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Molecular mechanisms of autism: a possible role for Ca^{2+} signaling

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Autism spectrum disorders (ASDs) are a group of developmental disorders characterized by social and emotional deficits, language impairments and stereotyped behaviors that manifest in early postnatal life. The molecular mechanisms that underlie ASDs are not known, but several recent developments suggest that some forms of autism are caused by failures in activity-dependent regulation of neural development. Mutations of several voltage-gated and ligand-gated ion channels that regulate neuronal excitability and Ca^{2+} signaling have been associated with ASDs. In addition, Ca^{2+}-regulated signaling proteins involved in synapse formation and dendritic growth have been implicated in ASDs. These recent advances suggest a set of signaling pathways that might have a role in generating these increasingly prevalent disorders.

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Introduction

Recent epidemiological studies have reported a dramatic increase in the prevalence of autism spectrum disorders (ASDs) over the past 15 years. As many as 1 in every 166 children is diagnosed with ASDs, and yet the etiology of these disorders remains largely unknown [1]. Neuropathological studies of autistic patients suggest a widespread defect in neuronal development that manifests in the early postnatal years. These developmental defects can lead to disruptions in the connectivity between brain areas involved in higher-order associations (see also review by Geschwind and Levitt, in this issue). Genetic studies suggest that autism has a strong genetic component that might involve the interaction of many different genes [2]. Many ASD candidate genes have been identified through genome scans for susceptibility loci on human chromosomes and through linkage and association studies [3]. In a few cases, specific mutations in individual genes have been found to cause both syndromic and non-syndromic forms of ASD [2]. Despite these advances, the molecular and cellular pathways that are perturbed in ASDs and the nature of these perturbations remain poorly understood.

A considerable challenge in deciphering the causes of ASDs lies in understanding how mutations in many different genes can ultimately produce specific deficits in cognitive and social behavior. One approach to tackle this problem is to look for signaling cascades that involve multiple ASD candidate genes. Recent studies have identified many such genes that either directly or indirectly control intracellular Ca^{2+} levels or are regulated by elevations in neuronal Ca^{2+} levels. These genes encode ion channels, neurotransmitter receptors and Ca^{2+}-regulated signaling proteins that are crucial for development of the central nervous system.

Early in neural development, spontaneous and sensory-driven electrical activity leads to increased intracellular Ca^{2+} levels and to activation of signaling pathways that are important in regulating processes such as neuronal survival, differentiation, migration and synaptogenesis (for reviews, see [4–9]). Defects in these developmental processes could give rise to some of the neuroanatomical abnormalities identified in ASD patients, including increases in cell-packing density, decreases in neuron size and arborization, and alterations in connectivity (for reviews, see [10,11]). Here, we review genetic evidence from the past two years that supports the hypothesis that defects in activity-dependent signaling events are a molecular cause of autism. We also discuss a possible link between environmental factors that might lead to autism, and Ca^{2+} signaling pathways in neurons that have been implicated in ASDs.

Voltage-gated ion channels

Three recent studies [12••,13•,14•] show that functional mutations in genes encoding voltage-gated Ca^{2+} channels can lead to ASDs. Point mutations in the gene encoding the L-type voltage-gated Ca^{2+} channel $Ca_V1.2$ (*CACNA1C*) cause Timothy syndrome, a multisystem disorder that includes cardiac abnormalities and autism [12••,15]. $Ca_V1.2$ channels are expressed predominantly in the dendrites and cell bodies of mature neurons, where they regulate both neuronal excitability and the activation of various Ca^{2+}-regulated signaling cascades [16,17]. $Ca_V1.2$ is particularly important for activation of transcription factors that have key roles in promoting neuronal survival and

dendritic arborization, such as cAMP-response-element binding protein (CREB) and myocyte enhancer factor 2 (MEF2) [18]. The mutations associated with Timothy syndrome prevent voltage-dependent inactivation of $Ca_V1.2$, which causes the channels to have longer open periods and carry more Ca^{2+} than wild type channels [12••]. However, the developmental and cell-biological consequences of these mutations in neurons are not known.

A similar mutation in *CACNA1F*, which encodes the L-type voltage-gated Ca^{2+} channel $Ca_V1.4$, causes autistic symptoms in a New Zealand family of patients who have stationary night blindness [13•,19]. Similar to the Timothy syndrome mutation, this mutation also prevents voltage-dependent inactivation of the channel and is predicted to produce excessive Ca^{2+} influx. Interestingly, the phenotype of patients who have *CACNA1F* loss-of-function includes night blindness but not autism, suggesting that a gain-of-function in the channel is necessary for the development of autism.

In addition to the L-type Ca^{2+} channels, a T-type voltage gated Ca^{2+} channel has also been implicated in ASDs [14•]. In one group of autistic patients, missense mutations in the gene encoding $Ca_V3.2$ (*CACNA1H*) were associated with decreased channel activity. The function of T-type channels in the brain is not completely understood, but they are known to regulate the oscillatory behavior of neurons in the cortex and thalamus [20]. Although the mutations in T-type channel genes alone were not responsible for ASDs, they were significantly more common in ASD patients than in controls, providing further evidence for an involvement of Ca^{2+} channels and signaling in autism.

ASD-associated mutations have been identified not only in genes encoding Ca^{2+} channels themselves but also in genes encoding ion channels whose activity is directly modulated by Ca^{2+}. Several point mutations in *SCN1A* and *SCN2A*, which encode the voltage-activated Na^+ channels $Na_V1.1$ and $Na_V1.2$, respectively, are associated with childhood epilepsy and autism [21]. Activity-dependent phosphorylation of *MECP2* was recently found to regulate the expression of BDNF in response to increases in neuronal activity [22,23•]. The effects of these mutations on channel function are not known, but one of the mutations in *SCN1A* lies in the calmodulin binding site of the channel and reduces its affinity for Ca^{2+}-bound calmodulin [21]. This mutation also produced a large Ca^{2+}-dependent conformational change in the $Na_V1.2$–calmodulin complex, which might confer upon $Na_V1.2$ a novel sensitivity to Ca^{2+} signaling [24]. In addition, mutations in a similar C-terminal region of other voltage-gated Na^+ channels reduce the amount of channel inactivation, raising the possibility that excessive ion channel activity leads to ASDs in these patients [24,25].

A recent report [26•] suggests that there is also an association between a Ca^{2+}-activated K^+ channel (BK_{Ca}) and ASDs. Disruption of the BK_{Ca} gene *KCNMA1* led to haploinsufficiency and reduced BK_{Ca} activity. BK_{Ca} channels are expressed throughout the brain and are localized mainly to the presynaptic active zone, where they help to regulate synaptic transmission and neuronal excitability [27]. These channels are directly activated by increased Ca^{2+} levels or depolarization and they rapidly hyperpolarize the membrane and terminate Ca^{2+} influx through voltage-gated Ca^{2+} channels. Thus, the reported decrease in BK_{Ca} channel activity, together with the reduced inactivation of voltage-gated Ca^{2+} channels in autistic patients, suggests that some forms of autism are due to abnormally sustained increases in intracellular Ca^{2+} levels.

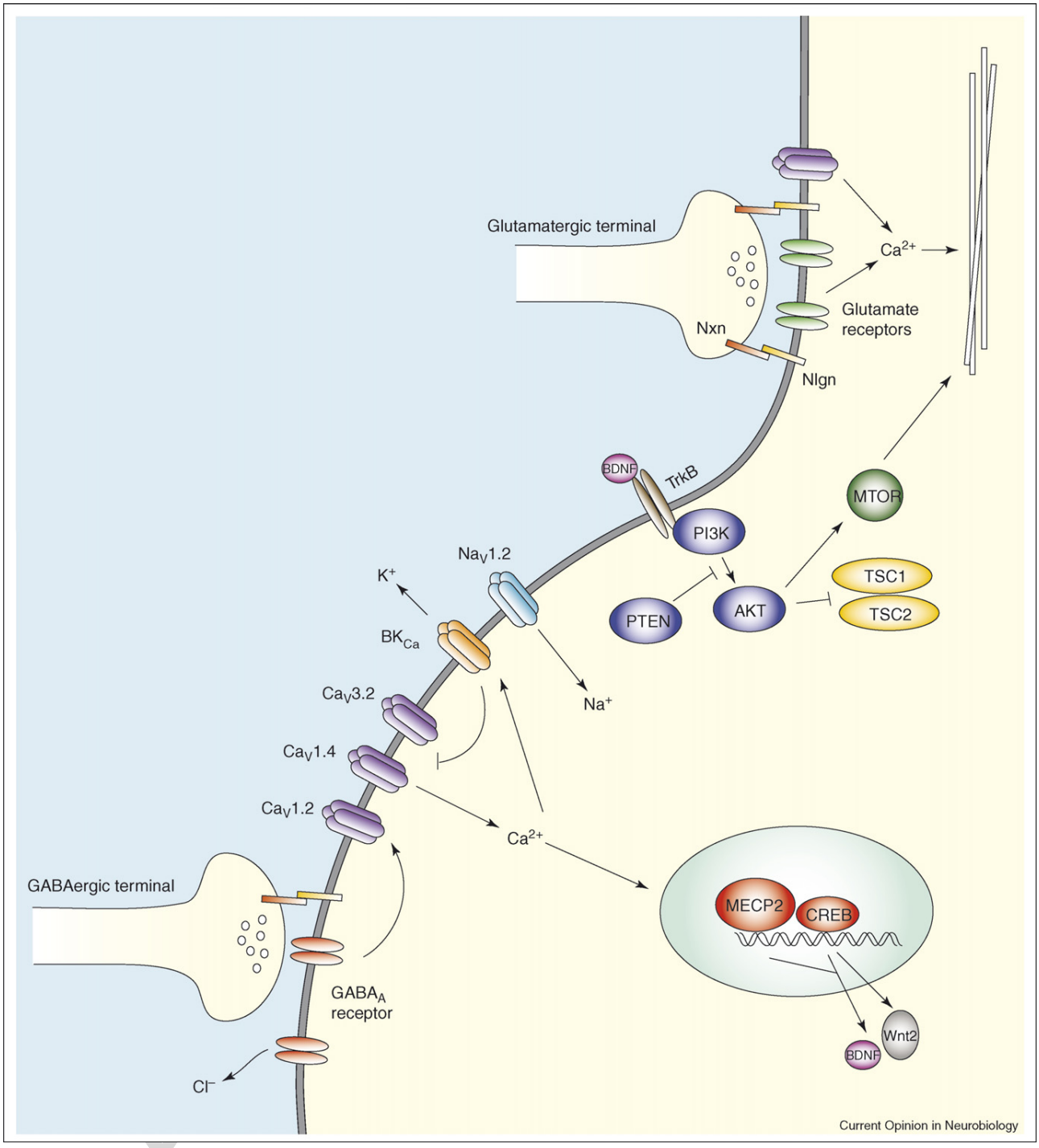
Neurotransmitter receptors

Abnormalities in neuronal excitability during development could also be caused by alterations in neurotransmitter systems. Glutamate receptors are ligand-gated Ca^{2+} channels that are important in many activity-dependent developmental processes. Polymorphisms in *GRIN2A*, which encodes an NMDA receptor subunit, have been associated with ASDs [28]. ASD-associated polymorphisms have also been reported in the gene encoding subunit 2 of the kainate ionotropic glutamate receptor (*GRIK2*) and in metabotropic glutamate receptor genes [29,30]. The effects of these polymorphisms are not yet known but they raise the possibility that alterations in glutamate signaling underlie some forms of ASD.

Among neurotransmitter systems, some of the strongest evidence supports defects in the GABAergic inhibitory system in autism. Autistic patients are frequently reported to have rearrangements in chromosome 15q11-13, an area known to contain a cluster of GABA receptor genes that includes *GABRA5*, *GABRG3* and *GABRB3* [31–33]. Polymorphisms in *GABRA4* have also been associated with autism [34,35]. Angelman syndrome, a severe developmental seizure disorder that includes mental retardation and autism, results from a deletion of chromosome 15q11-13a, which includes the *GABRB3* gene [33,36]. Mouse models of Angelman syndrome have epilepsy and could be useful for understanding how alterations in the GABAergic system at different points in development could result in ASDs [37].

In addition to the GABA receptor genes themselves, genes involved in the differentiation and migration of GABAergic interneurons have also been associated with ASDs. The transcription factor ARX is expressed in several cell types throughout the forebrain and is thought to regulate the development of GABAergic neurons in the basal ganglia and cortex [38]. Mutations of *ARX* can lead to epilepsy, movement disorders, cortical malformations, mental retardation and, in some cases, autism [39,40].

Figure 1



Activity-related signaling proteins implicated in ASDs. Membrane depolarization activates voltage-gated Na^+ channels ($Na_V1.2$) and voltage-gated Ca^{2+} channels ($Ca_V1.2$, $Ca_V1.4$ and $Ca_V3.2$), which leads to an increase in neuronal excitability. Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCCs) can in turn increase the inactivation of $Na_V1.2$ and activate Ca^{2+}-activated K^+ channels (BK_{Ca}), which hyperpolarize the membrane and inhibit VGCC activity. Early in development, activation of $GABA_A$ receptors depolarizes the membrane and causes Ca^{2+} influx through VGCCs, particularly $Ca_V1.2$. Ca^{2+} influx through glutamate receptors (NMDA receptor 2a subunit, GluR6 kainate receptor and metabotropic glutamate receptor 6) and VGCCs leads to increased neurite growth by acting on the cytoskeleton and can activate Ca^{2+}-regulated transcriptional regulators such as CREB and MECP2. Activity-dependent phosphorylation of MECP2 and CREB increases transcription of the growth factor BDNF, and CREB also increases transcription of the secreted morphogen Wnt2. BDNF activates TrkB receptors and the

DLX-family transcription factors also regulate the longevity and differentiation of inhibitory GABAergic interneurons, and polymorphisms in *DLX2*, in *DLX5* and in an intragenic enhancer of *DLX5* and *DLX6* have been found in families with ASDs [41]. A recent study has also linked decreased expression of the MET receptor tyrosine kinase gene with autism susceptibility [42•]. The MET receptor responds to hepatocyte growth factor and has many neurodevelopmental functions, including regulating the migration of GABAergic interneurons into the cortex [43,44]. Unlike pyramidal neurons, GABAergic interneurons are thought to migrate tangentially for considerable distances, making their migration more susceptible to genetic or environmental disturbances during early development [45].

Together, these results suggest that autism is due to deficiencies not only in GABA receptor expression or function but also in the differentiation and migration of GABAergic neurons into the cortex. These findings are consistent with the hypothesis that autism is caused by suppression of GABAergic pathways, leading to hyperexcitability in the brain and problems in filtering out excess stimuli from environmental and intrinsic sources [46,47]. However, GABA is a principal excitatory neurotransmitter during early development, when it leads to neuronal depolarization and Ca^{2+} influx through voltage-gated Ca^{2+} channels. Such GABA-evoked Ca^{2+} transients are thought to be important in many neurodevelopmental processes, including cell proliferation and migration, dendritic arborization and Purkinje cell development (reviewed in [7]). Activation of GABA receptors is involved in the generation of spontaneous synchronous network activity and corresponding Ca^{2+} waves in the developing cortex [48–50]. Thus, defects in GABA receptor function could lead to autism by perturbing patterns of spontaneous activity and related activity-dependent events in early cortical and cerebellar development.

Signaling proteins

ASDs are also associated with mutations in Ca^{2+}-regulated signaling pathways (Figure 1). Mice deficient in the Ca^{2+}-dependent chromatin regulator methyl CpG-binding protein 2 (Mecp2) display delayed neuronal maturation in the cerebral cortex, with reduced dendrite growth and dendritic spine density and a thinner cortex at four weeks of age compared with wild type mice [51,52]. This defect might be linked to reduced levels of brain-derived neurotrophic factor (BDNF), which has effects on neuronal morphology.

BDNF has a key role in development of the cortex and the cerebellum, in part by activating the phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt) signaling pathway, which affects dendritic arborization and local protein synthesis in dendrites [53,54]. Patients who have mutations in phosphatase and tensin homolog on chromosome ten (*PTEN*), a tumor suppressor gene that encodes a lipid phosphatase that reverses phosphorylation of Akt by PI3K, are more prone to tumors and neurological defects such as autism [55,56]. Conditional mouse knockouts of *Pten* displayed hypertrophic and ectopic dendritic and axonal arbors, and an increase in spine and synapse number [57••]. The GTPase-activating proteins TSC1 and TSC2 are repressed by Akt phosphorylation [58–60], an event that leads to activation of the mammalian target of rapamycin (mTOR; also known as FRAP1) and to increases in dendritic arborization and local protein synthesis. Heterozygous mutations in *TSC1* or *TSC2* cause tuberous sclerosis, an autosomal-dominant neurocutaneous disorder that leads to high rates of autism and epilepsy [61], and loss of a single copy of *Tsc1* is sufficient to perturb dendritic spine morphology and density in mice [62•].

In addition to BDNF another secreted protein, WNT2, has also been implicated in ASDs. Two families with mutations in *WNT2* have been identified, and a polymorphism in an upstream region of *WNT2* has been associated with families defined by severe language abnormalities [63]. Increases in Ca^{2+} concentrations have recently been shown to enhance Wnt synthesis and release, through activity of the Ca^{2+}-regulated transcription factor CREB [64•]. Wnt genes could therefore contribute susceptibility to autism, through disruptions in early patterning and/or through alteration of activity-dependent processes later in development. As further support for a role of Wnt signaling pathways in ASDs, mice lacking Dishevelled 1, a crucial component of the Wnt signaling cascade, show abnormalities in development of social behaviors and in sensorimotor gating, making these mice a promising model for ASDs [65].

Several ASDs have been linked to genes that regulate synaptogenesis. Neuroligins are a family of cell-adhesion molecules that bind to the postsynaptic organizer protein PSD95 and help orchestrate recruitment of neurotransmitter receptors to the postsynaptic site [66] (see also review by Craig and Kang, in this issue). Some neuroligins seem to be important for regulating the balance of excitatory to inhibitory synapses [67]. Two studies have found point mutations in *NLGN3* and *NGLN4* in autistic patients and in non-autistic mentally retarded males [68,69]. When introduced into cultures of hippocampal neurons, these mutations cause defects in protein trafficking and

(Figure 1 Legend Continued) PI3K–AKT signaling pathway, which also regulates neurite growth and synaptogenesis. Neuroligins (Nlgn) and their presynaptic adhesion partners the neurexins (Nxn) regulate excitatory and inhibitory synapse formation and maturation and can also be regulated by Ca^{2+}.

severe impairment of presynaptic differentiation [70]. These observations suggest that defects in synaptogenesis are strongly correlated with ASDs. Interestingly, neuroligins contain two putative, Ca^{2+}-binding EF-hand domains, and the binding of neuroligins to their cognate adhesion molecules (β-neurexins) on presynaptic sites depends on Ca^{2+} [71]. Thus, defects in Ca^{2+} signaling affect synaptogenesis in addition to dendritic arborization, cell survival and gene expression.

Environmental influences

Although autism has the strongest genetic component of any neuropsychiatric disorder, the discordance in autism diagnosis between some monozygotic twins and the recent sharp rise in ASD prevalence suggest that the environment might also have an effect [2]. One of the most controversial and widely debated environmental influences is ethyl mercury, which is a component of some vaccinations [72]. Recent epidemiological studies suggest that these vaccines do not have a large effect on the development of ASDs, but a role for other sources of ethyl and methyl mercury cannot be discarded [73]. Both ethyl and methyl mercury increase Ca^{2+} signals by altering ryanodine receptors and other Ca^{2+} signaling mechanisms in neurons, suggesting that they affect development by altering Ca^{2+}-dependent pathways [74,75]. In addition to mercury, embryonic exposure to the anticonvulsant valproic acid has been shown to lead to ASDs in a substantial percentage of patients [76–78]. Exposure of embryonic rats to valproic acid can lead to neuroanatomical and behavioral deficits similar to those seen in ASDs [79•,80,81]. Valproic acid is a common antiepileptic drug that can increase GABA production and so might increase electrical activity in the developing embryonic brain.

Conclusion

Ca^{2+} signaling pathways integrate environmental stimuli with genetic programs to sculpt the adult nervous system. A body of recent genetic evidence suggests that some ASDs result from a failure in Ca^{2+}-dependent development of the central nervous system. Whether this is a common cause of ASDs will need to be determined, both by epidemiological studies, to correlate ASDs with measures of cellular excitability such as cardiac QT intervals and electroencephalograms (EEGs), and by looking for additional ASD candidate genes. Much is known about the molecules that regulate activity-dependent development of the central nervous system and these will be a fertile ground to search for additional ASD candidate genes. Most of the ASD-associated mutations in genes that encode Ca^{2+}-regulatory molecules lead to an increase in Ca^{2+} signaling, suggesting that ASDs might arise from excessive activation of Ca^{2+}-dependent processes. The specific mechanisms that connect misregulation of Ca^{2+} signaling to the complex phenotype of ASD patients is a key question that will need to be addressed. Finally it will be interesting to test whether existing FDA-approved drugs that modify Ca^{2+} signaling are effective pharmacological agents to treat or prevent ASDs.

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