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Heterogeneity and convergence: the synaptic pathophysiology of autism

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Abstract

Autism is a developmental disorder characterised by a high heterogeneity of clinical diagnoses and genetic associations. This heterogeneity is a challenge for the identification of the pathophysiology of the disease and for the development of new therapeutic strategies. New conceptual approaches are being used to try to challenge this complexity and gene cluster analysis studies suggest that the pathophysiology of autism is associated with a dysregulation of specific cellular mechanisms. This review will present the experimental evidence for a convergence of synaptic pathophysiology between syndromic and non-syndromic forms of autism, grouped under the generic term of autism spectrum disorders. In particular I will highlight the results from genetic mouse models identifying a convergence of dysregulation of the synaptic type I metabotropic glutamate receptor pathway in mouse models for autism spectrum disorders. These results help to build a new conceptual framework for the study of the synaptic phenotype of autism, which is important for the identification of new therapeutic strategies.

Introduction

Autism is a developmental disorder defined by decreased sociability and communication, and the recurrence of ritualistic behaviors. The variability in the degree of severity of these symptoms is very high, and in about 15% of cases the patients also present additional unrelated symptoms. As a consequence, the disease affects heterogeneous panels of patients, and the different forms of autism are grouped under the generic term of autism spectrum disorders (ASD). ASD is also a highly heritable disease, and genome-sequencing studies continue to identify genetic mutations associated with these disorders (Gilman *et al.*, 2011; Neale *et al.*, 2012). To date, about 500 genes have been associated with various forms of ASD (for a complete list see the Simons Foundation database: https://gene.sfari.org), each of them accounting for only a small fraction of the cases of ASD. These results point to the fact that, at the genetic level, ASD is a constellation of rare monogenic disorders that converge to a narrower set of behavioral symptoms.

The pathway hypothesis of autism spectrum disorders

One explanation of the apparent contradiction between the various genetic underpinnings and convergent symptomatology is that these different mutations all impinge on a common set of cellular signaling pathways and their dysregulation triggers a common constellation of symptoms. From this hypothesis, Iossifov *et al.* (2008) postulated that the classical linkage of a gene would be enhanced if its involvement in a molecular network is taken into account. To test this, they re-evaluated *in-silico* the linkage of known ASD-related genes using a new method combining classical linkage with the knowledge of direct molecular interactions. When they compared the classical linkage of two genes, TJP1 and UBE3A, with their probabilistic method they found a dramatic increase in the *P*-value of the association of TJP1 and UBE3A with ASD, confirming their hypothesis. Within the clusters of genes interacting with TJP1 and UBE3A, they unraveled genes that had at this date not been identified as linked to ASD. The authors analysed their genetic linkage with ASD and identified 48 new candidate genes for autism, confirming the validity of their approach. Based on the same conceptual approach, a study by Sakai *et al.* (2011) has built an interactome of ASD-associated proteins employing a yeast two-hybrid screen assay to identify molecular clusters associated with ASD. The authors used known ASD candidate-gene products as bait for their screen to identify new molecular partners and discovered previously unknown interactions between proteins associated with ASD. For example, they discovered that both Prosap2/Shank3 and NL3 (two autism-associated proteins) can interact with proteins associated with the ASD-associated disease tuberous sclerosis (TSC), Tsc1 and Akt, respectively. This result unravels a potential link between different forms of ASD. They subsequently evaluated the genetic association of newly identified genes with ASD. They found three *de-novo* mutations implicating the genes NECAB2, PKM2, and FLNA, and thereby demonstrated the validity of their method. These two studies validate the hypothesis of interconnections between ASD-associated genes and suggest the existence of co-dysregulations of clusters of genes in ASD. Among the genes linked with ASD, many code for proteins involved in neuronal development, leading to the hypothesis of dysregulation of pathways specifically associated with neuronal functions (Bourgeron, 2009). To directly address this question, Voineagu *et al.* (2011) compared the regulation of clusters of genes involved in the cortical development of typically developing controls and patients with ASD. By postmortem tissue analysis, they found a

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cluster of 174 genes differentially regulated between the frontal and temporal cortices of typically developing controls, a cluster likely to be important for the differentiation of these cortical regions. When they analysed the same cluster of genes in patients with ASD they found a smaller difference of regulation during cortical development. Importantly, when they analysed the genetic cluster in more detail, they found an enrichment of genes associated with synaptic functions and neuronal signaling. In particular, they identified a dysregulation of A2BP1/FOX1, a gene coding for a splicing factor and previously known to be associated with ASD. The results of this study support the existence of co-deregulated cluster genes in ASD. In addition, the authors identified a new cluster of genes, associated with a synaptic molecular pathway, involved in cortical development dysfunction in ASD.

These human genetic studies propose a basis for the dysfunction of specific molecular pathways in ASD, affecting in particular neuronal development and functions. The study of molecular and cellular mechanisms is limited in human, making it challenging to identify the precise nature of the pathophysiology of ASD. The development of animal models is therefore a critical step to describe these molecular mechanisms in detail and to identify potential pharmacological targets.

The synaptic pathophysiology of autism spectrum disorders: the Prosap/Shank and Neuroligin case studies

It is very likely that a general synaptic defect is not systematically associated with ASD; genes coding for general synaptic proteins (e.g. DLG4 or GPHN) and their respective mouse models have little to no association with ASD (https://gene.sfari.org). Consequently, only a subset of genes coding for specific synaptic proteins, their synaptic networks and their related functions are relevant for ASD. To illustrate this point I will highlight the analysis of the relationship between ASD and the different Prosap/Shank and Neuroligin (NL) protein isoforms, two families of synaptic genes with the highest linkage with ASD (for comparative linkage analysis see the Peking University database: http://autismkb.cbi.pku.edu.cn).

The Prosap/Shank family

The PROSAP/SHANK gene family consists of three members: PROSAP3/SHANK1, PROSAP1/SHANK2 and PROSAP2/SHANK3, which have various degrees of genetic linkage with ASD (for an exhaustive review on the link between PROSAP/SHANK genes see Jiang & Ehlers, 2013). Mutations in 22q13.3, containing in particular the PROSAP2/SHANK3 locus, are known to cause the Phelan–McDermid syndrome, a profound neurological disorder including symptoms of autism-like behavior. In addition, many mutations have been found in PROSAP2/SHANK3 to be causative for an ASD phenotype. In the majority of the cases these mutations lead to a PROSAP2/SHANK3 haploinsufficiency. Four point mutations of PROSAP1/SHANK2 have been found in patients with ASD, which lead to a disruption of the protein. So far, none have been identified in PROSAP3/SHANK1. Corresponding mouse models were generated based on the knockout of the genes, leading to the absence of the majority of the PROSAP1/SHANK2 and PROSAP2/SHANK3 isoforms. In accordance with their genetic associations, the different mouse models show different ASD-related behavior. Whereas PROSAP1/SHANK2 and PROSAP2/SHANK3 knockout mouse models show strong autism behavior, PROSAP3/SHANK1 knockout mice remain largely unaffected (Bozdagi *et al.*, 2010; Peca *et al.*, 2011; Wang *et al.*, 2011; Schmeisser *et al.*, 2012; Won *et al.*, 2012; Yang *et al.*, 2012). Although these results point to a differential role of the PROSAP/SHANK isoforms, in fact only their brain localisation differs. All isoforms are subjected to alternative splicing and contain five conserved domains. Through these different domains, Prosap/Shank proteins interact with more than 30 synaptic proteins, including NLs, the type I metabotropic glutamate (mGluR1 and 5) and the *N*-methyl-D-aspartate (NMDA) receptors, and mediate their localisation and function (Boeckers *et al.*, 2002). To our current knowledge, only the protein and mRNA localisations of PROSAP/SHANK are isoform-dependent. *In situ* hybridisation and northern blot analyses revealed that PROSAP3/SHANK1 mRNA is expressed in many brain regions to various levels. High levels are found in the cortex, hippocampus and amygdala, moderate levels in the thalamus and substantia nigra, and low or undetectable levels in the cerebellum, caudate nucleus, corpus callosum and subthalamic nucleus. PROSAP1/SHANK2 mRNA is expressed in many brain regions without noticeable differences in levels, with the exception of the mesencephalon where PROSAP1/SHANK2 is absent. PROSAP2/SHANK3 mRNA expression is high in the hippocampus, cerebellum, caudate putamen and thalamus, moderate in the cortex and low to absent in the hypothalamus, mesencephalon and amygdala. This localisation can also be dependent on the cell type, in the cerebellum PROSAP3/SHANK1 and PROSAP1/SHANK2 mRNAs are expressed in Purkinje cells, whereas PROSAP2/SHANK3 mRNA is found only in the granule cell layer. This expression is also developmentally regulated. PROSAP3/SHANK1 and PROSAP1/SHANK2 transcript levels are high at birth and decrease slightly during development. On the contrary, PROSAP2/SHANK3 mRNA expression is low after birth and increases during development (Boeckers *et al.*, 1999a,b; Lim *et al.*, 1999; Petralia *et al.*, 2005). In the mature brain, all of the isoforms remain expressed, Prosap3/Shank1 being the most abundant (Peca *et al.*, 2011). In addition, it has been suggested that alternative splicing can influence expression levels as the pattern of Prosap/Shank immunoblot bands can vary between different brain regions (Sheng & Kim, 2000). The localisation of Prosap/Shank isoforms as well as their interactions might therefore be influenced by alternative splicing. These results allow the hypothesis that only specific neurons and synapses containing Prosap1/Shank2 and Prosap1/Shank3 are associated with the pathophysiology of ASD.

The Neuroligin family

Four Neuroligin genetic isoforms exist in mice and five in humans (Neuroligin gene (NLGN)1-3, 4X and 4Y coding for the proteins NL1-3, 4X and 4Y, respectively). Genetic studies show a much higher genetic linkage for NLGN3 and NLGN4 with autism compared with NLGN1 and NLGN2 [for an comprehensive review see Xu *et al.* (2012) and http://autismkb.cbi.pku.edu.cn]. Series of mutations have been found in NLGN3 and NLGN4, which primarily lead to a decrease or an absence of protein expression. One mutation has been associated with NLGN1 and three with NLGN2 without any clear evidence on the impact on the protein level. In line with this, only NLGN3 and NLGN4 mouse models present autism-related behavioral phenotypes (Tabuchi *et al.*, 2007; Chadman *et al.*, 2008; Jamain *et al.*, 2008; Radyushkin *et al.*, 2009; Etherton *et al.*, 2011; Ey *et al.*, 2012). By contrast, the different NLGN isoforms are well described as sharing similar structures, functions and

brain localisation patterns. All NLGN isoforms are subjected to alternative splicing and code for type I membrane proteins containing two conserved domains. Their generic function is to form and maintain synapses through their interactions with presynaptic and postsynaptic proteins; they bind to adhesion and scaffolding proteins and receptors to organise presynaptic and postsynaptic compartments (Sudhof, 2008; Baudouin & Scheiffele, 2010). In addition, studies showed that NL3 can form heterodimers with NL1 and co-immunoprecipitates with NL2, further suggesting an overlap of functions between the different isoforms (Budreck & Scheiffele, 2007; Poulopoulos *et al.*, 2012). The different isoforms are mainly expressed in the brain and their mRNA is found in all of the different brain areas. In addition, their expression is developmentally regulated. NL1, NL2, NL3 and NL4 are detectable from embryonic day 12. Their expression increases throughout development, peaking at around postnatal day 14, and is maintained in the adult (> postnatal day 90) (Song *et al.*, 1999; Varoqueaux *et al.*, 2004; Budreck & Scheiffele, 2007). What are the NL isoform specificities that could be relevant for ASD? Apart from death at perinatal stages, the triple-knockout mice for NLGN1, NLGN2 and NLGN3 show very subtle synaptic phenotypes, demonstrating that the overlap of functions between at least these three different isoforms is probably not as large as previously thought (Varoqueaux *et al.*, 2006). At the subcellular level, NL1 and NL2 have a restricted expression pattern, specific to excitatory or inhibitory synapses, respectively (Song *et al.*, 1999; Varoqueaux *et al.*, 2004), whereas NL3 and NL4 can be expressed at both depending on the neuronal subtype (Graf *et al.*, 2004; Budreck & Scheiffele, 2007; Hoon *et al.*, 2011; Baudouin *et al.*, 2012). At the functional level, only a few isoform-specific functions have been found. Of the different isoforms, NL1 is the only one to interact with extracellular NMDA receptors to stabilise them at the synapse and control NMDA-dependent synaptic transmission and plasticity (Budreck *et al.*, 2013). As opposed to NL1 and NL3, NL2 and NL4 can both interact with gephyrin and activate collybistin to induce receptor clustering at inhibitory synapses (Poulopoulos *et al.*, 2009). NL3, but not NL1, regulates mGluR protein levels at excitatory synapses and controls mGluR-dependent long-term depression (mGluR-LTD) (Baudouin *et al.*, 2012). Both NLGN3 loss-of-function and gain-of-function mutations are associated with ASD. Interestingly, the respective mouse models (i.e. Nlgn3 knockout mice and mice carrying an amino acid substitution, R451C, associated with ASD) show a dysfunction of the endocannabinoid signaling but at different synaptic subtypes (Foeldy *et al.*, 2013). In addition, the behavioral alterations in these two models do not entirely overlap. These results highlight the different impact of specific synapse populations in the pathophysiology of ASD. Altogether these data illustrate the divergence of function between the different NLGN isoforms. The specific role of NLGN isoforms in ASD probably resides in their subcellular localisation and their ability to interact with different types of synaptic receptors to control synaptic transmission and plasticity. They further suggest that the different NL isoforms can be part of distinct gene and protein interaction networks; identification of the respective interactome of NL3 and NL4 will be informative to determine the molecular mechanisms defective in ASD.

A convergent Prosap/Shank–Neuroligin pathway in autism spectrum disorders? Evidence and consequences

By analysing biochemical and functional data an attractive hypothesis emerges with the possibility that Prosap/Shank and NL are part of a common molecular pathway associated with ASD. Data from a yeast two-hybrid screen showed that NLs bind to the PDZ domain of Prosap3/Shank1 and Prosap2/Shank3. This interaction is specific, as it is not shared by other adhesion molecules such as Sidekick2 or Ephrin B2 (Meyer *et al.*, 2004). More recently, a study by Arons *et al.* (2012) addressed the relevance of the interaction between Prosap2/Shank3 and NLs for synapse formation and function. Arons *et al.* (2012) found that, although Prosap2/Shank3 is not essential for the synaptic localisation of NL1 and NL3 (NL2 and NL4 were not tested in this study), the ability of Prosap2/Shank3 to promote presynaptic vesicle clustering and to control excitatory synaptic function is dependent on NL interactions with presynaptic adhesion proteins. Although a recent yeast two-hybrid study failed to show any direct interaction between Prosap2/Shank3 and NL3 (Sakai *et al.*, 2011), these functional data validate a model where specific Prosap/Shank and NL isoforms are part of a common pathway.

The analysis of Prosap/Shank and NL functions leads to the idea that the localisation of the protein and its interaction network are two crucial elements relevant for the pathophysiology of ASD. The idea of a different role of specific brain regions in ASD is already apparent in results from imaging studies where only selective brain regions are found to be associated with the disease (i.e. cerebellum, cortex and amygdala for the most frequently associated) (Amaral *et al.*, 2008). Nothing is known to date about the PROSAP/SHANK-NLGN-containing neuronal network associated with ASD. Using mouse genetics to control the expression of these genes in specific neurons of precise brain regions will aid in defining the different cell types important for the ASD phenotype. Human brain postmortem analysis showed that Purkinje cells in the cerebellum are affected in the disease (Bauman & Kemper, 2005). In correlation with these data, Tsai *et al.* (2012) demonstrated that specific deletion of TSC1 in Purkinje cells in mice is sufficient to induce ASD-related phenotypes, confirming the involvement of these cells in the pathology of ASD. I propose similar approaches to define the neuron and synapse types associated with PROSAP/SHANK- and NLGN-related ASD. When postulating that PROSAP/SHANK and NLGN are part of the same ASD-associated gene cluster, a different hypothesis can emerge with regards to the function of this cluster. The two gene families code for proteins with synaptic functions; in particular, Prosap/Shank and NLs can control different aspects of synaptic plasticity. Through their interaction with type I mGluR, Prosap2/Shank3 and NL3 can control mGluR-LTD (Verpelli *et al.*, 2011; Baudouin *et al.*, 2012; Foeldy *et al.*, 2013). In addition, all Prosap/Shank isoforms and NL1 can control NMDA signaling and function. Alternative splicing and local translation are particularly interesting functions because they represent complex modes of regulation giving rise to variation in the localisation and function of specific proteins. As a consequence, these mechanisms represent an attractive source of heterogeneity that could explain the different magnitude of the symptom manifestations in ASD. Notably, both the PROSAP/SHANK and NLGN gene families undergo alternative splicing. The splicing factor A2BP1/FOX1 has been shown at the center of a gene cluster that is dysregulated in ASD (Voineagu *et al.*, 2011). Binding motifs for A2BP1/FOX1 and FOX2 have been found in the NLGN3 RNA sequence, and interestingly this sequence is variable between different human populations (Zhang *et al.*, 2008). None of the mRNAs of the different PROSAP/SHANK isoforms has been found to bind to A2BP1/FOX1 or FOX2 but also to date nothing is known about the splicing factors associated with PROSAP/SHANK. Conversely, Prosap/Shank and NLs can interact with splicing factors and are part of the molecular pathways involved in protein translation. A yeast two-hybrid screen study found that Prosap2/Shank3 and NL3 can interact with the

splicing factors FOX2 and KHDRBS1, respectively. In the same screen, the authors showed an interaction of Prosap2/Shank3 and NL3 with members of the ASD-associated mammalian target of rapamycin (mTOR) pathway, Tsc1 and Akt, respectively, which regulates protein translation (Sakai *et al.*, 2011). Conversely, the Fragile X mental retardation protein (FMRP) or mTOR (for NLGN only) regulate PROSAP/SHANK and NLGN translation. Through their interaction partners, NLs and Prosap/Shank can therefore influence neuronal activity, splicing activity and regulate translation rate, candidate mechanisms implicated in the pathophysiology of ASD.

Convergence of pathophysiology in mouse models for autism spectrum disorders

Dysregulation of type I metabotropic glutamate receptor signaling in mouse models of syndromic autism spectrum disorders

The first genetic mouse models for ASD were developed with the discovery of monogenic forms of ASD. The recapitulation of ASD-associated mutations in mice and the development of standardised behavioral tests related to ASD allowed the generation and analysis of a large number of animal models (Silverman *et al.*, 2010). In the following section I will describe the results obtained in mouse models of syndromic ASD and the evidence for the role of type I mGluR (mGluR1 and 5) signaling in the pathophysiology of the disease.

For 15% of patients with ASD the diagnosis is associated with other features unrelated to the core symptoms of autism, such as morphological abnormalities. These forms of autism are termed syndromic and include Fragile X, Rett or Phelan–McDermid syndromes or TSC among others. These syndromes have in common that a large proportion of the patients are diagnosed for autism. The analysis of the convergent phenotypes between these different syndromes is the approach of choice to identify the pathophysiology of ASD. Importantly, for most of the cases, these syndromes are monogenic [Fragile X mental retardation (FMR1) is linked to Fragile X, TSC1 and TSC2 to TSC, and methyl C—phosphate—G binding protein 2 to Rett syndrome]. Although it is more difficult to draw a direct causal link between the genetic mutation and the autism phenotype in syndromic forms of ASD, the direct relationship in monogenic cases validates the associated mouse models. Early work from the laboratories of Broadie, Bear and Jongens identified an increase in type I mGluR-LTD (in animal models for Fragile X syndrome, Fmr1 knockout *Drosophila* and mice) (Zhang *et al.*, 2001; Huber *et al.*, 2002; McBride *et al.*, 2005), which has been linked to a hypersensitivity of mGluR5 (Osterweil *et al.*, 2010). Based on these results, the authors postulated that one of the core features of the disease consists of a dysregulation of mGluR5 (Bear *et al.*, 2004). Additional studies looked at a potential dysregulation of type I mGluR signaling in other mouse models for ASD. The analysis of mice lacking PTEN or TSC1, two members of the mTOR pathway related to TSC syndrome, revealed a defect in mGluR-LTD (Bateup *et al.*, 2011; Takeuchi *et al.*, 2013). Interestingly, PTEN and TSC2 knockout mice show a phenotype opposite to FMR1 knockout, with a decreased mGluR-LTD. In addition, mGluR5 protein is increased in TSC2 heterozygous mice, showing that TSC2 negatively controls the mGluR5 protein level (Potter *et al.*, 2013). To test the hypothesis that the FMR1 and TSC2 knockout mice phenotypes are related, Auerbach *et al.* (2011) created FMR1–TSC2 double knockout mice and found a normal mGluR-LTD in these mice. Additional data showed an occlusion of mGluR-dependent activation of mTOR in FMR1 knockout mice (Sharma *et al.*, 2010), confirming that Fmrp (the protein encoded by FMR1) and mTOR act in a common pathway that is dysregulated in ASD. Several studies used pharmacological approaches (i.e. positive or negative modulators) to control mGluR5 activity and generated compelling evidence showing that this strategy is successful to correct FMR1 or TSC2 knockout mice phenotypes (Auerbach *et al.*, 2011; Michalon *et al.*, 2012). These results are important as they identify, for the first time, a potential shared molecular pathway between unrelated forms of ASD, leading to a potential common treatment.

Type 1 metabotropic glutamate receptor signaling defect: a convergent pathway in autism spectrum disorders?

As presented in the previous paragraph, the dysregulation of Fmrp or mTOR pathways leads to syndromic forms of ASD associated with a defect in mGluR-LTD. From these results the remaining question is whether this defect is a secondary consequence of a broad cellular dysfunction or whether the core of the phenotype arises directly from a synaptic dysfunction. A key piece of evidence for a synaptic dysregulation of type I mGluR in ASD comes from the study of the synaptic protein NL3 and its associated knockout mouse model, which related to a form of autism not associated with any other symptoms (i.e. the non-syndromic form of ASD). Our study identified an increased level of mGluR1 at parallel fiber to Purkinje cell synapses in the cerebellum. In correlation with this we found an occlusion of the mGluR-LTD at these synapses, confirming a convergence of the phenotype with syndromic forms of ASD (Baudouin *et al.*, 2012). These results confirm that a strictly synaptic dysfunction, the absence of NLGN3, can lead to dysregulation of type I mGluR function, confirming the hypothesis of synaptic type I mGluR dysfunction in ASD. Some evidence also points to a defect in mGluR5 signaling associated with a lack of PROSAP/SHANK. Results from *in vitro* data show that a knockdown of PROSAP2/SHANK3 in hippocampal neurons induces a decrease in the mGluR5 protein expression level. This was correlated with a decrease in mGluR-LTD (Verpelli *et al.*, 2011). This result failed to be reproduced *in vivo* in PROSAP2/SHANK3 knockout mice where mGluR-LTD is unaffected (Bozdagi *et al.*, 2010; Yang *et al.*, 2012). In PROSAP1/SHANK2 knockout mice mGluR-LTD is also unaffected, but a study by Won *et al.* (2012) showed that pharmacological treatment of PROSAP1/SHANK2 knockout mice with an antagonist of mGluR5 is able to correct ASD-related behavioral phenotypes. These seeming contradictions can be due to the complexity of the Prosap/Shank structure, which is subjected to extensive splicing and is therefore difficult to completely knockout, or to a compensation from other isoforms, as the Prosap2/Shank3 and Prosap1/Shank2 protein levels are increased in PROSAP1/SHANK2 and PROSAP2/SHANK3 knockout mice, respectively.

Conversely, results show that type I mGluR could control both NL3 and Prosap/Shank protein levels through Fmrp and mTOR-dependent translation. NLGN3 and PROSAP3/SHANK1, PROSAP1/SHANK2 and PROSAP2/SHANK3 mRNA have been found in dendrites (Boeckers *et al.*, 2002; Cajigas *et al.*, 2012). An unbiased screen showed that FMRP binds to the mRNA of NLGN2 and NLGN3 and of all PROSAP/SHANK isoforms (Darnell *et al.*, 2011). In addition, Gkogkas *et al.* (2013) showed that the mTOR complex can control the translation of all NLGN isoforms through activation of the eukaryotic initiation factor 4E. FMRP or mTOR activity is known to be controlled by type I mGluR, through a sequence of phosphorylation events (Hou & Klann, 2004; Hou *et al.*, 2006; Nalavadi *et al.*, 2012). These data identify a potential

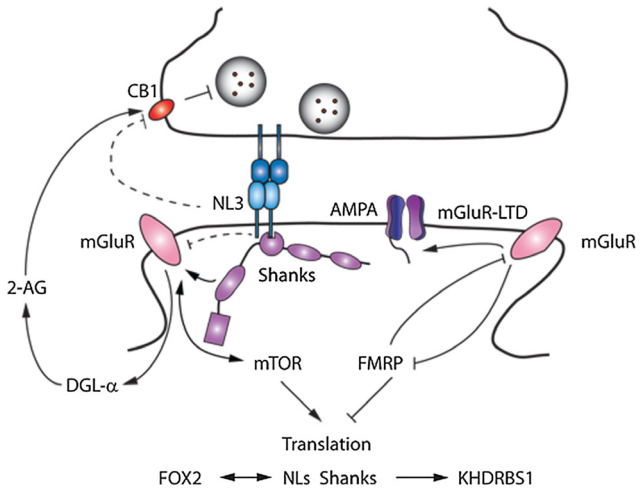

FIG. 1. Type I mGluR pathway in ASD. ASD-related proteins regulate type I mGluR function. NL3 or mTOR negatively controls the mGluR protein level, and Fmrp or Prosap/Shank regulates its function. Conversely, type I mGluR control the activation of the mTOR and Fmrp pathway, thereby regulating the translation of NLs and Prosap/Shank. These interactions represent the core mechanism by which type I mGluR are associated with ASD. Type I mGluR activation induces internalisation of AMPA receptors and promotes postsynaptic long-term depression. In addition, type I mGluR controls CB1-dependent presynaptic plasticity through the activation of diacylglycerol lipase-α (DGL-α)-dependent production and secretion of the CB1 ligand 2-arachidonoyl glycerol (2-AG). In addition to their effect on mGluR, NLs or Prosap/Shank can interact and thereby potentially modulate activity of the splicing factors FOX2 and KHDRBS1, respectively.

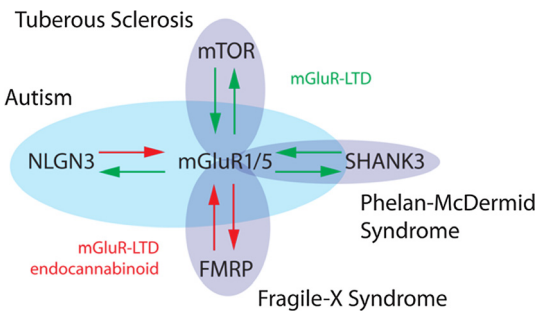

FIG. 2. Type I mGluR signaling: a point of convergence in the pathophysiology of ASD. At the clinical level, the Phelan–McDermid and Fragile X syndromes and TSC share a common diagnosis with autism; at the genetic level they are associated with mutations in PROSAP2/SHANK3, FMRP and mTOR, respectively. The analysis of the function of these different genes and NLGN3, which is associated with non-syndromic forms of autism, in mouse models reveals a common dysregulation of type I mGluR (mGluR1/5). Both mTOR- and PROSAP2/SHANK3-related knockout mice share similar phenotypes with increased mGluR-LTD. On the contrary, FMRP- and NLGN3-related knockout mice show a defect in mGluR-LTD and an alteration of endocannabinoid signaling. As a consequence, type I mGluR is a point of convergence for the pathophysiology of ASD and, together with the members of its associated signaling cascade, a primary target for the development of therapeutic strategies.

convergence of interaction between NLs, Prosap/Shank, mTOR and Fmrp orchestrated by type 1 mGluR. Conversely, mutations of these genes converge to similar dysfunction in type I mGluR function, leading to speculation that these genes function together as a dysregulated cluster in ASD (Fig. 1).

The consequences of the dysregulation of type I mGluR within this cluster might be broader than solely affecting mGluR-LTD. It has to be taken into account that the molecular signaling associated with type I mGluR is complex and controls several forms of synaptic plasticity that can be implicated in ASD. For example, type I mGluR also regulate endocannabinoid-mediated presynaptic plasticity. Upon activation, neurons can secrete the 2-arachidonoyl glycerol endocannabinoid, which binds its cannabinoid receptor CB1 on the presynaptic side to suppress presynaptic vesicle release and plasticity (Fig. 1). Type I mGluR induce the production of diacylglycerol, which is converted to 2-arachidonoyl glycerol by a diacylglycerol lipase (Kano *et al.*, 2008). A study by Jung *et al.* (2012) has found that FMR1 knockout mice show an absence of 2-arachidonoyl glycerol-dependent mGluR-LTD. They demonstrated that the absence of FMR1 induces the disruption of the macrocomplex containing mGluR5 and diacylglycerol lipase, impairing the production of 2-arachidonoyl glycerol. In a follow-up study, Busquets-Garcia *et al.* (2013) treated FMR1 knockout mice with CB1 and CB2 receptor antagonist and found a partial reversion of the FMR1 knockout-associated behavior in both cases. In NLGN3 knockout mice, Foeldy *et al.* (2013) found a defect in endocannabinoid-dependent neurotransmission at cholecystokinin to pyramidal neurons in the hippocampus, which they suggest is independent of mGluR signaling. These data support the idea that a dysregulation of type I mGluR signaling is associated with various forms of ASD and that defects in the type I mGluR interactome are a candidate risk factor for ASD.

Conclusion and perspectives

Human genetic studies demonstrate that a diagnosis of ASD can be associated with the dysregulation of gene clusters important for normal brain development. By piecing together the findings of many independent studies we find support for the existence of such clusters and identify type I mGluR as a point of convergence (Fig. 2). The type 1 mGluR pathway is the first candidate cluster identified on the basis of results from animal models, but additional pathways relevant for ASD, and potentially other psychiatric disorders, have started to be investigated. For example, the specific link between NL1, Prosap/Shank and the NMDA receptor function is of particular interest as it may be relevant for different psychiatric disorders including schizophrenia. Altogether, these works establish a new framework for the study of the pathophysiology of ASD. The role of a particular ASD-associated gene should not be studied in isolation but rather in its interaction with a complex set of proteins. As exemplified through this review, analysis of such clusters will lead to the identification of new pharmacological targets relevant for various forms of ASD. Heterogeneity in the genetic underpinnings and diagnosis of ASD is an obstacle to our understanding of the disease. The identification of a convergence in the pathophysiology of different forms of ASD represents a decisive step in narrowing down this complexity and will give new insight into unraveling the biological mechanisms that are defective in the disease.

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Abbreviations

ASD, autism spectrum disorders; FMR1, Fragile X mental retardation; FMRP, Fragile X mental retardation protein; mGluR-LTD, metabotropic glutamate receptor-dependent long-term depression; mGluR, metabotropic glutamate

receptor; mTOR, mammalian target of rapamycin; NL, Neuroligin; NLGN, Neuroligin gene; NMDA, *N*-methyl-D-aspartate; TSC, tuberous sclerosis.

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