Int J Physiol Pathophysiol Pharmacol 2011;3(3):223-235
www.ijppp.org /ISSN:1944-8171/IJPPP1008005

Review Article

The role of GABAergic system in neurodevelopmental disorders: a focus on autism and epilepsy

Paola Sgadò[1], Mark Dunleavy[1,2], Sacha Genovesi[1], Giovanni Provenzano[1], Yuri Bozzi[1,3]

[1]Laboratory of Molecular Neuropathology, Centre for Integrative Biology (CIBIO), University of Trento, Italy; [2]Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland; [3]CNR Neuroscience Institute, Pisa, Italy.

Received August 24, 2011; accepted September 5, 2011; Epub September 9, 2011; Published September 30, 2011

Abstract: Autism spectrum disorders (ASD) and epilepsy are very common neurological disorders of childhood, with an estimated incidence of about 0.5 – 1 % in worldwide population. ASD and epilepsy are often associated, suggesting that common neurodevelopmental bases may exist for these two disorders. The neurodevelopmental bases of both ASD and epilepsy have been clearly showed by a number of genetic, neuroimaging and neuropathological studies. In recent years, dysfunction of inhibitory GABAergic circuits has been proposed as a cause for both disorders. Several studies performed on both animal models and postmortem human samples indicate that GABAergic neurons and circuits are altered in both ASD and epilepsy, suggesting that the excitation/inhibition imbalance resulting from neurodevelopmental defects in GABAergic circuitry might represent a common pathogenetic mechanism for these disorders. Here, we will review the most significant studies supporting this hypothesis.

Keywords: Seizure, mental retardation, neurological disorder, neurotransmission

Introduction

Autism spectrum disorders (ASD) and epilepsy are among the most devastating and common neurological disorders of childhood, affecting about 0.5-1% of the population. ASD and epilepsy are often associated. Moreover, evidence from clinical studies identifies a high rate of seizures and EEG abnormalities in children with ASD, and autistic patients develop epilepsy in a large proportion of cases [1,2]. The incidence of epilepsy in ASD has been reported to be between 5 – 40% [3]. Epilepsy diagnose in autistic patients is made more difficult because the behavioural abnormalities associated with seizure are often attributed to ASD. There is no primary seizure type or syndrome associated with ASD. Complex partial, absence, generalized tonic-clonic have all been reported [4-8]. In contrast, the frequency of ASD in patients with epilepsy remains to be established; factors such as referral criteria, age and severity of cognitive impairments all contribute to the variability in report rate [3,9]. Children that co-express ASD and epilepsy show a poorer outcome in cognitive behaviour than those with either epilepsy or autism [6,7].

Epilepsy and ASD are both heterogeneous disorders with multiple etiologies and pathophysiology. The frequent co-occurrence of these two diseases suggests – at least in certain cases – common neurodevelopmental bases [10]. Both ASD and epilepsy may result from common developmental pathophysiological mechanisms leading to abnormalities in connectivity, imbalances in excitation/inhibition and disrupted synaptic plasticity. These alterations can be of genetic origin resulting in both ASD and epilepsy as for fragile X syndrome (FXS), Rett syndrome (RTT) and tuberous sclerosis (TSC).

γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain. A number of studies have implicated the GABAergic system dysfunction in epilepsy and ASD. An increasing body of evidence suggests that a downregulation of GABAergic function is critical in ASD-associated epilepsy. Quantitative autoradiographic studies examining the density and distri-

bution of GABAergic receptor subunits indicated a downregulation of GABAergic function in the hippocampus of ASD patients with seizures [11]. Furthermore, altered packing of GABAergic interneurons in the CA1 and CA3 hippocampal subfields where malformations are associated with the generation of seizures have been described [12]. Several studies have demonstrated a loss of inhibitory interneurons in the epileptic brain [13,14]. Human genetics studies clearly indicate an association between ASD and genes for GABA receptor subunits as well as genes controlling GABAergic neuron development or GABAergic synapse structure. Moreover, recent studies, performed on both animal models and postmortem human samples, suggest that GABAergic neurons and circuits may be altered in ASD patients. Here we examine the link between ASD and epilepsy with particular focus on the role of GABAergic dysfunction in the pathogenesis of these diseases. In particular, we will describe some of the key human and animal studies further outlining the link between epilepsy and ASD and the potential mechanistic role of GABA dysfunction.

Genetics of ASD

ASD represent a very heterogeneous group of neurodevelopmental disabilities of proven genetic origin, with an incidence of about 60-70/10,000 [15]. A gender distortion is observed in ASD (4:1 males to females ratio) [16], reflecting a possible involvement of the X chromosome or imprinting mechanisms. The genetic factors play an important role in the pathogenesis of these diseases [17], as documented by the recurrence risk in families and twin studies. These studies show a concordance rate of 82–92% in monozygotic versus 1–10% in dizygotic twins. Heritability is estimated above 90% and sibling recurrence risk is above 6–20% [16,18]. About 10%–20% of individuals with an ASD have an identified genetic etiology. Chromosomal alterations have been reported in ~5% of cases. Genetic forms of ASD include monogenic and complex disorders. The most common single gene mutation in ASD is FMR1 associated with FXS, present in ~2% of cases. Other monogenic disorders include neurofibromatosis (NF1), tuberous sclerosis (TSC1, TSC2), Angelman syndrome (UBEA3A) and Rett syndrome (MECP2), covering only the 2–5% of the ASD cases [19].

Cytogenetic investigations and genome-wide scans have been performed to identify chromosomal regions containing ASD susceptibility genes [20,21]. The most common chromosomal rearrangement is the maternal duplication of 15q11-q13, which accounts for approximately 1-2% of ASD cases [22]. Recent genome-wide association (GWA) studies have identified novel candidate loci between the cadherin genes CDH9 and CDH10 (5p14.1) [23] and between the SEMA5A and TAS2R1 genes (5p15.2) [24]. In addition, the Autism Genome Project (AGP) Consortium has genotyped 1,558 ASD families for one million single nucleotide polymorphisms (SNPs), identifying a novel locus near the gene MACROD2 (20p12.1) [25]. Syndromic forms of ASD have been associated with both copy number variations (CNVs) and rare mutations in several genes, including SHANK3, NLGN3, NLGN4, NRXN1 and HOXA1 [26]. A recent extensive metanalysis of the literature [27] allowed identification of 103 disease genes and 44 genomic loci reported in subjects with ASD or autistic behaviour. The vast majority of these genes and loci have been also causally implicated in epilepsy [27]. For example, chromosome 15q, which contains genes coding for GABA receptor subunits, has been reported to be a common site for mutations in ASD and syndromic epilepsy [28].

GABAergic dysfunction in ASD and epilepsy

Abnormalities in GABAergic interneuron function and/or connectivity might represent the anatomical substrate of an unbalanced ratio of excitation/inhibition in sensory, mnemonic, social and emotional systems, that has been postulated to occur in the autistic brain [29]. Indeed, several studies showed that impaired m aturation of the GABAergic circuitry results in an immature structure and function of the cerebral cortex, that remains more plastic and sensitive to alterations in sensory inputs [30-32]. An immature structure and function of the cerebral cortex is considered a major feature of neurodevelopmental disorders [29].

Several studies have described alterations in the GABAergic system in post-mortem brains of autistic patients. A 50% reduction in GAD65/67 protein levels was reported in the cerebellum and parietal cortex of ASD patients [33]. Reduced levels of GAD67 and GAD65 mRNAs were also detected in Purkinje cells and dentate nuclei neurons in the cerebellum from ASD cases [34]. Interestingly, the same authors also re-

ported an increase of GAD67 mRNA levels in GABAergic basket cells that was interpreted as a compensatory up-regulation to supply the loss of Purkinje cells in ASD brains [35]. Several studies showed a significant decrease in $GABA_A$ receptor α4, α5, β1 and β3 subunits [11,36,37], as well as a significant reduction of benzodiazepine binding sites in ASD brains [38]. $GABA_B$ receptors were also reduced in restricted regions of the cerebral cortex in ASD patients [39]. Recently, a transcranial magnetic stimulation study detected a reduced cortical inhibition (interpreted as a possible disruption of $GABA_A$ receptor activity) in the brain of a subset of ASD patients [40]. Taken together, these data support the hypothesis of a GABAergic signalling deficit in ASD.

As for ASD, several evidence link the pathophysiology of epilepsy with GABA neurotransmission and imbalanced excitation/inhibition. Abnormalities of GABAergic function have been observed in several genetic and experimental animal models of epilepsy [41]. In addition, reductions of GABA-mediated inhibition, activity of glutamate decarboxylase, binding to $GABA_A$ and benzodiazepine sites, GABA in cerebrospinal fluid and brain tissue have all been reported in studies of human epileptic brain tissue [42]. Furthermore, GABA agonists suppress seizures, and GABA antagonists produce seizures [43]. There are two main aspects to the role of GABA dysfunction in the pathogenesis of epilepsy in ASD. First, absence of GABA signaling results in loss of inhibitory neuronal firing that normally prevents the spread of paroxysmal discharge. Furthermore, normal GABAergic function is crucial in brain development, and alteration in this function may significantly affect neuronal migration, differentiation, synaptogenesis and circuit formation. In addition to the direct effect of altered GABA system on the ability of interneurons to inhibit the generation of synchronized discharges, there is a vast array of ASD candidate genes involved in secondary regulation of the GABAergic system during development that may play a role in the pathogenesis of epilepsy-autism disorders.

Taken together, these data provide a sound rationale for proposing GABA dysfunction, (primarily through loss of GABA transmission, and secondarily through altered circuit formation in development) as a potential link between epilepsy and ASD, possibly even a common pathology. The range of genes involved may reflect the spectrum of pathologies associated with ASD and epilepsy and warrant more detailed investigation. Overall, these data indicate that the ASD-epilepsy condition is a spectrum disorder within itself. The severity of the ASD condition (e.g., presence or absence of mental retardation) is closely associated with the severity of the epilepsy phenotype (e.g., seizure frequency, severity and intractability). Early diagnosis and suitable treatment protocols are vital for successful outcomes [44,45]. Properly constructed prospective clinical studies, aimed at profiling the progression of the disease in a growing cohort of cases, could provide vital insights required to develop successful therapeutic approaches for epilepsy in ASD.

ASD and epilepsy: common neurodevelopmental bases

The co-occurrence of ASD and epilepsy has been well studied in genetic conditions that result in abnormal excitability and disrupted synaptic plasticity in the developing brain such as fragile X syndrome (FXS), Rett syndrome (RTT), tuberous sclerosis complex (TSC), all of which include ASD and epilepsy, and in several animal models lacking genes involved in brain development such as DLX, NRP2, ARX and EN2.

Fragile X syndrome

Fragile X syndrome (FXS) is the most frequent form of inherited mental retardation and often presents with ASD and epilepsy. FXS results from the expansion of triplet repeats in the untranslated region of the FMR1 gene, preventing synthesis of the FMR1 gene product FMRP. FMRP regulates mRNA transport and local protein synthesis in neuronal dendrites and spines. Patients with FXS show increased spine density in the neocortex and the abundance of long, thin dendritic spines with an immature morphology [46,47]. Fmr1 knockout mice show a phenotype resembling the human disease: altered learning and behaviour, greater susceptibility to seizures, altered synaptic plasticity [48] and an excess of long, thin dendritic spines [49]. Recently, an increased intrinsic excitability of the excitatory neurons was described in Fmr1 knockout mice supporting a connection to epileptogenesis [50]. Alterations in the glutamatergic system in FXS could disrupt the normal actions of inhibitory GABAergic neurons, resulting

in downregulation of GABA receptor subunits [51-53] and altered expression of a number of enzymes involved in the metabolism of GABA [54]. Several studies show a strong reduction in the expression of $GABA_A$ receptor subunit mRNAs and proteins in adult Fmr1 knockout mice [54,55], that is accompanied by abnormal GABAergic transmission [56,57], deficits of parvalbumin (PV) but not calbindin (CB) or calretinin (CR) cortical interneurons and increased audiogenic seizure susceptibility [58].

Rett Syndrome. Rett syndrome (RTT) is a postnatal neurodevelopmental disorder typically emerging between 6 – 18 months of age consisting of progressive loss of cognitive and motor function and the emergence of epilepsy [59]. Seizures have been reported in 50 – 90% of RTT patients [60,61]. RTT is caused by mutations in the gene encoding for the methyl-CpG binding protein 2 (MeCP2), a transcriptional regulator involved in chromatin remodeling and splicing. MeCP2 regulates gene expression by binding to methylated CpG dinucleotides leading to chromatin compaction and transcriptional repression [62]. Recent evidence suggests that in same cases MeCP2 can also act as a transcriptional activator [63]. Reduced brain size and increased neuronal density have been observed in several brain regions of RTT patients, including the cerebral cortex, hypothalamus and the hippocampus [64]. Studies of RTT autopsy material revealed a reduction of the size and complexity of pyramidal neurons dendritic trees in the cortex and in the hippocampus [65]. In addition, autoradiography studies in the frontal cortex and basal ganglia of autopsy RTT brains revealed abnormalities in the density of neurotransmitter receptors, such as excitatory NMDA, AMPA, kainate and metabotropic glutamate receptors as well as inhibitory GABA receptors [66]. Mice with truncated MeCP2 recapitulate many RTT features [67]. Mecp2-null mice are behaviourally normal until 5 weeks of age, when they begin to show behavioural deficits resembling RTT patients' symptoms. Initially body tremors, motor abnormalities and spontaneous seizures appear. Later on the mutant mice show pronounced stereotypic forelimb motions and clasping when suspended by the tail, resembling the typical hand-wringing seen in RTT patients [67]. Despite the evident behavioural abnormalities, Mecp2-mutant mice show no major neuropathological phenotype. They show only a reduction in brain weight and a simplified dendritic tree of pyramidal neurons, neuronatomical features observed alson in many RTT patients [68]. Electrophysiological studies showed a reduction in excitatory synaptic strength and glutamatergic synapse numbers [69] and alteration inhibitory strength in the cortex, hippocampus and brainstem of MeCP2 null mice [70]. More recently, the observation that cortical wildtype GABAergic neurons express 50% more MeCP2 than non-GABAergic neurons suggested a specific role of MeCP2 in GABAergic function [71]. Loss of Mecp2 in inhibitory neurons expressing the vesicular GABA transporter (VIAAT/VGAT) resulted in ASD-like repetitive and stereotyped behaviours, EEG abnormalities and seizures. Interneurons immunolabelling in VGAT-Mecp2 mutants also showed a reduction of GAD65 and GAD67 mRNA in the cerebral cortex. Moreover, electrophysiological recordings indicated that Mecp2 deficiency in GABAergic neurons determines a reduction the neurotransmitter release due to a reduction of the enzyme GAD in presynaptic terminals [71]. Interestingly, loss of MeCP2 in GABAergic neurons, both globally and in a subset, reveals a multitude of neuropsychiatric phenotypes encompassing social behaviour, learning/memory, motor function, stereotyped behaviours and sensorimotor gating [71].

Tuberous Sclerosis. Tuberous sclerosis complex (TSC) is a neurocutaneous syndrome characterized by benign tumors, early onset epilepsy, intellectual disability, and autism. TSC results from mutations of TSC1 (hamartin) or TSC2 (tuberin) genes that lead to the formation of hamartomatous growths in one or more body systems (skin, central nervous system, kidneys, heart, lungs, and retina). Hamartin and tuberin form a protein complex that inhibits the phosphatidyl inositol 3-kinase (PI3) and the mTOR signaling pathways. Mutations of hamartin or tuberin in TSC lead to hyperactivation of the mTOR, and other downstream signaling pathways resulting in increased cell growth, proliferation, and abnormal gene expression. Mice with a heterozygous inactivating mutation in the TSC genes show deficits in learning and memory and abnormal social behaviour [72]. Furthermore Tsc1 or Tsc2 loss increases spine length and decreases dendritic spines density in hippocampal slice cultures [73] . In addition to structural changes in dendrites, the mTOR pathway is reported to play a role in post-synaptic AMPA receptor expression [74]. Alterations in expression of specific glutamate and GABA receptor subunits have been also described in

cortical tubers from human TSC patients indicating that imbalances of excitation and inhibition may contribute to the TSC phenotype [75,76].

Neurexins and neuroligins

Neurexins (NRXNs) are presynaptic proteins, binding postsynaptic neuroligins. This interaction is thought to trigger postsynaptic differentiation and control the balance of inhibitory GABAergic and excitatory glutamatergic inputs [77,78]. There are three NRXN genes (NRXN 1–3) in mammals; among these, mutations and chromosomal rearrangements in NRXN1 has been associated with ASD [79-81]. Recently it has been shown that NRXNs can bind not only neuroligins but also $GABA_A$ receptors [82]. The effect of this ligand-receptor interaction decreases GABAergic transmission. Neuroligins (NLGNs) are neural cell adhesion molecules, which act as ligands for neurexins [77,78]. NLGNs play a key role in the formation, organization, and remodeling of synapses and different isoforms are associated with different synaptic types. NLGN1, NLGN4X and NLGN4Y are localized at glutamatergic synapses [17,83]. NLGN3 is present in both excitatory and inhibitory synapses [84,85], whereas NLGN2 is located in GABAergic synapses [17,83,86]. Mutations in NLGN1, 3 and 4X genes have been identified in patients with familial ASD [87-89]. Mice carrying the R451C mutation in the Nlgn3 gene show behavioural phenotypes related to ASD (lack of social behaviours, reduced ultrasound vocalization; [90,91]). In addition, Nlgn3 R451C knock-in mice present an increase in the number of GABAergic synapses (as evaluated by vesicular GABA transporter and gephyrin immunostaining) and in the amplitude of inhibitory currents, suggesting that the R451C mutation switches Nlgn3 synaptic specificity from glutamatergic to GABAergic [91]. Further characterization of these mutants demonstrated that loss of PV-positive basket cells is detectable across the two hemispheres in these mice [92]. As neurorexins and neuroligins control the formation of both excitatory and inhibitory synapses they may contribute to alter the balance between excitatory synapses and inhibitory synapses that in turn could affect cognition and social behaviour as well as contribute to epilepsy.

Genes regulating brain development

Evidence is accumulating that several genes associated with both ASD and epilepsy play roles in the development of cortical inhibitory interneurons including the members of the distal-less homeobox (DLX) family, neuropilin 2 (NPN2), reelin (RELN), aristaless-related homeobox (ARX) and engrailed 2 (EN2) [93-97]. The human DLX genes are located head-to-head on chromosome 2q31 (DLX1 and DLX2) and 7q21.3 (DLX5 and DLX6), two regions previously associated to ASD susceptibility in several genome-wide linkage studies [98]. Two studies examining SNPs in the DLX1 and DLX2 genes have found an association with ASD, suggesting that common genetic variations in these genes play a critical role in the disease [99,100]. DLX genes encode homeodomain transcription factors key regulators in forebrain and basal ganglia development. The DLX genes are mainly expressed in the basal ganglia, the amygdala, the hypothalamus and in local circuit neurons of the cerebral cortex [29]. The Dlx transcription factors regulate the development of basal ganglia GABAergic projection neurons and of the cortical inhibitory interneurons [101]. Mice lacking Dlx1 display a selective loss of somatostatin (SST), neuropeptide Y (NPY), CR and RELN expressing interneurons accompanied by reduced GABAergic inhibitory transmission and late-onset epilepsy [102]. More recently, additional behavioural abnormalities (such as conditioned fear response) linked to impairment of GABAergic systems were described in Dlx1-null mice [103]. While the role of Dlx1/2 in brain development is well established, little information is available on the function of Dlx5/6. Dlx5 is known to promote differentiation of olfactory bulb interneurons [104,105]. Because Dlx1/2 are required to induce expression of Dlx5/6 in the lateral and medial ganglionic eminence [106,107], the contribution of Dlx5/6 to the Dlx1/2 phenotype has not been established. Recently a role of Dlx5/6 in the development of PV interneurons has been described [108].

Deficits in inhibitory interneurons and reduced seizure threshold were observed in NPN2 deficient mice [109]. The gene for NPN2 (also known as NRP2) is coded for at 2q34, a region known to be strongly associated with ASD [94]. NPN2 functions as a chemorepulsive receptor for the axon guidance molecule Semaphorin 3F, regulate neuronal migration and differentiation, contributing to brain development and network formation. NPN2 deficient mice had shorter seizure latencies, increased vulnerability to seizure-induced neuronal death and developed chemically-induced spontaneous recurrent sei-

zures [109]. Importantly, NPN2 null mice had a reduced number of GABA, PV and NPY interneurons [109].

GABAergic neuron development dysfunction may also occur in conjunction with abnormalities in the RELN gene, coding for the extracellular matrix glycoprotein Reelin which is involved in neuronal migration and lamination of the cerebral cortex during embryogenesis [110]. Reelin binding to membrane receptors enhances signal transduction pathways leading to synaptic plasticity and axonal growth (Beffert, 2005). RELN maps to 7q22 human chromosome [111]. Linkage in this region is among the most robust genetic findings in ASD. In family-based and case–control studies the 5′-untranslated region (5′-UTR) GGC repeat alleles was associated with ASD [112]. Mice lacking the Reelin gene (*reeler* mice) show a disorganization of laminated brain regions such as cerebral cortices and cerebellum, resulting in a dramatic impairment of neuronal migration in the cerebral cortex [113]. Reeler mice also show a decrease of dentritic spine density and a decreased GABA turnover [114]. Recently, ASD-like behaviours and loss of PV interneurons was reported in Reeler mice [115].

The aristaless-related homeobox gene (ARX), in chromosome Xp22, is a transcription factor that belongs to a family of paired- class homeobox genes, and plays a pivotal role in the development of the central nervous system. ARX large deletions, protein truncating mutations and missense mutations in the homeobox region lead to X-linked lissencephaly with abnormal genitalia, while other ARX mutations cause intellectual disability, ASD and epilepsy without cortical malformations. Arx expression has been detected in the developing lateral and medial ganglionic eminence (LGE and MGE) and later in cortical progenitors and migrating interneurons [116-119]. Arx expression is controlled by the Dlx genes in cells derived from basal ganglia progenitor domains [120]. Studies in animal models showed that ARX is critical for radial migration of cortical progenitors and is crucially involved in the development and migration of GABAergic interneurons [96]. In Arx-null embryos, migration from the LGE and the MGE are nearly absent, whereas migration through the cortical layers is only partially impaired. As a consequence, CB- and CR-expressing cells are severely reduced and NPY interneurons are nearly absent throughout the brain [118,121]. Defective tangential migration is similarly observed after electroporation into the MGE of rat brain slices [122]. Studies on Arx knock-out mice described abnormal expression of several transcription factors, potentially important for migration and differentiation of certain population of neurons [118,121].

Among the numerous ASD associated genes EN2 (coding for the homeobox-containing transcription factor Engrailed-2) was originally shown to be involved in posterior brain (midbrain/hindbrain) embryonic development [123]. EN2 maps to a region of chromosome 7 implicated in ASD susceptibility, and GWA studies indicated EN2 as a candidate gene for ASD [124]. Namely, two SNPs in the human En2 gene have been associated to ASD, one of which (rs1861973, A-C haplotype) is functional: when tested in a luciferase reporter assay in rat, mouse and human cell lines, this SNP markedly affected En2 promoter activity [124]. En2 null mice display cerebellar hypoplasia and a reduced number of Purkinje cells [123]. Importantly, ASD-like behaviours such as decreased play, reduced sociality, and impaired spatial learning and memory were described in these mutants [125]. Recently we showed that En2 is also expressed in telencephalic structures involved in epileptogenesis. Accordingly, an increased susceptibility to seizures was detected in En2 null mice, that was accompanied by reduced PV immunostaining on cell bodies of CA3 pyramidal neurons and reduced SST immunostaining in the the stratum lacunosum moleculare of the hippocampal formation [126]. These findings suggest that the En2 gene may be involved in GABAergic system development and maintenance, and altered En2 function may be a common cause of ASD and seizures.

Conclusions

Several studies suggest that an impairment of inhibitory neurotransmission and the subsequent imbalance in excitation/inhibition in the developing brain may constitute a fundamental event in the development of both ASD and epilepsy. ASD and epilepsy are often associated, and defects in the development, maintenance and function of GABAergic interneurons have been postulated as a pathogenic mechanism of ASD-epilepsy syndromes. However, a direct, causal demonstration of a GABAergic dysfuction

in the brain of ASD patients is still lacking. Conversely, data from mouse models of ASD strongly support the hypothesis of GABAergic dysfunction in ASD-epilepsy; further analyses on these and novel models will contribute to unravel the common neurodevelopmental basis of ASD and epilepsy.

Acknowledgements

P.S. and M.D. are postdoctoral fellows, respectively supported by Provincia Autonoma di Trento (Italy) and IRCSET (Ireland) under the Marie Curie-People cofunding action of the European Community. This work was funded by the Italian Ministry of University and Research (PRIN 2008 grant # 200894SYW2_002), the Italian Ministry of Health (grant RF-TAA-2008-1141282) and the University of Trento (CIBIO start-up) grants to Y.B.

Please address correspondence to: Yuri Bozzi, Laboratory of Molecular Neuropathology, Centre for Integrative Biology (CIBIO), University of Trento, via delle Regole 101, 38123 Mattarello, Trento, Italy. Ph: +39 461 283651; Fx: 39 461 283937; Email: bozzi@science.unitn.it

References

[1] Chez MG, Chang M, Krasne V, Coughlan C, Kominsky M, and Schwartz A. Frequency of epileptiform EEG abnormalities in a sequential screening of autistic patients with no known clinical epilepsy from 1996 to 2005. Epilepsy & behavior : E&B 2006; 8:267–271.

[2] Kim HL, Donnelly JH, Tournay AE, Book TM, and Filipek P. Absence of seizures despite high prevalence of epileptiform EEG abnormalities in children with autism monitored in a tertiary care center. Epilepsia 2006; 47:394–398.

[3] Canitano R. Epilepsy in autism spectrum disorders. European child & adolescent psychiatry 2007; 16:61–66.

[4] Steffenburg S, Steffenburg U, and Gillberg C. Autism spectrum disorders in children with active epilepsy and learning disability: comorbidity, pre- and perinatal background, and seizure characteristics. Developmental medicine and child neurology 2003; 45:724–730.

[5] Tuchman RF, Rapin I, and Shinnar S. Autistic and dysphasic children. II: Epilepsy. Pediatrics 1991; 88:1219–1225.

[6] Danielsson S, Gillberg IC, Billstedt E, Gillberg C, and Olsson I. Epilepsy in young adults with autism: a prospective population-based follow-up study of 120 individuals diagnosed in childhood. Epilepsia 2005; 46:918–923.

[7] Hara H. Autism and epilepsy: a retrospective follow-up study. Brain & development 2007; 29:486–490.

[8] Parmeggiani A, Posar A, Antolini C, Scaduto MC, Santucci M, and Giovanardi-Rossi P. Epilepsy in patients with pervasive developmental disorder not otherwise specified. Journal of child neurology 2007; 22:1198–1203.

[9] Tuchman R, Moshé SL, and Rapin I. Convulsing toward the pathophysiology of autism. Brain & development 2009; 31:95–103.

[10] Brooks-Kayal A. Epilepsy and autism spectrum disorders: are there common developmental mechanisms? Brain & development 2010; 32:731–738.

[11] Blatt GJ, Fitzgerald CM, Guptill JT, Booker AB, Kemper TL, and Bauman ML. Density and distribution of hippocampal neurotransmitter receptors in autism: an autoradiographic study. Journal of autism and developmental disorders 2001; 31:537–543.

[12] Bauman ML and Kemper TL. Neuroanatomic observations of the brain in autism: a review and future directions. International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience 2005; 23:183–187.

[13] Zhu ZQ, Armstrong DL, Hamilton WJ, and Grossman RG. Disproportionate loss of CA4 parvalbumin-immunoreactive interneurons in patients with Ammon's horn sclerosis. Journal of neuropathology and experimental neurology 1997; 56:988–998.

[14] Wittner L, Maglóczky Z, Borhegyi Z, Halász P, Tóth S, Eross L, Szabó Z, and Freund TF. Preservation of perisomatic inhibitory input of granule cells in the epileptic human dentate gyrus. Neuroscience 2001; 108:587–600.

[15] Fombonne E. Epidemiology of pervasive developmental disorders. Pediatric research 2009; 65:591–598.

[16] Abrahams BS and Geschwind DH. Advances in autism genetics: on the threshold of a new neurobiology. Nature reviews Genetics 2008; 9:341–355.

[17] Persico AM and Bourgeron T. Searching for ways out of the autism maze: genetic, epigenetic and environmental clues. Trends in neurosciences 2006; 29:349–358.

[18] Toro R, Konyukh M, Delorme R, Leblond C, Chaste P, Fauchereau F, Coleman M, Leboyer M, Gillberg C, and Bourgeron T. Key role for gene dosage and synaptic homeostasis in autism spectrum disorders. Trends in genetics : TIG 2010; 26:363–372.

[19] Hatton DD, Sideris J, Skinner M, Mankowski J, Bailey DB, Roberts J, and Mirrett P. Autistic behavior in children with fragile X syndrome: prevalence, stability, and the impact of FMRP. American journal of medical genetics Part A 2006; 140A:1804–1813.

[20] Freitag CM. The genetics of autistic disorders and its clinical relevance: a review of the literature. Molecular Psychiatry 2007; 12:2–22.

[21] Yang MS and Gill M. A review of gene linkage, association and expression studies in autism and an assessment of convergent evidence. International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience 2007; 25:69–85.
[22] Vorstman JAS, Staal WG, van Daalen E, van Engeland H, Hochstenbach PFR, and Franke L. Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. Molecular Psychiatry 2006; 11:1, 18–28.
[23] Wang K, Zhang H, Ma D, Bucan M, Glessner JT, Abrahams BS, Salyakina D, Imielinski M, Bradfield JP, Sleiman PMA, Kim CE, Hou C, Frackelton E, Chiavacci R, Takahashi N, Sakurai T, Rappaport E, Lajonchere CM, Munson J, Estes A, Korvatska O, Piven J, Sonnenblick LI, Alvarez Retuerto AI, Herman EI, Dong H, Hutman T, Sigman M, Ozonoff S, Klin A, Owley T, Sweeney JA, Brune CW, Cantor RM, Bernier R, Gilbert JR, Cuccaro ML, McMahon WM, Miller J, State MW, Wassink TH, Coon H, Levy SE, Schultz RT, Nurnberger JI, Haines JL, Sutcliffe JS, Cook EH, Minshew NJ, Buxbaum JD, Dawson G, Grant SFA, Geschwind DH, Pericak-Vance MA, Schellenberg GD, and Hakonarson H. Common genetic variants on 5p14.1 associate with autism spectrum disorders. Nature 2009; 459:528–533.
[24] Weiss LA, Arking DE, Gene Discovery Project of Johns Hopkins & the Autism Consortium, Daly MJ, and Chakravarti A. A genome-wide linkage and association scan reveals novel loci for autism. Nature 2009; 461:802–808.
[25] Anney R, Klei L, Pinto D, Regan R, Conroy J, Magalhaes TR, Correia C, Abrahams BS, Sykes N, Pagnamenta AT, Almeida J, Bacchelli E, Bailey AJ, Baird G, Battaglia A, Berney T, Bolshakova N, Bölte S, Bolton PF, Bourgeron T, Brennan S, Brian J, Carson AR, Casallo G, Casey J, Chu SH, Cochrane L, Corsello C, Crawford EL, Crossett A, Dawson G, de Jonge M, Delorme R, Drmic I, Duketis E, Duque F, Estes A, Farrar P, Fernandez BA, Folstein SE, Fombonne E, Freitag CM, Gilbert J, Gillberg C, Glessner JT, Goldberg J, Green J, Guter SJ, Hakonarson H, Heron EA, Hill M, Holt R, Howe JL, Hughes G, Hus V, Igliozzi R, Kim C, Klauck SM, Kolevzon A, Korvatska O, Kustanovich V, Lajonchere CM, Lamb JA, Laskawiec M, Leboyer M, Le Couteur A, Leventhal BL, Lionel AC, Liu X-Q, Lord C, Lotspeich L, Lund SC, Maestrini E, Mahoney W, Mantoulan C, Marshall CR, McConachie H, McDougle CJ, McGrath J, McMahon WM, Melhem NM, Merikangas A, Migita O, Minshew NJ, Mirza GK, Munson J, Nelson SF, Noakes C, Noor A, Nygren G, Oliveira G, Papanikolaou K, Parr JR, Parrini B, Paton T, Pickles A, Piven J, Posey DJ, Poustka A, Poustka F, Prasad A, Ragoussis J, Renshaw K, Rickaby J, Roberts W, Roeder K, Roge B, Rutter ML, Bierut LJ, Rice JP, Salt J, Sansom K, Sato D, Segurado R, Senman L, Shah N, Sheffield VC, Soorya L, Sousa I, Stoppioni V, Strawbridge C, Tancredi R, Tansey K, Thiruvahindrapduram B, Thompson AP, Thomson S, Tryfon A, Tsiantis J, van Engeland H, Vincent JB, Volkmar F, Wallace S, Wang K, Wang Z, Wassink TH, Wing K, Wittemeyer K, Wood S, Yaspan BL, Zurawiecki D, Zwaigenbaum L, Betancur C, Buxbaum JD, Cantor RM, Cook EH, Coon H, Cuccaro ML, Gallagher L, Geschwind DH, Gill M, Haines JL, Miller J, Monaco AP, Nurnberger JI, Paterson AD, Pericak-Vance MA, Schellenberg GD, Scherer SW, Sutcliffe JS, Szatmari P, Vicente AM, Vieland VJ, Wijsman EM, Devlin B, Ennis S, and Hallmayer J. A genome-wide scan for common alleles affecting risk for autism. Human molecular genetics 2010; 19:4072–4082.
[26] Lintas C and Persico AM. Autistic phenotypes and genetic testing: state-of-the-art for the clinical geneticist. Journal of medical genetics 2009; 46:1–8.
[27] Betancur C. Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting. Brain research 2011; 1380:42–77.
[28] Schroer RJ, Phelan MC, Michaelis RC, Crawford EC, Skinner SA, Cuccaro M, Simensen RJ, Bishop J, Skinner C, Fender D, and Stevenson RE. Autism and maternally derived aberrations of chromosome 15q. American journal of medical genetics Part A 1998; 76:327–336.
[29] Rubenstein JLR and Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes, Brain and Behavior 2003; 2:255–267.
[30] Huang ZJ, Kirkwood A, Pizzorusso T, Porciatti V, Morales B, Bear MF, Maffei L, and Tonegawa S. BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell 1999; 98:739–755.
[31] Hensch TK. Critical period plasticity in local cortical circuits. Nature Reviews Neuroscience 2005; 6:877–888.
[32] Hensch TK, Fagiolini M, Mataga N, Stryker MP, Baekkeskov S, and Kash SF. Local GABA circuit control of experience-dependent plasticity in developing visual cortex. Science (New York, NY) 1998; 282:1504–1508.
[33] Fatemi SH, Halt AR, Stary JM, Kanodia R, Schulz SC, and Realmuto GR. Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. Biological psychiatry 2002; 52:805–810.
[34] Yip J, Soghomonian JJ, and Blatt GJ. Decreased GAD67 mRNA levels in cerebellar Purkinje cells in autism: pathophysiological implications. Acta neuropathologica 2007; 113:559–568.
[35] Yip J, Soghomonian JJ, and Blatt GJ. Increased GAD67 mRNA expression in cerebellar interneurons in autism: implications for Purkinje cell dysfunction. Journal of Neuroscience Re-

search 2008; 86:525–530.
[36] Fatemi SH, Reutiman TJ, Folsom TD, Rooney RJ, Patel DH, and Thuras PD. mRNA and protein levels for GABAAalpha4, alpha5, beta1 and GABABR1 receptors are altered in brains from subjects with autism. Journal of autism and developmental disorders 2010; 40:743–750.
[37] Samaco RC, Hogart A, and LaSalle JM. Epigenetic overlap in autism-spectrum neurodevelopmental disorders: MECP2 deficiency causes reduced expression of UBE3A and GABRB3. Human molecular genetics 2005; 14:483–492.
[38] Oblak AL, Gibbs TT, and Blatt GJ. Reduced GABAA receptors and benzodiazepine binding sites in the posterior cingulate cortex and fusiform gyrus in autism. Brain research 2011; 1380:218–228.
[39] Oblak AL, Gibbs TT, and Blatt GJ. Decreased GABA(B) receptors in the cingulate cortex and fusiform gyrus in autism. Journal of neurochemistry 2010; 114:1414–1423.
[40] Enticott PG, Rinehart NJ, Tonge BJ, Bradshaw JL, and Fitzgerald PB. A preliminary transcranial magnetic stimulation study of cortical inhibition and excitability in high-functioning autism and Asperger disorder. Developmental medicine and child neurology 2010; 52:e179–83.
[41] Olsen RW and Avoli M. GABA and epileptogenesis. Epilepsia 1997; 38:399–407.
[42] Treiman DM. GABAergic mechanisms in epilepsy. Epilepsia 2001; 42 Suppl 3:8–12.
[43] Macdonald R. Anticonvulsant drugs: mechanisms of action. [Adv Neurol. 1986] - PubMed result. Advances in neurology 1986.
[44] Tuchman R. Treatment of seizure disorders and EEG abnormalities in children with autism spectrum disorders. Journal of autism and developmental disorders 2000; 30:485–489.
[45] Bombardieri R, Pinci M, Moavero R, Cerminara C, and Curatolo P. Early control of seizures improves long-term outcome in children with tuberous sclerosis complex. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society 2010; 14:146–149.
[46] Irwin SA, Galvez R, and Greenough WT. Dendritic spine structural anomalies in fragile-X mental retardation syndrome. Cerebral cortex (New York, NY : 1991) 2000; 10:1038–1044.
[47] Grossman AW, Aldridge GM, Weiler IJ, and Greenough WT. Local protein synthesis and spine morphogenesis: Fragile X syndrome and beyond. Journal of Neuroscience 2006; 26:7151–7155.
[48] Penagarikano O, Mulle JG, and Warren ST. The pathophysiology of fragile x syndrome. Annual review of genomics and human genetics 2007; 8:109–129.
[49] Comery TA, Harris JB, Willems PJ, Oostra BA, Irwin SA, Weiler IJ, and Greenough WT. Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. Proceedings of the National Academy of Sciences of the United States of America 1997; 94:5401–5404.
[50] Gibson JR, Bartley AF, Hays SA, and Huber KM. Imbalance of neocortical excitation and inhibition and altered UP states reflect network hyperexcitability in the mouse model of fragile X syndrome. Journal of neurophysiology 2008; 100:2615–2626.
[51] Idrissi El A, Ding X-H, Scalia J, Trenkner E, Brown WT, and Dobkin C. Decreased GABA(A) receptor expression in the seizure-prone fragile X mouse. Neuroscience letters 2005; 377:141–146.
[52] D'Hulst C, De Geest N, Reeve SP, Van Dam D, De Deyn PP, Hassan BA, and Kooy RF. Decreased expression of the GABAA receptor in fragile X syndrome. Brain research 2006; 1121:238–245.
[53] Gantois I, Vandesompele J, Speleman F, Reyniers E, D'Hooge R, Severijnen L-A, Willemsen R, Tassone F, and Kooy RF. Expression profiling suggests underexpression of the GABA (A) receptor subunit delta in the fragile X knockout mouse model. Neurobiology of disease 2006; 21:346–357.
[54] D'Hulst C, Atack JR, and Kooy RF. The complexity of the GABAA receptor shapes unique pharmacological profiles. Drug discovery today 2009; 14:866–875.
[55] Adusei DC, Pacey LKK, Chen D, and Hampson DR. Early developmental alterations in GABAergic protein expression in fragile X knockout mice. Neuropharmacology 2010; 59:167–171.
[56] Centonze D, Rossi S, Mercaldo V, Napoli I, Ciotti MT, De Chiara V, Musella A, Prosperetti C, Calabresi P, Bernardi G, and Bagni C. Abnormal striatal GABA transmission in the mouse model for the fragile X syndrome. Biological psychiatry 2008; 63:963–973.
[57] Curia G, Papouin T, Séguéla P, and Avoli M. Downregulation of tonic GABAergic inhibition in a mouse model of fragile X syndrome. Cerebral Cortex 2009; 19:1515–1520.
[58] Musumeci SA, Calabrese G, Bonaccorso CM, D'Antoni S, Brouwer JR, Bakker CE, Elia M, Ferri R, Nelson DL, Oostra BA, and Catania MV. Audiogenic seizure susceptibility is reduced in fragile X knockout mice after introduction of FMR1 transgenes. Experimental neurology 2007; 203:233–240.
[59] Chahrour M and Zoghbi HY. The story of Rett syndrome: from clinic to neurobiology. Neuron 2007; 56:422–437.
[60] Witt Engerström I. Age-related occurrence of signs and symptoms in the Rett syndrome. Brain & development 1992; 14 Suppl:S11–20.
[61] Steffenburg U, Hagberg G, and Hagberg B. Epilepsy in a representative series of Rett syndrome. Acta paediatrica (Oslo, Norway : 1992) 2001; 90:34–39.
[62] Chen WG, Chang Q, Lin Y, Meissner A, West AE,

Griffith EC, Jaenisch R, and Greenberg ME. Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science (New York, NY) 2003; 302:885–889.
[63] Chahrour M, Jung SY, Shaw C, Zhou X, Wong STC, Qin J, and Zoghbi HY. MeCP2, a key contributor to neurological disease, activates and represses transcription. Science (New York, NY) 2008; 320:1224–1229.
[64] Bauman ML, Kemper TL, and Arin DM. Microscopic observations of the brain in Rett syndrome. Neuropediatrics 1995; 26:105–108.
[65] Armstrong DD. Neuropathology of Rett syndrome. Journal of child neurology 2005; 20:747–753.
[66] Blue ME, Naidu S, and Johnston MV. Altered development of glutamate and GABA receptors in the basal ganglia of girls with Rett syndrome. Experimental neurology 1999; 156:345–352.
[67] Shahbazian M, Young J, Yuva-Paylor L, Spencer C, Antalffy B, Noebels J, Armstrong D, Paylor R, and Zoghbi H. Mice with truncated MeCP2 recapitulate many Rett syndrome features and display hyperacetylation of histone H3. Neuron 2002; 35:243–254.
[68] Kishi N and Macklis JD. Dissecting MECP2 function in the central nervous system. Journal of child neurology 2005; 20:753–759.
[69] Dani VS, Chang Q, Maffei A, Turrigiano GG, Jaenisch R, and Nelson SB. Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. Proceedings of the National Academy of Sciences of the United States of America 2005; 102:12560–12565.
[70] Medrihan L, Tantalaki E, Aramuni G, Sargsyan V, Dudanova I, Missler M, and Zhang W. Early defects of GABAergic synapses in the brain stem of a MeCP2 mouse model of Rett syndrome. Journal of neurophysiology 2008; 99:112–121.
[71] Chao H-T, Chen H, Samaco RC, Xue M, Chahrour M, Yoo J, Neul JL, Gong S, Lu H-C, Heintz N, Ekker M, Rubenstein JLR, Noebels JL, Rosenmund C, and Zoghbi HY. Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. Nature 2010; 468:263–269.
[72] Goorden SMI, van Woerden GM, van der Weerd L, Cheadle JP, and Elgersma Y. Cognitive deficits in Tsc1+/- mice in the absence of cerebral lesions and seizures. Annals of neurology 2007; 62:648–655.
[73] Tavazoie SF, Alvarez VA, Ridenour DA, Kwiatkowski DJ, and Sabatini BL. Regulation of neuronal morphology and function by the tumor suppressors Tsc1 and Tsc2. Nature Neuroscience 2005; 8:1727–1734.
[74] Wang Y, Barbaro MF, and Baraban SC. A role for the mTOR pathway in surface expression of AMPA receptors. Neuroscience letters 2006; 401:35–39.
[75] White R, Hua Y, Scheithauer B, Lynch DR, Henske EP, and Crino PB. Selective alterations in glutamate and GABA receptor subunit mRNA expression in dysplastic neurons and giant cells of cortical tubers. Annals of neurology 2001; 49:67–78.
[76] Wong M, Ess KC, Uhlmann EJ, Jansen LA, Li W, Crino PB, Mennerick S, Yamada KA, and Gutmann DH. Impaired glial glutamate transport in a mouse tuberous sclerosis epilepsy model. Annals of neurology 2003; 54:251–256.
[77] Graf ER, Zhang X, Jin S-X, Linhoff MW, and Craig AM. Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. Cell 2004; 119:1013–1026.
[78] Scheiffele P, Fan J, Choih J, Fetter R, and Serafini T. Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. Cell 2000; 101:657–669.
[79] Feng J, Schroer R, Yan J, Song W, Yang C, Bockholt A, Cook EH, Skinner C, Schwartz CE, and Sommer SS. High frequency of neurexin 1beta signal peptide structural variants in patients with autism. Neuroscience letters 2006; 409:10–13.
[80] Kim H-G, Kishikawa S, Higgins AW, Seong I-S, Donovan DJ, Shen Y, Lally E, Weiss LA, Najm J, Kutsche K, Descartes M, Holt L, Braddock S, Troxell R, Kaplan L, Volkmar F, Klin A, Tsatsanis K, Harris DJ, Noens I, Pauls DL, Daly MJ, MacDonald ME, Morton CC, Quade BJ, and Gusella JF. Disruption of neurexin 1 associated with autism spectrum disorder. American journal of human genetics 2008; 82:199–207.
[81] Wiśniowiecka-Kowalnik B, Nesteruk M, Peters SU, Xia Z, Cooper ML, Savage S, Amato RS, Bader P, Browning MF, Haun CL, Duda AW, Cheung SW, and Stankiewicz P. Intragenic rearrangements in NRXN1 in three families with autism spectrum disorder, developmental delay, and speech delay. American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 2010; 153B:983–993.
[82] Zhang C, Atasoy D, Araç D, Yang X, Fucillo MV, Robison AJ, Ko J, Brunger AT, and Südhof TC. Neurexins physically and functionally interact with GABA(A) receptors. Neuron 2010; 66:403–416.
[83] Craig AM and Kang Y. Neurexin-neuroligin signaling in synapse development. Current opinion in neurobiology 2007; 17:43–52.
[84] Chih B, Engelman H, and Scheiffele P. Control of excitatory and inhibitory synapse formation by neuroligins. Science (New York, NY) 2005; 307:1324–1328.
[85] Budreck EC and Scheiffele P. Neuroligin-3 is a neuronal adhesion protein at GABAergic and glutamatergic synapses. The European journal of neuroscience 2007; 26:1738–1748.

[86] Varoqueaux F, Aramuni G, Rawson RL, Mohrmann R, Missler M, Gottmann K, Zhang W, Südhof TC, and Brose N. Neuroligins determine synapse maturation and function. Neuron 2006; 51:741–754.
[87] Jamain S, Quach H, Betancur C, Råstam M, Colineaux C, Gillberg IC, Soderstrom H, Giros B, Leboyer M, Gillberg C, Bourgeron T, Paris Autism Research International Sibpair Study. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nature genetics 2003; 34:27–29.
[88] Laumonnier F, Bonnet-Brilhault F, Gomot M, Blanc R, David A, Moizard M-P, Raynaud M, Ronce N, Lemonnier E, Calvas P, Laudier B, Chelly J, Fryns J-P, Ropers H-H, Hamel BCJ, Andres C, Barthélémy C, Moraine C, and Briault S. X-linked mental retardation and autism are associated with a mutation in the NLGN4 gene, a member of the neuroligin family. American journal of human genetics 2004; 74:552–557.
[89] Lawson-Yuen A, Saldivar J-S, Sommer S, and Picker J. Familial deletion within NLGN4 associated with autism and Tourette syndrome. European journal of human genetics : EJHG 2008; 16:614–618.
[90] Radyushkin K, Hammerschmidt K, Boretius S, Varoqueaux F, El-Kordi A, Ronnenberg A, Winter D, Frahm J, Fischer J, Brose N, and Ehrenreich H. Neuroligin-3-deficient mice: model of a monogenic heritable form of autism with an olfactory deficit. Genes, Brain and Behavior 2009; 8:416–425.
[91] Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, and Südhof TC. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science (New York, NY) 2007; 318:71–76.
[92] Gogolla N, Leblanc JJ, Quast KB, Südhof T, Fagiolini M, and Hensch TK. Common circuit defect of excitatory-inhibitory balance in mouse models of autism. Journal of neurodevelopmental disorders 2009; 1:172–181.
[93] Hamilton SP, Woo JM, Carlson EJ, Ghanem N, Ekker M, and Rubenstein JLR. Analysis of four DLX homeobox genes in autistic probands. BMC genetics 2005; 6:52.
[94] Wu S, Yue W, Jia M, Ruan Y, Lu T, Gong X, Shuang M, Liu J, Yang X, and Zhang D. Association of the neuropilin-2 (NRP2) gene polymorphisms with autism in Chinese Han population. American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 2007; 144B:492–495.
[95] Holt R, Barnby G, Maestrini E, Bacchelli E, Brocklebank D, Sousa I, Mulder EJ, Kantojärvi K, Järvelä I, Klauck SM, Poustka F, Bailey AJ, Monaco AP, EU Autism MOLGEN Consortium. Linkage and candidate gene studies of autism spectrum disorders in European populations. European journal of human genetics : EJHG 2010; 18:1013–1019.
[96] Marsh E, Fulp C, Gomez E, Nasrallah I, Minarcik J, Sudi J, Christian SL, Mancini G, Labosky P, Dobyns W, Brooks-Kayal A, and Golden JA. Targeted loss of Arx results in a developmental epilepsy mouse model and recapitulates the human phenotype in heterozygous females. Brain 2009; 132:1563–1576.
[97] Wang L, Jia M, Yue W, Tang F, Qu M, Ruan Y, Lu T, Zhang H, Yan H, Liu J, Guo Y, Zhang J, Yang X, and Zhang D. Association of the ENGRAILED 2 (EN2) gene with autism in Chinese Han population. American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 2008; 147B:434–438.
[98] International Molecular Genetic Study of Autism Consortium (IMGSAC). A genomewide screen for autism: strong evidence for linkage to chromosomes 2q, 7q, and 16p. American journal of human genetics 2001; 69:570–581.
[99] Liu X, Novosedlik N, Wang A, Hudson ML, Cohen IL, Chudley AE, Forster-Gibson CJ, Lewis SME, and Holden JJA. The DLX1and DLX2 genes and susceptibility to autism spectrum disorders. European journal of human genetics : EJHG 2009; 17:228–235.
[100] Chang S-C, Pauls DL, Lange C, Sasanfar R, and Santangelo SL. Common genetic variation in the GAD1 gene and the entire family of DLX homeobox genes and autism spectrum disorders. American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 2011; 156:233–239.
[101] Pleasure SJ, Anderson S, Hevner R, Bagri A, Marín O, Lowenstein DH, and Rubenstein JL. Cell migration from the ganglionic eminences is required for the development of hippocampal GABAergic interneurons. Neuron 2000; 28:727–740.
[102] Cobos I, Calcagnotto ME, Vilaythong AJ, Thwin MT, Noebels JL, Baraban SC, and Rubenstein JLR. Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. Nature Neuroscience 2005; 8:1059–1068.
[103] Mao R, Page DT, Merzlyak I, Kim C, Tecott LH, Janak PH, Rubenstein JLR, and Sur M. Reduced conditioned fear response in mice that lack Dlx1 and show subtype-specific loss of interneurons. Journal of neurodevelopmental disorders 2009; 1:224–236.
[104] Levi G, Puche AC, Mantero S, Barbieri O, Trombino S, Paleari L, Egeo A, and Merlo GR. The Dlx5 homeodomain gene is essential for olfactory development and connectivity in the mouse. Molecular and Cellular Neuroscience 2003; 22:530–543.
[105] Long JE, Garel S, Depew MJ, Tobet S, and Rubenstein JLR. DLX5 regulates development

of peripheral and central components of the olfactory system. Journal of Neuroscience 2003; 23:568–578.
[106] Anderson SA, Qiu M, Bulfone A, Eisenstat DD, Meneses J, Pedersen R, and Rubenstein JL. Mutations of the homeobox genes Dlx-1 and Dlx-2 disrupt the striatal subventricular zone and differentiation of late born striatal neurons. Neuron 1997; 19:27–37.
[107] Long JE, Garel S, Alvarez-Dolado M, Yoshikawa K, Osumi N, Alvarez-Buylla A, and Rubenstein JLR. Dlx-dependent and -independent regulation of olfactory bulb interneuron differentiation. Journal of Neuroscience 2007; 27:3230–3243.
[108] Wang Y, Dye CA, Sohal V, Long JE, Estrada RC, Roztocil T, Lufkin T, Deisseroth K, Baraban SC, and Rubenstein JLR. Dlx5 and Dlx6 regulate the development of parvalbumin-expressing cortical interneurons. Journal of Neuroscience 2010; 30:5334–5345.
[109] Gant JC, Thibault O, Blalock EM, Yang J, Bachstetter A, Kotick J, Schauwecker PE, Hauser KF, Smith GM, Mervis R, Li Y, and Barnes GN. Decreased number of interneurons and increased seizures in neuropilin 2 deficient mice: implications for autism and epilepsy. Epilepsia 2009; 50:629–645.
[110] Förster E, Tielsch A, Saum B, Weiss KH, Johanssen C, Graus-Porta D, Müller U, and Frotscher M. Reelin, Disabled 1, and beta 1 integrins are required for the formation of the radial glial scaffold in the hippocampus. Proceedings of the National Academy of Sciences of the United States of America 2002; 99:13178–13183.
[111] DeSilva U, D'Arcangelo G, Braden VV, Chen J, Miao GG, Curran T, and Green ED. The human reelin gene: isolation, sequencing, and mapping on chromosome 7. Genome research 1997; 7:157–164.
[112] Persico AM, D'Agruma L, Maiorano N, Totaro A, Militerni R, Bravaccio C, Wassink TH, Schneider C, Melmed R, Trillo S, Montecchi F, Palermo M, Pascucci T, Puglisi-Allegra S, Reichelt K-L, Conciatori M, Marino R, Quattrocchi CC, Baldi A, Zelante L, Gasparini P, Keller F, Collaborative Linkage Study of Autism. Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. Molecular Psychiatry 2001; 6:150–159.
[113] D'Arcangelo G and Curran T. Reeler: new tales on an old mutant mouse. BioEssays : news and reviews in molecular, cellular and developmental biology 1998; 20:235–244.
[114] Carboni G, Tueting P, Tremolizzo L, Sugaya I, Davis J, Costa E, and Guidotti A. Enhanced dizocilpine efficacy in heterozygous reeler mice relates to GABA turnover downregulation. Neuropharmacology 2004; 46:1070–1081.
[115] Macrì S, Biamonte F, Romano E, Marino R, Keller F, and Laviola G. Perseverative responding and neuroanatomical alterations in adult heterozygous reeler mice are mitigated by neonatal estrogen administration. Psychoneuroendocrinology 2010; 35:1374–1387.
[116] Colombo E, Galli R, Cossu G, Gécz J, and Broccoli V. Mouse orthologue of ARX, a gene mutated in several X-linked forms of mental retardation and epilepsy, is a marker of adult neural stem cells and forebrain GABAergic neurons. Developmental dynamics: an official publication of the American Association of Anatomists 2004; 231:631–639.
[117] Friocourt G, Poirier K, Rakić S, Parnavelas JG, and Chelly J. The role of ARX in cortical development. The European journal of neuroscience 2006; 23:869–876.
[118] Kitamura K, Yanazawa M, Sugiyama N, Miura H, Iizuka-Kogo A, Kusaka M, Omichi K, Suzuki R, Kato-Fukui Y, Kamiirisa K, Matsuo M, Kamijo S-I, Kasahara M, Yoshioka H, Ogata T, Fukuda T, Kondo I, Kato M, Dobyns WB, Yokoyama M, and Morohashi K-I. Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. Nature genetics 2002; 32:359–369.
[119] Poirier K, van Esch H, Friocourt G, Saillour Y, Bahi N, Backer S, Souil E, Castelnau-Ptakhine L, Beldjord C, Francis F, Bienvenu T, and Chelly J. Neuroanatomical distribution of ARX in brain and its localisation in GABAergic neurons. Brain research Molecular brain research 2004; 122:35–46.
[120] Cobos I, Broccoli V, and Rubenstein JLR. The vertebrate ortholog of Aristaless is regulated by Dlx genes in the developing forebrain. The Journal of comparative neurology 2005; 483:292–303.
[121] Colombo E, Collombat P, Colasante G, Bianchi M, Long J, Mansouri A, Rubenstein JLR, and Broccoli V. Inactivation of Arx, the murine ortholog of the X-linked lissencephaly with ambiguous genitalia gene, leads to severe disorganization of the ventral telencephalon with impaired neuronal migration and differentiation. Journal of Neuroscience 2007; 27:4786–4798.
[122] Friocourt G, Kanatani S, Tabata H, Yozu M, Takahashi T, Antypa M, Raguénès O, Chelly J, Férec C, Nakajima K, and Parnavelas JG. Cell-autonomous roles of ARX in cell proliferation and neuronal migration during corticogenesis. The Journal of neuroscience : the official journal of the Society for Neuroscience 2008; 28:5794–5805.
[123] Joyner AL, Herrup K, Auerbach BA, Davis CA, and Rossant J. Subtle cerebellar phenotype in mice homozygous for a targeted deletion of the En-2 homeobox. Science (New York, NY) 1991; 251:1239–1243.
[124] Benayed R, Choi J, Matteson P, Gharani N, Kamdar S, Brzustowicz L, and Millonig J. Autism

-Associated Haplotype Affects the Regulation of the Homeobox Gene, ENGRAILED 2. Biological psychiatry 2009.

[125] Cheh MA, Millonig JH, Roselli LM, Ming X, Jacobsen E, Kamdar S, and Wagner GC. En2 knockout mice display neurobehavioral and neurochemical alterations relevant to autism spectrum disorder. Brain research 2006; 1116:166–176.

[126] Tripathi PP, Sgado P, Scali M, Viaggi C, Casarosa S, Simon HH, Vaglini F, Corsini GU, and Bozzi Y. Increased susceptibility to kainic acid-induced seizures in Engrailed-2 knockout mice. Neuroscience 2009; 159:842–849.