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Roberto Toro[1,2], Marina Konyukh[1,2], Richard Delorme[1,2,3,6], Claire Leblond[1,2], Pauline Chaste[1,2,3], Fabien Fauchereau[1,2,4] , Mary Coleman[5], Marion Leboyer[,6,7, 10], Christopher Gillberg[8,9] and Thomas Bourgeron[1,2,4,6*]

[1] *Human Genetics and Cognitive Functions, Institut Pasteur, 75015 Paris, France*

[2] *CNRS URA 2182 "Genes, synapses and cognition", Institut Pasteur, Paris, France*

[3]*Department of Child and Adolescent Psychiatry, Hôpital Robert Debré, Assistance Publique-Hôpitaux de Paris, 75019 Paris, France*

[4]*University Denis Diderot Paris 7, 75005 Paris, France*

[5]*Foundation for Autism Research*

[6]*Foundation FondaMental French National Science Foundation*

[7]*INSERM U955, IMRB, Psychiatry Genetic team, Faculy of Medecine, Université Paris Est-Créteil I, Créteil, France*

[8] *Department of Child and Adolescent Psychiatry, Göteborg University, 41119 Göteborg, Sweden*

[9] *Institute of Child Health, London, United Kingdom*

[10,] *AP-HP, Henri Mondor-Albert Chenevier Hospitals, Department of Psychiatry, Creteil, F-94000, France*

* Correspondence should be addressed to Thomas Bourgeron, PhD, Human Genetics and Cognitive Functions, Institut Pasteur, 25 rue du Docteur Roux, 75015 Paris, France. Tel: 33-1-40-61-32-16. Fax: 33-1-40-61-39-53. E-mail: thomasb@pasteur.fr

Abstract

Autism spectrum disorders (ASD) are characterized by impairments in reciprocal social communication, and repetitive, stereotyped verbal and non-verbal behaviors. Genetic studies have provided a relatively large number of genes that constitute a comprehensive framework to better understand this complex and heterogeneous syndrome. Based on the most robust findings, three observations can be made: first, genetic contributions to ASD are highly heterogeneous with most likely a combination of alleles with low and high penetrance. Second, the majority of the mutations apparently affect a single allele, suggesting a key role for gene dosage in the susceptibility to ASD. Finally, the broad expression and function of the causative genes suggest that alteration of synaptic homeostasis could be a common biological process associated with ASD. Understanding the mechanisms that regulate synaptic homeostasis should shed new light on the causes of ASD and may provide means to modulate the severity of the symptoms.

Introduction

The diagnosis of autism is based on impairments in two major domains – reciprocal social communication, and repetitive, stereotyped and ritualistic verbal and non-verbal behaviors. The term "autism spectrum disorders" (ASD) is used as a shorthand to refer to any patient that meets these diagnostic criteria. But beyond this unifying definition lies an extreme degree of clinical heterogeneity, ranging from profound to moderate impairments, but always with functional disability. Indeed, autism is not a single entity, but rather a complex phenotype thought to be caused by different types of defects in common pathways, producing similar behavioral phenotypes. The prevalence of ASD overall is about 1/100, but closer to 1/300 for typical autism [1]. ASD are more common in males than females with a 4:1 ratio [2, 3]. Twin and family studies have conclusively described ASD as the most "genetic" of neuropsychiatric disorders, with concordance rates of 82-92% in monozygotic twins versus 1-10% in dizygotic twins; sibling recurrence risk is 6% [2, 3].

From 15 to 70% of children diagnosed as suffering from ASD have intellectual disabilities [4], and it is now understood that, like intellectual disability, autism symptoms can be caused either by gene mutations or by chromosomal aberrations (Box 1). In approximately 10-25% of the affected individuals, autism is "syndromic", ie. occurring in a child with a known genetic or environmental toxin disorder, such as fragile X, tuberous sclerosis, neurofibromatosis, valproic syndrome, or autism caused by brain herpes simplex infection[2, 4].

In the last years, various independent studies and large scale international efforts have identified a growing number of candidate genes for ASD and suggest a set of mechanisms that could underlie the ASD phenotype.

Genetic variations and the modes of inheritance of ASD

Due to the absence of classical Mendelian inheritance, ASD were first thought to be a polygenic trait involving many loci. Therefore, model free linkage studies, such as affected sib-pair analyses, were performed to identify susceptibility genes. Many genomic regions were detected, but only a restricted number of loci were replicated in independent scans (e.g., chromosome 7q31 and 17q11). To homogenize the genetic and phenotypic data and to gain higher statistical power, collaborative efforts

were initiated, such as the autism genome project (AGP), that genotyped 1496 sib-pair families using the Affymetrix 10K single nucleotide polymorphisms (SNP) array [5]. Nevertheless, no genome-wide significant loci could be detected, and the signals on chromosome 7q31 and 17q11 were lost. The absence of relevant targets identified by linkage studies prompted geneticists to use an alternative method: association studies with dense SNP arrays. In theory, association studies are sensitive to allelic heterogeneity whereas linkage studies are not. Nevertheless, association studies provide major advantages compared with linkage studies. First, studies can include large sample of patients since they are not restricted to multiplex families with two or more affected children. Second, the genomic regions associated with the trait are much narrower than in linkage studies, due to loss of strong linkage disequilibrium between relatively close genomic regions. In addition, SNP arrays can be used to detect structural variants such as copy number variations (CNVs) [6].

By using these approaches, several genes were associated with ASD. A list of 190 genes is available at AutDB, a public, curated, web-based, database for autism research (http://www.mindspec.org/autdb.html). However, it should be noted that most of these genes remain only candidates since their association was not always confirmed by replication and/or functional validation. In Tables 1 and 2, we present a list of some of these genes, and we consider the mechanisms that might be affected. Depending on the penetrance of the mutation on the risk for ASD, we derive two main categories of genes or loci. In the first (Table 1), genes or loci appear to have a high penetrance, but are mutated in a limited number of individuals (sometime a single individual). In this category, variations are mostly composed of *de novo* or rare point mutations, CNVs and cytogenetically detected deletions/duplications (Box 1). The second category of genes includes the so-called susceptibility genes to ASD (Table 2). Here, the variations are mostly composed of SNPs or inherited CNVs observed in the general population and associated with low risk for ASD. Especially, in this category of genes, the association with ASD should be taken with great care, since the three largest genome-wide association studies (GWAS) performed on more than 1000 patients in each study could not detect the same genes associated with ASD [7-9].

Two opposite models have been proposed to explain the inheritance of ASD. Some researchers have considered autism as a polygenic trait (i.e., a group of patients with a relatively

homogeneous phenotype produced by multiple genes, each of low effect). Others have considered autism as a very phenotypically heterogeneous group of different disorders (autisms), thought to be caused by different genes in common pathways (in this model, eventually a single highly penetrant mutation would be sufficient to produce autism). The heterogeneous model was recently supported by both the absence of strong linkage and/orassociation loci replicated by whole genome scans and by the identification of apparently monogenic forms of autism, each time affecting a limited number of patients, 1-2% for the most replicated genes. Nevertheless, the polygenic model of autism cannot be excluded for several reasons. First, it is now well established that the clinical outcome of most of the monogenic disorders are actually modulated by additional genetic variations. Second, in polygenic traits such as blood pressure and height for example, the effect of a single common variant on the phenotype seems to be very low (OR<2) and requires large sample size (>15000) to be detected [10]. In ASD, the current genome scans – the largest were performed on cohorts of less than 2000 cases – might lack the statistical power necessary to detect such common alleles with low effect. Finally, the number of deleterious mutations within the human genome of one individual remains difficult to establish, but it is most likely that, in a large proportion of patients, the combination of multiple rare variants such as CNVs, coding and regulatory variations could affect specific biological pathways and therefore increase the risk for autism.

Abnormal gene dosage in ASD

In recent years, enormous progress has been achieved in detecting rare and frequent structural variants of the human genome, such as deletions, duplications and inversions [11]. At least eight studies have searched for such genomic imbalances in patients with ASD [5, 12-19]. Differences in patient inclusion criteria, genotyping methodologies and algorithms to detect deletions and duplications make the comparison of these results difficult. Nevertheless, it seems that there is a significant increase in rare inherited and *de novo* CNVs in the ASD population compared with the general population. The overall rate of *de novo* CNVs in ASD could range from 5-10% compared with 1% in the general population [15]. Not surprisingly, the frequency of rare and *de novo* CNVs increases for simplex families with one affected child (7-10%) compared with multiplex families with at least two affected children (2-3%) [15].

The presence of dysmorphic features in the patient also increases the odds of detecting a rare or *de novo* CNV in up to 27.5% [12]. Based on the current findings, the majority of the CNVs apparently affect only one copy of the gene (which can be either deleted or duplicated), suggesting that abnormal gene dosage or expression might play a key role in the susceptibility to ASD.

Several mechanisms could explain why abnormal gene dosage would increase the risk for ASD. First, deletions may reveal a mutation on the second allele (Fig. 1A), as it has been recently shown for *NRXN1* and *CNTNAP2,* two genes associated with ASD. Patients with Pitt-Hopkins-like syndrome (severe mental retardation, autistic behavior, epilepsy, and breathing anomalies) have been reported to carry one CNV affecting either *NRXN1* or *CNTNAP2,* and a deleterious point mutation affecting the same genes on the second allele [20]. Therefore, this apparently dominant trait was actually a recessive trait when a mutation screening of the remaining allele was performed. In theory, the second mutation could be present only in some tissues, maybe in specific parts of the brain (Fig. 1B). This situation has not been observed in ASD yet, but is well known in familial forms of cancer where both an inherited and a somatic mutation are required to develop the disease (the "two hit" or Knudson model). Gene dosage problems may also appear even if the second copy does not present a mutation, but is silenced by epigenetic hallmarks, as it has been observed to be the case in disorders affecting imprinted loci, such as Angelman or Prader-Willi syndromes [21]. Similarly, allelic exclusion (i.e., the expression of a single allele in one cell) could turn off the unaffected allele in all cells or in specific cell types (Fig. 1C). Allelic exclusion was well characterized for immunoglobulins and olfactory receptors and is one of the mechanisms ensuring that a single antibody or receptor is expressed per cell [22]. This mechanism, first considered as rare, could be in fact more frequent than originally thought [23]. Especially in the brain, allelic exclusion has been observed for cell adhesion molecules such as protocadherins [24]. If the cadherins and protocadherins associated with ASD [7, 14, 25] were subject to allelic exclusion, then it would be possible that turning off the expression of the unaffected copy could totally deplete the cell of the protein product.

Second, abnormal gene dosage might by itself represent a risk factor for ASD. It is known from work in yeast that genes with high sensitivity to dosage are enriched in regulatory and structural proteins [26], that need to interact with precise stoichiometry. Thus, lower or higher levels of a single

component of these complexes might disorganize the assembly of the machinery and alter its biological function. In particular, scaffolding synaptic proteins such as SHANK involved in specific protein-protein interactions are expected to be especially sensitive to dosage (Figure 1D). Finally, some mutation associated with ASD could act as gain of function. Indeed, the knock-in mice carrying the Nlgn3 R451C mutation – previously identified in one patient with autism and his brother with Asperger syndrome [27]– displayed different synaptic and behavioral phenotypes compared to the complete Nlgn3 knock-out [28].

Abnormal level of synaptic proteins

Several lines of evidence indicate that mutations in genes regulating various aspects of synaptogenesis and neuronal circuit formation (Fig. 2) are associated with an increased risk for ASD. Among these, several genes seem to regulate the level of proteins at the synapse. Two X-linked genes, *MeCP2* and *FMR1,* are involved in autism "secondary" to Rett and fragile X syndromes, respectively. MeCP2 (Fig 2B) is a protein that directly and/or indirectly regulates neurotrophic factors, such as Brain Derived Neurotrophic factor (BDNF), by binding to methylated DNA [29]. Deletions or mutations of *MECP2* are associated with Rett syndrome in females, whereas duplications of *MeCP2* are associated with mental retardation and ASD in males and psychiatric symptoms, including generalized anxiety, depression, and compulsions in females [30]. FMRP (Fig 2A, B) is a selective RNA-binding protein that transports mRNA into dendrites and regulates the local translation of some of these mRNAs at synapses in response to activation of metabotropic glutamate receptors (mGluRs). In the absence of FMRP, there is an excess and a dysregulation of mRNA translation leading to altered protein synthesis dependent plasticity [31].

Mutations of other genes associated with ASD seem to affect the level of synaptic proteins by dysregulating overall cellular translation [31]. Patients with neurofibromatosis, tuberous sclerosis, or Cowden/Lhermitte Duclos syndromes have higher risk than the general population to have ASD. These disorders are caused by dominant mutations in the tumor suppressor genes *NF1*, *TSC1/TSC2* and *PTEN* (Fig. 2). These proteins act in a common pathway as negative effectors of the rapamycin-sensitive mTOR-raptor complex, a major regulator of mRNA translation and cellular growth in mitotic

cells [31]. The mutations observed in ASD have been predicted to enhance the mTORC1 complex, which could lead to abnormal synaptic function due to an excess of protein synthesis. Interestingly loss of *Tsc1/Tsc2* or *Pten* in mice results in neuronal hypertrophy [32], and patients presenting mutations in *NF1*, *TSC1/TSC2* and *PTEN* have a higher risk for macrocephaly. Further modulation of the PTEN and MTOR pathways is exerted by serotonin and the proto-ocongene cMET, two pathways that were associated with ASD [33, 34].

Consistent with the hypothesis of a relationship between abnormal levels of synaptic proteins in ASD, many studies have reported mutations in genes involved in synaptic protein ubiquitination, including *UBE3A*, *PARK2*, *RFWD2* and *FBXO40* [13] (Fig. 2A). Protein degradation through ubiquitination proceeds through the ligation of ubiquitin to the protein to be degraded. This post-translational modification directs the ubiquitinilated proteins to cellular compartments or to degradation into the proteasome. The ligation of ubiquitin is reversible and could be used to regulate specific protein levels at the synapse. In mice, many proteins of the post-synaptic density, including the mouse orthologs of the ASD-associated SHANK proteins, have been demonstrated to be targeted by ubiquitination in an activity-dependent homeostatic manner [35]. Ubiquitination involves activating enzymes (E1), conjugating enzymes (E2) and ligases (E3). Substrate specificity is usually provided by the E3 ligases, which typically have substrate-binding sites. UBE3A (also called E6-AP) is an E3 ligase encoded by an imprinted gene (only expressed from the maternal copy) and is responsible for Angelman syndrome [21]. In ASD, *de novo* maternal duplications of chromosome 15q11-q13 including *UBE3A* have been observed in 1-3 % of the patients [21]. It is still not clear whether *UBE3A* alone contributes to the risk of ASD, since other candidate genes are also duplicated on chromosome 15q11-q13, however, its role at the synapse has been recently demonstrated in mice [36, 37]. In cultured hippocampal neurones *Ube3a* is localized at the pre- and post-synaptic compartments, but also at the nucleus. Experience-driven neuronal activity induces *Ube3A* transcription, and then, Ube3A regulates excitatory synapse development by controlling the degradation of Arc, a synaptic protein that promotes the internalization of the AMPA subtype of glutamate receptors [37]. This might have many consequences for synaptic structure, as suggested by Ube3a maternal-deficient mice, which exhibit abnormal dendritic spine development, including spine morphology, number and length [36], and a

reduced number of AMPA receptors at excitatory synapses [37].

Finally, the transcription factor *MEF2C* (Fig. 2B), involved in the regulation of the number of synapses appears to be a risk factor for intellectual disability [38], and could therefore also be associated with ASD. Taken together, the genetic results obtained in humans and the functional studies mostly obtained in mice suggest that different independent mechanisms could alter the level of synaptic proteins; however, the actual nature of the impaired synaptic function(s), and its association with ASD phenotype remains to be characterized.

Abnormal formation of neuronal circuits in ASD

The main category of genes associated with ASD is related to the development and the function of neuronal circuits [39]. At the synaptic membranes, cell adhesion molecules, such as NLGNs and NRXNs (Fig. 2) are major organizers of excitatory glutamatergic and inhibitory GABAergic synapses, and contribute to the activity-dependent formation of neuronal circuits in mice [40]. Mutations identified in patients with ASD were found to alter the ability of NLGNs to trigger synapse formation in cultured neuronal cells [41, 42]. The disorders associated with NLGN-NRXN mutations can greatly vary among individuals, and this appears to be the case even for subjects of the same family, carrying the same mutation Mutations of the X-linked *NLGN4X* have been associated with mental retardation [43], typical autism [42, 44], Asperger syndrome [27] and more recently with Tourette syndrome [45]. In one case, a *NLGN4X* deletion was observed in a male with normal intelligence and apparently no autistic features [46]. *NRXN1*, by contrast, has been implicated in disorders such as schizophrenia and Pitt-Hopkins-like syndrome, but has been also found in asymptomatic carriers [20].

Interestingly, NLGN and NRXN might also play a role in social interaction in other species than humans without affecting overall cognitive functions. Mutant mice carrying a R451C *Nlgn3* mutation displayed an increased number of GABAergic synapses and inhibitory currents [28], normal [47] to reduced social interaction [28] and a reduction of ultrasonic vocalization in pups [47]. The knockout mice for *Nlgn4* displayed reduced social interactions and ultrasonic vocalizations at the adult stage [48]. By contrast, mutant knockin *Nlgn3* and knockout *Nlgn4* displayed enhanced to normal learning compared with wild-type mice [28, 48]. Furthermore, in the mouse model for fragile X, an enhanced

Nlgn1 expression improved social behavior, whereas no effect on learning and memory was observed [49]. Finally, in the honeybee, sensory deprived animals had a lower level of Nlgn1 expression, but a generally increased level of the Nlgn2-5 and NrxnI expression compared with hive bees [50].

The postsynaptic density plays a major role in the organization and plasticity of the synapse, and mutations affecting scaffolding proteins, such as SHANK2, SHANK3 and DLGAP2, are recurrently found in ASD [19, 51, 52]. Deletions at 22q13 and mutations of *SHANK3* could be present in more than 1-2% of ASD patients (Box 1) [51-53]. Shank proteins are a family of three members, which are crucial components of the postsynaptic density. Together with their binding partners, they have been shown, *in vitro*, to regulate the size and shape of dendritic spines [54]. They also link glutamate receptors to the cytoskeleton and variations in genes regulating cytoskeletal dynamics were associated with mental retardation and ASD [19, 55].

The role of neurotransmitter transporters and receptors in the susceptibility to ASD is still unclear. Because of the abnormally high levels of serotonin in ASD patients [33], the serotonin transporter *SLC6A4* was extensively analyzed, and the results pointed toward dimensional rather than categorical roles for *SLC6A4* in stereotypic behaviors [56]. For glutamate, only weak associations for *GRIK2* were detected [57], and a duplication of the X-linked *GRIA3* receptor gene was observed in a patient presenting typical autism [12]. Concerning GABA, the most robust findings concern the duplication of the GABA receptor subunit gene-cluster on chromosome 15q11-13 and the observation of maternal over-transmission of a rare variant of the GABA(A) receptor beta3 subunit gene (GABRB3) [58].

Finally, proteins, related to axonal growth and synaptic identity, are now also suspected to play a role in ASD. Semaphorins are membrane or secreted proteins (Fig. 2) that influence axon outgrowth and pruning, synaptogenesis and the density and maturation of dendritic spines. SNPs located close to the semaphorin *SEMA5A* were associated with ASD in a large cohort [8]. Independently, the level of *SEMA5A* mRNA was found to be lower in brain-tissue and B-lymphoblastoid cell lines from patients with ASD compared with controls [59]. The contactin family of proteins is involved in axonal guidance as well as in the connection between axons and glial cells, and ASD patients have been found to have deletions of the contactin genes *CNTN3* and *CNTN4* and the contactin associated

protein *CNTNAP2* [25, 60-63]. In addition, inherited CNVs or SNPs have been found in other cell-adhesion proteins – cadherins (*CDH9*, *CDH10*, *CDH18*) and protocadherins *PCDH9 and PCDH10* [7, 14, 25] – which might contribute to the susceptibility to ASD by altering neuronal identity.

Abnormal synaptic homeostasis in ASD

Different homeostatic mechanisms allow neuronal cells to maintain an optimal level of neuronal activity despite global changes in the overall activity of the network [64-66]. Recent evidence suggests that homeostasis plays a role in the adaptation of synaptic plasticity by changing levels of activity [35, 64], and might be also associated with the downscaling of synaptic weights during sleep [65]. During development and the first years of life, activity plays an important role in the refinement of brain connections, and many results suggest that these processes are under homeostatic control at the synapse [67]. The genes and the mechanisms that we have surveyed in this article might disrupt synaptic homeostasis at various levels [68]. The synthesis and degradation of different postsynaptic density proteins has been shown to vary as a function of activity [35]. Mutations in ubiquitin-dependent degradation could directly interfere with this process, as would also be the case if mutations are present in scaffolding genes such as the Shank family. Synaptic homeostasis has been shown to depend on local protein synthesis, on Ca^{2+} concentration and on a tight regulation between the pre- and the post synaptic sides of the synaptic contact mediated by cell adhesion molecules such as NLGN and NRXN [69]. Finally, synaptic homeostasis is not independent from cellular homeostasis and therefore should be affected by mutations altering gene expression level as well as neuronal numbers and shape such as mutations related to the mTOR pathway.

If synaptic homeostasis is altered in ASD, environmental factors that influence this regulatory process could also modulate its severity. As reviewed elsewhere [33, 70], abnormal serotonin and/or melatonin levels and altered sleep or circadian rhythms might constitute risk factors for ASD [71]. Sleep has been proposed as an important mechanism to regulate synaptic homeostasis. During wakefulness there appears to be a global increase in the strength of excitatory synapses, scaled down during sleep to a baseline level [65, 72], a mechanism that can play an important role in learning and memory [73]. In addition to mutations of genes directly involved in synaptic processes, we have recently proposed that,

in some cases, ASD could result from the interplay between abnormalities in synaptic and clock genes, and that restoring circadian rhythms might therefore be beneficial for the patients and their families [70].

Most of the genes considered in this review are thought to be expressed throughout the brain, however, neuroimaging studies seem to converge into a stereotypical network of brain regions where differences between ASD and control populations can be detected. These two results would not need to be in contradiction, if different brain networks were differently resilient to variations in synaptic homeostasis (Fig. 3). From an evolutionary standpoint, brain networks involved in more recently acquired cognitive skills, such as language or complex social behavior, might have less compensatory mechanisms compared with more ancient biological functions that have been shaped by a much stronger selective pressure.

Concluding remarks and perspectives

It is a matter of time for geneticists to be able to obtain whole genome sequences of ASD patients. Exploring epigenetic alterations should be also more feasible in the near future, thanks to the availability of brain tissue samples and stem cells from patients. Animal models based on genetic results are now under scrutiny in many laboratories and the consequence of the mutations and their reversibility is analyzed from cell to behavior. However, more than ever we need to recognize the inherent heterogeneity of the genetic correlates of ASD. A true understanding of the relationship between genetic mutations and ASD phenotype will not be possible if we persist in considering autism as a binary value in our analyses. Advancement in the research on ASD requires the expertise from different fields, but only clinicians and psychiatrists will be able to determine what we are actually looking at (i.e. the autism phenotype or, rather, the different autism phenotypes). If to date common variants seem to be difficult to identify in heterogeneous samples of less than 1000 families, future studies should tell if increasing sample size or meta-analyses, phenotypic stratification, pathway analyses and SNP x SNP interactions can identify common variants associated with sub groups of patients with ASD.

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Box 1. Matched syndromes of reciprocal imprinting or deletions and duplications

A series of matched syndromes have been found which have aberrations in identical or very similar genomic regions. These syndromes usually contain subgroups with autistic features, but each matched pair of syndromes might have different symptoms in other clinical categories.

On chromosome 7q11, a microdeletion causes the Williams-Beuren syndrome, which is the opposite of autism: if anything, they are overly friendly and have strengths in language, face processing, and sociability. Strikingly, the same genomic region was found duplicated in several patients with severely impaired articulation and expressive speech abilities[74].

On chromosome 15q11-13, maternal deletions cause Angelman syndrome (40-80% autism), which has mental retardation, handflapping and mild spasticity or ataxia[13, 21]. Paternal deletions or maternal UPD cause the Prader-Willi syndrome of hypotonia, obesity, short stature with small hands and feet and hypogonadotropic hypogonadism. Regarding duplications, maternal duplications are a relatively common cause of ASD with autistic features found in 40% of patients with the duplications [21].

On chromosome 16p11.2, deletions and duplications of ~600kb are inherited or *de novo* and were identified in 0.5-1% of the patients with ASD [14, 75, 76]. The duplication of the same region is also associated with schizophrenia [14, 75-77].

On chromosome 17p11.2, deletions cause Smith-Magenis syndrome characterized by infant hypotonia, short stature, a dysmorphic face, mental retardation, congenital cardiac defects, inverted melatonin cycles and often sleep disorders and self-abusive behaviors [78]. Duplication of the same genomic region causes the Potocki-Lupski syndrome where about two-thirds of the children meet ASD criteria [79].

On chromosome 22q11.2, deletions are mostly *de novo* and encompass 1.5 to 3 Mb of genomic DNA including 24-30 genes. The patients present with the DiGeorge 1 syndrome, where 50% meet autism criteria [80]. Other individuals might have learning disabilities or schizophrenia [80]. Reciprocal duplications are mostly inherited from healthy parents and these children also have a higher risk for ASD with hypotonia and both mental and growth retardation [81].

On chromosome 22q13.3, the microdeletion syndrome is characterized by normal to accelerated growth combined with neonatal hypotonia, minor dysmorphic features, absent to severely delayed speech and global developmental delay [51]. Up to 44% of these children meet autistic criteria and a duplication of the same region, including the *SHANK3* gene, was observed in a boy with Asperger syndrome who learned language preciously, in a girl with ADHD and in several patients with schizophrenia [51, 52, 82].

Figure legends

Figure 1. Figure 1. Possible consequences of abnormal gene dosage. Abnormal gene dosage seems to play a key role in the susceptibility to ASD. Several mechanisms can explain how abnormal gene dosage can alter brain development. In panel A, a gene has two alleles, A1 and A2 (in yellow and blue respectively, left column). The deletion of allele A1 could completely devoid the cell of the gene product if the other allele A2 is mutated (middle column) as it has been shown for patients with NRXN1 and CNTNAP2 deletions; or silenced by epigenetic hallmarks as in Angelman or Prader-Willi syndromes (right column). The second mutation could devoid the brain from the gene product only in specific regions (panel B) if, as in the "two-hits" or Knudson model, the first allele presented a germinal mutation while the mutation in the second allele (somatic mutation) occurred later during development. Another mechanism involves allelic exclusion, which has been already observed for cell adhesion molecules (panel C). For example, if the protocadherins involved in ASD were subject to allelic exclusion, brain cells would produce either allele A1 or A2 in the normal case (left side of panel C). If allele A1 was deleted, then no protein would be produced in the cells where A2 is silent, and only A2 would be present in the brain, produced by the remaining cells. Finally, an imbalance of gene product due to a deletion or a duplication could lead to disorganized protein complexes. Scaffold proteins (such as the SHANK family) at the post-synaptic density assemble with other proteins in a precise stoichiometry (panel D, left). Both the absence (middle) or excess (right) of protein could affect this equilibrium.

Figure 2. Biological processes involved in ASD. The genes associated with ASD appear to participate into three main biological processes. First (panel A), at the synapse cell adhesion proteins such as cadherins (CDH), protocadherins (PCDH), neuroligins (NLGN) and neurexins (NRXN) are involved in synaptic recognition and assembly. Within the postsynaptic density, scaffold proteins such as SHANK3 and DLGAP2 assemble the synaptic components and provide a link between membrane proteins and the actin skeleton. FMRP transports mRNA at the dendrites and regulates local translation of synaptic proteins. In the cytoplasm, the mTOR pathway regulates translation and is influenced by proteins such as PTEN, NF1, TSC1/TS2 and c-MET. The E3 ligase UBE3A is involved in the

targeting of synaptic proteins to the proteasome. Receptors for glutamate (GLUR) and GABA (GABAR) are playing a central role in producing excitatory and inhibitory currents, respectively. IMPP2L is a peptidase within the inner membrane of the mitochondria. Second (panel B), in the nucleus the methyl binding protein MECP2 and transcription factors such as MEF2C regulate the expression of neuronal genes involved in the formation of neuronal circuits and synaptic functions. The FMRP protein transports and regulates the translation of mRNA at the synapse. Finally (panel C), at the nodes of Ranvier proteins such as CNTN and CNTNAP2 organize the tight junctions between the axon and the myelinating glia. At the membrane or in the intercellular space, cell adhesion molecules and secreted proteins such as NRCAM or SEMA5A act as guidance cues for axonal outgrowth.

Figure 3. Different resilience of brain networks to gene dosage. Depending on their different resilience, the functional effects of abnormal gene-dosage could seem localized, even if the genetic abnormalities are widespread. Brain networks involved in evolutionary older biological processes might have developed more compensatory mechanisms than those supporting more recent cognitive functions. In the illustration we distinguish four possibilities, networks insensitive to gene-dosage (dark blue), networks only sensitive to duplications (light blue) or deletions (orange), and finally, those unable to compensate for gene-dosage abnormalities (red). Nodes represent brain regions, and edges between nodes represent their functional link. Wavy edges represent sub-optimal functional links.

Databases used in this review

DECIPHER v4.3 https://decipher.sanger.ac.uk/application/

Autism Genetic Database (AGD) http://wren.bcf.ku.edu/

Autism CNV Database http://projects.tcag.ca/autism_500k/

AutDB http://www.mindspec.org/autdb.html

BioGPS http://biogps.gnf.org/#goto=welcome

UCSC Genome browser http://genome.ucsc.edu

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Table 1

Table 1. Genes associated with high risk for ASD

Gene	Chromosome	Function	Evidence	Inheritance	Diagnosis	Reference
FMR1	Xq27	Synaptic translation	Mutations	de novo (permutations)	ASD, Fragile X syndrome	83
MECP2	Xq26	Chromatin remodeling	CNV, mutations	de novo (rarely inherited)	ASD, Rett syndrome	84
TSC1	9q34.13	mTOR / PI3K pathway	CNV, mutations	de novo, inherited	ASD, Tuberous sclerosis	85
TSC2	16p13.3	mTOR / PI3K pathway	CNV, mutations	de novo, inherited	ASD, Tuberous sclerosis	85
NF1	17q11.2	mTOR / PI3K pathway	CNV, mutation	de novo, inherited	ASD, Neurofibromatosis	86
PTEN	10q23.31	mTOR / PI3K pathway	CNV, mutations	de novo, inherited	ASD, Cowden syndrome	87
CACNA1C	12p13.33	Calcium channel	Mutation	de novo	ASD, Timothy syndrome	88
DPYD	1p21.3	Pyrimidine base biosynthesis	CNV	de novo	ASD	14
RFWD2	1q25.1-q25.2	Ubiquitination	CNV	de novo, inherited	ASD	13
NRXN1	2p16.3	Synaptic CAM	CNV, mutations, SNP	de novo,inherited	ASD, SCZ	5, 89
CNTN4	3p26.3	Synaptic CAM	CNV	Inherited	ASD, MR	13, 25, 63, 90
MEF2C	5q14.3	Transcription factor	CNV, mutations	de novo	MR, Seizures	38
SYNGAP1	6p21.3	Synaptic Ras GAP	CNV	de novo	ASD, MR	19
CNTNAP2	7q35-7q36.1	Synaptic CAM	CNV, rare variants *	Inherited	ASD, MR, SCZ, TS,	20, 60-62, 91, 92
DPP6	7q36.2	dipeptidyl-peptidase activity	CNV	de novo, inherited	ASD	14
DLGAP2	8p23.3	Synaptic scaffold	CNV	de novo	ASD	14
ASTN2	9q33.1	Neuron-Glial Interaction	CNV	Inherited	ASD, SCZ, ADHD	13
SHANK2	11q13	Synaptic scaffold	CNV	De novo	ASD	19
NBEA	13q13.2	Synaptic protein	Translocation	de novo	ASD	93
UBE3A	15q11-q13	Ubiquitination	CNV	de novo, inherited	ASD	13
SHANK3 (del 22q13)	22q13	Synaptic scaffold	CNV, mutations	de novo, inherited	ASD, MR, SCZ	51, 52 94
NLGN3	Xq13.1	Synaptic CAM	Mutation	Inherited	ASD	95
IL1RAPL1	Xp21.3-p21.2	Synaptic receptor	CNV, mutations	de novo, inherited	ASD, MR	96
NLGN4	Xp22	Synaptic CAM	CNV, mutations	de novo, inherited	ASD, MR, TS	95
PTCHD1	Xp22.11	Hedgehog receptor activity	CNV	Inherited	ASD	14
GRIA3	Xp25	Synaptic receptor	CNV	Inherited	ASD	12

ASD Autism Spectrum Disorder ; SCZ Schizophrenia; MR Mental Retardation; ADHD Attention-Deficit Hyperactivity Disorder ; MDC1D congenital muscular dystrophy ; BP Bipolar, TS Tourette syndrome; * in contrast to mutations, the functional role of the rare variants was not confirmed

Table 2

Table 2. Proposed susceptibility genes for ASD

Gene	Chromosome	Function	Evidence	Diagnosis	References
ASMT	PAR1	Melatonin pathway	Inherited CNV, SNPs, mutations	ASD	81, 97, 98
DISC1/DISC2	1q42.2	Axonal growth	Inherited CNV	ASD, SCZ	99
TSNAX	1q42.2	Cell differentiation	Inherited CNV	ASD, SCZ	99
DPP10	2q14.1	Dipeptidyl-peptidase activity	Inherited CNV	ASD	14
CNTN3	3p12.3	Synaptic CAM	Inherited CNV	ASD	25
FBXO40	3q13.3	Unknown function	Inherited CNV P = 3.3 x 10^{-3}	ASD	13
SLC9A9	3q24	Transporter	Inherited CNV, mutations	ASD, ADHD, MR	25
PCDH10	4q28	Synaptic CAM	Inherited CNV	ASD	25
PARK2	6q26	Ubiquitination	Inherited CNV P = 3.3 x 10^{-3}	ASD, PD	13
IMMP2L	7q31.1	Mitochondrial protease	Inherited CNV	ASD, TS, ADHD	100
PCDH9	13q21	Synaptic CAM	Inherited CNV	ASD	14, 25
MDGA2	14q21.3	GPI anchor protein	Inherited CNV P = 1.3 × 10^{-4}	ASD	18
BZRAP1	17q22	Benzodiazepine receptor binding	Inherited CNV P = 2.3 × 10^{-5}	ASD	18
PLD5	1q43	Phospholipase D	SNP rs2196826 P = 1.1 × 10^{-8}	ASD	9
SLC25A12	2q31.1	Synaptic receptor	SNP rs2056202 P = 1 x 10^{-3}	ASD	101, 102
CDH9/CDH10	5p14.2	Synaptic CAM	SNP rs4307059 P = 3.4 x 10^{-8}	ASD	7
SEMA5A	5p15.2	Axonal guidance	SNP rs10513025 P =2 x 10^{-7}	ASD	8
TAS2R1	5p15.2	Receptor	SNP rs10513025 P =2 x 10^{-7}	ASD	8
GRIK2	6q16.3	Synaptic receptor	SNP rs3213607 P = 0.02	ASD, SCZ, OCD, MR	57
POU6F2	7p14.1	Transcription factor	SNP rs10258862 P = 4.4 x 10^{-7}	ASD	9
RELN	7q22.1	Axonal guidance	GGC repeat in the 5' UTR P <0.05	ASD, BP	103
NRCAM	7q31.1	Synaptic receptor	SNP rs2300045 P =0.017	ASD	104
MET	7q31.2	Tyrosine kinase	SNP rs1858830 P = 2 x 10^{-3}	ASD	34
EN2	7q36.3	Transcription factor	SNP rs1861972 P = 9 x 10^{-3}	ASD	105
ST8SIA2	15q26.1	N-glycan processing	SNP rs3784730 P = 4 x 10^{-7}	ASD	9
GRIN2A	16p13.2	Synaptic receptor	SNP rs1014531 P =2.9 x 10^{-7}	ASD, SCZ	106
ABAT	16p13.2	Enzyme	SNP rs1731017 P = 1 x 10^{-3}	ASD, GABA-AT Deficiency	106
SLC6A4	17q11.2	Serotonin Transporter	Meta analysis P >0.05	ASD, OCD	107
ITGB3	17q21.3	Cell-matrix adhesion	SNP Leu33Pro P = 8.2 x 10^{-4}	ASD	108
TLE2 / TL6	19p13	Wnt receptor signaling pathway	SNP rs4806893 P =7.8 x 10^{-5}	ASD, FHM2, AHC	109
MACROD2	20p12	Unknown function	SNP rs4141463 P = 2 x 10^{-8}	ASD	9

ASD Autism Spectrum Disorder ; SCZ Schizophrenia; PD Parkinson Disease; TS Tourette syndrome; ADHD Attention-Deficit Hyperactivity Disorder ; MR Mental Retardation; FHM2 Familial Hemiplegic Migraine 2 ; AHC Alternating Hemiplegia of Childhood ; OCD Obsessive-Compulsive Disorder ; BP Bipolar Disorder .

Figure1

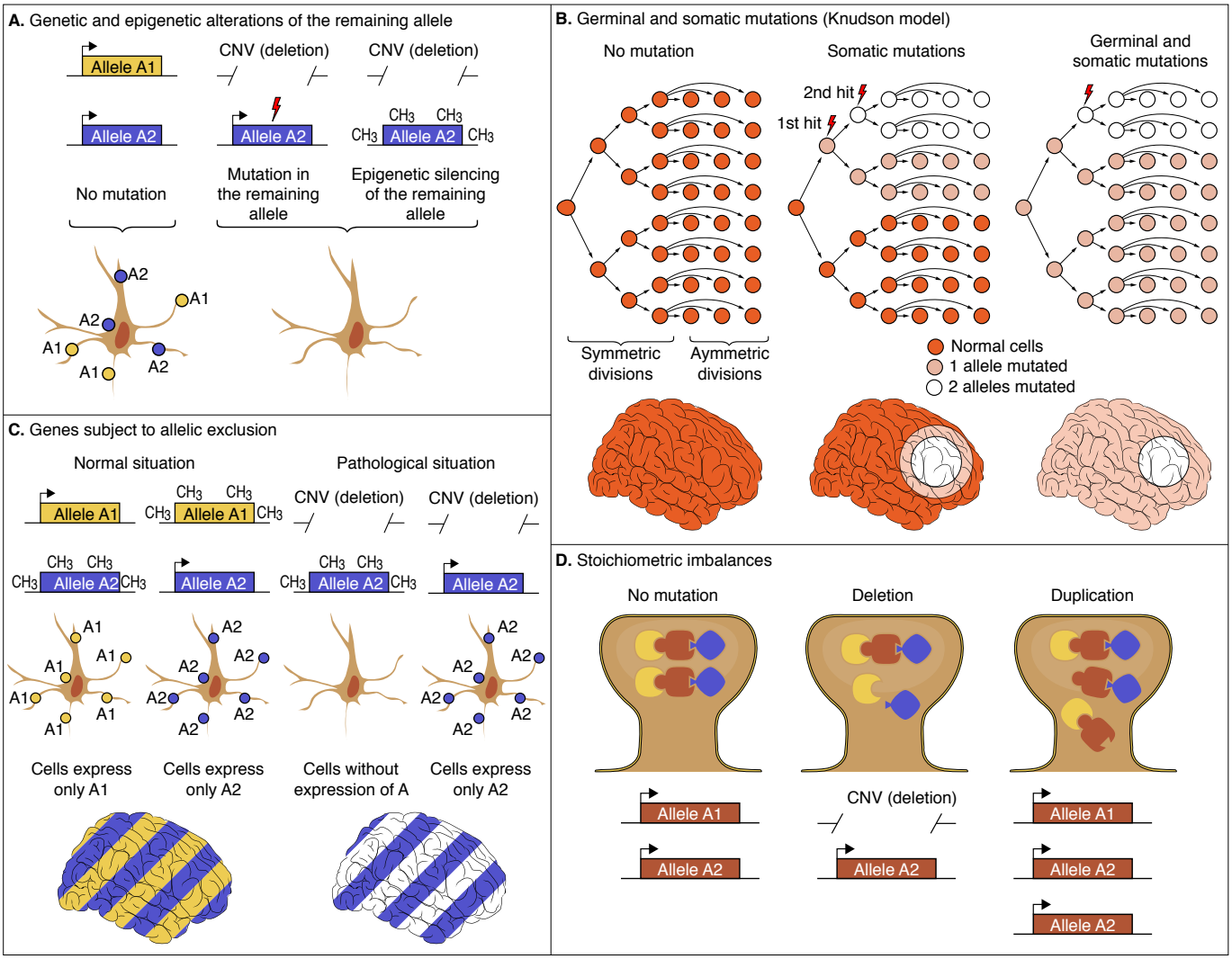

A. Genetic and epigenetic alterations of the remaining allele
Allele A1
Allele A2
CNV (deletion)
CNV (deletion)
CH3 CH3
CH3 Allele A2 CH3
No mutation
Mutation in the remaining allele
Epigenetic silencing of the remaining allele
A2
A1
A2
A1
A1
A2
B. Germinal and somatic mutations (Knudson model)
No mutation
Somatic mutations
Germinal and somatic mutations
2nd hit
1st hit
Symmetric divisions
Aymmetric divisions
Normal cells
1 allele mutated
2 alleles mutated
C. Genes subject to allelic exclusion
Normal situation
Pathological situation
Allele A1
CH3 CH3
CH3 Allele A1 CH3
CNV (deletion)
CNV (deletion)
CH3 CH3
CH3 Allele A2 CH3
Allele A2
A1
A2
Cells express only A1
Cells express only A2
Cells without expression of A
Cells express only A2
D. Stoichiometric imbalances
No mutation
Deletion
Duplication
Allele A1
Allele A2
CNV (deletion)
Allele A2
Allele A1
Allele A2
Allele A2

Figure2

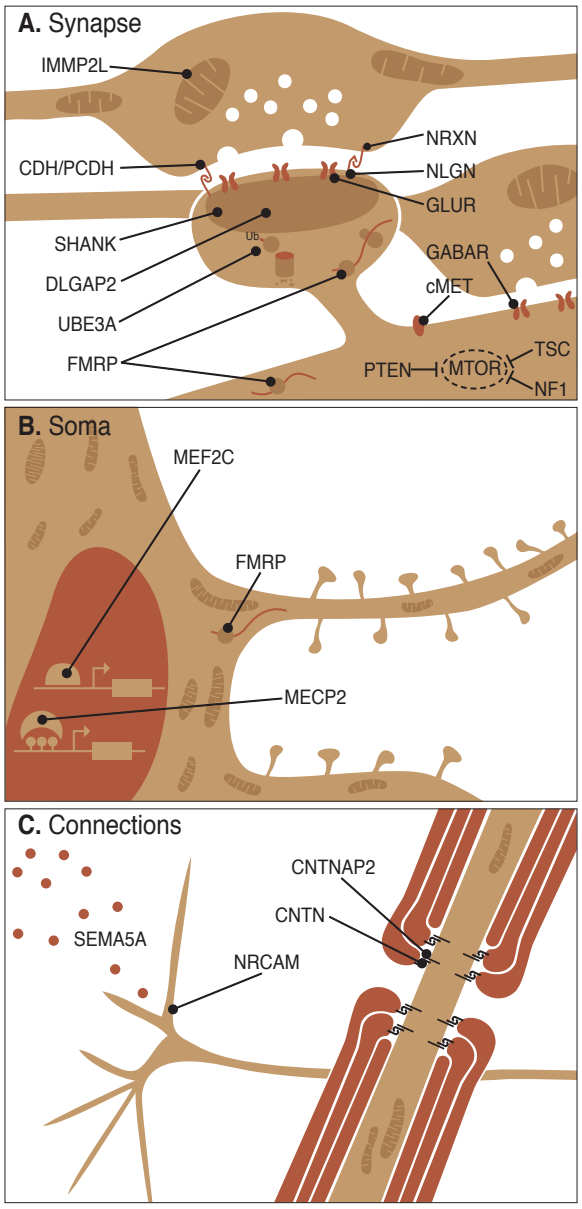
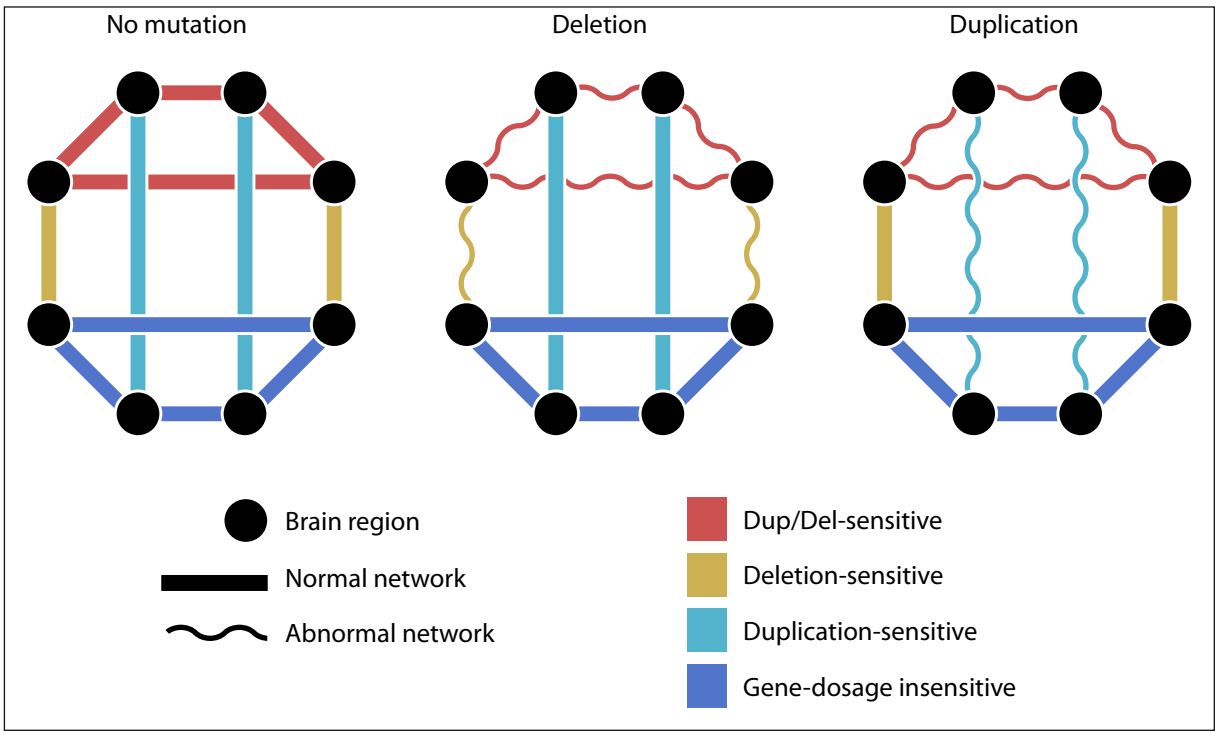

A. Synapse
IMMP2L
NRXN
CDH/PCDH
NLGN
GLUR
SHANK
Ub
GABAR
DLGAP2
cMET
UBE3A
FMRP
TSC
PTEN
MTOR
NF1
B. Soma
MEF2C
FMRP
MECP2
C. Connections
CNTNAP2
CNTN
SEMA5A
NRCAM

Figure3


No mutation
Deletion
Duplication
Brain region
Normal network
Abnormal network
Dup/Del-sensitive
Deletion-sensitive
Duplication-sensitive
Gene-dosage insensitive