isoforms and also of NMDA receptors [34]•. Together, these results establish the importance of ubiquitin ligases to the regulation of excitatory synapses, in the etiology of ASDs and also hint at overlap with the network surrounding Shank proteins.

Concluding remarks

Though recent years have seen important strides in the discovery of genes that may confer susceptibility to ASDs, it is increasingly important to further our grasp on the cellular and molecular consequences of putative candidate gene mutations. Additionally, some of the more auspicious results to date have come from studies showing that postnatal genetic rescue of certain genetic lesions can ameliorate phenotypical defects, as demonstrated for genetic mouse models of Rett syndrome, Fragile X syndrome and OCD-like behaviors [68–71]•. These results establish the prospect of reversibility of symptoms in some neurodevelopmental disorders, encompassing also reversibility at the level of cellular defects underlying aspects of these disorders. Thus, the continued accrual of information

across multiple animal models and the delineation of which nodes may be more amenable to intervention will be essential. This will require further experimental data confirming the relationship between these pathways and the identification of targets for therapeutical intervention.

Yet, one concern that arises as a corollary to the molecular interconnectedness of ASD gene pathways is as follows: mutations affecting ubiquitously expressed gene products may result in ASD specific phenotypes in an indirect manner, due to perturbation of functional interactions with affiliated gene products that are enriched or expressed only in specific brain regions. This could lead to a focal disruption of discrete brain circuits. As a putative example, FMRP mutations, while broadly affecting protein translation of many target transcripts, would only precipitate aberrant Shank3 mRNA translation in neurons and brain regions that normally express Shank3. Understanding this potential cellular asymmetry in brain disorders will require continued exploration of cell autonomous function but with additional consideration to the unique expression patterns of functionally linked genes, as well as higher order neural networks between brain regions. Thus, even if genetic mutations form the etiology of many ASDs, it is essential to understand the neuropathology in the context of the specific brain circuits underpinning the hallmark neurological and behavioral manifestations.

Acknowledgments

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Research Highlights

- Mutations in several genes encoding the core transsynaptic complex of Neurexin/Neuroligin/PSD-95/SAPAP/Shank confer susceptibility to ASDs

- Tuberous Sclerosis Complex, Fragile X syndrome, Angelman syndrome and synaptic ASD candidate genes have overlapping interaction networks

- Mouse models for ASDs have helped identify commonalities in glutamatergic dysfunction

- Identifying important nodes in interaction networks may facilitate therapeutical intervention

- ASD endophenotypes may be triggered by common neural circuit defects
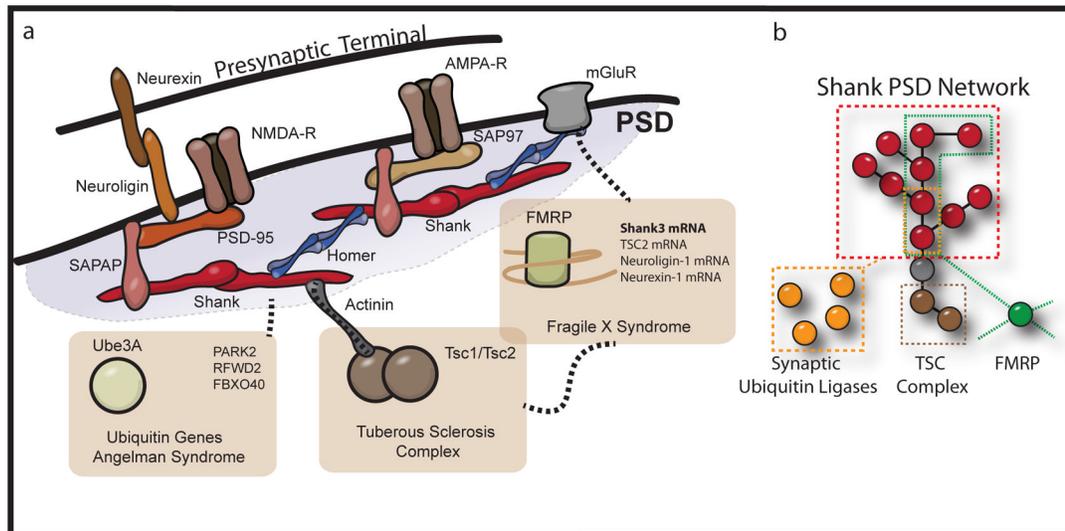
Figure 1.
Shank proteins at the center of an ASD disease-module. A model for the overlap between synaptic proteins involved in susceptibility to syndromic and non-syndromic autism. **(a)** Neurexin and Neuroligins are transsynaptic partners and candidate genes for susceptibility to autism [10–14], in the postsynaptic density these bind to the SAPAP family of proteins which have been linked to ASDs and OCD [17,72], PSD-95 and SAP97 which are involved in intellectual disability and autism [15,16] and Shank2 and Shank3 which are also candidate genes for autism [18,19]. Shank dimers are thought to organize a molecular platform in concert with Homer tetramers to stabilize the larger PSD, connecting AMPAR, NMDAR and mGluR into one protein hub. In the deeper synaptic compartment the control of PSD protein levels may be tightly controlled by independent complexes such as TSC1/2 through mTOR, or via FMRP regulation of synaptic transcripts, and most likely also through synaptic ubiquitin ligases. **(b)** Simplified putative disease-module network centering on Shank proteins at the PSD (red) displaying the overlap between synaptic ubiquitin ligases and ubiquitination of SAPAP/Shank (see text – orange), the Tuberous sclerosis genes TSC1/TSC2 (brown) and FMRP regulation of several PSD protein transcripts (green).