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The Neurobiology of Autism

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Improving clinical tests are allowing us to more precisely classify autism spectrum disorders and diagnose them at earlier ages. This raises the possibility of earlier and potentially more effective therapeutic interventions. To fully capitalize on this opportunity, however, will require better understanding of the neurobiological changes underlying this devastating group of developmental disorders. It is becoming clear that the normal trajectory of neurodevelopment is altered in autism, with aberrations in brain growth, neuronal patterning and cortical connectivity. Changes to the structure and function of synapses and dendrites have also been strongly implicated in the pathology of autism by morphological, genetic and animal modeling studies. Finally, environmental factors are likely to interact with the underlying genetic profile, and foster the clinical heterogeneity seen in autism spectrum disorders. In this review we attempt to link the molecular pathways altered in autism to the neurodevelopmental and clinical changes that characterize the disease. We focus on signaling molecules such as neurotrophin, Reelin, PTEN and hepatocyte growth factor, neurotransmitters such as serotonin and glutamate, and synaptic proteins such as neurexin, SHANK and neuroligin. We also discuss evidence implicating oxidative stress, neuroglial activation and neuroimmunity in autism.

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INTRODUCTION

Autism spectrum disorders (ASD) are the most devastating conditions in the broad range of developmental abnormalities known as "pervasive developmental disorders" (175). ASD comprise a complex and heterogeneous group of conditions that include autism, Rett and Asperger syndromes, and pervasive developmental disorder-otherwise nonspecified (2). The main clinical features of ASD are stereotypic behaviors and marked impairment in communication, social skills and cognition (129, 174). Clinical signs of ASD are frequently present at 3 years of age and recent prospective studies in toddlers indicate that abnormalities in social, communication and play behavior that may represent early indicators of autism can be detected as early as 14 months of age (124). Abnormalities in language development, mental retardation and epilepsy are frequent problems in the clinical profiles of patients with autism, and some patients may exhibit features of clinical regression, in which neurodevelopmental milestones are lost and/or other clinical signs worsen (174). ASD are clinically heterogeneous and can be associated in up to 10% of patients with well-described neurological and genetic disorders, such as tuberous sclerosis, fragile X, Rett's and Down syndromes, although in most patients the causes are still unknown (159, 176) (see review by London). The heterogeneity and clinical variability of autism has prompted some researchers to use the term *autisms* instead of autism (81).

The stereotypic behaviors and marked delay or disruption of communication and social behavior trajectories that characterize ASD indicate that crucial neuroanatomic structures and neurodevelopmental pathways may be affected during intra-uterine and/or early postnatal brain development. Several lines of research indicate that ASD are associated with disarrangement of neuronal organization, cortical connectivity and neurotransmitter pathways. While the causes of these abnormalities are still being identified, it is generally believed that genetic as well as environmental factors are involved in the pathogenesis of ASD (98, 147, 164). This review focuses on the current knowledge of molecular and cellular factors that may contribute to pathogenic mechanisms in ASD, and examines how they might affect the development and functioning of the central nervous system (CNS).

THE NEUROANATOMICAL AND NEURODEVELOPMENTAL BASIS OF ASD

Different approaches, including clinical assessment, neuroimaging and neuropathological studies have been used to assess the structural and morphological brain abnormalities in ASD. One consistent finding in ASD is altered brain growth, which has been extensively documented by Courchesne et al (54). The clinical onset of autism appears to be preceded by two phases of brain growth abnormalities: a reduced head size at birth, then a sudden and excessive increase between 1–2 months and 6–14 months of age (54, 57). Furthermore, these reports and other recent neuroimaging studies have shown that an abnormal pattern of brain overgrowth also occurs in areas of the frontal lobe, cerebellum and limbic structures between 2 and 4 years of age, a pattern that is followed by abnormal slowness in brain growth (54, 55, 57, 192). These brain regions are intimately involved in the development of social, communication and motor abilities that are impaired in ASD. For example, social orienting deficits in ASD were linked to abnormalities in frontal brain mechanisms involved in associating rewards with goal-directed activity (62, 201). A recent clinical study found that a head circumference >75th percentile is associated with more impaired adaptive

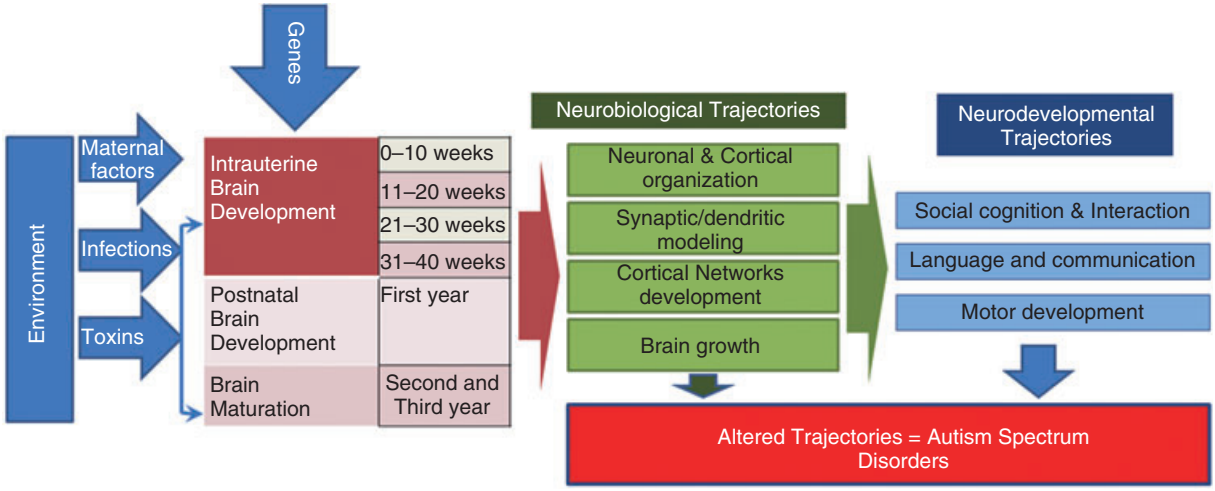

Figure 1. Genetic and environmental factors that influence intrauterine and early postnatal brain development likely alter neurobiological and neurodevelopmental trajectories that determine the clinical core of ASD.

behaviors and with less impairment in IQ measures and motor and verbal language development (182). Neuroimaging studies have also demonstrated an overall enlargement of brain volume associated with increased subcortical white matter in the frontal lobe, and abnormal patterns of growth in the cerebral cortex, amygdala and hippoccampal formations (see review by Herbert (95)). A detailed parcellation study of the cerebral white matter showed increased volume of the subcortical or outer radiate white matter in all lobes, but most remarkable in the frontal lobe, supporting the view that an overgrowth of intrahemispheric and cortico-cortical connections rather than interhemispheric connections occur in patient with autism and language-associated developmental disorders (96, 97). Other studies of cortical and cerebral white matter volumes are indicative of inter-regional disconnectivity (95–97), potentially resulting in poor integration within and across neurobehavioral developmental domains (56, 117).

Other novel neuroimaging approaches such as diffusion tensor imaging (DTI) and functional magnetic resonance imaging (fMRI) have also demonstrated disruption of white matter tracts and disconnection between brain regions in patients with autism. DTI of the brain reveals reduced fractional anisotropy values in white matter adjacent to the ventromedial prefrontal cortices, anterior cingulated gyrus and superior temporal regions, suggesting disruption of white matter tracts in brain regions involved in social functioning (9). Interestingly, fMRI of the brain has also shown abnormal patterns of activation and synchronization across different cortical and subcortical regions. This includes reduction in the functional connectivity and decreased correlation of the time series involved in higher order tasks that include language, working memory, problem solving and social cognition (reviewed by Minshew (147)).

Post-mortem neuropathological studies also show disturbances in neuronal and cortical organization (reviewed in this issue by Casanova). Indeed, cytoarchitectural organizational abnormalities of the cerebral cortex, cerebellum, and other subcortical structures appear to be the most prominent neuropathological changes in autism (7, 112). An unusual laminar cytoarchitecture with packed small neurons has been described in the classical neuropathological studies by Kemper and Bauman, but no abnormalities in the external configuration of the cerebral cortex were noted (112). Cerebellar and brainstem pathology was also prominent, with loss and atrophy of Purkinje cells, predominantly in the posterolateral neocerebellar cortex. Kemper and Bauman (11, 112) have delineated at least three different types of pathological abnormalities in autism: (i) a curtailment of the normal development of neurons in the forebrain limbic system, (ii) an apparent decrease in the cerebellar Purkinje cell population, and (iii) age-related changes in neuronal size and number in the nucleus of the diagonal band of Broca, the cerebellar nuclei and the inferior olive. Most recently, studies of the amygdala showed an abnormal pattern of growth with an overall decrease number of neurons (190, 191). These observations suggest that delays and disarrangements in neuronal maturation are important in the pathogenesis of autism (55), although the possibility that Purkinje cells or other neurons were initially present and subsequently degenerated must also be considered. In addition to these cytoarchitectural abnormalities, the structure and number of cortical minicolumns, narrow chains of neurons that extend vertically across layers 2–6 (151) to form anatomical and functional units, appear to be abnormal in ASD. Minicolumns in brain from patients with ASD are more numerous, smaller, and less compact in their cellular configuration in the frontal and temporal regions, as compared with controls ((34) and review by Casanova in this issue).

Taken together, clinical, neuroimaging and neuropathological studies support the hypothesis that autisms are disorders of neuronal-cortical organization that cause alterations of information processing at different levels of the nervous system, from synaptic and dendritic organization to pathway connectivity and brain structure (81, 147). These neurobiological alterations likely affect the developmental trajectory of social behavior and communication during early stages of childhood (124) and appear to be influenced by both genetic and environmental factors (Figure 1). Some of the morphological abnormalities (eg, minicolumnar disorganization) suggest the events involved in the

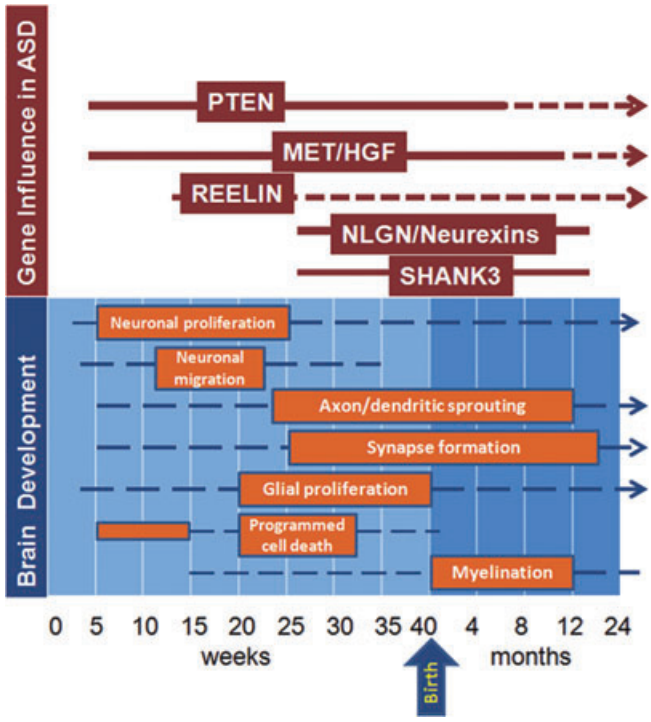

Figure 2. Multiple genes associated with autism spectrum disorders (ASD) appear to influence neurodevelopment at different stages of prenatal and postnatal life. These genes have specific periods of influence (red solid line) during defined stages of brain development (orange boxes), but their influence may extend to later stages of development including adult life (red broken lines). (Brain development graphic concept based on review by de Graaf-Peters and Hadders-Algra. (63))

pathogenesis of ASD occur early during neurodevelopment, perhaps during first and second trimester of gestation. However, there is still uncertainty about the precise timing of the neuronal and cortical changes in ASD. For example, there is lack of clear gyral or cortical lamination abnormalities (103), a common feature of neurodevelopmental disorders originating at early stages such as those that occur during the first or second trimester.

GENETICS AND NEUROBIOLOGY OF ASD

The major role of genetics in autism is clear, as a concordance rate of 60% to 92% is seen in monozygotic twins. Recent studies have further documented the genetic complexity of ASD, and highlight the polygenic nature of the disorder (160, 187, 194, 205, 220). From these and other analyses, it is clear that molecular pathways with the potential to disrupt neurodevelopmental trajectories *in utero* or after birth are involved in the pathogenesis of ASD. Such pathways may be associated with many different developmental processes, from neuronal migration and cortical organization to synaptic and dendritic conformation. Environmental factors (159), including both maternal/intrauterine and postnatal events, may well modify the underlying genetic substrate and lead to greater abnormalities in neuronal organization and cortical network development. In the sections below, we further discuss the range of neurobiological changes in ASD, and associate them when possible with potential genetic etiologies. We have attempted to use a neuroanatomical framework in organizing this part of the review (Figure 2), while recognizing that many of the molecular pathways implicated in autism have effects on multiple CNS processes.

Neuronal and cortical organization. Molecular pathways critical for normal neuronal and cortical organization that have been implicated in patients with ASD include those directed by growth factors such as hepatocyte growth factor (HGF) and its receptor MET, neurotrophic factors such as brain-derived neurotrophic factor (BDNF), serotonin and other neurotransmitters, and signaling proteins such as Reelin.

MET and the HGF pathway. Both genetic and protein expression studies have associated the receptor MET and its ligand HGF with ASD. A recent case–control study demonstrated a strong association of a single nucleotide polymorphism (G-to-C) in a common 5′ promoter of the MET gene with ASD. The relative risk of ASD diagnosis was 2.27 in subjects with the C/C as compared with the G/G genotype (32). This study is especially relevant because the MET gene is located at 7q31 in one of the regions most commonly associated by genetic linkage studies with ASD (104, 220). MET is a transmembrane receptor that possesses tyrosine kinase activity (14, 24) and is activated by binding to HGF, also termed scatter factor or hepatopoietin A. HGF and MET, are present in both developing and adult mammalian brains, suggesting important functions across a broad range of neurodevelopment (115). HGF acts as a neurotrophic factor for motor, sensory and parasympathetic neurons (203), and influences neuronal migration (169, 170) and dendritic development (91). The HGF/MET pathway also plays a role in regulating dendritic morphology in the developing cerebral cortex and promoting neurite outgrowth (170). Decreased levels of MET itself and altered levels of mRNA of proteins associated with the HGF/MET pathway have been documented in brain tissues from patients with ASD (33). In addition to these genetic observations and brain tissue findings, we have documented increased levels of HGF in cerebrospinal fluid (CSF) of patients with autism (211), suggesting a potential compensatory feedback mechanism.

Interestingly, the multifunctional roles of the HGF/MET pathway also involve the immune system, as studies have demonstrated expression of MET in dendritic cells (161) and during activation of monocytes (12). HGF-stimulated monocytes increased the expression of chemoattractant factors including MCP-1, MIP-2β, MIP-1α and IL-8 (13). HGF also exhibited immunosuppressive effects without up-regulation of IL-10 or TGF-β (161), findings that suggest HGF/MET signaling is involved in regulation of the inflammatory responses. Because some of the non-neurological manifestations of ASD include immune and gastrointestinal prob-

lems, the dysregulation of HGF/MET may provide a link between dysfunction of the CNS and other organs.

Reelin. RELN, which encodes the protein Reelin is another gene playing a critical role in cortical patterning that may be involved in autism. Reelin is a secreted extracellular matrix protein that controls neuronal migration, cortical layering and other aspects of brain development via interactions with lipoprotein receptors (reviewed by Forster (77)). It was initially implicated in ASD based on associations between a polymorphic GCG repeat immediately 5′ of the RELN gene and autism in both case-control and family-based studies in an Italian population (166). The fact that RELN is located on the distal long arm of chromosome 7 at a locus (7q22) associated with autism susceptibility added further support to the concept that Reelin function might be important, as did the reduced levels of Reelin found in post-mortem studies of autistic brains (73). Attempts to confirm these intriguing preliminary findings have yielded varied results. Some reports have supported an association between genetic changes in the RELN locus and autism (196, 199, 224), while others have not (22, 66, 118).Transgenic mouse studies are also suggestive, but not definitive, with some social changes and defects in cortical layering observed in mice mutant in RELN alleles (186).

Neurotrophins. Neurotrophic growth factors, or neurotrophins, are good candidates for involvement in ASD because of their fundamental roles in guiding CNS development and cortical organization, and their abnormal expression patterns in autistic individuals. The core functions of neurotrophins during neurodevelopment include regulation of cell proliferation, migration and survival, and extend to include the modulation of axonal and dendritic outgrowth, synapse formation and other neuroplastic processes (5). The neurotrophin family consists of at least four proteins, including nerve growth factor, BDNF, neurotrophin-3 and neurotrophin-4 (92). Their potential role in pathogenic ASD pathways has been examined in several studies involving a heterogeneous groups of neurodevelopmental disorders (146, 155, 179).

Neurotrophins and their receptors are expressed in the neocortex and hippocampus (102) and these patterns of neurotrophin expression are activity-dependent and regulated by sensory inputs, electrical activity and stimulation (102) (138). BDNF and its receptor, trkB, are densely expressed on cortical and hippocampal neurons, and influence both axonal and dendritic growth in a highly neuron-specific and age-dependent manner (139). In rodents, the expression of the trkB receptor peaks in the first 2 weeks postnatally, but BDNF action on cortical plasticity continues into adulthood (119, 139). With maturation, trkB becomes enriched at the site of glutamatergic synapses and therefore uniquely able to modulate experience-dependent plasticity (85).

Interestingly, abnormalities in neurotrophins, especially BDNF, have been implicated in the etiology of several brain disorders that show altered cortical maturation and plasticity, such as schizophrenia and depression (158, 197). Genetic studies and expression of BDNF in serum of patients with ASD have pointed out potential links to the pathogenesis of autism. Nelson et al found elevated levels of BDNF and NT4/5 by assessment of archived neonatal blood samples of ASD patients (155). Elevation of BDNF was also reported in a study of 18 Japanese children with ASD as compared with controls (148), and the authors suggested hyperactivity of this growth factor may be involved in neurobiological abnormalities in autism. Similar findings were reported in a study of American children with ASD, where elevation of BDNF was demonstrated along with the presence of auto-antibodies against BDNF (47, 153, 206).

It is still unknown how these observations fit into the neurodevelopmental pathogenesis of ASD, and it is unclear whether the increase in BDNF is a primary pathogenic mechanism or a secondary reaction to cortical abnormalities in ASD. However, one report suggesting that genetic changes in autistic individuals account for altered neurotrophin levels supports the notion that BDNF dysregulation could be a primary factor in the development of autism. CADPS2 is a gene found in the AUTS1 susceptibility locus for autism on 7q31 (42). Sadakata et al have recently shown that CADPS2 is aberrantly spliced in some autistic patients, and that Cadps2 knockout mice have autistic-like phenotypes. CADPS2 regulates the exocytosis of dense-core vesicles, including BDNF-containing vesicles. In addition, the cellular distribution of BDNF in the brain largely overlaps with that of CADPS2 (183, 184).

Neurotransmitters. Several lines of research suggest that abnormalities in serotoninergic, GABAergic and glutamatergic pathways occur in autism (reviewed by Zimmerman (225)). Neurotransmitter function in the CNS is linked not only to synaptic neuronal interactions, but also to other roles including brain maturation and cortical organization. Neurotransmitters and their receptors may act as paracrine signaling molecules in the immature brain and help control mechanisms that govern neuronal migration and positioning (134). It is well known that activation of specific GABA and glutamate receptors (GluRs) occurs during cell migration, and is involved in regulating radial and tangential migration (134). Because of these diverse functions, neurotransmitters and their receptors are clearly capable of playing central roles in the wide variety of neurobiological alterations associated with ASD.

The role of serotonin in autism has been explored using biomarker, neuroimaging and genetic approaches (193). The most relevant brain imaging studies used positron emission tomography to show that young children with autism lacked the developmental peak in brain 5-HT synthesis capacity seen in typically developing infants (36) (41). Reduced synthesis of 5-HT was observed in dentatothalamocortical pathways, with simultaneous increases in the contralateral dentate cerebellar nucleus (41). More recently, SPECT studies demonstrated significant reductions in 5-HT_{2A} binding in the cerebral cortex (152). Elevated levels of serotonin in the platelets of patients with autism has also been observed by a number of groups (29, 48, 123). In contrast, studies that assess changes in 5-HT receptors in platelets or whole blood of individuals with autism show decreased 5-HT_2 receptor binding (51, 140).

Genetic studies have also identified abnormalities in serotonin-related genes. Tryptophan hydroxylase-2 (TPH2) is the

rate-limiting enzyme in 5-HT synthesis in the CNS, and one group found a particular variant of TPH2 to be associated with autism (53). A second study, however, was not able to confirm this (181). Polymorphisms in the promoter region of the serotonin transporter gene SLC6A4 have also been reported to be associated with autism and cortical gray matter volume (39, 52, 67, 204, 213, 215). Finally, the gene ITGB3 has been proposed as a regulator of serotonin levels in autism based on genetic association studies (214, 215). Synergistic interaction between the SLC6A4 and ITGB3 loci has also been suggested (58).

Another line of research supporting serotonin as a neurobiological factor in ASD comes from pharmacological interventions. Drugs acting on the 5-HT2 receptor (28, 143) alter the serotonin system and have caused behavioral improvements in autistic patients (94, 101, 114, 150, 168). Specifically, the selective serotonin reuptake inhibitor fluoxetine causes improvements in social behavior while decreasing aggressive and stereotyped behaviors in children with autism (6, 27, 50, 64, 72, 82). Interestingly, approaches that decrease CNS serotonin such as tryptophan depletion exacerbated symptoms in patients with ASD (49, 142).

A wide range of studies suggest that changes in serotonin and other neurotransmitters can result in aberrant cortical development. 5-HT afferents from the brainstem raphe nuclei innervate cerebral cortex during a critical time in cortical morphogenesis. Similar to the peak in serotonin synthesis at 2 years of age in humans, rodents show a transient peak in serotonin levels in the first few days after birth (46, 100). At this time, layer IV of the sensory areas of cortex exhibits dense patches of staining for serotonin and 5-HTTs, particularly in the "barrel field" in primary somatosensory cortex (18, 60, 78, 178). *In vivo*, it appears that too little or too much serotonin is detrimental to cortical development. Experimental approaches in rodents with neonatal systemic 5-HT depletion reveal delayed development of several cortical layers (162), the aberrant appearance of thalamocortical afferent patterning in the barrel field (18) and an ultimate decrease in the size of the barrel field (156, 165). Altered dendritic and synaptic development appears to be at the root of serotonin's effects (137, 219), as barrel formation is restored in MAOA and 5-HTT single and double knockouts by the blockade of serotonin synthesis, or the additional knockout of 5-HT_{1B} receptors, which normally inhibit glutamate release (185).

The interaction of serotonin pathways with neurotrophins such as BDNF suggests a potential interplay between these factors in ASD pathogenesis. BDNF and serotonin show co-regulation in response to environmental factors (25, 136). During brain development, factors such as perinatal stress or environmental enrichment lead to long-term alterations in BDNF expression in brain and blood plasma (25, 79). In rodent models, maternal infection can cause long-term increases in BDNF within the cerebral cortex and other brain areas that eventually affect the development of serotoninergic pathways (83). Another example of this interaction comes from mice heterozygous for BDNF (BDNF+/–) that display premature, age-associated loss in forebrain serotonergic innervation (130). Similarly, 5-HTT function is impaired in the brains of BDNF+/– mice (61). Localized increases in BDNF expression promote 5-HT fiber sprouting after injury (88, 133). In turn, 5-HT depletion via inhibition of synthesis is accompanied by decreases in BDNF levels in the mature hippocampus (223). Such decreases in BDNF expression may be mediated by serotonergic mechanisms in that 5-HT2A receptor antagonists have been shown to block stress induced decreases in BDNF expression in the hippocampus and cortex (210).

Excitatory neurotransmitter signaling via glutamate receptors (GluRs) also likely plays a role in cortical development (134), and has the potential for involvement in the pathogenesis of ASD. Candidate genes-screening and association analyses showed that the kainate receptor GluR6 (105, 198, 202), metabotropic GluR8 (GRM8) (195) and one of four *N*-methyl-D-aspartate (NMDA) receptor 2 subunits, GRIN2A (8), appear to be associated with ASD. Interestingly, cDNA micro-array techniques along with other mRNA and protein studies of brain tissues from patients with autism identified significant increases in expression of several genes associated with glutamatergic pathways, including excitatory amino acid transporter 1 and glutamate receptor AMPA 1 (173). Such disturbances of the glutamatergic system may well affect cortical development and plasticity, as experimental evidence suggests that GluRs play roles in the activity-dependent refinement of synaptic connectivity (65). GluRs are classified broadly into two groups, ionotropic sites, linked to ion channels and metabotropic sites, linked to second messengers (144). The ionotropic sites include those activated by the exogenous agonists, NMDA, amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate (KA). NMDA receptors influence both the retraction of incorrectly placed axon arbors and synapses and the elaboration of correctly positioned terminals. NMDA receptors also have well-documented roles in cortical development and activity-dependent plasticity (89, 134).

GABAergic pathways also play important roles during brain development, and the interplay of glutamatergic and GABAergic systems facilitates modeling of the cerebral cortex by positioning of principal, pyramidal and interneurons (134). The establishment of the GABAergic system and the migration of GABAergic interneurons are crucial for the development of an inhibitory cortical system that regulates the excitatory processes mediated by glutamatergic pathways (127). A balance between excitation and inhibition is crucial for normal development, and its disruption may produce profound consequences for CNS function and homeostasis (126). GABAergic interneurons are also important for processing of information across cortical domains and are part of the structure of mini-columns, an essential module involved in the physiopathology of cortical dysfunction in autism (35). The potential involvement of the GABAergic system in the pathogenesis of ASD has been suggested by clinical, neuropathological and genetic studies. Elevated levels of GABA in platelets (180) and reduction in the GABAergic receptor system has been documented by studies of brain tissues from patients with autism (16, 17). The location of three genes for subunits of the $GABA_A$ receptor, GABRB3, GABRA5 and GABRG3 on the proximal 15q arm (189) prompted genetic studies in ASD that yielded inconsistent results (reviewed by Schmitz (188)). One study that evaluated fourteen GABA receptor subunit genes found an association between *GABRA4*

and a potential increase in the risk of autism through interaction with *GABRB1* (131).

Synaptic and dendritic changes. An early review focused on the neurobiology of autism and Rett syndrome helped introduce the concept that experience-dependent synaptic plasticity might be disrupted in such developmental disorders (227). Dendritic abnormalities can also be observed in ASD. Indeed, decreased dendritic branching in CA1 and CA4 was reported in one of the earliest analyses of pathological changes in autism (177). Several leads from genetic studies have also implicated synaptic changes in autism. These include alterations in the genes encoding Neuroligins 3 and 4, their binding partners Neurexins 1 and 3, SHANK and contactin-associated-protein-like 2 (CNTNAP2). The neuroligins, a family of five postsynaptic cell adhesion molecules, were the first of these to be associated with autism. In 2003, Jamain et al reported that Neuroligins 3 and 4 were mutated in ASD patients (106). They initially examined the locus because it is located at Xp22.3, a chromosomal region deleted in several autistic females. When they screened 36 pairs of affected siblings and 122 trios with autism, they found one Swedish family harboring a frameshift mutation leading to a premature stop codon in NLGN4, and another Swedish family with a mutation affecting a highly conserved residue in NLGN3. The NLGN4 mutation is predicted to represent a genetic null allele, while the changes in NLGN3 result in a protein that does not efficiently traffic to the cell surface and appears to have altered binding abilities (40, 44).

Subsequent attempts to confirm the role of neuroligin mutations in patients with ASD have yielded mixed results. Laumonnier et al reported a frameshift mutation in the NLGN4 gene in a large French family with mental retardation, some of whom also had autism (125). A mixed cohort of 148 autistic patients from the USA and Portugal contained about 3% with missense mutations in conserved regions of NLGN4, but no changes in NLGN3 (218). A functional analysis found that the R704C mutation described by Yan et al weakened the binding of neuroligin to syntrophin, suggesting they could be biologically significant (217). A Finnish study of 100 families with autism yielded a modest association of the disease symptoms with the NLGN1, three and four loci, but no functional mutations were identified in the 30 cases sequenced (221). It has also been suggested that the splicing pattern of the neuroligins is altered in autistic individuals (207). In contrast, however, studies of 96 autistic patients in Quebec (80), 196 in Toronto (212) and 124 from an international molecular genetic study of autism (15) did not identify any genetic alterations interpreted as being causally linked to autism. Furthermore, in at least one family deletion of NLGN4 was not associated with autistic symptoms (132).

Given these somewhat conflicting findings, the recent discovery that neurexin, a major protein partner of the neuroligin family, is altered in some autistic individuals provides key support for the concept that this synaptogenic pathway is involved in ASD development. Feng et al screened three beta-neurexin genes in 203 patients with autism, as well as in 535 controls (74). They found two putative missense mutations predicted to cause structural changes in four autistic cases, but in none of the controls. Neurexin are presynaptic proteins, and represent the binding partners for postsynaptic neuroligins. This interaction is thought to trigger postsynaptic differentiation and control the balance of inhibitory GABAergic and stimulatory glutamatergic inputs (87, 171).

SHANK3, another synaptic protein which can bind neuroligins, was also recently implicated in autism. It was initially investigated because of its location on chromosome 22 in a region lost or rearranged in patients with ASD. This microdeletion syndrome involving 22q13.3 is characterized by multiple developmental delays, dysmorphic features and autistic behavior (135). SHANK3, also known as ProSAP2, is one of three genes located in the minimal involved region. It encodes a type of protein found in excitatory synapses that serves as a scaffold and can bind to neuroligins (145). Shank proteins have been proposed as master organizers of postsynaptic density because of their ability to nucleate multimeric protein complexes in dendritic spines. Durand et al recently sequenced all SHANK3 exons in 227 individuals with ASD and in 190 controls (69). They identified alterations in a small percentage of patients, and showed that mutations in a single copy could be associated with language and/or social communication disorders.

Abnormalities in brain growth. Head circumference was found to be abnormally large in a subset of autistic patients by Kanner in 1943, and approximately 20% of children with autism have macrocephaly (76, 122). As described above, a wide range of imaging studies have more precisely delineated abnormalities in the growth of the brain as a whole, and of specific regions and structures. Potential molecular causes of these size changes are beginning to be discovered. For example, a polymorphism in the HOXA1 homeobox gene has been associated with increased head circumference in patients with autism (45). The cellular basis of brain overgrowth is not yet clear, but several theories have been advanced. One hypothesis is a reduction in the pruning and consolidation of synapses during development, leading to an increased number of neurites. Increased numbers of neurons or glia in the brain, either through initial overproduction or reduction of cell death, are additional possibilities. These and other theories are discussed in more detail in a recent review of brain growth in autism (141). Finally, it is possible that hypertrophy of individual cells may cause the brain size increase. An intriguing candidate potentially involved in the regulation of brain size in autism via this final mechanism is the gene PTEN (phosphatase and tensin homolog on chromosome 10).

PTEN was initially evaluated in ASD patients because it is mutated in Cowden syndrome, a rare autosomal dominant condition characterized by numerous hamartomas and an increased risk of cancer (167). Inherited PTEN mutations are also found in patients with Bannayan–Riley–Ruvalcavba (BRRS) and Proteus syndromes. Macrocephaly is a feature of Cowden syndrome patients, and some of these individuals were reported to be autistic (84, 167). Macrocephaly and autistic behavior has also been reported in a patient with BRRS (228). Given these commonalities between inherited PTEN syndromes and autism, Butler et al sequenced the

PTEN gene in 18 autistic patients with macrocephaly, and found three with heterozygous germline mutations (30). A more recent screen of 88 patients with ASD and macrocephaly identified one with a misssense mutation in PTEN, but no partial or whole gene deletions (31). Several additional cases of autistic individuals with PTEN mutations have also been reported recently, leading to the recommendation that such testing be routinely performed (19, 99). It is not yet clear if PTEN mutations in autistic individuals are always associated with increased head size, or if normocephalic autistic patients might also have disruptions in PTEN function. It will also be interesting to determine if other members of the signaling cascades regulated by PTEN are altered in autism.

PTEN is a phosphatase that regulates signaling through the phosphoinositol 3-kinase (PI3K) pathway. It has multiple downstream effects, and regulates cellular proliferation, differentiation and migration. In neoplasms, PTEN acts as a tumor suppressor, with loss of function mutations and deletions causing increased proliferation and decreased cell death. In postmitotic neurons, however, loss of PTEN function leads to the hypertrophic growth without proliferation, resulting in formation of aberrant ganglion cells and a phenotype highly similar to that seen in Lhermitte–Duclos disease, which is associated with Cowden syndrome (120).

PTEN has subsequently been deleted from postmitotic neurons of the cerebral cortex and dentate gyrus in transgenic mice, leading to some very interesting behavioral and neuropathological changes (121). These animals showed progressive macrocephaly, but also were impoverished in their social interactions. For example, while wild-type animals will preferentially interact with a mouse they have not previously encountered, PTEN deficient animals did not. Indeed, the transgenic animals were as likely to interact with an inanimate object as a social target animal. These behavioral changes may be caused by multifaceted neuropathological changes, as in addition to increased neuronal size the authors found alterations in axons, dendrites and synapses in the transgenic animals. Specifically, in mutant animals they documented enlargement of mossy fiber tracts, ectopic granule axons, dendritic hypertrophy and a dramatic increase in the number of presynaptic vesicle. These changes are consistent with a previous report implicating the AKT/mTOR pathway, which functions downstream of PTEN, in dendritic arborization (109).

Tuberous sclerosis (TS) is another genetically defined neurodevelopmental disorder caused by alterations in genetic signaling pathways that converge with those controlled by PTEN. TS patients are frequently also diagnosed with autism, with estimated rates of ASD ranging from 17% to 68% (200). Some investigators have found that the numbers or location of cortical tubers in TS is correlated with autistic behaviors, suggesting these discrete structural lesions might cause the association (20, 71). Others, however, did not find that the number or site of cortical tubers correlated with autistic behaviors (3, 21). In order to examine this pathway, we performed a preliminary immunohistochemical investigation of S6 ribosomal protein phosphorylation in post-mortem brains from five autistic children and an equal number of matched controls, but did not identify any major changes (70).

NEUROIMMUNITY, ENVIRONMENT AND NON-GENETIC FACTORS IN ASD

It is clear that genetics alone do not determine the entire ASD phenotype, and that other non-genetic factors must play roles as modifiers of processes determined by genetic susceptibility. Environment and epigenetic factors both have the ability to influence pathogenic mechanisms of cortical and neuronal function. Among environmental factors, maternal influences and exposure to neurotoxins and potential environmental pollutants have been the focus of attention in recent investigations. These may interact with the neuroimmune system and disrupt neurodevelopmental pathways resulting in alterations of neurobehavioral trajectories such as those that occur in ASD (124, 129). A recent study, for example, found that patients with autism and larger head sizes show a significant association with a history of allergic/immune disorders both in the patient and in first-degree relatives (182).

Neuroglia and neuroimmunity in ASD. Neuroglial cells such as astrocytes and microglia, along with perivascular macrophages and endothelial cells, play important roles in neuronal function and homeostasis (1, 10, 68, 157, 216). Both microglia and astroglia are fundamentally involved in cortical organization, neuroaxonal guidance and synaptic plasticity (75, 209). Neuroglial cells contribute in a number of ways to the regulation of immune responses in the CNS. Astrocytes, for example, play an important role in the detoxification of excess excitatory amino acids (154), maintenance of the integrity of the blood–brain barrier (172), production of neurotrophic factors (10) and the metabolism of glutamate (154). In normal homeostatic conditions, astrocytes facilitate neuronal survival by producing growth factors and mediating uptake/removal of excitotoxic neurotransmitters, such as glutamate, from the synaptic microenvironment (154). However, during astroglial activation secondary to injury or in response to neuronal dysfunction, astrocytes can produce several factors that may modulate inflammatory responses. For example, they secrete pro-inflammatory cytokines, chemokines and metalloproteinases that can magnify immune reactions within the CNS (10). Microglial and astroglial activation is an important factor in the neuroglial responses to injury or dysfunction. Microglia are involved in synaptic stripping, cortical plasticity and immune surveillance (1, 86). Changes in astroglia and microglia can therefore produce marked neuronal dysfunction that is likely to be associated with mechanisms of neuronal dysfunction observed in autism. These neuroglial changes are mediated by the production of oxidative species, cytokines, chemokines and other neuroactive substances (10).

There has been growing interest in the role of immunity and immunological dysfunction in the pathogenesis of ASD (reviewed by Pardo (163) and Ashwood (4)). Several reports link the presence of immunological dysfunction with autism, and some studies suggest that up to 60% of patients with ASD have various types of systemic immune dysfunction, either as part of cellular or humoral immune responses (116, 128, 208). A few earlier case reports found pathological evidence of immunological reactions within the CNS, such as lymphocyte infiltration and microglial nodules (7, 90). Several reports using

different methodologies and small patient populations have shown increases in pro-inflammatory cytokines in peripheral blood samples in ASD (see review by Ashwood (4)). Most recently, Molloy et al found an increased pattern of production of Th2-associated cytokines in leukocytes from autistic subjects (149).

Neuropathological studies of post-mortem brain tissues from autistic patients demonstrate an active and ongoing neuroinflammatory process in the cerebral cortex and white matter characterized by astroglial and neuroglial activation. These findings support a role for neuroimmune responses in the pathogenesis of ASD (211). As both astroglia and microglia are involved in pathogenic inflammatory mechanisms common to many different disorders of the CNS, it is possible that different factors (eg, genetic susceptibility, maternal factors, prenatal environmental exposures) may trigger the development of these neuroglial reactions. Furthermore, protein array techniques used to establish the profiles of immune mediators demonstrated that cytokines/chemokines such as MCP-1, IL-6 and TGFβ1, which are mainly derived from activated neuroglia, are the most prevalent cytokines in brain tissues (211). Similar findings were seen in CSF from autistic patients. Preliminary studies also show that serum concentrations of subsets of cytokines and chemokines, such as MCP-1 and IL-6, parallel the CSF levels, suggesting that serum levels may be useful as surrogate markers of neuroinflammatory activity in autistic subjects. These findings strongly suggest that neuroimmune reactions are part of the neuropathological processes in ASD, and that immune responses are among the factors that may contribute to CNS dysfunction. However, the significance of the neuroinflammatory response to the specific neuropathologies and behavioral disruptions in ASD, and its position in the etiology of ASD, requires further exploration.

Oxidative stress. Oxidative stress is another possible cause of Purkinje cell loss and other neuroanatomical changes described in autistic brains (reviewed in (37, 113)). Oxidative stress occurs when the levels of reactive oxygen species exceed the antioxidant capacities of a cell, often leading to cell death. Because of its very high oxygen demands and limited antioxidant capacity, the brain is thought to be relatively vulnerable to oxidative stress (111). Several studies have shown decreased levels of antioxidants such as superoxide dismutase, transferrin and ceruloplasmin in the blood or serum of patients with ASD (38, 108, 222). Significant elevations in biomarker profiles indicating increased oxidative stress, such as increased lipid peroxidation, have also been documented in autism (38, 107, 229). Interestingly, in one report the alterations in antioxidant proteins were linked specifically to regressive autism, suggesting a postnatal environmental effect (38). Polymorphisms in metabolic pathway genes may contribute to the increased oxidative stress in autism (108). Advanced glycation end products have also been reported to be elevated in both the brain tissue and serum of autistic patients, a change which can also lead to increased oxidative damage (23, 110).

Maternal factors. A final interesting area of research has focused on the potential role of maternal factors in the pathogenesis of autism. A study by Comi and Zimmerman (43) showed that the mean number of autoimmune disorders was greater in families with autism, and that 46% of ASD patient's families had two or more members with autoimmune disorders. As the number of family members with autoimmune disorders increased from one to three, the risk of autism was greater, with an odds ratio that increased from 1.9 to 5.5. The most common autoimmune disorders observed were type 1 diabetes, adult rheumatoid arthritis, hypothyroidism and systemic lupus erythematosus. However, a large population-based case–control study found no significant differences in the proportion of case and control mothers with a diagnosis of autoimmune disease in the 4-year period surrounding pregnancy (59). Tissue-based studies have also suggested a role for maternal autoimmune factors in the pathogenesis of autism. The presence of maternal auto-antibodies that cross-react with brain epitopes was demonstrated by two studies (26, 226). In one study by Zimmerman et al, serum from 11 mothers and their children with autism was compared with serum from controls in its ability to bind adult rat brain proteins using immunoblot techniques. In another study by Van de Water et al (26), of 61 mothers of patients with autism, seven of the plasma samples (11.5%) contained maternal antibody cross-reactive with human fetal brain proteins 73 kDa and 37 kDa in size. This was not observed in the control group of mothers with typical developing or non-autistic developmentally delayed children. The presence of such antibodies in the plasma of some mothers suggests the transfer of maternal antibodies during early development could interact with fetal CNS proteins, affecting neurodevelopmental pathways and increasing the risk of ASD.

SUMMARY

In this review, we have attempted to briefly summarize some of what is currently known about the neurobiological causes of autism. Autism and related developmental disorders are clinically heterogeneous, and are likely caused by a range of factors. This heterogeneity has made it difficult to tease out the individual causal elements of this devastating disease. Slowly, however, genetic and environmental alterations are being defined, including the molecular and genetic changes affecting brain growth and development described above. This improving understanding may ultimately lead to new strategies for the prevention or cure of ASD. An encouraging recent report provides an example of such therapeutic progress, as Hayashi et al have shown that the symptoms of fragile X syndrome in mice can be reversed by inhibition of a specific kinase (93). Similar studies targeting a broad range of molecular factors involved in autism will hopefully eventually allow us to treat the growing number of patients afflicted with ASD.

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