Etiology of infantile autism: a review of recent advances in genetic and neurobiological research

Geneviève Trottier, MSc; Lalit Srivastava, PhD; Claire-Dominique Walker, PhD

Department of Psychiatry, Douglas Hospital Research Centre, McGill University, Montreal, Que.

The etiology of autism is complex, and in most cases the underlying pathologic mechanisms are unknown. Autism is a hetereogeneous disorder, diagnosed subjectively on the basis of a large number of criteria. Recent research has investigated genetics, in utero insults and brain function as well as neurochemical and immunological factors. On the basis of family and twin studies, there appears to be a genetic basis for a wide "autistic syndrome." About a quarter of cases of autism are associated with genetic disorders such as fragile X syndrome or with infectious diseases such as congenital rubella. Genetic studies have shown an association between autism markers of brain development such as 3 markers of the c-Harvey-*ras* oncogene and the homeobox gene EN2. In some cases, autism is associated with insults early in gestation, including thalidomide embryopathy. Autism may arise from abnormal central nervous system functioning, since most autistic patients have indications of brain dysfunction, and about half of them have abnormal electroencephalograms. Similarly, the pattern of evoked response potentials and conduction time is altered in autistic children. There is substantial evidence from neuroimaging studies that dysfunctions in the cerebellum and possibly the temporal lobe and association cortex occur in autistic symptoms. Neurochemical studies have investigated the role of serotonin, epinephrine and norepinephrine, since levels of these neurotransmitters are altered in autism, although other hypotheses implicate overactive brain opioid systems and changes in oxytocin neurotransmission. Autoimmunity may also play a role; antibodies against myelin basic protein are often found in children with autism, who also have increased eosinophil and basophil response to IgE-mediated reactions. In summary, the prevailing view is that autism is caused by a pathophysiologic process arising from the interaction of an early environmental insult and a genetic predisposition.

L'étiologie de l'autisme est complexe et, dans la plupart des cas, on ne connaît pas les mécanismes pathologiques sous-jacents. L'autisme est un trouble hétérogène diagnostiqué subjectivement en fonction de multiples critères. Des recherches récentes ont porté sur des aspects génétiques, les agressions in utero et le fonctionnement du cerveau, de même que sur des facteurs neurochimiques et immunologiques. Des études sur des membres de la famille et des jumeaux semblent indiquer qu'il existe des causes génétiques d'un vaste «syndrome autistique». On a établi un lien entre le quart environ des cas d'autisme et des troubles génétiques comme le syndrome de l'X fragile ou des maladies infectieuses comme la rubéole congénitale. Des études génétiques ont démontré l'existence d'un lien entre des marqueurs de l'autisme dans le développement du cerveau, tels trois marqueurs des oncogènes c-Harvey-*ras*,

Correspondence to: Dr. Claire-Dominique Walker, Department of Psychiatry, McGill University, Douglas Hospital Research Centre, 6875 Lasalle Blvd., Verdun QC H4H 1R3; fax 514 762-3034; waldom@douglas.mcgill.ca

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et le gène homéobox EN2. Dans certains cas, on a associé l'autisme à des agressions au début de la gestation, y compris l'embryopathie causée par la thalidomide. L'autisme peut découler d'un fonctionnement anormal du système nerveux central, car la plupart des patients autistes présentent des signes de dysfonction cérébrale et environ la moitié d'entre eux produisent des électroencéphalogrammes anormaux. De même, les courbes des potentiels évoqués et la durée de la conduction sont modifiées chez les enfants autistes. Des données probantes importantes découlant d'études de neuroimagerie révèlent que des dysfonctions du cervelet et, possiblement, du lobe temporal et du cortex associatif sont impliquées dans les symptômes de l'autisme. Des études de neurochimie ont porté sur le rôle de la sérotonine, de l'épinéphrine et de la norépinéphrine, puisque les niveaux de ces neurotransmetteurs changent dans les cas d'autisme, bien que d'autres hypothèses mettent en cause des systèmes opioïdes cérébraux hyperactifs et des variations de la neurotransmission des oxytocines. L'auto-immunité peut aussi jouer un rôle. On trouve souvent des anticorps de la protéine de base de la myéline chez les enfants atteints d'autisme, chez lesquels les éosinophiles et les basophiles répondent davantage aux réactions provoquées par l'IgE. En résumé, on pense actuellement que l'autisme est causé par un processus pathophysiologique qui découle de l'interaction entre une agression environnementale précoce et une prédisposition génétique.

Introduction

Autism is a severe behavioural disorder that develops in the first 3 years of life. It is characterized by pervasive impairments in social interaction, deficits in verbal and nonverbal communication, and stereotyped and repetitive patterns of behaviours and interests. The prevalence in the general population ranges from 0.04% to more than 0.1%,[1-4] and boys are 3 to 4 times more commonly affected than girls. While all children with autism are grossly impaired in their social relations, the severity and specific nature of cognitive impairments vary widely. The intellectual competency of children with autism ranges from profound mental deficiency to superior intelligence, although most such children suffer from mental retardation.

For more than 2 decades after Leo Kanner first described it in 1943, autism was believed to be psychogenic. However, the high incidence of mental deficiency and epileptic seizures pointed to biological determinants. Many studies have reported abnormalities in electroencephalograms (EEGs) of autistic subjects; more recently, structural abnormalities have been found in various regions of the brain.[5] Genetic influences have also been noted; there are high concordance rates in siblings of autistic probands, as well as in monozygotic twins.[6] Hence, it has now become generally accepted that autism has a biological basis.

Like most behavioural syndromes, autism is etiologically complex, and the underlying pathologic mechanisms are unknown in most cases. A number of causes have been proposed, including various medical conditions (fragile X syndrome, phenylketonuria, and tuberous sclerosis, among others), perinatal insult and autoimmune mechanisms. Like many other psychiatric conditions, such as bipolar disorder, panic disorder, alcoholism, Alzheimer's disease and schizophrenia,[7] autism has been shown to have a genetic component, which is now the focus of intensive investigation. There has also been substantial work to elucidate the neurobiological correlates of the disorder. In this review, recent advances in the understanding of the genetics and neurobiology of infantile autism will be discussed.

Genetic factors in autism

Findings from epidemiological, family and twin studies — modes of transmission

Epidemiological studies have determined that the risk of autism in the siblings of an autistic child is around 3%,[8] which is about 50 times higher than the prevalence rate for the whole population. Moreover, in same-sex twins, there is a much higher concordance rate in monozygotic (MZ) than in dizygotic (DZ) twins. A British twin study conducted in 1977 by Folstein and Rutter[9] found a 36% concordance for diagnosed autism in MZ twins, compared with no concordance in DZ twins. However, when the data were later reassessed using a broader phenotype of cognitive and social abnormalities, the concordance increased to 82% in MZ twins and 10% in DZ twins.[10] These results were confirmed recently in a large investigation by Bailey et al,[6] which also revealed that milder symptoms were more common in first-degree relatives of probands with autism than in relatives of nonautistic mentally retarded individuals (with Down syndrome). Indeed, a significant proportion of parents of autistic children ex-

hibit impaired executive function, as evidenced by poor planning skills and attentional flexibility.[11] Studies also reported an aggregation of a variety of personality characteristics, language abnormalities and psychiatric disorders in families with autism. It has been hypothesized that this constellation of symptoms may be a manifestation of an underlying genetic susceptibility to autism.[10,12] Thus, the bulk of twin and family studies points to a genetic basis for an autistic spectrum extending beyond classic diagnosed cases.

A variety of mechanisms have been proposed to explain the etiology of autism, including an association with a known medical syndrome. A recent systematic review concluded that autism related to a medical disorder accounts for an average 24.4% of cases across studies.[13] Such disorders include genetic disorders such as fragile X syndrome (the chromosomal abnormality most frequently associated with autism), tuberous sclerosis, phenylketonuria and Rett syndrome, as well as nongenetic conditions such as infectious diseases (e.g., congenital rubella, acute encephalopathy, cytomegalovirus).[14,15] Although the frequency of these disorders in the population with autism is far greater than would be expected by chance, none of these disorders is consistently associated with typical or atypical autism, highlighting the heterogeneity of the autistic spectrum. Association of autism with genetic diseases could result from close linkage of the 2 genes that, separately, cause both disorders.[16] In this perspective, the variety of genetic defects associated with autism seems to indicate multiloci inheritance of the disorder, and the frequent association of fragile X syndrome with autism supports at least partial X-linked inheritance of a genetic predisposition to autism.[17,18] An association has also been reported between chronic intestinal inflammation (ileal lymphoid nodular hyperplasia) and cognitive and behavioural regression in boys with a history of normal development.[19] Onset of autistic-like symptoms has also been reported by parents to coincide with a vaccination or infection, highlighting the possible importance of inflammatory or immunological responses in the association between autism and other medical conditions.

It has been hypothesized that one of the underlying causes of autism is the brain abnormalities that accompany some of these medical conditions. If this is the case, clinical presentation and severity of autistic symptoms must depend on the brain systems specifically affected.[20,21] In support of this hypothesis, it was recently reported that some children with fetal alcohol syndrome (FAS), who are generally most impaired in the area of interpersonal skills,[22] have been given the diagnosis of autism. Interestingly, animal models of FAS exhibit cerebellar abnormalities,[23] which are a key finding in the neuropathology of autism.

In cases not related to a known medical condition, various mechanisms have been put forward to explain the patterns of familial aggregation. These include autosomal recessive inheritance, X-linked inheritance, and multifactorial and polygenic models.[10] Some authors have reported that autism sometimes presents characteristics of genetic anticipation — increased symptom severity and decreased age of onset with successive generations.[24,25] This indicates that transmission of autism in some cases may involve "unstable DNA" — that is, genes susceptible to trinucleotide repeat expansion. Interestingly, it was found that autistic individuals have increased expression of autosomal fragile (folate-sensitive) sites compared with control children.[26] Segregation analysis in families multiplex for autism (with at least 2 affected members) has suggested autosomal recessive transmission patterns; hence, differential penetrance in males and females could account for the unbalanced sex ratio in autism (with almost 4 times more males than females are affected).[16] However, reported recurrence rates do not seem to fit with single-gene models of inheritance.[27,28] Based on data from twin studies, Bailey et al[6] proposed that inheritance of the genetic susceptibility to autism is best explained by a multifactorial threshold model involving more than 1 locus, with a higher threshold in females. This type of model predicts a greater frequency of autistic behaviours in relatives of the least affected sex. Most epidemiological findings do not show a greater frequency in families of female autistic probands;[16] however, a recent study reported a higher rate of verbal anomalies in the male relatives of autistic girls,[28] providing partial support for the multifactorial inheritance model.

The mode of inheritance proposed by Bailey et al could explain familial aggregation in some pedigrees, but it remains highly probable that autism is genetically heterogenous. Environmental factors, in concert with genetic susceptibility, may play an important role in the determination of the autistic phenotype. Folstein and Rutter[9] suggested as early as 1977 that genotypic autism is usually expressed as a language disorder, and becomes clinical autism only with the co-occurrence of a secondary factor, such as perinatal brain damage.[9] Adoption studies have been valuable in determining the rela-

tive importance of environmental versus genetic factors in other conditions. The lack of such studies in autism may be explained by the rarity of the disorder and the intrinsic difficulties associated with adoption studies.

Genetic linkage studies

Genetic evidence from case reports points to a variety of chromosomal abnormalities that are occasional causes of autism.[14,20] The most common abnormalities arise from duplication of chromosomal material, frequently chromosome 15 or the sex chromosomes. The 15q region from the maternally derived chromosome was found to be the most commonly affected in autistic individuals. Of possible etiological relevance, this region has also been implicated in the genetic basis of dyslexia, one of the features of the autistic spectrum. As will be discussed later, potential candidate genes from this site include genes coding for 3 γ-aminobutyric acid (GABA)-A receptor subunits.[29–31]

A full genome screen was recently performed in 99 pairs of affected relatives to identify loci associated with autism.[32] There were significant association scores for regions on chromosomes 4, 7, 10, 18, 19 and 22, with the highest significance for region 7q. A more specific study investigated linkage of autism with a large number of red cell markers and serum enzymes, but found no positive association.[33] Because of the frequent association of autism with fragile X syndrome, a genetic linkage between autism and the gene responsible for fragile X (*FMR-1*) has been investigated in families multiplex for autism.[34] No involvement of the *FMR-1* region was found in autistic individuals who had no cytological evidence of fragile X expression. However, recent findings concerning other markers on the X chromosome support involvement of X-related factors in autism.[35] One potential problem with linkage analysis in autism is that such analysis requires homogeneous samples, along with a clear distinction between affected and unaffected individuals. This is clearly not the case in autism; hence, other associative approaches have been attempted. In particular, the association method investigates allele frequency of individual loci ("candidate genes") chosen for their potential "mapping" of specific features of the disorder.[7,36] For instance, autism has often been associated with biochemical abnormalities in the metabolism of serotonin, and drugs that target $(5\text{-HT})_T$ have been found to be effective in relieving autistic symptoms.[37] Recent results reveal an association between the autistic phenotype and variants of the serotonin transporter $(5\text{-HT})_T$ gene.[37] Similarly, Hérault et al[38] tested for an association between autism and a number of candidate genes, including the serotonin $(5\text{-HT})_{2A}$ receptor gene and genes coding for tyrosine hydroxylase (TH), dopamine ß-hydroxylase and tryptophan hydroxylase. Frequencies of these genes in autistic individuals were no higher than in normal children.[39] The lack of association for the TH gene, situated on the short arm of chromosome 11, was later confirmed by Comings et al[40] using a more variable gene polymorphism. In further investigations of the short arm of chromosome 11, no association between autism and the genes for insulin and insulin-like growth factor 2 were found, but a positive association was shown between autism and the c-Harvey-*ras* oncogene,[41] and this was confirmed by other investigators.[42] A subsequent study supported an association of autism with 2 more c-H-*ras* markers.[39] Important functions of *ras* protein in cell growth, signal transduction, cell architecture and intracellular transport suggest that a mutation of c-H-*ras* could contribute to autism. Furthermore, a marker of c-H-*ras* associated with autism was also found to be related to the expression of obsessive–compulsive and phobic symptoms in patients with Tourette's syndrome.[42] These association studies make a strong case for a general involvement of the 11p15.5 region in the etiology of childhood autism and other psychiatric disorders. It is noteworthy that the D_4 dopaminergic receptor gene has been localized to this same region of chromosome 11. Research shows that this gene is not related to schizophrenia,[43] but its relation to autism has not yet been studied.

Another line of research targeted genes involved in cell growth and development. Based on the hypothesis that a deficiency in adenylosuccinate lyase (ADSL), which is responsible for *de novo* purine synthesis, may be an etiological factor in certain cases of autism, investigators performed a preliminary genetic screening of 119 autistic patients for point-mutations in the *ADSL* gene.[44] However, they found no occurrence of mutations in this particular gene, and it was concluded that ADSL deficiency is, at best, a rare cause of autism. Allele frequencies were also studied for 2 probes of the homeobox gene EN2, a gene located on chromosome 2q13-q21 and involved in cerebellar development.[45] Significant differences were found between autistic and control subjects for 1 polymorphic site, which may prove especially meaningful in light of recent neuropathological findings of cerebellar abnormalities in autism.

Pre-, peri- and neonatal insults in the etiology of autism

As for other neuropsychiatric disorders, most notably schizophrenia, earlier lines of research focused on the influence of early environmental variables. It was repeatedly suggested that complications during pregnancy and delivery could be causal factors in autism. However, reviews of pre-, peri- and neonatal complications did not prove any consistent or specific association with the autistic syndrome,[46] although positive associations were found in some cases. For instance, various studies have suggested that autistic symptoms may be associated with events that occur in the first trimester of gestation. There is a solid association between autism and congenital rubella occurring before maturation of the fetal immune response.[14] Similarly, the frequent occurrence of autism in thalidomide-associated embryopathy (in 4% to 5% of cases) is probably related to damage to the central nervous system in the early stages of gestation.[47] In addition, in pairs of twins discordant for autism, the autistic twin is more frequently afflicted with minor congenital anomalies than the nonautistic twin,[6] which may reflect some early congenital insult. In addition, elevated maternal age was once considered a risk factor, but recent studies have found no significant differences in maternal age between autistic and control groups.[48]

Patients with many psychiatric conditions — including schizophrenia, schizoaffective disorder, major depression, bipolar disorder and autism — have a seasonal birth distribution.[49] Although the link between this seasonal birth excess and autism has not been firmly established, the effects of weather, differences in nutrition or maternal infections during pregnancy may be involved in the development of autism.[50]

In summary, autism seems to arise from environmental factors interacting with a genetic predisposition — as yet unidentified — to a broad "autistic syndrome." The most promising genetic associations thus far have involved genes that code for the serotonin transporter and the c-Harvey-*ras* oncogene. In addition to genetic investigation, studying the neurobiological correlates of autism could provide important insights to elucidate the etiological and therapeutic aspects of this severely debilitating disorder.

Neurobiological aspects of autism

It is now generally accepted that autism could arise from abnormalities in central nervous system functioning. A population-based study concluded that 85% to 90% of autistic subjects show some indication of underlying brain dysfunction.[20] A clue to the underlying central lesion is the high incidence of various types of epileptic seizures in autistic patients. As well, a substantial proportion (about 50%) of autistic patients have abnormal EEGs. When discussing putative neurological substrates of autistic pathology, it is important to remember that structural abnormalities do not necessarily reflect the site or cause of pathogenic processes. As the putative deficiencies in autism seem to be related to the sensory system, the integrity of sensory pathways has been the focus of a number of recent studies.

Auditory impairment and neurophysiological studies

Autism seems to profoundly affect the normal decoding of sensory information. Evoked response potentials (ERPs) were studied to determine whether autistic children have defects in immediate sensory processing at the brain stem level (evidenced by early ERP waves [I-IV]), or whether abnormalities lie in the delayed sensory processing in the cortex (evidenced by long latency potentials and their negative components). No differences between autistic children and controls were found in ERP waves (I-IV), but the amplitude of long latency auditory waves was reduced, and the negative component was absent in autistic subjects.[14] Since both types of affected waves originate in the association cortex, these findings argue for a defect in secondary sensory processing. In addition to differences in the amplitude of the ERP, their conduction time (CCT) can be altered in autism. Indeed, McClelland et al[51] found that the CCT was prolonged in autistic children aged 14 years and older compared with age-matched normal controls, and that the CCT was normal in younger autistic children. This prolonged duration of nervous conduction in older autistic children could be explained by a defect in myelination, since, in normal children, the process of myelination leads to progressive shortening of CCT with age.[52] A myelination defect could also account for the late occurrence of epilepsy in some autistic patients. Interestingly, 58% of autistic individuals, compared with only 9% of control subjects, have antibodies reactive to myelin basic protein (anti-MBP) in their serum.[53] An early anti-MBP autoimmune response could result in poor myelination or abnormal function of myelin. Myelination abnormalities could

also be caused by perinatal insults, such as anoxia, and this could be a critical factor in the development of the autistic syndrome.[53] However, a more precise electrophysiological recording technique revealed anomalies in conduction latency in most autistic individuals, regardless of age,[54] which puts into question the validity of the abnormal myelination hypothesis.

Neuroimaging and neuropathological studies

The investigation of central nervous system pathology in autism has intensified as a result of the increased availability of structural and functional neuroimaging techniques and detailed histopathology. Early neuroimaging studies have reported various anomalies, including dilation of cerebral ventricles, abnormalities of the basal ganglia, and diverse cortical malformations.[8] The heterogeneity and nonspecificity of these observations tended to disqualify them as causes or necessary correlates of autism. Although it remains difficult to integrate findings from different neuroimaging studies, the emerging concept is that dysfunctions in autism occur at several levels, including the brain stem, cerebellum, limbic system and association cortex.[45]

Cerebellum: There have been interesting findings from the study of cerebellar size and morphology in autistic individuals. Using magnetic resonance imaging (MRI), Courchesne et al[55] repeatedly showed hypoplasia of vermian lobules VI and VII in groups of autistic individuals, although these results could not be replicated by others.[56–58] A further meta-analysis revealed, however, that one subgroup of autistic individuals consistently shows significant vermian *hypo*plasia (in approximately 87% of patients), while the remaining 13% of patients show vermian *hyper*plasia.[59,60] Although abnormality of the cerebellar vermis is a consistent and well-replicated finding in autism, it is difficult to assign morphological changes to the single pathology of autism, since hypoplasia of cerebellar vermian lobules is also seen in various neurogenetic syndromes, with or without autistic behaviours.[61]

Postmortem studies of autistic brains have failed to detect any kind of gross pathology. However, recent investigations have reported alterations in neuronal size, density and dendritic branching in the cerebellum and limbic structures of autistic individuals.[62,63] The most consistent finding is a reduction in granule and Purkinje cell density in the neocerebellum.[60] Cell loss was not associated with gliosis, which indicates that the putative insult must have occurred in the early stages of embryonic development. Cerebellar abnormalities in autism appear to be progressive and age-related. An autopsy study by Bauman and Kemper[64] revealed age-dependent abnormalities in neuronal cell size in nuclei of the cerebellum and brain stem in autistic children. The authors speculated that early loss of Purkinje cells in the cerebellum may cause the persistence of fetal circuitry (large cells) in the olivary and cerebellar nuclei of younger subjects. In older autistic children, this circuitry would be progressively lost but not replaced by the adult neuronal pattern, leading to reduced cell size and number. Hashimoto et al[65] also observed a correlation between area of the vermis and age in autistic but not in control subjects, suggesting an abnormal pattern of vermian growth in autism.

Substantial evidence supports the involvement of the cerebellum in functions affected in the primary symptoms of infantile autism. Animal and human studies have indicated that the cerebellum, and more specifically the cerebellar vermis, is implicated in affect, motivation, social interaction, learning, and in the processing and modulation of sensory and motor information.[66] A recent neuroanatomical comparison of MZ twins discordant for strictly defined autism showed a markedly smaller caudate, amygdaloid and hippocampal volume, together with smaller cerebellar vermian lobules VI and VII, in the most severely affected twin, compared with his brother.[67] Finally, neurophysiological studies have revealed that cerebellar damage is associated with impairments in rapid mental attention-shifting in autism.[68] Positron-emission tomographic studies in autistic individuals have also shown deficiencies in coordinated interaction between the cortical and subcortical systems involved in directed attention.[69] Since coordination of attentional processes is important in the acquisition of social communication skills,[70] dysfunctions in these systems may explain the profound impairments in socialization associated with autism.

Temporal lobe: Research on nonhuman primates has demonstrated that insults to the temporal lobe during infancy lead to persistent socio-emotional abnormalities and selective deficits in learning and memory analogous to those seen in autism. There have also been a number of case reports of autistic behaviours in humans following temporal lobe damage,[71,72] including focal temporal lobe abnormality in patients with tuberous sclerosis.[73] Another line of evidence implicating temporal lobe dysfunction in autism is the high incidence of

temporal lobe epilepsy, as well as the finding of temporal lobe enlargement in autistic individuals.[74] Various authors[71,75–77] have postulated that a developmental lesion in the medial temporal lobe, and more specifically the amygdala, may be a cause of autism. According to this model, precise location and size of the lesion could determine the nature and severity of symptoms.

Association cortex: It is believed that the autistic syndrome cannot be fully explained by localized lesions to subcortical sites, since some autistic dysfunctions, such as social relatedness and complex reasoning, are governed by higher cortical functions. In contrast to other mental disorders, in which a degenerative course is often accompanied by brain atrophy, in autism about 25% of those affected have increased head circumference. This marker appears to be specific to autism, as it is not seen in normal mental retardation or in language disorders.[15] Megalencephaly is typically caused by thickening of the cerebral cortex, and a recent MRI study analyzing the size of the various cortical lobes concluded that autistic subjects exhibit a specific sex-dependent pattern of cortical lobe enlargement, accounting for increased brain size in autistic males.[74] MRI studies also showed a high incidence of gyri malformations in autistic subjects, while none were detectable in controls.[78] These cortical malformations, in the absence of gliosis, are thought to result from a defect in the migration of neurons during the first 6 months of fetal development. However, they are not specific to autism, having been reported in association with various other developmental disorders. It is noteworthy that similar migration anomalies have been observed in schizophrenia, in which it is hypothesized that misconnection of brain regions may underlie the neuropathology.[79] Indeed, some of the manifestations of schizophrenia are closely related to autistic symptoms (disturbed sense of self, social withdrawal and emotional detachment, formal thought disorder including repetitions and stereotypies); and, in fact, autism was once viewed as a form of childhood-onset schizophrenia.

In autism, abnormalities could exist within the frontal cortex or affect the functional linkage between frontal and parietal association cortices.[8,80] One preliminary study using magnetic resonance spectroscopy provided evidence of abnormal phosphorus metabolism and increased degradation of neuronal membranes in the frontal lobe of autistic subjects.[81] This study also showed that the amount of breakdown products from membranes increased as test performance decreased in the autistic group, but not in normal subjects. These findings are consistent with anatomical observations of truncated dendritic branching.[82,83] In addition, there are similarities in cognition, affect and behaviour between autistic individuals and patients with acquired lesions of the frontal lobes.[8] Frontal lobe processes are required for the formation of second-order cognitive representations, which are dramatically impaired in autism, as well as other high-order cognitive processes.[84] However, findings of frontal lobe abnormalities in autism are rare, which has led to the suggestion that there may be transient abnormalities during postnatal development. Regional cerebral blood flow (CBF) was studied in age-matched autistic and normal children at 3 to 4 and 6 to 7 years of age. At first examination, corresponding to the age of frontal maturation in normal children, the autistic group had lower CBF values in the frontal cortex. Three years later, however, their CBF values were not different from those of normal children of the same age.[84] A functional imaging study in normal volunteers performing specific story-comprehension tasks also provided a glimpse into the neural substrates of autism.[85] It pointed to a specific pattern of activation associated with normal mental state attribution (which is strikingly affected in autism), including the temporal poles, the left superior temporal gyrus, and the posterior cingulate cortex. These are likely to reflect areas that have abnormal structure or function in autistic brains, and are valuable targets for future research.

Neurochemical studies

The neurochemistry of childhood autism has been the focus of extensive research. The most often replicated finding is that about one-third of autistic individuals have hyperserotonemia.[86] However, investigation of various other hormone and neurotransmitter systems, as well as drug trials, provide indirect evidence of several other neurochemical abnormalities. Elevated serotonin levels are not specific to autism, being found as well in other neuropsychiatric disorders, such as chronic schizophrenia, and in severe mental retardation.[86] While an in-depth review of this literature is beyond the scope of this paper, the following discussion gives a brief overview of the salient points of neurochemical investigation in autism.

Serotonin: The interest in assessing serotonergic function in autistic individuals stems from the role of sero-

tonin in the perception and filtering of sensory signals, as well as in social attachment. (Animal research has indicated that hyperserotonemia could reduce the drive for social attachment by inhibiting separation distress.[87]) Several groups have reported high levels of serotonin in the blood and platelets in a subgroup of autistic patients,[88] and platelet serotonin levels have been correlated with certain autistic behaviours.[89]

However, Hérault et al[38] recently questioned the notion of elevated serotonin transmission; their study found evidence of reduced whole-blood serotonin levels in autistic children. As well, there were high urinary levels of 5-hydroxyindoleacetic acid (5-HIAA) in autistic compared with control individuals,[38] whereas another study found little or no difference in 5-HIAA concentrations in cerebrospinal fluid (CSF).[90] Furthermore, the ratio of 5-HIAA to homovanillic acid in CSF was shown to reflect functional competence in autistic children.[91] Todd and Ciaranello[92] suggested that autistic children may have an autoimmune dysfunction affecting brain serotonin receptors, since 7 out of 13 autistic children, but no control subjects, had CSF antibodies directed against brain 5-HT receptors. However, a more recent study found no differences in brain-tissue $(5\text{-HT})_{1A}$ and $(5\text{-HT})_2$ receptor inhibition, on th e basis of immunoglobulins isolated from the plasma of autistic subjects compared with unrelated controls.[93] Although disparate, these findings seem to argue for a serotonergic dysfunction in autism, with highly variable manifestations. Further evidence implicating serotonin in the pathology of autism was derived from clinical pharmacological trials. Administration of fenfluramine, a potent serotonin depletor, was associated with clinical improvement in about one-third of the patients.[94] Risperidone, a blocker of both serotonin and dopamine receptors, was also effective at relieving the behavioural symptoms of autism, including self-injury.[94] A recent report showed that tryptophan depletion (with a resultant reduction in serotonin precursor availability) exacerbates autistic symptoms in most patients.[95] By contrast, trials of selective serotonin reuptake inhibitors, which enhance central serotonergic transmission, also had favourable outcomes: both fluoxetine[96] and clomipramine[97] provided promising results in reducing repetitive and compulsive behaviours.

Catecholamines: Animal research has highlighted various functions of the noradrenergic system. In particular, noradrenergic cells from the locus ceruleus have been implicated in attentional processes, filtering of irrelevant stimuli, arousal, anxiety, and learning, which are all impaired functions in autistic patients.[91] Various independent studies have demonstrated that plasma epinephrine and norepinephrine levels are increased in autistic subjects. However, urinary metabolite determinations were much less consistent, and, unlike plasma levels, platelet concentrations of norepinephrine and epinephrine were found to be decreased in autism.[86] Similarly, dopamine levels were found to be elevated in plasma, but decreased in platelets, of autistic patients.[98] Investigations of plasma and urinary levels of homovanillic acid (the end-product of the catecholamine degradation pathway) were inconclusive, showing levels ranging from normal to elevated in autistic individuals.[90] Other evidence of a dopaminergic dysfunction in autism is a blunted or delayed growth-hormone response to levodopa administration,[99,100] which indicates decreased central dopamine transmission.

In terms of plasma monoamine levels, Martineau et al[101] found a significant age effect for dopamine, homovanillic acid, 3-methyltryptamine, norepinephrine, epinephrine and serotonin in autistic children. Based on these findings, the authors suggested that autism may be characterized by abnormal maturation of monoamine systems. A few studies have investigated key enzymes of monoaminergic metabolism, but, in general, did not find them to be affected.[86]

Other neurotransmitters and hormones: Preclinical studies in animals suggest that social attachment, an area of functioning severely impaired in autism, is mediated, at least in part, by serotonin, melatonin and opioids.[87] The opioid hypothesis of autism, supported by studies showing elevated plasma and CSF levels of opioid peptides in autistic individuals, states that overactive brain opioid systems may underlie symptoms of autism, such as lack of socialization and decreased sensitivity to pain.[102] This theory was the impetus for pharmacological trials of opiate blockers. Short-term administration of naltrexone acts to improve hyperactivity, stereotypies and social withdrawal, while both naltrexone and naloxone reduce self-injurious behaviours.[87,103] It is well known that oxytocin, in addition to opioids, is involved in the formation and maintenance of social bonds. Two independent groups have proposed that social deficits in autism could be mediated by oxytocin hypoactivity[104] or excess.[105] In accordance with the hypoactivity hypothesis, Modahl et al[106] recently found that autistic children have significantly lower plasma oxytocin concentrations than normal

children, and do not show the normal increase in oxytocin secretion with age.[106]

There are other hypotheses of neurotransmitter alterations. Cohen et al[107] suggested that GABA could mediate social avoidance behaviour in autistic individuals. This could be particular to GABAergic transmission in the Purkinje cells of the cerebellum, which has repeatedly been shown to be affected by a degenerative process in autistic individuals.[107]

Investigations into the neuroendocrinology of autism have provided inconclusive data. Cases of improvement after therapy with triiodothyronine (T_3) have been reported, and a high rate of abnormal reponse to thyrotropin-releasing hormone challenge has been demonstrated.[86] Marginal changes in circadian rhythms of thyroid-stimulating hormone and an abnormal circadian rhythm of melatonin have been seen in severely autistic young adults.[108] Despite normal basal hormonal levels, subgroups of autistic children have been shown to have a blunted or delayed growth hormone response to levodopa,[99,100] a blunted prolactin response to fenfluramine[109,110] and a high rate of cortisol nonsuppression after dexamethasone administration.[111] Chamberlain and Herman[87] have proposed a cascade model of biochemical dysfunctions in autism, starting with hypersecretion of pineal melatonin, and alternations in the serotonin system.

Studies of the immune system

Considering the high incidence of autoimmune disorders or abnormalities of the immune system in the autistic population, autoimmunity has been proposed as a possible mechanism underlying autism. Autistic children have been shown to exhibit many anomalies in cell-mediated immunity, including abnormal T cell activation,[112] decreased relative numbers of helper-inducer lymphocytes, and a lower helper-suppressor ratio.[113] These last 2 measures were inversely correlated with severity of autistic symptoms. Autistic children also demonstrate increased urinary levels of native neopterin, a marker of cellular immunity activation,[114,115] and abnormally elevated α-interferon levels in the plasma, which could influence cell growth, activation and proliferation.[116] Interestingly, withdrawal and loss of interest, resembling symptoms of autism, have been observed in children receiving interferon therapy for cancer.[117] Furthermore, as discussed earlier in this review, antibodies against myelin basic protein are frequently found in children with autism and might be implicated in abnormal myelination, leading to symptoms of autism.[51] One study reported that autistic subjects and their first-degree relatives have antibodies to neuron-axon filament proteins, and that treatment with immunomodulants resulted in symptom improvement in 6 of 8 autistic patients.[118]

Autoimmune diseases are also frequently triggered by micro-organisms, and it is thought that an immune response to brain antigens resulting from pre- or postnatal viral infection plays a role in the development of the autistic phenotype.[53] Since autistic subjects have an increased frequency of the null allele for the complement component 4-binding protein (C4bp),[119] partial complement deficiency could result in prolonged immunological stimuli and eventually trigger an autoimmune reaction. Allergic reactions may also play a role in autistic symptoms. Autistic children appear to have an increased eosinophil and basophil response to IgE-mediated reactions, which could explain the high prevalence of allergic disorders among autistic chidren.[120,121] Investigators have recently suggested that food allergy and toxicity of food peptides affecting the central nervous system may be involved in physiopathological processes leading to autism. Lucarelli et al[122] showed that antibody reactivity to certain common foods is higher in autistic than in normal children, and elimination of these foods from the diet of autistic subjects significantly improved their behavioural symptoms.[122]

While the significance of these immunological findings in the development of autism is unclear, evidence is mounting that some manifestations of an impaired immune response might contribute to some aspects of the symptoms. More efforts are required to understand the nature of the interactions between the immune and neurobiological systems in autism and to design treatment strategies that account for altered immune function in autistic individuals.

To summarize, it should be noted that the causes underlying neurochemical abnormalities in autism are rarely known and that the results of neurochemical studies can often be obscured by the various medications administered to relieve autistic symptoms. It is also important to keep in mind that abnormalities in neurochemical transmission during brain development could have complex and lasting imprinting effects. Thus, research would benefit from increased emphasis on the timing and localization of neurochemical dysfunctions, as well as relating these anomalies to neuropathological findings.

Conclusion

Autism is a developmental disorder involving changes in neural organization. Evidence from neuropathological as well as epidemiological studies suggests that autism is best viewed as a convergent behavioural manifestation of various brain dysfunctions with different causes. In spite of our limited knowledge regarding the etiology of autism, we can hypothesize that the pathophysiological process is initiated with the interaction of a highly variable early environmental insult and a general genetic predisposition. A very similar causal mechanism has been proposed to explain the etiology of schizophrenia.[79] A general liability to neuropsychiatric disorders could encompass a variety of diagnoses and give rise to defined phenotypes when paired with a lesion at a specific site and at a specific time during central nervous system development. The epidemiological link between autism and thalidomide embryopathy,[47] as well as neuropathological reports of cerebellar cell loss in the absence of gliosis,[60] suggest that the precipitating insult to the developing central nervous system likely occurs in early pregnancy. In fact, based on neuroanatomical data of cerebellar abnormalities, some investigators have proposed that the fifth week of pregnancy is a period of increased vulnerability.[123]

Although certain findings may be integrated into coherent models, heterogeneity remains a central feature of autism. The diagnosis of autism is and has always been subjective and relies on meeting a defined number of criteria out of a large set. No single manifestation is pathognomonic, and some symptoms are also seen in other mental disorders, such as mental retardation, developmental dysphasia, obsessive–compulsive disorder, and learning disabilities. It has been suggested that future research should focus on identifying homogenous groups in order to derive more conclusive results.[124] However, it might prove difficult to define boundaries between different "autistic diseases," be they defined by neuropsychological testing, degree of genetic loading, specific brain pathology, or other more or less artificial criteria. Identification of efficient strategies for the prevention and cure of the disorder is hampered by the substantial variation in autistic manifestations among patients. Further work is expected to provide glimpses into neuronal substrates and genetic determinants that will explain the heterogeneity of autism and thus provide hope of alleviating, if not curing, this deeply debilitating lifelong disorder.

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