Molecular and synaptic defects in intellectual disability syndromes
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The search for genetic causes of intellectual disability has identified, over the past twenty years, numerous mutated genes that code for proteins concerned with synapse function. Functional studies have shown that these genes may be involved in synapse formation, the synthesis and degradation of specific synapse proteins, the regulation of dendritic spine morphology, or regulation of the synaptic cytoskeleton. It is now clear that even mild alterations in synapse morphology and function can give rise to intellectual disability, and pharmacological agents able to counteract these morphological and functional anomalies – and improve the symptoms of some of these conditions – now appear feasible. This paper reviews recent findings on the functions of some of the genes responsible for intellectual disability syndromes.

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Introduction
Aberrant synaptic formation signalling and plasticity, or altered spine morphology, characterize several psychiatric and neurological diseases [1] and it is now clear that precise control of synaptic development is crucial for the formation of a normally active neuronal network allowing normal brain function.

Intellectual disability (ID), formerly mental retardation, is one of the most common neurodevelopmental disorders; it is characterized by an intelligence quotient of 70 or below and deficits in behaviors related to adaptive functioning, including autism spectrum disorders (ASD). Although in up to 60% of cases no cause has been identified, in over 25% of cases ID is caused by genetic factors [2]. A recent study found that ASD patients had a high global burden of rare copy number variants especially of loci known to be associated with ASD, and also of new genes previously unconnected with ASD [3**].

Over the past 15 years, many single-gene causes of syndromic or nonsyndromic ID have been identified, the most common of which are located on the chromosome X and are responsible for X-linked intellectual disabilities (XLID). Over 50% of the ID-related proteins that are not transcription or chromatin-remodelling factors, are conspicuously present in the pre-synaptic or post-synaptic compartments and seem to be involved in synaptic actin cytoskeleton rearrangement, synaptic plasticity or synapse formation [4].

It is useful to divide synapse-related proteins associated with ID into those that localize purely at synapses (mutations to which directly interfere with synaptic formation) and other proteins that regulate neuronal development and synapse formation indirectly by controlling the synthesis, degradation or modulation of synaptic proteins or the synaptic actin cytoskeleton. The molecular mechanisms (Figure 1) by which alterations in some of these proteins contribute to ID constitute the main topic of this review.

X-linked gene mutations
Fragile-X and Rett syndromes are responsible for most forms of XLID. However mutations to several other genes on chromosome X have been found strongly associated with ID, including mutations to the neuroligin genes NLGN3 and NLGN4, which were among the first to be clearly associated with synaptic function [5].

Neuroligins were first identified as binding partners of neurexins. Later neuroligins and neurexins were found to associate in synapses to form a trans-synaptic complex crucial for synapse formation and function.

IL1RAPL1
Mutations of the interleukin-1 receptor accessory protein-like 1 gene (IL1RAPL1) are associated with cognitive impairment ranging from nonsyndromic ID to ASD [6–9]. IL1RAPL1 belongs to new Toll/IL-1 receptor family and shares 52% homology with the IL-1 receptor accessory protein (IL-1RaCp). Like other members of this family, IL1RAPL1 has three extracellular Ig-like domains, a transmembrane domain, and an intracellular Toll/IL-1R homology domain (TIR domain). Unlike other family members, however, IL1RAPL1 has 150
additional amino acids at the C-terminus. It has been shown that IL1RAPL1 interacts with neuronal calcium sensor-1 through its intracellular region [10], and that this interaction mediates the regulatory effect of IL1RAPL1 overexpression on N-type voltage-gated calcium channel activity in PC12 cells [11].

We recently showed that IL1RAPL1 binds postsynaptic density protein 95 (PSD-95) and regulates its phosphorylation and synaptic association by activating c-Jun terminal kinase (JNK) [12]. We also found that the extracellular domain of IL1RAPL1 induces presynaptic differentiation by binding the receptor tyrosine phosphatase δ (PTPδ), localized at the presynaptic terminal, while the TIR domain binds to RhoGAP2 and regulates dendritic spine formation [13]. Yoshida et al. [14] also recently reported that IL1RAPL1 and PTP-DELTA interact, but showed in addition that the interaction was confined to particular splice variants of PTPδ. These findings suggest that the IL1RAPL1 mediates trans-synaptic signalling that regulates excitatory synapse and dendritic spine formation.

Thus it seems that even small changes in these synaptic adhesion proteins can cause major changes in connectivity, resulting in cognitive impairment. Interestingly, most of the adhesion molecules found associated with ID regulate excitatory synapse formation, and the resulting functional alteration or reduction in number of excitatory synapses arising from mutations, may alter the balance between excitatory and inhibitory synapses leading to a general over-inhibition within neuronal circuits as proximate cause of ID.

**Oligophrenin-1**

Mutations or deletions in the synaptic RhoGTPase-activating protein oligophrenin-1 are also associated with certain forms of XLID [15] and constitute the main evidence that signalling involving member A of the Ras homologue gene family (RhoA) is involved in ID. Oligophrenin-1 is a
negative regulator of RhoA and also interacts with the postsynaptic adaptor protein Homer [16]. Govek et al. [16] showed that oligophrenin-1 knockdown in CA1 pyramidal neurons significantly reduces spine length, an effect mimicked by a constitutively active form of RhoA, and rescued, in the presence of constitutively active RhoA, by inhibiting the RhoA effector Rho-kinase (ROCK1). Since ROCK1 plays key role in RhoA-induced actin reorganization, these findings implicate RhoA in regulating the actin cytoskeleton of spines, probably through effects on LIM kinase, myosin light chain (MLC), or MLC phosphatase [15,16]. Thus, loss of oligophrenin-1 probably causes changes in spine morphology during development due to alteration of the actin cytoskeleton caused by loss of repression of RhoA and ROCK1. Khelfaoui et al. [17] later showed that oligophrenin-1-deficient mice have behavioral deficits. A subsequent study showed that activation of synaptic NMDA receptors localizes oligophrenin-1 to dendritic spines, where it forms a complex with AMPA receptors and selectively enhances AMPA-receptor-mediated transmission and spine size by stabilising those receptors [18]. That oligophrenin-1 stabilizes AMPA receptors in synapses is suggested by reduced number and activity of such receptors in oligophrenin-1 KO mice, defects which are rescued by blocking AMPA receptor endocytosis – in turn indicating a link between oligophrenin-1/RhoA signalling and AMPA receptor endocytosis [18]. Another study has shown that oligophrenin-1 is concentrated at endocytic sites and regulates AMPA receptor endocytosis at excitatory synapses by RhoA/ROCK signalling [19].

Very recently oligophrenin-1 has been shown to interact with the circadian clock protein Rev-erβ regulating its circadian activity, suggesting that interaction between synaptic activity and circadian oscillators can be involved in the etiology of intellectual disability [20**]. Thus oligophrenin-1 seems to have multiple functions in regulating synaptic activity and plasticity.

**Fmrp**

The *fmr1* gene encodes the fragile X mental retardation protein (FMRP). Failure to express FMRP results in fragile X syndrome, leading cause of congenital ID in humans. Patients with this syndrome have more, longer and thinner dendritic spines than normal [21]. FMRP belongs to the heterogeneous nuclear ribonucleoprotein family of RNA-binding proteins; it regulates the transport to synapses and translation of a subset of neuronal mRNAs [21–23] and its expression and localization to dendrites increase after synaptic stimulation, suggesting a direct link between FMRP and synaptic plasticity [24–26]. The translational dysregulation of target mRNAs that occurs when FMRP is not expressed appears to be the main cause of the dendritic spine and synapse alterations that characterize fragile X syndrome [23].

In *fmr1* KO mice, DHPG-induced long term depression (LTD) is strongly increased [27] and at the same time metabotropic glutamate receptor (mGluR)-dependent local protein synthesis is deregulated [27–30] The latter finding has inspired the ‘mGluR theory’ of fragile X syndrome: that alterations in mGluR-mediated signalling might underlie the cognitive deficits of the syndrome, so mGluR inhibitors might be useful as treatment [31].

The translation of several proteins is increased in *fmr1* KO mice, particularly in purified synaptosomes [29,30,32–35]. Schutt et al. [36] found that expression levels of the postsynaptic scaffold proteins SAPAP1-3, Shank1, Shank3, and IRSp53, as well as of the NR1 and NR2B subunits of the NMDA receptor and GluR1 subunit of the AMPA receptor are increased in cortex and hippocampus of *fmr1* KO mice. FMRP is also a negative regulator of transcripts of the NR2A subunit of NMDA – a regulation influenced by the microRNA miR-125b. These findings suggest that FMRP absence alters synaptic plasticity in fragile X syndrome by altering NMDA receptor subunit composition [37*].

PSD-95 – key regulator of synaptic signalling and learning – may be one of the more important synaptic proteins regulated by FMRP. In *fmr1* KO mice, PSD-95 mRNA and protein levels are lowered in hippocampus but not cortex [28]. However FMRP appeared to exert its effect by stabilising PSD-95 mRNA, a new FMRP function [28].

Another study indicated that FMRP regulates mGluR activation-dependent and microRNA dependent translation of PSD-95, CaMKIIα, and GluR1/2 in cortex [30]. Phosphorylated FMRP promotes the formation of an AGO2-miR-125a inhibitory complex on PSD-95 mRNA that is released by mGluR1 activation, causing FMRP to dephosphorylate. These findings indicate that FMRP phosphorylation provides a reversible switch for AGO2 and microRNA to selectively regulate mRNA translation at synapses in response to receptor activation [30]. Dysregulated PSD-95 translation at synapses could, therefore, be responsible for the altered synaptic plasticity and dendritic spine morphology of *fmr1* KO mice [21]. However proteomic analysis of the entire synaptosome is required to fully elucidate the role of FMRP and its mRNA targets at synapses.

**MECP2**

Over 90% of Rett syndrome cases are caused by a mutation on the *MECP2* gene, which encodes methyl-CpG-binding protein-2 (MeCP2), a protein that binds methylated DNA and thereby influences gene transcription. Early functional studies suggested that MeCP2 functioned as a molecular link between DNA methyl-ation, chromatin remodelling and subsequent gene silencing [38]. More recent studies indicate that MeCP2 represses the transcription of some genes yet promotes the transcription of others; [39] it may even control the AKT/mTOR signalling pathway and protein translation,
suggesting that defects in the AKT/mTOR pathway are responsible for altered translational control in MeCP2 mutant neurons [40*].

Whatever MeCP2’s precise function, experimental models involving both loss and gain of function of the mouse mecp2 gene are characterized by numerous changes in the morphology and function of neurons and synapses, accompanied by severe neurodevelopmental defects and behavioral alterations analogous to those in humans [41–43]. Furthermore, direct evidence of MeCP2 involvement in the regulation of synaptic connectivity comes from post-mortem studies [42,44], which reveal that hippocampal CA1 pyramidal neurons from Rett syndrome females have lower spine density than age-matched non-Rett syndrome female controls [42,44], and that the syndrome is associated with abnormalities in the expression of molecules crucial for excitatory and inhibitory synaptic transmission [42,44].

MeCP2 also appears important for activity/experience-dependent synaptic remodelling. Li et al. generated knock-in mice that lacked activity-induced MeCP2 phosphorylation and found that the mice did better in hippocampus-dependent memory tests, had enhanced long-term potentiation and had increased excitatory synaptogenesis [45*]; the phospho-mutant MeCP2 protein also bound more tightly to several MeCP2 target gene promoters altering the expression of the genes. In mecp2-deficient mice, retinogeniculate synapses developed similarly to wild-type littersmates between postnatal days 9 and 21, indicating that initial phases of synapse formation, elimination, and strengthening were not affected by MeCP2 absence [46*]. However, in the subsequent experience-dependent phase of synapse remodelling, the circuit became abnormal in mutants and synaptic plasticity in response to visual deprivation was disrupted [46*]. These findings point to MeCP2 as crucially involved in experience-dependent refinement of synaptic circuits, in line with the clinical course in Rett syndrome patients, who after near-normal early development reach a plateau followed by severe regression.

Synaptic scaffold proteins
Since PSD-95 is the most abundant scaffold protein at the PSD, its gene – DLG4 in humans – has been extensively studied for polymorphisms and mutations associated with neurodevelopmental diseases. Only one study, however, indicates an association between DLG4 gene variation and ASD and Williams syndrome [47], while a haplotype derived from 2 polymorphic markers at the core promoter has been linked to schizophrenia [48].

By contrast, the human DLG3 gene, which encodes synapse-associated protein 102 (SAP102) is clearly associated with ID [49,50]. The mutations identified introduce premature stop codons within or before the third PDZ domain, probably impairing the ability of the truncated SAP102 to interact with the NMDA receptor and other proteins involved in NMDA receptor signalling pathways. A recent publication demonstrated that SAP102 links NMDA receptor activation to alterations in spine morphology [51].

SHANK/ProSAP family
Phelan-McDermid syndrome (PMS, also called 22q13.3 deletion syndrome) is characterized by intellectual impairment, absent or delayed speech, and autistic-like behavior, as well as hypotonia and mild dysmorphic features [52–55]. The deletion can be small but always involves a crucial region that includes SHANK3 encoding the Shank3/ProSAP2 postsynaptic scaffold protein. Loss of Shank3 is now considered to cause the neurobehavioral symptoms of PMS, although other genes may also be lost [52,54,56,57]. De novo mutations in SHANK3 [57–59] and also in SHANK2 [60] have been identified in individuals with ASD and ID.

Mice lacking Shank1 have small dendritic spines, weakened synaptic transmission, enhanced learning [61] and defects in social communication [62]. Recent studies in mice highlight the importance of Shank3 haploinsufficiency [63,64*,65*,66*]. Thus male mice with heterozygous or homozygous disruption of Shank3 had abnormal behavior, learning and memory, compared to wild-type littersmates [64*,66*]. At the level of the synapse, these animals had markedly impaired basal synaptic transmission in CA3-CA1 connections, reduced GluR1 clusters and protein levels in hippocampus, and altered activity-dependent AMPAR synaptic plasticity [64*,66*].

Mice with genetic deletion of two major Shank3 splice variants exhibit self-injurious repetitive grooming and deficits in social interaction correlating with major alteration in striatal synapses and cortico-striatal circuits, but not in hippocampus, suggesting that the remaining Shank3 splice variant(s) may be sufficient to maintain normal synapse function and structure in hippocampus [65*].

A mutated Shank3 protein that lacks the Homer-binding C terminus induces a gain-of-function phenotype, that reduces Shank3 expression at synapses by >90% owing to greater polyubiquitination. The NR1 subunit of the NMDA receptor is also reduced at synapses by greater polyubiquitination, while AMPAR function and composition are not affected [63*].

We knocked down all major Shank3 splice variants in rodent neuronal cultures by RNA interference [67] and found that Shank3 knockdown in hippocampal cells reduced the expression of mGluR5 receptors, and also reduced DHPG-induced phosphorylation of ERK1/2 and CREB. The overall result was reduced mGluR5-dependent synaptic plasticity and modulation of neural network activity.
Together these studies show that mutations in Shank3 cause alterations in both synaptic morphology and signalling.

Therapeutic prospects

Molecular mechanisms contributing to the pathogenesis of various types of genetically determined ASD and ID suggest new targets for the development of drugs to ameliorate these conditions. Since ProSAP/Shank disruption seems to lead to a hypoglutamatergic state, upregulation of the glutamatergic system may be a promising therapeutic approach. In particular, agents that activate synaptic currents mediated by AMPA-type glutamate receptors (AMPARs) could prove useful [68]. AMPARs improve the induction of long-term potentiation and exert a positive effect on excitatory transmission by upregulating the production of regulated brain-derived neurotrophic factor (BDNF) which is involved in synaptic plasticity and memory consolidation [69]. In view of the deficit in mGluR5-mediated intracellular signalling found in Shank3 knockout neurons, use of positive allosteric modulators of mGluR5 – for example CDPPB – suggests itself as a pharmacological approach to these conditions [67].

Environmental enrichment is a radical non-pharmacological approach to ASD. Animals reared under environmental enrichment conditions have enhanced synapse formation and plasticity and increased BDNF expression [70] and environmental enrichment promotes synaptic plasticity and regulates synapse stability in the cerebral and cerebellar cortex of MeCP2 null mice [71].

Another possible approach to rescuing synaptic defects in patients with ID is to use human induced pluripotent stem cells (hiPSC) as suggested by the work of Marchetto et al. [72**]. These authors cultured hiPSCs from the fibroblasts of patients with Rett syndrome and tested drugs on the culture with the aim of rescuing the evident synaptic defects in the cells.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- - of outstanding interest


This paper analyzed the genome-wide characteristics of rare copy number variation in ASD using dense genotyping arrays. Many new genetic and functional targets in ASD were uncovered.


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45. Li H, Zhong X, Chau KF, Williams EC, Chang Q: Loss of activity-induced phosphorylation of MeCP2 enhances synaptogenesis, LTP and spatial memory. Nat Neurosci 2011. This study shows that the knockout mice that lack activity-induced phosphorylation of MeCP2 perform better in hippocampus-dependent memory tests, present enhanced long-term potentiation in the hippocampus and increased excitatory synaptogenesis. These findings suggest that activity-induced phosphorylation of MeCP2 is required for modulating dynamic functions in the adult mouse brain.

46. Noutel J, Hong YK, Leu B, Kang E, Chen C: Experience-dependent retinogeniculate synapse remodeling is abnormal in MeCP2-deficient mice. Neuron 2011, 70:35-42. The study investigates retinogeniculate synapse maturation in MeCP2-deficient mice showing that synapse development in these mutants is similar to that of wild-type animals. However during the subsequent experience-dependent phase of synapse remodeling, the circuit becomes abnormal in mutants and synaptic plasticity, in response to visual deprivation, is disrupted.


This and the following three studies describe multiple synaptic and behavioral defects in mice in which major splice variants of Shank3 have been knocked out.


See ref. [63].


See ref. [63].


This study demonstrates for the first time the use of hiPSC to recapitulate early stages of human Rett syndrome and shows how this tool can be used as a cellular model for drug screening.