From the genetic architecture to synaptic plasticity in autism spectrum disorder

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Abstract | Genetics studies of autism spectrum disorder (ASD) have identified several risk genes that are key regulators of synaptic plasticity. Indeed, many of the risk genes that have been linked to these disorders encode synaptic scaffolding proteins, receptors, cell adhesion molecules or proteins that are involved in chromatin remodelling, transcription, protein synthesis or degradation, or actin cytoskeleton dynamics. Changes in any of these proteins can increase or decrease synaptic strength or number and, ultimately, neuronal connectivity in the brain. In addition, when deleterious mutations occur, inefficient genetic buffering and impaired synaptic homeostasis may increase an individual's risk for ASD.

Dysmorphic features
Differences in body structure compared with that in the general population. Dysmorphic features can be isolated or multiple and vary from mild anomalies, such as minor malformations of the fingers, to more severe differences, such as microcephaly.

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Autism spectrum disorder (ASD) is an early onset neuropsychiatric disorder that is characterized by impaired social communication, restricted interests and stereotyped and repetitive behaviours[1–3]. These traits exhibit a wide, continuous distribution both in the general population and in individuals who have been diagnosed with ASD[4–6], and epidemiological studies estimate that more than 1% of the world's population could receive a diagnosis of ASD[7,8]. The core symptoms of autism rarely emerge in isolation; they usually coexist with other psychiatric and medical conditions, including intellectual disability (ID), epilepsy, motor control difficulties, attention-deficit hyperactivity disorder (ADHD), tics, anxiety, sleep disorders and gastrointestinal problems[9,10]. ASD affects more males than females; this is especially true among individuals with a normal intelligence quotient (>5 males/1 female affected) but less so in populations with ID (2 males/1 female affected), and the ratio becomes more balanced if patients present with dysmorphic features[11]. Individuals with ASD also exhibit alterations in sensory processing, including difficulties in the integration of information across different sensory modalities[12,13].

The brain regions that are involved in ASD remain difficult to determine, and the neural mechanisms that underlie the altered social communication and integration of sensory information in this disorder remain largely unknown. Abnormal brain growth and connectivity between regions (for example, too many short-range and too few long-range neuronal connections) have been proposed as a cause of ASD[14,15], but these findings remain controversial and could be due to methodological artefacts[16,17]. To better understand the pathophysiology of ASD, much research has focused on identifying genetic mutations associated with ASD and studying their effects in animal models.

A growing number of the genes that have been associated with ASD seem to have a role in synaptic plasticity[18–24]. Indeed, animal studies have shown that many ASD-risk genes are regulated by neuronal activity and that their gene products modulate synaptic strength or number[18,25,26–29]. It was therefore hypothesized that mutations could lead to the distortion of typical neuronal connectivity[30], increasing the risk of ASD[26,31] and the difficulties in adapting to external stimuli, such as those received during social interactions[27–29].

In this Review, I first depict the complex genetic architecture of ASD and emphasize the crucial role of both rare and common genetic variants in this disorder. I then examine how ASD-linked mutations might affect synaptic plasticity at different levels. Finally, I reassess and expand the synaptic homeostasis hypothesis of ASD[27,28], building on recent data. I propose that genetic mutations will either reduce or increase the synapse strength or number through the pathways that are typically induced by neuronal activity and sensory inputs. When compensatory mechanisms such as synaptic homeostasis fail, mutations could induce atypical brain connectivity and symptoms of ASD.

The genetic architecture of ASD

Before summarizing epidemiological and molecular genetic studies of ASD, it is important to consider our current understanding of human genome diversity[32–34].

An individual carries, on average, 3 million genetic variants that differ from the reference human genome sequence. These variants are mainly single-nucleotide variants (SNVs), short insertions and deletions and copy-number variants (CNVs). The vast majority of these variants (>95%) are shared with more than 5% of the population worldwide (the so-called common variants). Approximately 130,000 of the variants (~4% of the genome) are shared with only 0.5–5% of the population (these are known as rare variants), and less than 1% of the variants are either unique to the individual or shared by a very small number of relatives[32–34]. Finally, any one individual carries, on average, 18–74 *de novo* SNVs in their genome, including 1–4 located in the exons of their genes — these are mutations that occurred either early during development or in the germinal cells of the individual's parents (in most cases, the father)[20,21,35].

Remarkably, the range of mutations that affect protein sequences is considerably different today from the range that existed as recently as 200 to 400 generations ago[33]. Of the putatively deleterious protein-coding variants, the vast majority (>86%) are rare and arose in the last 5,000 to 10,000 years[33]. It is estimated that each individual carries, on average, 40–110 variants that are classified by the Human Gene Mutation Database as disease-causing mutations[32]. Thus, carrying a disease mutation is not the exception but the rule. The central question for epidemiologists and molecular geneticists is therefore: how are low-, medium- and high-risk mutations distributed in the population and how can certain individuals remain unaffected despite carrying deleterious ones? As described below, the genetic architecture of ASD is heterogeneous. In one subset of patients, a single fully penetrant mutation can be enough to cause ASD, but in another an accumulation of many low-risk alleles (>1,000) could be the origin of this disorder[36,37].

Epidemiology and heritability of ASD. Social skills and general intelligence, as measured by psychological and cognitive tests, are quantitative traits that show a normal distribution in the general population[4–6]. Individuals diagnosed with ASD or ID are considered to be at the lower end of this distribution (FIG. 1a). However, the demarcation between affected and non-affected individuals based on these traits is difficult to draw[4]. In addition, although people with clinical ASD and individuals with autistic-like traits differ from each other in the severity and/or degree of functional impairment, twin studies have shown that they could share the same susceptibility genes[38,39]. The relative influence of genetic, epigenetic and environmental factors on the risk of ASD remains under debate, but all epidemiological studies point to there being a strong genetic contribution to the risk of ASD[40–42]. In all twin studies conducted to date on ASD, monozygotic twins have higher concordance rates for ASD (ranging from 60% to 90%) than dizygotic twins have (from 0% to 30%). The estimated heritability of ASD differs from one study to another, but genetic factors always account for at least 38% and up to 90% of the phenotypic variance[40–42]. In families with a history of ASD, the likelihood of having a child with ASD increases with the proportion of the genome that the child shares with one affected sibling or parent[41,43,44] (FIG. 1b). In a population-based sample of 14,516 children diagnosed with ASD[41], the relative risk (compared with that for the general population) for ASD was estimated to be 153.0 (95% confidence interval (CI): 56.7–412.8) for monozygotic twins, 8.2 (95% CI: 3.7–18.1) for dizygotic twins, 10.3 (95% CI: 9.4–11.3) for full siblings, 3.3 (95% CI: 2.6–4.2) for maternal half siblings, 2.9 (95% CI: 2.2–3.7) for paternal half siblings and 2.0 (95% CI: 1.8–2.2) for cousins. In this study, ASD heritability was estimated to be 0.50 (95% CI: 0.45–0.56) and the non-shared environmental influence was also 0.50 (95% CI: 0.44–0.55). Surprisingly, both shared environment and dominance components seemed to have only a small role in the heritability of ASD[41]. Twin studies further revealed that a substantial proportion of the genetic contribution to ASD was shared with other neurodevelopmental disorders, such as ADHD (>50%) and ID (>40%)[40,45–47].

In summary, epidemiological studies provide crucial information on the genetic contribution to ASD. However, they do not inform us on the genes involved or on the number and frequency of their variants. In the past 15 years, candidate-gene and whole-genome analyses have been performed to address these questions.

The molecular genetics of ASD. Based on studies using quantitative molecular genetics[48], the proportion of ASD explained by common genotyped single-nucleotide polymorphisms (SNPs) has been estimated to be between 17% and 60%[49–51]. The contribution of common variants to these disorders is therefore important and should not be neglected. Unfortunately, the causative SNPs remain unknown, as they are numerous (>1,000) and each is associated with only a low risk. To date, the largest genome-wide association studies (GWASs) performed on <5,000 families with ASD were not sufficiently powered to identify a single SNP with genome-wide significance[52,53]. Hence, the majority of what we know about the genes for ASD has come from studies that identified *de novo*, rare and highly penetrant variants in clinical cases (Supplementary information S1 (table))[36,37].

Indeed, in 10–25% of individuals with ASD, a single genetic alteration (for example, a chromosomal rearrangement, CNV, short insertion, short deletion or SNV) seems to be penetrant enough to cause the main symptoms of these disorders. Overall, 5–15% of individuals with ASD carry a *de novo* CNV versus 1–2% in the general population[54–56]. This enrichment in CNVs applies to both deletions and duplications. Interestingly, in some cases, both deletions and duplications of the same locus could increase the risk of ASD, illustrating the importance of gene dosage in ASD[27]. The majority of causative CNVs are observed in only one individual, but some loci (for example, loci at chromosomal regions 15q11-q13, 16p11 and 22q11) are recurrently found to be deleted or duplicated. These recurrent deletions or duplications are often caused by recombination events occurring in the genomic regions made of low-copy repeats that flank the CNV[54–56]. In some individuals with ASD, more than one deleterious CNV has been identified, suggesting a

Synaptic homeostasis
Crosstalk between the presynaptic and the postsynaptic sides of the synapse that allows the tight regulation of synaptic strength and thus maintains excitability within a narrow range. It stabilizes neuronal circuits and ensures the fidelity of communication within the neuronal network despite sensory and/or growth-dependent changes.

Single-nucleotide variants
(SNVs). DNA-sequence variations occurring within a population. SNV is the general term for all such variations. Single-nucleotide polymorphism is usually used for SNVs occurring in > 1 % of the population.

Copy-number variants
(CNVs). Variations in the number of copies of one segment of DNA. CNVs include deletions and duplications.

Psychological and cognitive tests
Various self- and parent-reports designed to reliably quantify autistic traits in the general population and in clinical cases. Examples of theses scales are the social responsiveness scale and the autism spectrum quotient.

Heritability
The proportion of the phenotypic variance that is due to genetic factors. In the narrow sense, heritability includes only the additive genetic component. However, in the broad sense, heritability includes both the additive and the dominance genetic components.

Dominance components
Part of the genetic contribution to a phenotype, the other part of which is the additive component. Some genes have an additive effect on the quantitative trait, whereas other genes may exhibit a dominant gene action, which will mask the contribution of the recessive alleles at the locus.

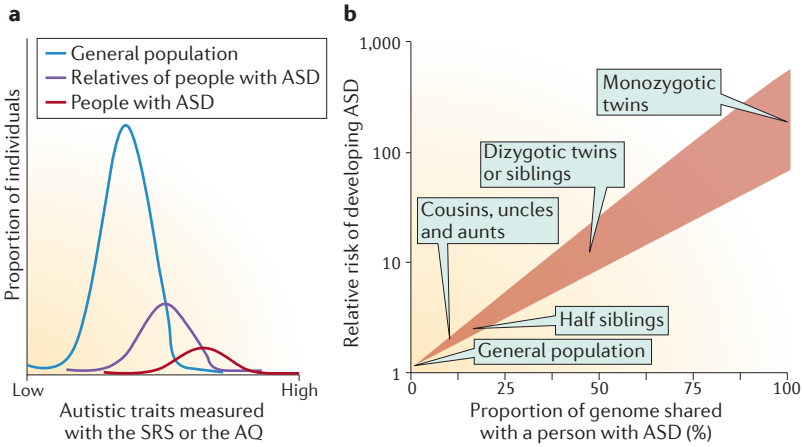

Figure 1 | **The genetic risk of ASD. a** | Autistic traits can be measured using tests such as the social responsiveness scale (SRS) and the autism spectrum quotient (AQ). The SRS and the AQ are questionnaires that can measure the severity of social impairment across clinical cases and in the general population. A high SRS or AQ score indicates the presence of autistic-like traits. The scores are normally distributed in the general population and in individuals diagnosed with autism spectrum disorder (ASD) and their relatives, although these scores may be shifted to the right and thus to higher values in the latter two groups. **b** | Twin and familial studies revealed that the relative risk of an individual developing ASD is proportional to the percentage of the genome shared with an individual diagnosed with ASD.

Quantitative molecular genetics
A branch of population genetics that assesses the heritability of continuously distributed phenotypes based on their molecular genetic signatures. For example, the Genome-Wide Complex Trait Analysis (GCTA) software estimates genomic relationships between pairs of conventionally unrelated individuals using single-nucleotide polymorphism (SNP) data.

Gene dosage
The number of copies of a gene that are present in a cell. An abnormal gene dosage (by gene deletion or duplication) can result in abnormal levels of gene product formation. Gene dosage compensation to adjust the normal level of gene product can occur at different levels (transcription, translation and degradation).

Genetic buffer
The process by which an individual's genetic background can moderate or counteract the phenotypic effect of deleterious mutations.

'multiple-hit model' for the disorders in these people[56–59]. For example, in 2,312 children with neurodevelopmental disorders known to carry a CNV associated with ID and congenital abnormalities, 10% carried a second large CNV[58]. No parental transmission bias was observed for the primary *de novo* or inherited CNV, but 72% of the second CNVs were inherited from the mother[58].

Large-scale exome-sequencing studies have found only a slightly elevated rate of *de novo* SNVs in ASD probands compared with their unaffected siblings (1.02 versus 0.79 mutations per offspring), but the probands had two- to threefold more disruptive *de novo* mutations than their siblings or than the expected probability to observe such *de novo* events[19–24]. Interestingly, *de novo* mutations are not restricted to sporadic cases of ASD, they also seem to contribute to the risk of ASD in families with two affected children[60]. The *de novo* SNVs arise three times as often in the paternal background, and mutation rates rise with age of either parent[20,21,35], whereas CNVs originated on the paternal and maternal chromosomes with equal likelihood[54–56]. Interestingly, some regions of the genome are more likely to harbour mutations than other regions (by 100-fold), and genes associated with ASD seem to fall into such regions of hypermutability[61]. This hypermutability of ASD-risk genes was not observed in controls from ASD exome-sequencing studies, and the mutations identified in ASD are markedly enriched in brain-expressed exons that are, from an evolutionary perspective, under negative selective pressure[62].

Importantly, the *de novo* loss-of-function mutations identified in individuals with ASD were not distributed equally in males and females and across the whole spectrum of ASD. They were more enriched in females than males and they were frequently associated with comorbidities, such as ID or epilepsy[22–24]. In individuals with normal IQ or Asperger syndrome, causative *de novo* mutations are rarely found[63–65]. Hence, as previously suggested, these rare and penetrant variants might not directly cause ASD, but they might 'reveal' autism by decreasing an individual's IQ and hence their ability to compensate for the effects of a high-risk genetic background for ASD[66]. Indeed, as pointed by Skuse, low levels of autistic vulnerability are more likely to lead to a recognizable ASD syndrome among individuals with ID[66]. Consequently, the apparent association between ID and ASD would not be because they usually have common causes but rather because the presence of both features greatly increases the probability of clinical ascertainment[66].

In summary, common, rare and *de novo* mutations all contribute to the genetic risk of ASD. The ASD-risk genes (including the level of confidence for the associations with ASD) can be found at the Simons Foundation Autism Research Initiative or at the AutismKb websites. Overall, none of the genes identified so far accounts for more of 1% of all cases of ASD[36,67]. The number of genes responsible for 'monogenic' forms of ASD (for example, those caused by a *de novo* deleterious mutation) is estimated to be more than 400 (REFS 19–21,68), and these forms of the disorder account for 10–20% of all ASD cases. Monogenic autosomal recessive forms of ASD could account for an additional 3–6% of the cases[69]. Altogether, karyotypes, CNV analysis and exome sequencing can detect a genetic cause for ASD in almost 25% of the cases. We can expect that whole-genome sequencing will increase the number of cases with a genetic cause of ASD by identifying deleterious mutations that are not screened for yet, such as those affecting regulatory elements of the genes located outside the coding regions[35,61,70].

The interplay between common and rare variants in ASD. The epidemiological and molecular data summarized above suggest that the genetic contribution to ASD may be shaped by a combination of rare deleterious variants and a myriad of low-risk alleles (also defined as the genetic background made of common SNPs). The interplay between rare deleterious variants and common low-risk alleles will therefore influence the phenotypic diversity observed in the population. Depending on the ability of the common low-risk alleles to buffer the impact of rare deleterious variants, a very different phenotype can be produced. A genetic background with a strong genetic buffer will cope with the effect of rare genetic variations by canalizing the phenotypic outcome[71,72] (BOX 1). By contrast, a genetic background with low level of genetic buffering in the child could reveal the effect of mutations that were kept silent in the parents. In this rare deleterious variants versus common low-risk alleles model, we can illustrate three extreme situations (FIG. 2).

In the first situation, an individual has a high genetic buffer and therefore is at low risk for ASD, even if they have a relatively high burden of rare deleterious ASD-risk genes (FIG. 2a). In this situation, only a highly penetrant mutation appearing *de novo* in the proband would be able to cause ASD (FIG. 2b). This model might

Box 1 | **Genetic buffering and synaptic function**

Genetic buffering is one process to cope with the effect of deleterious genetic variations. Several forms of genetic buffering could alleviate the consequence of a genetic mutation affecting synaptic function.

Complete gene redundancy
As humans carry two copies of each autosomal gene, the effect of a mutation in one of the two copies can be compensated for by the other gene copy. This type of buffer applies to the majority of human genes but there are exceptions, such as the X-linked genes in males (and to a certain extent in females due to the process of X inactivation), imprinted genes (with only one active copy because the other copy is epigenetically 'silenced') and genes subject to allelic exclusion (such as genes encoding for olfactory receptors and protocadherins). The finding that autism spectrum disorder (ASD) is less common in females could be due to buffering of X-linked risk alleles in females, which does not occur in males.

Partial gene redundancy
Several synaptic genes (for example, those encoding SH3 and multiple ankyrin repeat domains protein 1 (SHANK1)–SHANK3, contactin 1 (CNTN1)–CNTN6 and contactin-associated protein-like 1 (CNTNAP1)–CNTNAP5) are members of a family of genes that have been duplicated during evolution. The duplicate gene products acquired new specific functions but some functional overlap might still persist. In this case, a member of the gene family could buffer the presence of a mutation in one of its family members (that is, compensate for the effect of the mutation). In some cases, overexpression of one member of the family could also compensate for the loss of another. Such functional compensation may occur in mice lacking *Shank2*, as these mice had elevated levels of SHANK3 (REF. 122).

Negative feedback
Certain types of metabolic pathways are relatively insensitive to changes in expression level or enzyme concentration. By contrast, genes encoding proteins involved in protein complexes are more sensitive to gene dosage[27]. Synaptic scaffolding proteins such as SHANK3 seem to be very sensitive to gene dosage, as missing one copy of *SHANK3* (or having an extra copy) has a severe and penetrant clinical impact[64].

Alternative pathways
Several signalling pathways can be involved in increasing or decreasing synaptic efficacy or synapse number. These signalling pathways could compensate for each other if the activity of one pathway is altered by a mutation in a gene encoding a protein in that pathway.

Chaperone proteins
Chaperone proteins aid the folding of nascent polypeptide chains or denatured proteins and thereby prevent their aggregation. These proteins have been shown to be able to refold mutated proteins[166] and thus are crucial for genetic buffering. A low level of 14-3-3 chaperone proteins was found in the blood of patients with ASD[155], and a *de novo* mutation affecting this chaperone was found in a patient with ASD[167].

account for 10–25% of ASD cases, especially those with ID. Indeed, as previously indicated, *de novo* truncating mutations are observed in the majority of individuals with ID[19–24]. In the second situation, an individual has a genetic background that acts as a moderate genetic buffer for ASD. As a consequence, they will develop ASD when receiving only a relatively moderate burden of rare mutations (FIG. 2a). This model could account for the observation of multiple hits in some patients with ASD[56–59] (FIG. 2b).

Finally, in the third situation, the individual inherits a genetic background with a very low genetic buffer and is therefore at high risk for ASD. In this case, there is no need of a burden of rare deleterious mutations (FIG. 2a); the accumulation of a high load of low-risk alleles will be enough to cause ASD (FIG. 2b). This model might account for families in which parents have subclinical autistic traits[4].

In summary, the genetic landscape of ASD varies greatly between individuals. However, as discussed below, a majority of the mutated genes converge on specific biological pathways that influence synaptic plasticity and connectivity at different levels.

Synaptic plasticity in ASD

Synaptic plasticity is the property of synapses to strengthen or weaken in response to changes in both the amplitude and the temporal dynamics of neuronal activity. Sensory inputs and intrinsic brain activity can effect long-term changes in synaptic efficacy and eventually increase or decrease neuronal connectivity by modulating the number of synapses. Many mutations associated with ASD are predicted to influence the structure and the turnover of synapses at different levels (BOX 2) because they encode proteins involved in chromatin remodelling and transcription, protein synthesis and degradation, actin cytoskeleton dynamics or synaptic transmission (Supplementary information S2 (figure)). Below, I discuss the involvement of ASD-risk genes in these biological processes *in vitro* and in animal models of ASD.

Chromatin remodelling and transcription. Mutations in genes encoding key regulators of chromatin remodelling and gene transcription (for example, methyl-CpG-binding protein 2 (*MECP2*), myocyte-specific enhancer factor 2C (*MEF2C*), histone deacetylase 4 (*HDAC4*), chromodomain-helicase-DNA-binding protein 8 (*CHD8*) and catenin β1 (*CTNNB1*)) have been reported in individuals with ASDs (Supplementary information S1 (table)). Interestingly, a subset of these genes is regulated by neuronal activity and influences neuronal connectivity and synaptic plasticity[73–75].

An example of a relevant protein involved in chromatin remodelling is MECP2. Females with a deletion of, or point mutation in, the X-linked *MECP2* gene display Rett syndrome[76], whereas boys or girls with duplication of *MECP2* develop an ASD and ID[77] (Supplementary information S1 (table)). MECP2 is a transcription repressor that coats almost the entire genome[78]. A lack of MECP2 causes a modification of the global chromatin structure, with enhanced histone acetylation levels[79] and elevated transcriptional noise[80] (FIG. 3a). Interestingly, MECP2 was shown to act as a transcriptional repressor of long genes[81]. In response to activity, MECP2 is phosphorylated, which allows or facilitates long-gene transcription. In the absence of MEPC2, these genes are derepressed[81]. MECP2 should therefore be seen not as a single-gene regulation complex, but more as a genome-wide constituent of the neuronal chromatin that facilitates neuronal activity-dependent gene, especially long gene, regulation[73,81]. Post-mortem brain samples from patients with Rett syndrome and MECP2-deficient mice show postsynaptic morphological defects such as reduced dendritic branching, reduced dendritic spine density and defects in spine morphology[78]. Conversely, increasing MECP2 level in neuronal culture was associated with greater spine density[82].

An example of a relevant protein involved in transcriptional regulation is MEF2C. *De novo MEF2C* mutations cause a severe form of ASD[83] (Supplementary

Chromatin remodelling
The dynamic modification of chromatin architecture to allow or deny access of condensed genomic DNA to the regulatory transcription machinery proteins, thereby controlling gene expression.

X inactivation
A process by which one of the two copies of the X chromosome present in female mammals is inactivated.

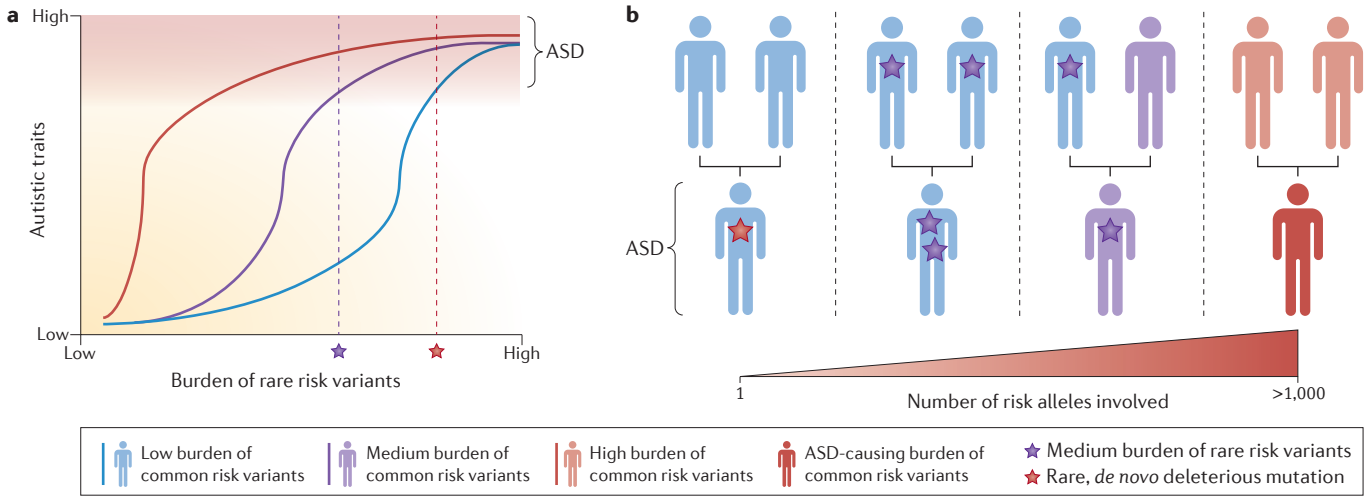

Figure 2 | **The interplay between rare mutations and genetic background.** **a** | For an individual with a genetic background harbouring a high load of common autism spectrum disorder (ASD)-risk variants, only a small burden of rare risk variants will cause ASD. By contrast, for an individual with a low burden of common risk variants, only a high burden of deleterious mutations will cause ASD. **b** | The transmission of ASD may occur through one of various paths. A *de novo* highly penetrant mutation can cause ASD even in individuals with a high genetic buffer for ASD (far left). ASD may also arise in children if both parents have a medium burden of rare variants (left-of-middle) or if one parent has a medium load of common risk variants for ASD and one has medium burden of rare risk variants (right-of-middle). Finally, children might develop ASD if both parents have a high load of common risk variants (far right).

information S1 (table)). MEF2C is a member of the MEF2 transcription factor family and a negative regulator of synapse number[84] (FIG. 3b). In mice, haploinsufficiency of MEF2C is associated with increased synaptic density and impaired hippocampus-dependent learning and memory, whereas expression of a superactivating form of MEF2C results in a reduction of the number of excitatory postsynaptic terminals without changes in learning and memory performance[84]. Interestingly, in a mouse model of fragile X syndrome, the process of MEF2C-mediated synapse elimination is repressed[85]. Individuals with mutations in *MEF2C* and people with fragile X syndrome could therefore present with a similar increase in the level of synaptic density that is caused by a common mechanism[85].

Other transcription factors encoded by ASD-risk genes, such as CHD8 and CTNNB1, contribute to early brain development[19,20,86–88]. *CHD8* is one of the major risk genes for ASD and is identified in 0.2% of patients (mostly those with ID). This transcription factor acts as a negative regulator of the WNT signalling pathway by repressing CTNNB1 activity (FIG. 3c). Mutations in *CHD8* and *CTNNB1* have opposite effects on brain development: individuals with *CHD8* mutations have larger heads than members of the general population, whereas people with mutations in *CTNNB1* have microcephaly[87,88]. These developmental abnormalities are consistent with the observation that zebrafish with reduced CHD8 expression have a larger brain[88] and that transgenic mice overexpressing CTNNB1 in neural precursors have increased cerebral cortical surface area and folding[89]. In this situation, the change of the head size is probably due to a change in the proliferation of neuronal progenitors in the brain[88,89]. However, it is important to note that, in contrast to the severe macrocephaly observed in patients with phosphatase and tensin homologue (*PTEN*) mutations, probands carrying *de novo* *CHD8* mutations have larger heads than members of the general population but they are not always very different from their siblings when adjusting for weight, height and parental head circumference[16].

Protein synthesis and degradation. Levels of synaptic proteins can be influenced by neuronal activity through global and local synaptic mRNA translation[90]. Indeed, glutamate and brain-derived neurotrophic factor (BDNF) induce a cascade of phosphorylation of the mammalian target of rapamycin (mTOR) and fragile X mental retardation protein 1 (FMRP; also known as FMR1 protein) pathways, leading to an increase in mRNA translation (FIG. 4). The newly translated proteins then help to locally increase synaptic strength or number. Many genes involved in such activity-driven regulation of synaptic proteins have been found to be mutated in individuals with ASD (Supplementary information S1 (table))[91].

The mTOR pathway controls global mRNA translation (FIG. 4), and its deregulation not only causes diseases associated with increased cell proliferation and loss of autophagy, including cancer[90], but also increases the risk for ASD (Supplementary information S1 (table)). Interestingly, mutations in genes encoding repressors of the mTOR pathway, such as neurofibromin (NF1), PTEN and synaptic RAS GTPase-activating protein 1 (SYNGAP1), cause an increase in translation in neurons and at the synapse[26] (with the exception of tuberous sclerosis 2 (*TSC2*) mutations, which lead to reduced protein synthesis in the hippocampus). This hyperactivation of mTOR leads to impaired autophagy and to an increase in

Box 2 | **Birth, life and death of synaptic proteins**

Many autism spectrum disorder (ASD)-risk genes and their protein products are regulated by neuronal activity at different levels (for example, at the level of transcription, splicing, translation or degradation). As many of these genes encode proteins that modulate synaptic plasticity, an abnormal turnover of synaptic proteins might be one of the mechanisms that increase the risk of developing ASDs.

Transcription

Neuronal activity can influence transcription through complex cellular signalling networks (reviewed in REF. 18). Neuronal genes are among the largest genes in the human genome and, with a transcription rate of approximately 3.8 kb per minute, a gene with a length of 1.5 Mb like contactin-associated protein-like 2 (*CNTNAP2*; one of the largest genes in the genome) would be transcribed in approximately 6.6 hr[168]. Interestingly, two proteins encoded by ASD-risk genes, methyl-CpG-binding protein 2 (MECP2) and DNA topoisomerase 1 (TOP1), facilitate the transcription of long genes[19,23,81]. Given that many ASD genes are exceptionally long, this could explain why mutations in *MECP2* or *TOP1* are risk factors for ASD[169]. The transcription of many synaptic genes (for example, SH3 and multiple ankyrin repeat domains protein 3 (*SHANK3*) and synaptic RAS GTPase-activating protein 1 (*SYNGAP1*)) can start at different alternative promoters and some are regulated by synaptic activity[170].

Editing

Nuclear pre-mRNA editing by selective adenosine deamination (adenosine-to-inosine editing) occurs in all organisms, from *Caenorhabditis elegans* to humans. This rare post-transcriptional mechanism can alter codons and hence the structure and function of proteins. Editing controls the maturation, intracellular trafficking and assembly of several neurotransmitter-gated ion channel subunits, including some linked to ASD or intellectual disability (ID), such as glutamate receptor ionotropic, kainate 2 (GRIK2)[171].

Splicing

Alternative pre-mRNA splicing has an important role in the control of neuronal gene expression[172]. Many neuronal proteins, especially cell-adhesion molecules (for example, protocadherins, neurexins (NRXNs) and neuroligins (NLGNs)), are structurally diversified through the differential inclusion and exclusion of exons[104]. This alternative splicing can be activity dependent, for example, in the case of *NRXN1*, mammalian target of rapamycin (*mTOR*) and several genes linked to ASD[103]. In addition, splicing dysregulation has been observed in the brains of individuals with ASD[173]. 5-hydroxymethylcytosine, a derivative of 5-methylcytosine, is abundant in DNA isolated from brains, especially at the exon–intron boundaries of synaptic genes[174], and it will be interesting to see whether changes in the distribution of this derivative are observed in ASD.

Translation, post-translational regulation and degradation

The extracellular signal-regulated kinase (ERK)–mTOR and fragile X mental retardation protein (FMRP)–eukaryotic translation initiation 4E (EIF4E)–cytoplasmic FMRP-interacting protein 1 (CYFIP1) pathways are the two major regulators of mRNA translation. Mutations in the genes encoding proteins in these pathways are strongly associated with ASD[91]. Synaptic plasticity is regulated by a cascade of phosphorylation and dephosphorylation of different signalling pathways, such as phosphoinositide 3-kinase (PI3K)–AKT and RAS–ERK pathways in response to neuronal activity. Proteinases also act at the post-translation level by regulating the activity-dependent cleavage of several synaptic cell adhesions molecules, such as NLGNs and NRXNs[175,176]. The ubiquitin–proteasome system is required for the degradation of AMPA receptors that influence synaptic plasticity, as well as for the turnover of many synaptic proteins[99]. Mutations in genes that encode key players in this process, such as ubiquitin–protein ligase E3A (*UBE3A*) and *PARK2*, have been found in individuals with ASD[54].

dendritic spine density in layer V of the temporal lobes, as seen in post-mortem brains from patients with ASDs[92]. These reductions in autophagy and developmental spine pruning were suggested to be the origin of the macrocephaly that is present in the majority of the individuals with such mutations[93].

Mutations affecting the FMRP–eukaryotic translation initiation 4E (EIF4E)–cytoplasmic FMRP-interacting protein 1 (CYFIP1) complex cause fragile X syndrome and increase the risk of ASD[94] (Supplementary information S1 (table)). This protein complex controls local translation of mRNA at the synapse and acts downstream of the RAS–extracellular signal-regulated kinase (ERK) signalling pathway (FIG. 4). As for mutations affecting the mTOR pathway, mutations affecting the FMRP–EIF4E–CYFIP1 complex were shown to increase mRNA translation instead of decreasing it. Interestingly, inhibition of RAS or ERK activity corrects the excessive protein synthesis observed in the fragile X mouse model, whereas rapamycin, an mTOR inhibitor, does not. The FMRP–EIF4E–CYFIP1 complex regulates the translation of more than 1,000 genes, many of which are ASD-risk genes[95–98]. An alteration in the level of expression of the FMRP–EIF4E–CYFIP1 complex should therefore create an imbalance in the level of many synaptic proteins that are associated with ASD.

The ubiquitin–proteasome system (UPS) is central to the degradation of proteins and, consequently, for the regulation of synapse composition, assembly and elimination[99] (FIG. 4). The gene encoding ubiquitin–protein ligase E3A (UBE3A) is mutated in patients with Angelman syndrome and is duplicated on maternal chromosome 15q11 in some individuals with ASD (Supplementary information S1 (table)). Neuronal activity increases *UBE3A* transcription through the MEF2 complex and regulates excitatory synapse development by controlling the degradation of activity-regulated cytoskeleton-associated protein (ARC), a synaptic protein that decreases long-term potentiation (LTP) by promoting the internalization of AMPA receptors (AMPARs)[100]. Disruption of UBE3A function in neurons leads to an increase in ARC expression and a concomitant decrease in the number of AMPARs at excitatory synapses. However, it is not clear whether a duplication of UBE3A (as observed in patients with ASD and duplication of chromosome 15q11) could lead to the inverse synaptic phenotype (a decrease in ARC expression and an increase in AMPARs at the membrane).

In summary, although there are exceptions, most of the mutations that have been identified in the mTOR, the FMRP–EIF4E–CYFIP and the UPS pathways result in an abnormally high level of synaptic proteins and an increase in synaptic density, as reported in several animal models[92,97,98] and in the brains of patients with ASD[92].

Synaptic receptors and cell adhesion molecules. Many proteins encoded by ASD-risk genes participate in different aspects of neuronal connectivity, such as glutamatergic (for example, glutamate receptor ionotropic, NMDA 2B (GluN2B)), GABAergic (for example, $GABA_A$ receptor subunit α3 (GABRA3) and GABAR β3 (GABRB3)) and glycinergic (for example, glycine receptor subunit α2) neurotransmission, neuritogenesis (for example, contactin 6 (CNTN6)), the establishment of synaptic identity (for example, cadherins and protocadherins), neuronal conduction (for example, contactin-associated protein-like 2 (CNTNAP2)) and permeability to ions (voltage-dependent calcium channel subunit α1 (CACNA1) proteins, CACNA2 δ3 (CACNA2D3) and sodium channel protein type 1 subunit-α (SCN1A)). Some of these

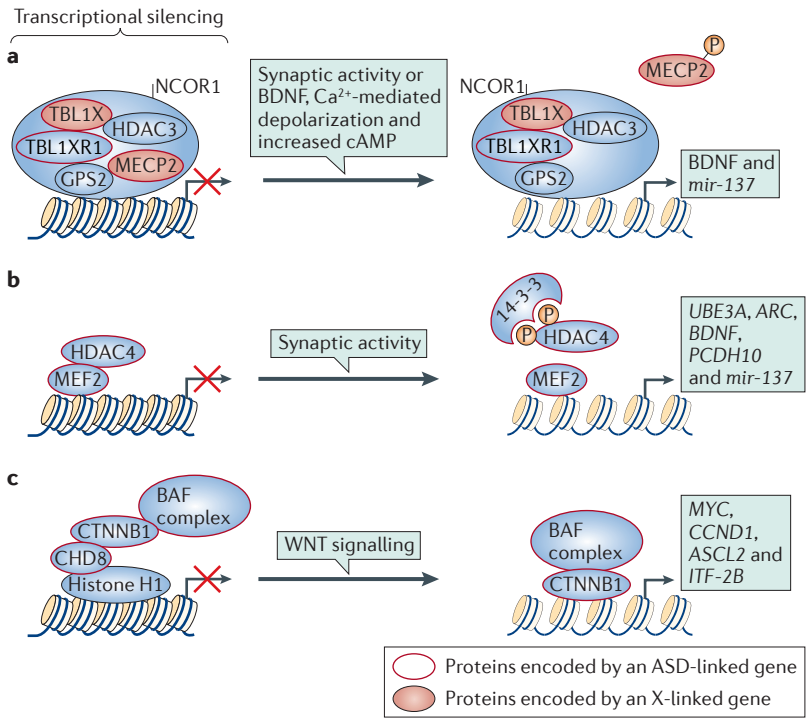

Figure 3 | **Chromatin remodelling and transcription factors associated with ASD.** On the left-hand side of the figure, the chromatin is condensed and transcription is repressed, whereas on the right-hand side the chromatin is open, facilitating transcription. **a** | The lack of methyl-CpG-binding protein 2 (MECP2 is) associated with an ~2.5-fold increase in the size of the nucleus of neurons[78]. This is accompanied by a modification of the global chromatin structure, with enhanced histone-acetylation levels[79] and elevated transcriptional noise[80]. Neuronal activity (or increased levels of brain-derived neurotrophic factor (BDNF)) induces the phosphorylation and the release of MECP2 from the nuclear receptor co-repressor (NCOR) complex to enable transcription[73,74], especially of long genes[81]. X-linked genes encoding components of the NCOR complex, such as transducin-β-like 1X (TBL1X) and TBLX1-related 1 (TBL1XR1) have also been found to be mutated in autism spectrum disorder (ASD)[36]. **b** | Myocyte-specific enhancer factor 2C (MEF2C) belongs to the MEF2 family and is a negative regulator of synapse number[84]. The interaction of MEF2C with histone deacetylase 4 (HDAC4), another protein essential for synaptic plasticity[75], is regulated by neuronal activation. When MEF2 is activated, synapses are eliminated through the expression of the synaptic cell adhesion molecule protocadherin 10 (PCDH10), which stimulates ubiquitylation of PSD95 (also known as DLG4), one of the major scaffolding proteins at glutamatergic synapses[85]. MEF2C also controls the expression of other genes related to synaptic plasticity and ASD, such as ubiquitin–protein ligase E3A (*UBE3A*) and activity-regulated cytoskeleton-associated protein (*ARC*)[100]. **c** | The DNA helicase chromodomain-helicase-DNA-binding protein 8 (CHD8) acts as a transcription repressor by recruiting histone H1 to target genes[88]. Through this mechanism, CHD8 acts as a negative regulator of the WNT signalling pathway by repressing β-catenin activity. In the same pathway, the genes encoding activity-dependent neuroprotector homeobox protein (ADNP) and AT-rich interactive domain-containing 1B (ARID1B), two members of the BRG1- or HRBM-associated factor (BAF) complex, are mutated in ASD[88]. ASCL2, Achaete–Scute homologue 2; CTNNB1, catenin β1; CCND1, cyclin D1; GPS2, G protein pathway suppressor 2; *mir-137*, microRNA 137.

proteins, such as the neurexins (NRXNs) and neuroligins (NLGNs), are directly involved in activity-driven synapse formation (FIG. 4).

NRXNs and NLGNs are binding partners that are located at the presynapse and postsynapse, respectively[25]. The three *NRXN* genes undergo extensive alternative splicing that can produce up to 3,000 putative distinct isoforms with specific biochemical interactions and synapse assembly functions[25,101–104]. Interestingly, neuronal activity can modulate the alternative splicing of *NRXN* genes via calcium/calmodulin-dependent kinase IV (CaMKIV) signalling[103]. Specific NLGN subtypes are located at the postsynaptic membrane of glutamatergic (NLGN1 and NLGN3), GABAergic (NLGN2 and NLGN4) and glycinergic (NLGN4) synapses[25]. The intracellular, carboxy-terminal part of the NLGNs binds to several scaffolding proteins of the postsynaptic density that are encoded by certain ASD-risk genes (Supplementary information S2 (figure))[105,106]. NLGNs can trigger the formation of glutamatergic and GABAergic synapses, but only in an activity-dependent manner[107–110]. Indeed, inhibition of NMDA receptors (NMDARs) or CaMKII (which signals downstream of NMDARs) suppresses the formation of glutamatergic synapses through the activity of NLGN1, whereas chronic inhibition of synaptic activity suppresses the formation of GABAergic synapses through the activity of NLGN2 (REF. 110). As such, NLGNs and NRXNs could regulate synaptic density to maintain local synaptic homeostasis[111]. Consistent with this idea, mice lacking NLGN3 exhibit extensive misplaced synapse formation and perturbed metabotropic glutamate receptor-dependent synaptic plasticity, similar to that observed in an animal model of fragile X syndrome[112].

Scaffolding proteins and the actin cytoskeleton. The positioning of cell-adhesion molecules and neurotransmitter receptors at the synapse involves a complex architecture of postsynaptic proteins, including scaffolding proteins, signalling molecules and the actin cytoskeleton[113,114] (FIG. 4). Many of these proteins are encoded by ASD-risk genes. For example, deletions, duplications and coding mutations in the three SH3 and multiple ankyrin repeat domains protein (SHANK) genes (*SHANK1*, *SHANK2* and *SHANK3*) have been recurrently reported in individuals with ASD[115] (Supplementary information S1 (table)). SHANKs assemble into large molecular platforms that interact with glutamate receptors and actin-associated proteins[105]. *In vitro*, *SHANK3* mutations identified in individuals with ASD reduce actin accumulation in spines, affecting the development and morphology of dendrites as well as axonal growth cone motility[116]. Neurons derived from induced pluripotent stem cells of patients with 22q13 deletions (which include the deletion of *SHANK3*) display major defects in excitatory, but not inhibitory, synaptic transmission[117]. In mice, mutations in *Shank3* decrease spine density in the hippocampus and increase dendritic arborizations in striatal neurons[118]. Mice with mutations in SHANK genes also present with behaviour resembling that in humans with ASDs. *Shank1*-knockout mice display increased anxiety-like behaviour, decreased vocal communication, decreased locomotion and, remarkably, enhanced working memory but decreased long-term memory[119–121]. *Shank2*-knockout mice present with hyperactivity, increased anxiety-like behaviour, repetitive grooming and abnormalities in vocal and social behaviours[122,123]. *Shank3*-knockout mice show self-injurious repetitive grooming and deficits in social interaction and communication[118,124–126].

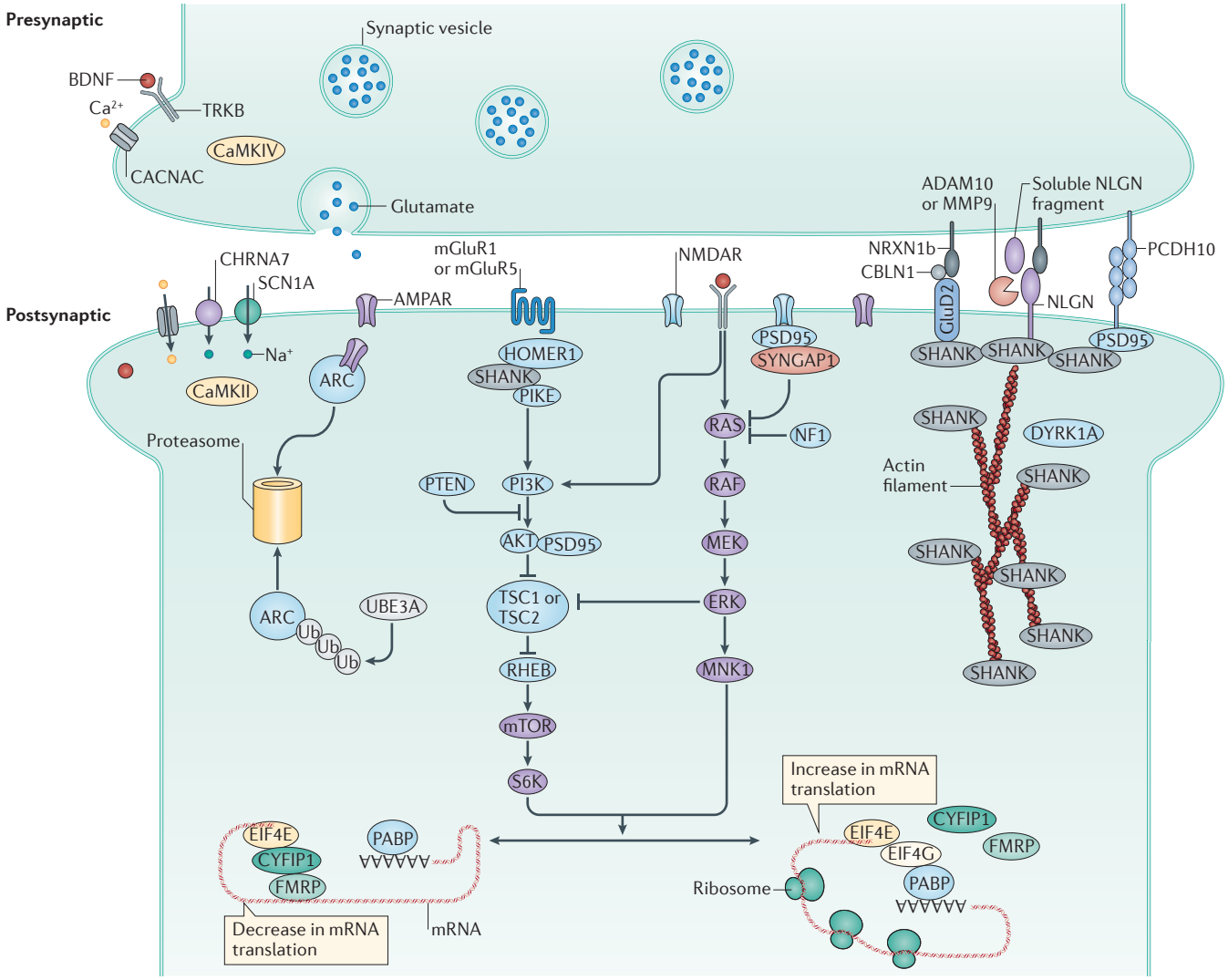

Figure 4 | **Main synaptic functions associated with ASD.** The mammalian target of rapamycin (mTOR) signalling pathway is a major regulator of cellular growth and is activated by a sequential kinase cascade downstream of the phosphoinositide 3-kinase (PI3K) pathway. Deregulated mTOR signalling increases the risk of autism in patients with mutations in neurofibromin (*NF1*), tuberous sclerosis 1 (*TSC1*), *TSC2* or phosphatase and tensin homologue (*PTEN*) (Supplementary information S1 (table)). NF1, TSC1, TSC2 and PTEN act as negative effectors of mTOR. Mutations in *NF1* or *PTEN* enhance mTOR activity, leading to an increase in mRNA translation. The mTOR pathway is modulated by neuronal activity or brain-derived neurotrophic factor (BDNF). The fragile X mental retardation protein (FMRP)–eukaryotic translation initiation 4E (EIF4E)–cytoplasmic FMRP-interacting protein 1 (CYFIP1) complex controls the translation of mRNA at the synapse and is modulated by neuronal activity through metabotropic glutamate receptor 1 (mGluR1), mGluR5 or BDNF signalling pathways[177]. When neurons are activated, CYFIP1 dissociates from EIF4E, thereby resulting in local synaptic protein synthesis and remodelling of the actin cytoskeleton[96,177] (FIG. 3). Many proteins regulating guanosine nucleotide-binding proteins and the actin cytoskeleton dynamics are associated with intellectual disability (ID) and autism spectrum disorders (ASDs)[56]. Neuronal activity increases ubiquitin (Ub)–protein ligase E3A (*UBE3A*) transcription through the myocyte-specific enhancer factor 2 (MEF2) pathway and regulates excitatory synapse development by controlling the degradation of activity-regulated cytoskeleton-associated protein (ARC), a synaptic protein that promotes the internalization of AMPA receptors (AMPARs)[100]. Mutations affecting glutamatergic signalling components (for example, glutamate receptor ionotropic, NMDA 2B (GluN2B)), cell-adhesion molecules (for example, neurexins (NRXNs) and neuroligins (NLGNs)) and scaffolding proteins (for example, SH3 and multiple ankyrin repeat domains proteins (SHANKs) and Disks large homologue-associated protein (DLGAP)) have been identified in ASD[63,64]. Neuronal activity or binding with NRXN1B leads to the release of a soluble extracellular fragment of NLGN[111,175,176]. SHANKs assemble into large molecular platforms in interaction with glutamate receptors and actin-associated proteins[105]. Mutations in ion channels, such as voltage-dependent calcium channel subunit α1C (CACNA1C) and sodium channel protein type 1 subunit-α (SCN1A), greatly increase the risk of epilepsy in patients with Timothy or Dravet syndromes, respectively[178]. Given their functions, these proteins could participate in the process of synaptic homeostasis[131–135]. ADAM10, a disintegrin and metalloproteinase domain-containing protein 10; CaMK, calcium/calmodulin-dependent kinase; CHRNA7, neuronal acetylcholine receptor subunit α7; DYRK1A, dual specificity tyrosine-phosphorylation-regulated kinase 1A; ERK, extracellular signal-regulated kinase; MEK, MAPK/ERK kinase; MMP9, matrix metalloproteinase 9; MNK1, MAPK signal-integrating kinase 1 (also known as MKNK1); PABP, poly(A)-binding protein; PCDH10, protocadherin 10; PIKE, PI3K enhancer (also known as AGAP2); RHEB, RAS homologue enriched in brain; S6K, S6 kinase; SYNGAP1, synaptic RAS GTPase-activating protein 1; TRKB, tropomyosin-related kinase B.

Synaptic homeostasis and ASDs

The sections above consider several examples of synaptic changes that could be caused by some of the rare or *de novo* mutations identified in individuals with ASD. Interestingly, mice carrying mutations in these genes often display ASD-like behaviours, such as self-injurious repetitive grooming and deficits in social interaction and communication[118–126] (see REF. 127 for a review). However, a gene mutation will not always manifest behaviourally in the same way in a mouse (or a fly, worm or fish) and a human. Thus, any robust phenotype found in these models could be relevant to understanding the biological roles of the ASD-risk genes. In some models, pharmacological intervention has been shown to reduce these behavioural changes, even in adult animals (see REF. 128 for a review). That a late-onset drug treatment can correct a behavioural phenotype in mice carrying ASD-risk gene mutations is actually very encouraging news for the treatment of humans. However, it is not clear whether these positive results obtained in animal models can be translated to positive results in humans.

Based on the different pathways associated with ASD depicted above, two main observations can be made regarding the shared properties of the ASD-risk genes. First, they participate in synaptic plasticity by modulating synaptic strength and/or number. Second, mutations in these genes seem to affect neuronal networks in animal models, resulting in either activity that is too high and increased synaptic density or activity that is too low and decreased synaptic density (FIG. 5). Surprisingly, the majority of ASD-related mutations — specifically, mutations in *MEF2C*, *FMR1*, *NF1*, *PTEN*, *SYNGAP1*, *EIF4E* and *CYFIP1* — result in increased gene transcription and mRNA translation; effects that are also seen with increased neuronal activity. These mutations therefore could lead to an abnormal increase in the strength and/or number of synapses within specific neuronal networks. This could account for the observation of ectopic synapses in mouse models of ASD[112] and in the brains of patients with ASD[129,130], as well as for the higher risk of epilepsy in patients with ASD (for example, in patients with *SCN1A* or *SCN2A* mutations).

To prevent neuronal networks from reaching such extreme states (network activity that is too low or too high), homeostatic regulation mechanisms adjust synapse strength, size and number to stabilizing firing[131–136] (FIG. 5). Many mechanisms are used to achieve this. One is the process of synaptic scaling[131], whereas another is metaplasticity[136]. Other mechanisms to achieve network activity regulation do not involve synapses and are mediated by changes in neuronal excitability[137].

The first report of synaptic scaling at central synapses revealed that activity blockade increased excitatory synaptic transmission through a simple change in the accumulation of postsynaptic glutamate receptors, with no changes in presynaptic function[131]. Although studies agree that synaptic scaling is induced by changes in AMPAR accumulation, there is less agreement on the subunit composition of the newly accumulated receptors. The signalling pathways involved in this synaptic scaling process include voltage-gated calcium channels and proteins such as the cytokine tumour necrosis factor, BDNF and ARC[132].

Metaplasticity is induced by synaptic or cellular activity, but it is not necessarily expressed as a change in the efficacy of normal synaptic transmission. Instead, it manifests as a change in the ability to induce subsequent synaptic plasticity, such as LTP or long-term depression (LTD). For example, if activity in the cortex or hippocampus is reduced for a period of time, LTP is enhanced and induced at lower stimulation frequencies, and LTD is repressed[136,138]. One molecular basis for this is known to be the upregulation of the NMDAR subunit GluN2B (encoded by the ASD-risk gene *GRIN2B*)[139,140]. Another basis for this metaplasticity is the downregulation of GABAergic inhibition (regulated by the ASD-risk genes *GABRA3* and *GABRB3*)[141,142].

Such homeostatic mechanisms might be especially crucial for restraining network activity and avoiding potential epileptogenic states. There is emerging evidence that sleep is necessary for synaptic homeostasis[143]. For example, in *Drosophila melanogaster*, the number and size of synapses in neuronal circuits increase after a few hours of waking and decrease only after sleep[144]. Interestingly, the NLGNs play an important part in this sleep-dependent synaptic homeostasis in both flies and rodents by regulating levels of glutamatergic and GABAergic currents after sleep deprivation[144–147]. Such problems could lead to the abnormal sleep and circadian rhythms that are present in a majority of individuals with ASD[148].

BDNF release from the postsynaptic neuron was shown to be essential for homeostatic retrograde enhancement of presynaptic function[149]. Several studies[150–152], which have not all been replicated[153], have reported an increase in BDNF levels in the blood of patients with ASD. In addition, high plasma levels of serotonin and *N*-acetylserotonin, as well as low plasma levels of melatonin, are more frequently observed in individuals with ASD than in controls[154,155]. Given that *N*-acetylserotonin is a potent agonist of the BDNF receptor tropomyosin-related kinase B (TRKB; also known as NTRK2)[156], an excess of this molecule could increase TRKB-induced phosphoinositide 3-kinase signalling, thus leading to higher protein synthesis, similarly to the effect of mutations affecting regulators of the mTOR pathway.

In summary, it can be hypothesized that, in a subset of patients with ASDs who carry genetic mutations, the process of synaptic homeostasis might not be able to counter the effects of the mutations on synaptic activity, leading to neuronal activity that is too high or too low. Abnormal sprouting (or pruning) of synapses as a result of the mutations could therefore lead to abnormal coordination and competition between neuronal networks; this could explain the problems in integrating information across different sensory modalities that are observed in individuals with ASD[12,13]. This abnormal interference between sensory stimuli could be revealed especially during critical phases of cognitive development, when a tight balance between sensory signals is essential[157].

Synaptic scaling
A form of synaptic plasticity that adjusts the strength of a neuron's excitatory synapses up or down to stabilize firing.

Metaplasticity
The plasticity of synaptic plasticity (that is, the prior history of activity of a synapse determines its current plasticity).

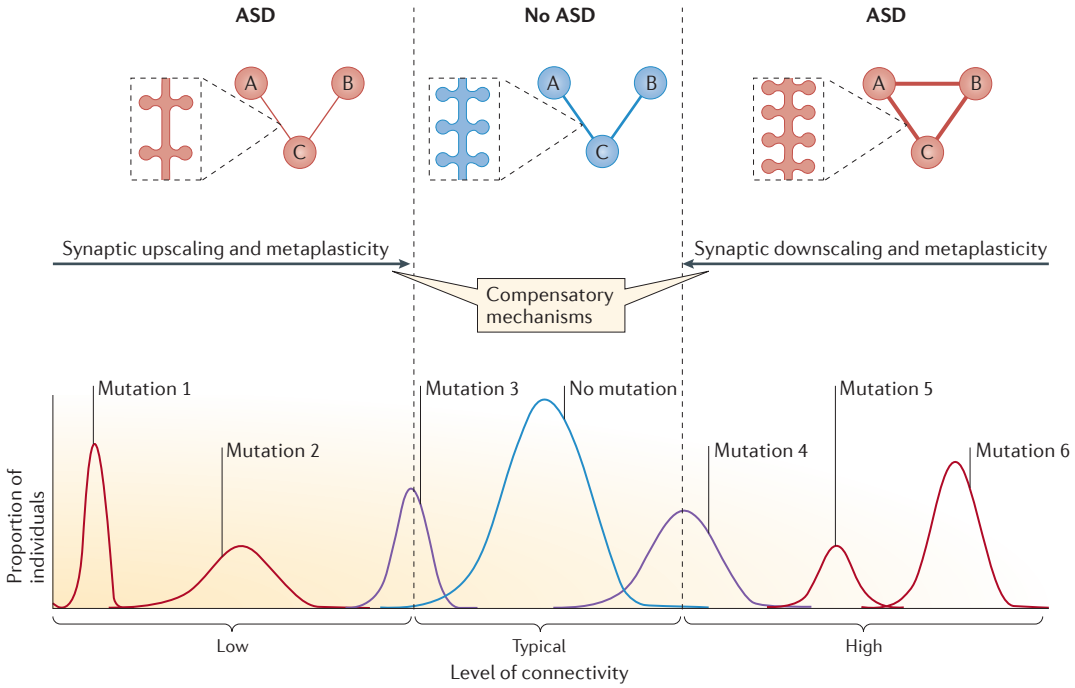

Figure 5 | **Possible effects of genetic mutations on neuronal connectivity in ASD.** In the 'typical' neuronal network, neurons A and B are equally connected to neuron C (centre). However, the presence of an autism spectrum disorder (ASD)-linked mutation may decrease (left) or increase (right) synaptic strength or efficacy and alter neuronal connectivity. In some cases, an abnormal increase of synapse number will create ectopic synaptic connections between A and B that are not observed in the typical neuronal network (right). The synaptic homeostasis process is able to scale up or down synapses to set the firing rate in a normal range. In the schematic, mutation 1 causes a severe loss of synaptic connectivity and all carriers of this mutation are affected (in this case, there is a low buffering capacity for this mutation). For mutation 2, there are diverse levels of genetic buffering capacity in the population. Thus, the phenotype associated with this mutation can range from very severe (low buffer) to almost normal (high buffer). Mutations 3 and 4 are observed in the general population. Depending on an individual's genetic buffering capacity, these mutations may be associated with typical or somewhat low or high connectivity. Mutations 5 and 6 cause effects that are opposite to those caused by mutations 1 and 2, and lead to higher synaptic connectivity with different levels of phenotypic severity.

Perspectives

In this Review, I discussed how an individual's genomic background made of common SNPs may buffer the deleterious effect of rare variants and the ability of the nervous system to scale up or down synapses to prevent neuronal networks from reaching extreme states. It is therefore tempting to unify both proposals and to hypothesize that the *de novo* and rare mutations associated with ASD strongly affect synaptic plasticity and that an individual's genetic background will determine their ability to maintain synaptic homeostasis. We have very little information on the common SNPs associated with ASD. Nevertheless, the common SNPs shared by different neuropsychiatric disorders, such as bipolar disorder and schizophrenia, have started to be identified from large-scale GWASs of >30,000 cases and controls. Interestingly, genes encoding voltage-gated calcium channel subunits CACNA1C, CACNB2 and CACNA1I are key players in synaptic homeostasis[132] and were among the top 108 loci identified in schizophrenia[158].

Two main questions that are not addressed in this Review relate to where and when in the brain autism 'emerges' (REFS 159,160). Almost all brain regions seem to be implicated in ASD, from the cortex to the cerebellum. Although glutamatergic synapses seem to have a major role in the susceptibility to ASD, GABAergic and glycinergic synapses might also be affected in a subset of patients[161,162]. As has been suggested by Waterhouse and Gillberg[163], it might be better to abandon the belief that there is a single defining dysfunction in the ASD brain and, instead, try to understand the diversity of ASD. The variety of ASD phenotypes may be linked to the many different mutations linked to these disorders but also to the capacity of each individual's genetic background to buffer the effect of their mutations on synaptic function and each individual's capacity to maintain synaptic homeostasis. The question of whether people with ASD are living in an 'intense world' (REF. 29) or a 'disconnected world' (REF. 15) might be valid for a subset of individuals, but not necessarily for all. Consideration of autism not as a single entity (affected versus non-affected) but as a continuum of human diversity, and identification of this heterogeneity through the use of information coming from different fields of research (including direct information from the affected individuals and their family[164]), should allow better diagnosis, care and integration into society of individuals with ASD[165].

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