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Abstract

Recent advances in genetics and genomics have unveiled numerous cases of autism spectrum disorders (ASDs) associated with rare, causal genetic variations. These findings support a novel view of ASDs in which many independent, individually rare genetic variants, each associated with a very high relative risk, together explain a large proportion of ASDs. Although these rare variants impact diverse pathways, there is accumulating evidence that synaptic pathways, including those involving synaptic cell adhesion, are disrupted in some subjects with ASD. These findings provide insights into the pathogenesis of ASDs and allow for the development of model systems with construct validity for specific causes of ASDs. In several neurodevelopmental disorders frequently associated with ASD, including fragile X syndrome, Rett syndrome, and tuberous sclerosis, animal models have led to the development of new therapeutic approaches, giving rise to optimism with other causes of ASDs.

Recent advances in the genetics of autism

Autism and autism spectrum disorders (ASDs) are neurodevelopmental disorders with characteristic behavioral abnormalities, including deficits in reciprocal social interactions and communication, and the presence of repetitive behaviors and/or restricted interests. Onset occurs during the first three years of life and there is a male preponderance, with a sex ratio of 4:1. The incidence of ASDs is now estimated at 6 in 1000 [1]. Family and twin studies have demonstrated that ASDs have a very high heritability. ASDs are etiologically heterogeneous, with an underlying genetic disorder identified in 10%-25% of cases, including monogenic disorders (e.g., fragile X syndrome [FXS], tuberous sclerosis, Rett syndrome), chromosomal aberrations and genomic imbalances [2]. It has been widely assumed that the remaining cases for which a causal factor has not been identified (referred to as “idiopathic” ASDs) are the result of interactions of multiple genetic variants of weak effect. This has led to the use of linkage and association studies to identify potential susceptibility loci of weak effect, few if any of which, however, have been consistently replicated [2].

Recent technological developments, including advances in the detection of copy number variations (CNVs) (deletions and duplications) and rare point mutations have begun to alter the conceptualization of ASDs, as the results indicate that an ever increasing proportion of ASDs are due to rare, causal, single locus genetic changes that can be either inherited or *de novo* in origin. Recent findings point to the existence of many distinct autism loci, with *de novo* CNVs and mutations playing important roles (*de novo* CNVs have been identified in 10% of sporadic cases and in 2%-3% of families having two or more affected children [3, 4]). The proportion of ASDs attributed to causal loci are likely to increase with the use of higher resolution microarrays and high-throughput sequencing.

This conceptual shift in ASDs can be summarized as a move from a 'common disease-common variant' perspective to a 'multiple rare variants' perspective (Box 1). The multiple rare variants model in ASD is reminiscent of the current conceptualization of intellectual disability (ID) [5, 6], in which defects in many different genes cause a similar behavioral phenotype. In fact, numerous genetic syndromes that cause ID can also present with ASD, suggesting the existence of common pathways in these two disorders. More broadly, variation in a given locus can lead to other neuropsychiatric disorders beyond ASDs and ID, suggesting that clinical diverse phenotypes may share similar genetic underpinnings.

Interestingly, many of the rare variants identified in ASDs relate to synaptic cell adhesion molecule (CAM) pathways, shedding light on the pathogenesis of ASDs and contributing to the identification of further candidate genes for ASDs. We review the genetic evidence for alterations in synaptic CAM pathways in ASDs and discuss how they might in turn result in cellular deficits based on studies on genetically modified mice and *in vitro* experiments. While these findings indicate that ASDs are much more heterogeneous in terms of etiology than previously thought, we note that the identification of any causal rare variant in ASDs has

immediate clinical relevance for genetic counseling and that these rare variants are amenable to modeling in experimental systems.

Synaptic cell adhesion molecules

Within the synapse, a complex molecular interplay takes place between functional domains of the pre and postsynaptic elements, including the postsynaptic density, actin cytoskeleton, and membrane trafficking domains, supporting the formation and maintenance of synapses. Structural and functional alterations of synaptic connections, including abnormal density and morphology of dendritic spines, synapse loss, and aberrant synaptic signaling and plasticity, have all been suggested to be involved in psychiatric and neurological disorders [7]. Molecular components of synapses, including synaptic CAMs and scaffolding proteins, play crucial roles in the structure and function of synaptic connections.

CAMs are involved in the initial contact between pre- and post-synaptic cells, in maintaining synaptic adhesion, and as anchors for scaffolding proteins (Figure 1). These scaffolding proteins in turn assemble signaling molecules, neurotransmitter receptors, and proteins in the actin cytoskeleton, all working together in the development and plasticity of synapses [8-10]. Recent genetic studies support the involvement of genes coding for synaptic CAMs, scaffolding proteins, and downstream signaling molecules, in developmental delay and ASD, both as causal genes and as potential susceptibility genes (Table 1).

Neuroligins, neurexins and SHANK3

The best-characterized synaptic CAM pathways implicated in ASDs are those involving neuroligins and neurexins, pathways also supported by findings with the SH3 and multiple ankyrin repeat domains 3 (SHANK3) scaffolding protein. The evidence for rare variants in each of these genes will be summarized here, before discussing less well-characterized synaptic CAM pathways involved in ASDs.

Neuroligins

Neuroligins (NLGNs) are postsynaptic CAMs that support synapse formation of both excitatory and inhibitory synapses *in vitro* [11]. There are five *NLGN* genes, including two X-linked genes (*NLGN3* and *NLGN4X*) and one Y-linked gene (*NLGN4Y*). A screen of the sex-linked *NLGN* genes in 158 ASD families identified a frameshift mutation in *NLGN4X* and a non-synonymous change in *NLGN3* in two families having each two affected brothers, one with autism and the other with Asperger syndrome [12], implicating for the first time these two genes in the etiology of ASDs. These mutations in *NLGN3* and *NLGN4X* result in intracellular retention of the mutant proteins in the endoplasmic reticulum *in vitro*, and the reduced levels of proteins that reach the cell surface show decreased affinity to neurexin [13-15]. Furthermore, when overexpressed in hippocampal neurons, the ASD-associated mutations fail to support the formation of presynaptic terminals [13]. The introduction of the *NLGN3* mutation into *Nlgn1*

drastically reduces spiny density and suppresses endogenous excitatory synapse function in cultured neurons [16].

Subsequently, a mutation in *NLGN4X* resulting in a premature stop codon was identified in a multi-generational pedigree with 13 affected males having either non-specific intellectual disability (ID) (10 individuals), ID with ASD (2 individuals), or ASD without ID (1 individual) [17]. Mutation screening of *NLGN3* and *NLGN4X* in independent ASD samples failed to identify other clear pathogenic mutations [18-22], although several *NLGN4X* missense variants [23] and *NLGN3* and *NLGN4X* splice isoforms [24] of unknown clinical significance were identified. These results indicate that although mutations in *NLGNs* are not frequent causes of ASDs, disruption of *NLGNs* can lead to rare cases of ASDs and/or ID. Additional studies have found *NLGN4X* deletions associated with ASDs as well as with a wider variety of neuropsychiatric conditions (including Tourette syndrome, attention deficit-hyperactivity disorder, learning disorders, anxiety and depression) [4, 25-28] while other studies have shown that loss of *NLGN4X* is not associated with a phenotype in certain cases [26, 29].

Some of these causal mutations in *NLGNs* have been directly modeled in mice. *Nlgn3* R451C knock-in mice, which replicate the amino acid change found in a human *NLGN3* mutation [12], were reported to show impaired social interactions, enhanced spatial learning abilities, and enhanced inhibitory synaptic transmission in the somatosensory cortex with no apparent effect on excitatory synapses [30]. This study did not report an analogous phenotype in *Nlgn3*-knockout animals and the authors thus proposed that the R451C mutation may represent a gain-of-function mutation. However, the interpretation is complicated by a recent report that could not replicate the observed changes in independently-generated R451C knock-in mice, although there were differences in the genetic background [31]. Recently, another group reported that their *Nlgn3* knockout mice show reduced ultrasound vocalizations and lack of social novelty preference, probably related to an olfactory deficit [32]. *Nlgn4* knockouts show selective deficits in reciprocal social interactions and communication that are reminiscent of ASDs [33]. Note, however, that in these and other studies with knockout mice, the relevance of models lacking two copies of a gene to a condition that is heterozygous in patients is questionable.

Whereas *in vitro* data suggest that neuroligins play a role in synapse formation [11], studies with triple *Nlgn1-Nlgn2-Nlgn3* knockout mice show that elimination of neuroligins does not affect synapse numbers in the brain but alters the recruitment of postsynaptic receptors to glutamatergic, γ-aminobutyric acid (GABA)ergic, and glycinergic synapses [34]. These findings indicate that neuroligins are essential for proper synapse maturation and function but not for the initial formation of synaptic contacts [35]. Based on comprehensive studies using both overexpression and genetically modified mice, it is becoming clear that neuroligins differentially specify synapse neurochemistry (excitatory or inhibitory), with different neuroligins acting on distinct types of synapses via activity-dependent mechanisms [16]. Thus, when Nlgn1 is overexpressed in cultured neurons, it increases excitatory but not inhibitory

synaptic responses, whereas Nlgn2 overexpression selectively enhances inhibitory synapses [16]. Accordingly, *Nlgn1* knockout mice exhibit impaired *N*-methyl-D-aspartate (NMDA) receptor signaling, whereas *Nlgn2* knockout mice have reduced inhibitory synaptic transmission [16]. Notably, the synapse-boosting activity of Nlgn1 and Nlgn2 is suppressed by inhibition of synaptic activity, indicating that the effects of neuroligins are dependent on synaptic signaling [16].

Neurexins

Neurexins (NRXN1–NRXN3) are predominantly presynaptic CAMs and are binding partners of the postsynaptic neuroligins [36]. Neuroligins induce presynaptic differentiation in contacting axons [37], a response mediated by neurexin [38]. Conversely, neurexin induces postsynaptic differentiation in glutamatergic synapses through interactions with Nlgn1, 3, 4 and in GABAergic synapses through interactions with Nlgn2 [39]. Although previously considered presynaptic molecules, neurexins are also expressed postsynaptically, where they block the synaptogenic activity of neuroligins by modulating the strength of neuroligin/neurexin interactions [40]. In addition, neurexins regulate postsynaptic NMDA receptor function through a cell-autonomous postsynaptic mechanism [41].

There are three neurexin genes (*NRXN1–NRXN3*), each of which encodes two major variants, α (long) and β (short), differing in their extracellular domains. CNV analysis using single nucleotide polymorphism (SNP) arrays in ~1200 families with at least two individuals with ASD identified a 2p16.3 deletion involving *NRXN1* in two sisters [42]. Subsequent studies also identified rare *NRXN1* deletions [4, 43, 44] and chromosomal abnormalities involving 2p16.3/*NRXN1* in ASDs [45]. Rare sequence variants in *NRXN1* have also been reported in ASD, but their clinical significance is unclear at present [45-47]. It should be noted however that both deletions and chromosomal aberrations disrupting *NRXN1* have been reported in healthy carriers [45, 48, 49], suggesting that heterozygous inactivation of *NRXN1* is not by itself sufficient to cause ASD and that other factors are required to produce the ASD phenotype. Furthermore, *NRXN1* deletions have recently been shown to confer risk for schizophrenia, suggesting an etiological overlap between this disorder and ASD (Box 2).

Double and triple α-Nrxn knockout mice show synaptic transmission defects without any obvious impairment in axon guidance or synapse formation [50, 51]. Ca^{2+}-triggered neurotransmitter release is severely depressed in these animals due to altered functional coupling of Ca^{2+} channels to the presynaptic membrane, an effect specifically rescued by α-Nrxn1 but not by β–Nrxns [52]. Interestingly, *CDK5* and *CASK*, two intracellular mediators of NRXN/NLGN-induced synaptogenesis [53], have been found to be either mutated or deleted in ID [54-56].

SHANK3

SHANK3 is a member of the SHANK synaptic scaffolding proteins that are abundant in the postsynaptic density [57-59], where they interact with neuroligins directly [60] or indirectly [61].

SHANK3 has multiple protein interaction domains, interfacing between glutamate receptor complexes and actin regulatory proteins, and is therefore well suited to play a role in spine morphogenesis and synaptic plasticity, functioning as a "master" scaffolding protein [58]. When overexpressed in cultured hippocampal neurons, Shank3 promotes the maturation and enlargement of dendritic spines [62]. Knock-down of Shank3 in hippocampal neurons decreases spiny density while transfection of Shank3 in aspiny neurons induces the formation of dendritic spines with functional synapses [63].

The 22q13.3 deletion syndrome is a microdeletion disorder characterized by global developmental delay, hypotonia, markedly delayed or absent speech, autistic behavior, and minor dysmorphic features [64]. Molecular characterization of individuals with the 22q13.3 deletion syndrome identified a minimal deleted region of 100 kb containing three genes, *SHANK3*, *ACR*, and *RAB2LB* [65]. Evidence for the involvement of *SHANK3* as the critical gene responsible for the ID and speech deficits associated with deletion of 22q13.3 first came from a patient with a balanced chromosomal translocation disrupting *SHANK3* [66]. Further support for this gene came from a small number of individuals with minimal 22q13.3 deletions who were shown to have breakpoints situated within *SHANK3*, including several with a recurrent breakpoint within intron 8 [67-69]. Morever, individuals with a ring chromosome 22 typically have deletions of the long arm of the chromosome and exhibit the 22q13 deletion phenotype, but subjects with an intact *SHANK3* gene are phenotypically normal [70].

Given the presence of autistic behavior in patients with 22q13.3 deletions [64, 69-71], variations in the *SHANK3* gene were studied in 227 subjects with ASD using fluorescent in situ hybridization (FISH) and direct sequencing [68]. Alterations in *SHANK3* were identified in three families: 1) a *de novo* 22q13 terminal deletion with the breakpoint in intron 8 of *SHANK3* in a boy with autism and ID; 2) a nucleotide insertion creating a frameshift mutation in two affected brothers, likely resulting from germinal mosaicism in the mother; and 3) a terminal 22q13 deletion in a girl with autism and a reciprocal duplication in her brother with Asperger syndrome, both resulting from a paternal chromosomal translocation [68]. This study confirmed that haploinsufficiency of *SHANK3* accounts for the 22q13 deletion phenotype. In addition, the ASD phenotype associated with both loss and gain of *SHANK3* supports a critical role for *SHANK3* gene dosage in neurodevelopment. The *SHANK3* frameshift mutation (3680insG) results in a truncated protein lacking crucial protein-protein interaction domains (Homer-binding, cortactin-binding, Abp1-binding, and SAM domains) involved in glutamate receptor and actin binding, spine formation and maturation and synaptic targeting. Accordingly, no synaptic localization was observed after overexpression of the mutant protein in rat hippocampal neurons [68].

Two recent studies confirmed the role of *SHANK3* in ASDs, identifying one *de novo* mutation and two deletions in 400 subjects [72] and one *de novo* splice site mutation in 427 subjects [73] (note that the latter study did not screen for deletions). These findings, together

with those reported initially [68] lead to an estimated prevalence of at least 0.47% (7/1054) *SHANK3* pathogenic variants in ASDs.

Knockout mice of the related molecule *Shank1* show smaller dendritic spines of CA1 pyramidal neurons and weaker synaptic transmission, along with altered learning and memory [74]. Our own preliminary studies with heterozygous *Shank3*-knockout mice support a clear role for *SHANK3* in synaptic transmission and plasticity, as evidenced by deficits in hippocampal long-term potentiation (T. Sakurai, J.D. Buxbaum et al., unpublished).

Other synaptic CAMs and scaffolding proteins associated with ASD and ID

The cadherin (CDH) and protocadherin (PCDH) families include a large number of proteins that are typical synaptic CAMs whose differential expressions may be important for specific synaptic formations [75, 76]. This family of proteins usually undergoes homophilic interactions to support cell adhesion. N-cadherin (*CDH2*), a prototypical cadherin, has been shown to be associated with both excitatory and inhibitory synapses in cultured hippocampal neurons [77]. Protocadherins have also been shown to be present at synaptic sites, where they may contribute to synaptic specificity in concert with classical cadherins [78, 79].

A recent study identified CNVs involving the *PCDH9* gene and a *de novo* translocation deleting the *CDH18* gene in ASDs [4]. Moreover, in a study of consanguineous families with children with ASD, homozygous deletions implicated *PCDH10*, whose expression is regulated by neuronal activity, in ASDs [80]. Epilepsy, female-restricted, with mental retardation (EFMR) is a disorder with an unusual mode of inheritance, affecting females. Very recently, mutations in the X-linked *PCDH19* gene were identified in 7 families with EFMR, some leading to premature termination codon and others disrupting adhesion of PCDH19 [81]. The restriction of EFMR to females has been suggested to be due to X inactivation of the gene, with hemizygous males having a homogeneous population of PCDH19-negative cells and affected females being mosaics for PCDH19-negative and wild-type cells. As the PCDH19 cell adhesion is possibly homophilic, this mosaicism might disrupt cell-cell communication more severely than a simple loss of PCDH19.

Two other genes encoding CAMs of the cadherin and immunoglobulin (Ig) superfamilies, *CDH15* and *KIRREL3,* were recently shown to be disrupted by a chromosomal translocation in a patient with ID [82]. Subsequent mutation screening in a cohort of patients with ID identified several rare missense variants in both genes [82]. The *CDH15* variants alter the cell-cell adhesion properties of the protein *in vitro*, while KIRREL3 was shown to interact in neurons with the synaptic scaffolding protein CASK, recently implicated in X-linked ID [56].

One recent discovery in ASD is the presence of recurrent 16p11.2 microdeletions and microduplications in as much as 1% of cases [4, 44, 83, 84]. The critical interval, which is flanked by low copy repeats, contains ~30 genes, including *TAOK2* and *MAPK3*, which are part of a signaling pathway directly linked to the synaptic CAMs N-cadherin and PCDH8 [85]. PCDH8 cell surface expression is modulated by neuronal activity via a mechanism that involves

activation of the kinases TAOK2 and MAPK3, which in turn leads to the internalization of PCDH8 and subsequent weakening of synapses. It would be interesting to determine if specific disruption of *TAOK2* and/or *MAPK3* is associated with ASD.

The Ig superfamily of CAMs is a large family of CAMs that are classified into subfamilies based on their structure. They interact both homophillically and heterophillically to support neuronal cell adhesion [86]. The L1 CAM (L1CAM), a member of the L1 subfamily, is an important mediator of neuronal migration, process outgrowth and synaptic targeting [87], and was the first CAM to be associated with a genetic disorder. Mutations in the *L1CAM* gene are responsible for a syndromic form of X-linked ID [88].

The contactins (CNTNs 1-6) are glycosyl phosphatidylinositol-linked Ig superfamily CAMs, which have been shown to be involved in axon growth, guidance, and synapse formation and plasticity [89]. Disruption of *CNTN4* (3p26.3) results in 3p deletion syndrome, which includes ID, growth retardation and dysmorphic features, and can be associated with ASD [90]. Array-based comparative genomic hybridization identified paternally-inherited CNVs in the *CNTN4* gene in ASDs, including a deletion in 2 siblings and a duplication in an unrelated individual [91]. Because the parents were reportedly healthy, these findings suggest either incomplete penetrance or a polymorphic (i.e. neutral) variant. More recently, compelling evidence for a role for *CNTN4* deletions and duplications in ASDs was observed in a CNV analysis involving over 2000 cases [44]; here too the CNVs were most commonly inherited. In addition, small deletions close to *CNTN3* (3p12.3) have been identified in ASDs [80]. Since studies in rodents show that both CNTN3 and CNTN4 are highly expressed in the developing brain at the time of synaptogenesis [92], these contactins may be associated with ASDs and ID through alterations in synapse formation and function.

The contactin associated proteins (CNTNAP1-5) show structural similarity to neurexin, even though they belong to different families. It is not yet known if CNTNAPs have a synaptic function like contactins or neurexins [93], although CNTNAP2 has been shown to be present in the synaptic plasma membrane fraction of brain lysates [94]. CNTNAP2 interacts with CNTN2 and localizes at the juxtaparanode of myelinated nerves [95, 96]. Moreover, CNTNAP2 is enriched in language-related circuits involving the frontal and anterior temporal lobes, striatum, and dorsal thalamus [97]. Homozygous *CNTNAP2* mutations were described in Amish children with cortical dysplasia-focal epilepsy syndrome, characterized by neuronal migration abnormalities, seizures, ID and ASD [98]. A *de novo* chromosomal inversion disrupting *AUTS2* and *CNTNAP2* was recently described in a child with cognitive and social delay and autistic traits [94]. Because polymorphic CNVs of the *AUTS2* locus had been described in healthy individuals, the authors focused on *CNTNAP2,* sequencing the gene in 635 patients with ASD and 942 controls. Among patients, they identified a total of 27 nonsynonymous changes; 13 were rare and unique to patients and 8 of these were predicted to be deleterious by bioinformatic approaches and/or altered residues conserved across all species. Overall, this resequencing data demonstrated a moderate increase in the burden of rare variants in cases

versus controls, suggesting that the contribution of rare variants of *CNTNAP2* may be modest in ASD [94]. However, the role of *CNTNAP2* in ASD received support by the recent report of a *CNTNAP2* deletion in a female with autism [99]. Consistent with a variable expressivity associated with many genetic and genomic variants, *CNTNAP2* was also reported to be disrupted by a chromosomal aberration in a family with Gilles de la Tourette syndrome and obsessive compulsive disorder [100] (and see Box 2).

As more rare causal variants are identified, we will gain further insight into CAMs and CAM-signaling pathways in ID and ASD. For instance, the recently described 3q29 microdeletion ID syndrome [101] includes *PAK2* (a synaptic signaling molecule) and *DLG1* (a synaptic scaffolding protein), which are homologous to two X-linked ID genes, *PAK3* and *DLG3*. These synaptic scaffolding proteins appear to bind to neuroligin [60] as well as to nectins, which are Ig superfamily CAMs [102] involved in synapse formation [103].

Common variants in CAMs and susceptibility to ASDs

Although this review focuses on the most recent discoveries of rare variants in ASDs, there remains empirical support for a role for common variants in the disorder, most notably because of the presence of a subtle phenotype in a large proportion of family members, the so-called broad autism phenotype [104], more commonly observed in multiply affected families [105]. Association studies of some synaptic CAMs have suggested that common variants in such genes might represent susceptibility loci. Positive, replicated evidence for association of ASDs with common variants in CAMs has been described for *CNTNAP2* [97, 106], *NRCAM* [107-109], and reelin [110]. However, recent genome-wide association analyses in considerably larger samples do not provide support for a strong association between ASDs and these genes. In contrast, genome-wide association analysis across multiple samples, totaling over 2600 ASD subjects, has identified six SNPs that lie between the cadherin 9 (*CDH9)* and cadherin 10 (*CDH10)* genes on 5p14.1 as associated with ASDs with an important population attributable risk [111], suggesting that common variants in certain neuronal CAMs can increase risk for ASDs.

CAMs and ASDs: emerging pathogenetic mechanisms

Neuropathological and imaging studies in autism have failed to identify consistent abnormalities of brain structure, except for brain overgrowth in about 20% of cases. Similarly, there is not as yet an accepted microscopic neuropathology in autism, although only a few postmortem studies have been performed. The genetic studies reviewed here implicate synaptic CAM pathways as etiological factors in ASD, but the nature of the underlying pathogenetic mechanisms and how they lead to the development of the social and communication deficits in individuals with ASDs remains largely unknown. Nevertheless, the results obtained in mutant mice as well as *in vitro* experiments provide a glimpse of some of the cellular events that might be involved in the development of ASD, including altered

synaptic maturation, connectivity and stabilization. The *in vitro* data with the scaffolding protein SHANK3 as well as *in vivo* data in *Shank1*-knockout mice depicting dramatic alterations in spine morphology [62, 63, 74], represent good examples of how altered expression of such genes can disrupt critical synaptic function. Similarly, the importance of the trans-synaptic neuroligin-neurexin complex for proper synaptic function is clear from the marked deficits in synaptic transmission in mice lacking *Nlgns* or *Nrxns* [35]. These findings are particularly interesting in view of previous studies showing abnormal development of dendritic spines in various neurodevelopmental disorders associated with ASD, including fragile X syndrome, Rett syndrome, Angelman syndrome, tuberous sclerosis, Down syndrome, and William's syndrome [112, 113]. In addition, the onset of autism occurs before the age of three years, during a critical period of synapse formation and maturation, and this time course is consistent with a role of synaptic dysfunction in the pathogenesis of autism. Recent studies showing that neuroligins validate transient synapses in an activity dependent manner [16] also support the hypothesis that ASD might result from disruption of postnatal experience-dependent synaptic plasticity [114].

The observation of epilepsy in about a third of patients with autism has prompted speculation that ASDs may be due to an enhanced excitation/inhibition (E/I) ratio resulting from aberrant formation and function of excitatory versus inhibitory cortical synapses [115]. Genetic variants that disrupt *SHANK3, NLGN3, NLGN4X* or *NRXN1* genes implicate alterations in glutamatergic synapse development. However, NRXN/NLGN interactions are also involved in inhibitory synapse development in ASD [11, 39, 116], and therefore could also play a role in ASD and ID through disruption of inhibitory synapses. *In vivo* studies with *Shank1* knockout mice show reduced excitatory synaptic strength [74], something we also observe in *Shank3* heterozygotes (T. Sakurai, J.D. Buxbaum et al., unpublished), so we must also consider decreased E/I ratios in a subset of ASDs. Brain imaging studies suggest that the core features of autism could be related to disturbed connectivity, both interhemispheric and intracortical [117]. It will be interesting to determine whether disruption of the CAM pathways leads to alterations in E/I balance, creating a noisy or muted system and contributing to altered connectivity. Certainly, the elegant neurobiological investigations on neuroligins and neurexins illustrate how mutations in these genes might alter the balance between excitatory and inhibitory neurotransmission [35], ultimately disrupting the cortical and subcortical brain networks involved in social cognition and language.

There is, of course, still a huge amount of work to be done to understand the role of synaptic CAMs and related proteins in the pathophysiology of ASDs. The identification of highly penetrant mutations in these genes is just a first step, providing molecular targets for neurobiological studies to unravel the complex pathogenesis of ASDs.

Novel therapeutic approaches in ASDs

FXS, which is the most common inherited cause of cognitive impairment, is found in about 2% of individuals with ASD [118], and, conversely, ~50% of individuals with FXS have autism or ASD [119]. FXS is caused by pathological trinucleotide expansions in the *FMR1* gene, which codes for the Fragile X mental retardation protein (FMRP). FMRP functions as a repressor of mRNA translation and participates in the transport of mRNA species that play a role in synaptic plasticity in an activity-dependent manner [120]. The reduction of FMRP expression in FXS leads to enhanced synaptic protein synthesis after stimulation of metabotropic glutamate receptors (mGluR) [121]. Remarkably, blocking the function of mGluR by either genetic means or by pharmacological agents reverses some of the synaptic abnormalities and higher-order phenotypes observed in model systems of FXS, including those in mice, flies, and worms [122-125]. This hypothesis has led to a clinical trial currently underway of an mGluR5 reverse agonist in FXS. Recent studies of mouse models of other genetic disorders associated with autism have also shown striking and dramatic reversal of neurological defects. Rett syndrome, caused by mutations in the X-linked *MECP2* gene, a transcriptional repressor, is considered a disorder of synapse development. Abnormal phenotypes in Mecp2-deficient mice can be rescued by reactivation of MeCP2 expression after birth [126, 127] or by administration of insulin-growth factor, which strongly promotes synaptic maturation [128]. Similarly, in *Tsc1* or *Tsc2* knockout mice (models of tuberous sclerosis), and *Pten*-knockout mice (which model the macrocephaly and behavioral abnormalities in ASD patients with *PTEN* mutations), the neurological abnormalities can be reversed by pharmacological inhibition of the mammalian target of rapamycin (mTOR) pathway [129-132]. Rapamycin improved survival, reduced seizures, and reversed cognitive abnormalities and associated cellular deficits, including neuronal hypertrophy and altered hippocampal synaptic plasticity. A multi-site clinical trial of rapamycin in individuals with tuberous sclerosis is ongoing, and interim reports have been positive for short-term memory effects. Smaller scale studies with other etiological subgroups are also underway, including intranasal insulin in children with 22q13 deletion syndrome [133].

The surprising phenotypic reversibility observed in these animal models after birth —or even in the adult— suggests that these "developmental" disorders could at least in part be due to the ongoing lack of functional protein and/or that the brain remains extremely plastic even after an impaired neurodevelopmental history. The importance of the results summarized here cannot be overstated as they imply that identifying causal forms of ASDs and ID might lead to successful targeted therapies for these disorders, some of which possibly being specific for a subtype of the disorders (“personalized medicine”).

Concluding remarks

During the past few years, researchers have identified several causal genes for ASD and ID encoding synaptic CAMs and associated molecules involved in synaptic function. We should note that many of these findings will need to be replicated in additional, large samples. In

addition, the functional relevance of some of the variants needs to be critically examined. In parallel, as causal genes for ASD and ID are identified and validated in genetic studies, we will need to clarify how these genes cause the ASD and/or ID phenotype, making use of *in vitro* and *in vivo* model systems. Causal variants are readily studied in model systems as the technology to introduce or disrupt genes in cellular and animal models is widespread. The understanding of these pathways in neurodevelopmental disorders will likely lead to novel, targeted therapeutic strategies, with the recent developments in experimental treatments in fragile X syndrome and other ASD-associated disorders representing important paradigms.

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Table 1. Genes involved in synaptic cell-adhesion pathways implicated in ASDs and ID

Gene	Name	Chromosomal locus	Evidence	Phenotype	Function	Reference
Cell adhesion molecules						
CDH9/CDH10	Cadherin 9/Cadherin 10	5p14.1	common SNPs	ASD	CAM	[111]
CDH15	Cadherin 15	16q24.3	chromosomal abnormality, rare mutations	ID	CAM	[82]
CDH18	Cadherin 18	5p14.3	chromosomal abnormality	ASD	CAM	[4]
CNTN3	Contactin 3	3p12.3	homozygous deletion	ASD	CAM	[80]
CNTN4	Contactin 4	3p26.3	deletion, chromosomal abnormalities, CNVs	3p deletion syndrome, ASD, ID	CAM	[44, 90, 91]
CNTNAP2	Contactin associated protein 2	7q36.1	rare mutations, deletion, chromosomal abnormality, common SNPs	ASD, language deficits, schizophrenia, cortical dysplasia, epilepsy, ID, Tourette syndrome, OCD	CAM	[94, 97-100, 106]
KIRREL3	Kin of IRRE like 3	11q24.2	chromosomal abnormality, rare mutations	ID	CAM	[82]
L1CAM	L1 cell adhesion molecule	Xq28	mutations, deletions	syndromic ID (hydrocephalus, spastic paraplegia)	CAM	[88]
NLGN3	Neuroligin 3	Xq13.1	rare mutations	ASD	CAM	[12]
NLGN4X	Neuroligin 4	Xp22.32-p22.31	rare mutations, CNVs	ASD, ID; in the case of deletions: Tourette syndrome, ADHD, learning disorders, anxiety and depression (including healthy carriers)	CAM	[4, 12, 17, 25-28]
NRCAM	NgCAM related cell adhesion molecule	7q31.1	common SNPs	ASD	CAM	[107-109]
NRXN1	Neurexin 1	2p16.3	CNVs, chromosomal abnormalities	ASD, schizophrenia, CNVs also in healthy carriers	CAM	[4, 42-45, 49]
PCDH9	Protocadherin 9	13q21.32	CNVs	ASD, CNVs also in healthy carriers	CAM	[4]
PCDH10	Protocadherin 10	4q28.3	homozygous deletion	ASD	CAM	[80]
PCDH19	Protocadherin 19	Xq22.1	rare mutations	epilepsy, female-restricted, with mental retardation (EFMR)	CAM	[81]
Additional molecules involved in synaptic cell adhesion pathways						
CASK	Calcium/calmodulin-dependent serine protein kinase	Xp11.4	rare mutations, deletion	ID, brain malformation	Scaffolding protein/kinase	[55, 56]
CDK5R1	Cyclin-dependent kinase 5, regulatory subunit 1	77q11.2	rare mutations	non syndromic ID	Kinase	[54]
DLG1/PAK2	Discs large (Drosophila) homolog 1/p21-activated kinase 2	3q29	deletion	3q29 microdeletion syndrome, ID, ASD	Scaffolding protein/kinase	[101]
SHANK3	SH3 and multiple ankyrin repeat domains 3	22q13.3	deletions, chromosomal abnormalities, rare mutations	22q13 deletion syndrome, ASD, ID	Scaffolding protein	[66-68, 72, 73]

Known disease genes are indicated in blue, whereas the remaining genes would still warrant further investigation. Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorders; CAM, cell adhesion molecule; CNVs, copy number variations; ID, intellectual disability; OCD, obsessive compulsive disorder; SNPs, single nucleotide polymorphisms.

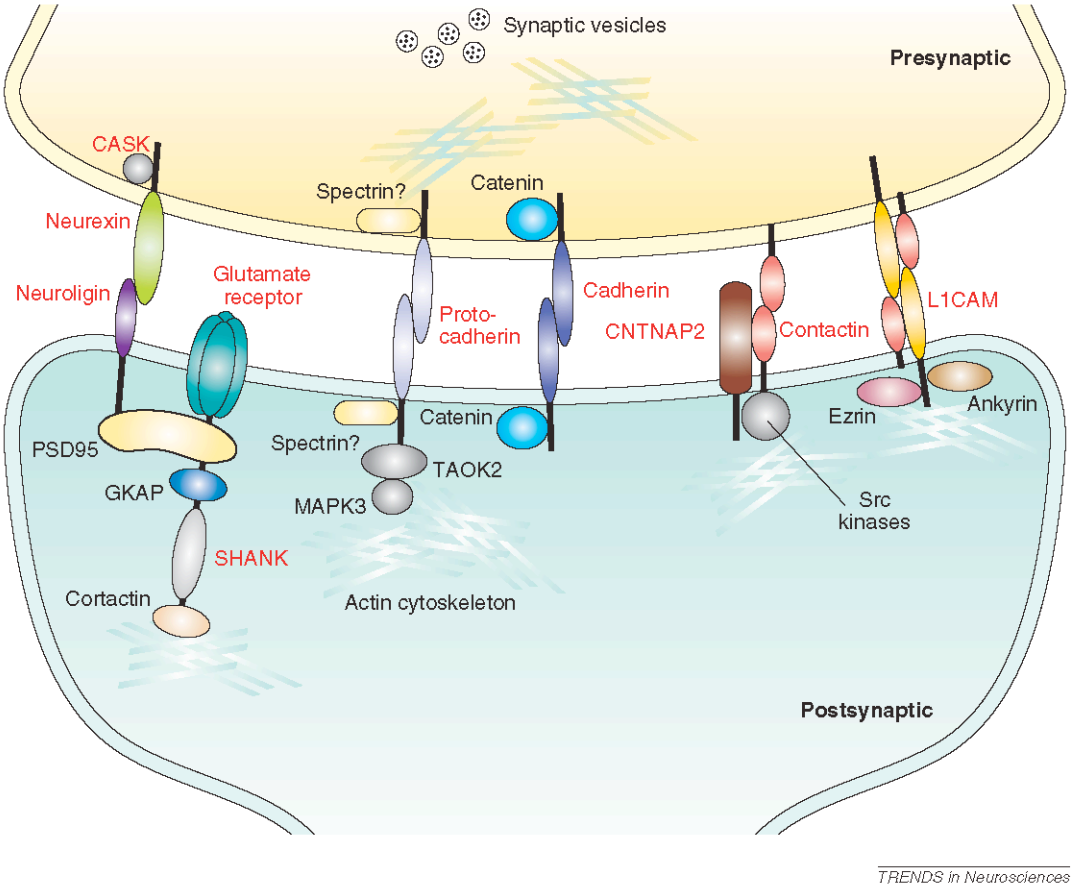

Figure 1. At the synapses, cell adhesion molecules connect presynaptic and postsynaptic sites through homophilic (e.g., caherins, protocadherins and L1CAM) and heterophilic (e.g., neuroligin-neurexin, CNTNAP2 and contactins) interactions, and play roles in synapse development, function and plasticity. These cell adhesion molecules also interact with several cytoplasmic proteins, namely synaptic scaffolding proteins (e.g., postsynaptic density protein 95 [PSD95] and SHANK, which in turn serve as platforms for signaling molecules), cytoskeletal proteins (e.g., ankryin, ezrin, catenin, actin and spectrin) and signaling molecules (e.g., calmodulin-dependent serine kinase [CASK], src family kinases, and mitogen-activated protein kinase [MAPK] cascade proteins including TAO kinase 2 [TAOK2] and MAPK3), all of which are involved in synapse assembly and shape, function, and plasticity. Expression of cell adhesion molecules can determine the chemical nature of synapses and the downstream signaling. The expression and localization of cell adhesion molecules are modulated by neuronal activity, leading to regulation of synaptic strength and sculpting of the molecular components of the synapse. The proteins whose genes have been shown to be involved in ASD and/or ID are indicated in red. The question marks shown after spectrin highlight the fact that the functional meaning of the interaction between this protein and protocadherins is unclear.

Box 1. The 'common disease-common variant' versus the 'multiple rare variants' perspectives

In many genetic diseases, including ASDs, there have been two models for explaining genetic etiology, which are not mutually exclusive. These are the 'common disease/common variant' and the 'common disease/rare variant' models, the latter also called the 'multiple rare variant' or 'rare alleles of major effect' model [134-137]. Common variants are genetic polymorphisms (susceptibility alleles) that are found widely distributed in the population and are associated with a modest increase in disease risk, typically associated with odds ratios (ORs) less than 2 (often in the ~1-1.3 range). In this model, a given disorder stems from the interaction of multiple common genetic variants and possibly other nongenetic factors. The common disease/common variant hypothesis has been challenged from a population genetic perspective because if one assumes any sort of selection against even moderately deleterious variants, the rates of the variants would be very low [134-136]. In contrast, in the multiple rare variant model, a large number of rare, and even very rare, variants underlie the disorder. These rare variants are typically highly penetrant, associated with ORs that can be substantial and may contribute the major part of the susceptibility for a given individual. At the extreme, rare variants have high ORs and are equivalent to rare deleterious mutations. These two models have very different implications regarding gene discovery strategies. While association studies are suitable to identify common susceptibility alleles present in large numbers of patients compared to controls, they fail to identify rare, causal mutations. Similarly, linkage studies are unable to identify mutations in critical genes in highly heterogeneous disorders involving many different genes and chromosomal loci such as ASDs.

Box 2. ASD and schizophrenia genetics

While ASDs are clearly distinct from schizophrenia, CNVs in identical genes have been identified in both disorders during the last years. For example, some of the synaptic CAMs noted in the current review have also been implicated in schizophrenia through CNVs and chromosomal abnormalities, including *NRXN1* [49] and *CNTNAP2* [138], as well as other CAMs, including protocadherin α [139] and *NCAM* [140]. Similarly, recent large-scale genome-wide studies identified CNVs at 1q21.1, 15q11.2, 15q13.3, and 16p11.2 in schizophrenia, ASD and ID. While at first surprising, these findings are not inconsistent with the well-known observation that the 22q11.2 deletion syndrome (velo-cardio-facial syndrome) increases risk for schizophrenia and for autism and suggest that neurodevelopmental abnormalities can lead to profoundly different manifestations depending on other factors that are as yet unknown. One can envision two scenarios [141]. In one case the CNV induces a mediating phenotype, such as cognitive impairment, which in turn increases risk for both ASD and schizophrenia. Alternatively, there are pleiotropic effects of the CNV without a mediating intermediate phenotype. Understanding the influence of CNVs and specific susceptibility genes in various clinical disorders will benefit from an explicit study of the relationship of the genetic variation to potential intermediate phenotypes, including detailed phenotypic assessment of apparently "unaffected" carriers.