Molecular Neurobiology

ISSN0893-7648/03/28(1): 1–22/$25.00

The Neurobiological Context of Autism

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Abstract

Autistic disorder (AD) is a complex neuropsychiatric disorder of neurodevelopmental origin, where multiple genetic and environmental factors may interact, resulting in a clinical continuum. The genetic component is best described by a multilocus model that takes into account epistatic interactions between several susceptibility genes. In the past ten years enormous progress has been made in identifying chromosomal regions in linkage with AD, but moving from chromosomal regions to candidate genes has proven to be tremendously difficult. Neuroanatomical findings point to early dysgenetic events taking place in the cerebral cortex, cerebellum, and brainstem. At the cellular level, disease mechanisms may include altered cell migration, increased cell proliferation, decreased cell death, or altered synapse elimination. Neurochemical findings in AD point to involvement of multiple neurotransmitter systems. The serotoninergic system has been intensively investigated in AD, but other neurotrasmitter systems (e.g., the GABAergic and the cholinergic system) are also coming under closer scrutiny. The role of environmental factors is still poorly characterized. It is not clear yet whether environmental factors act merely as precipitating agents, always requiring an underlying genetic liability, or whether they represent an essential component of a pathogenetic process where genetic liability alone does not lead to the full-blown autism phenotype. A third potential player in the pathogenesis of autism, in addition to genetic and environmental factors, is developmental variability due to "random" factors, e.g. small fluctuations of gene expression and complex, non-deterministic interactions between genes during brain development. These considerations suggest that a non-deterministic conceptual framework is highly appropriate for autism research.

Index Entries: Autism; development; genetics; serotonin; GABA; acetylcholine; oxytocin; reelin; wnt-2; hoxa1; ARX; animal models.

Received 12/06/02; Accepted 1/27/03
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Introduction

The purpose of this paper is to critically discuss available evidence from a perspective of autistic disorder (AD) as a complex disorder of neurodevelopmental origin, resulting from the interaction of genetic liability with nongenetic factors. We have selected, among the enormous body of available genetic, neuroanatomical, and functional data, those that can be tied together meaningfully with developmental mechanisms, because we think that this will turn out to be a most useful way to increase the understanding of AD. In addition, we are proposing new avenues of research that could turn out to be fruitful for our understanding of the pathogenesis of autism.

AD is currently defined as a developmental disorder with onset by 3 yrs of age and is characterized by impairments in the domains of social ability and language, and by a restricted pattern of interests, often accompanied by motor, language, and other types of stereotypies. AD and Asperger syndrome, together with Rett's syndrome and childhood disintegrative disorder, are classified in DSM IV *(1)* within the category of the pervasive developmental disorders (PDD). AD has been introduced as a new clinical syndrome, distinct from mental retardation and schizophrenia, by the child psychiatrist Leo Kanner at Johns Hopkins University in 1943 *(2)*, starting from his observations of 11 children, 8 boys and 3 girls. In his original description, Kanner identified the "pathognomonic," fundamental disorder as "the children's inability to relate themselves in the ordinary way to people and situations from the beginning of life" *(2)*. Interestingly, Kanner correctly recognized that delay in language development is not part of the core disorder. A year later, the Austrian pediatrician, Hans Asperger published an independent paper describing a similar "autistic psychopathy," on the basis of investigations of more than 200 children seen in the play-pedagogic station at the university children's clinic in Vienna *(3)*. The paper by Asperger was written in German and became widely known only after its English translation was published, in the 1980s *(4)*. The term "Asperger's syndrome" was first introduced by the British psychiatrist Lorna Wing, in substitution for the term "autistic psychopathy" used by Asperger, in a paper discussing 34 cases from the point of view of Asperger's accounts, 19 of which had the history and clinical picture of the syndrome in more or less typical form *(5)*. The two entities, AD and Asperger's syndrome, are now grouped within the "autism spectrum disorders" (ASD), although there is a very large overlapping area.

Not all domains need to be affected by the same degree in autism, but most authors agree that the deficit in social ability has to be present to allow the diagnosis of autism. In fact, already the original report by Kanner stresses the inability to relate socially as a core symptom. In recent years, the category of "broad autism phenotype" has been introduced, mainly as a consequence of studies showing that autism-like traits, if not the full-blown syndrome, are frequently present in first-degree relatives of autistic patients. The implication of the "broad autism phenotype" is that first-degree relatives may share some, but not all factors conferring susceptibility to autism. It has been reported that mental retardation is often associated with autism, perhaps in >70% of the cases *(6)*. However, there is a large body of evidence that mental retardation does not belong to the core clinical entity of AD. An exception are single gene diseases resulting in a clinical syndrome where mental retardation and autism are frequently associated, e.g., untreated phenylketonuria, or ARX gene abnormalities, which are associated with mental retardation, autism, and infantile spasms *(7)*. There is little doubt that, in these cases, mental retardation and autism are caused by the same genetic alteration.

Estimates of the prevalence of autism vary widely, depending on factors such as diagnostic criteria, type of population assessed, completeness of ascertainment, etc. A mean

prevalence of autism worldwide of 1 every 500–1000 children is probably reasonable, with a prevalence of autism spectrum disorder as high as 1:160 *(8).* There is presently some concern about the apparent increase in the frequency of autism diagnosis made in the past decade, which has led some to speak of an "autism epidemics." In the view of many authors, this phenomenon is probably due to higher public awareness for AD, more frequent referral of children with abnormal behavior, broader diagnostic criteria, more complete ascertainment of cases in epidemiologic studies, etc. *(9,10),* but a true increase in autism prevalence cannot be ruled out.

The sex ratio in autism is heavily skewed toward males, the male:female ratio being on average, across published studies, about 4:1. It should be noted, however, that the sex ratio varies among different subpopulations of patients. For example, the sex ratio is closer to normal in microcephalic autistic patients, while it is highly skewed toward males in normo- and macrocephalic patients *(11).* Furthermore, the male excess is less pronounced among patients with more severe disabilities than among those with higher abilities *(12).* Also, if one isolates cases that do not present any dysmorphic traits and have normal MRI, the sex ratio appears to be heavily skewed toward males *(13).* Thus, male sex can be considered as a risk factor of variable weight for different subpopulations of individuals. The variability of the sex ratio is a clear indicator of the etiological heterogeneity of AD.

The diagnosis of AD is based solely upon clinical observation: no laboratory tests (genetic, biochemical, neuroimaging) are currently available to support this diagnosis. Laboratory tests can contribute to diagnose known clinical conditions resulting in AD. In about 20% of cases, in fact, AD originates from specific diseases, most often represented by tuberous sclerosis, fragile-X syndrome, 15q duplications, or untreated phenylketonuria *(14).* However, one should be aware that there are many clinical conditions leading to AD.

Developmental Regression and the Role of Environmental Factors in Autism

Many cases of AD are brought to the attention of health professionals because the child fails to reach the normal developmental milestones. In other cases, the child shows developmental regression after a period of apparently normal development. Estimates of the frequency of developmental regression in AD lie between 30 and 50%, and the mean age at regression in autism is 18–24 mo *(15–18).* The phenomenon of autistic regression has led to the postulation of environmental factors role in autism. It is not uncommon for parents to report that regression was preceded by a stressful event, such as an intercurrent infection, trauma, operation, or vaccination. This phenomenon either suggests random co-occurrence or may suggest that environmental factors could act as *precipitating* agents in AD. Following an original report of children (age range 3–10 yr) undergoing autistic regression after measles-mumps-rubella (MMR) vaccination *(19),* the issue whether MMR vaccination is a risk factor for AD has become the subject of hot debates. Recent epidemiological evidence strongly argues against a causal relationship between vaccination and AD *(10).* Nevertheless, the immature brain, when there is a genetic predisposition, may be susceptible to diverse nonspecific environmental events (infections, toxic chemicals, etc.) that precipitate autistic regression *(18).* Environmental factors that may *precipitate* autistic regression should be clearly differentiated from environmental factors that could *cause* the disease by interacting with genetic liability during critical periods of development (*see* below). There have been several reports of children affected with AD having a history of prenatal exposure to various drugs (valproic acid, alcohol, thalidomide), environmental toxins (mercury), infectious agents (cytomegalovirus, rubella virus), or decreased levels of thyroid hormones (*see* ref. *20* for a review). These prenatal insults

probably do not play a specific pathogenetic role *per se,* in the absence of genetic liability. Finally, one should be aware that regression does not necessarily prove the role of environmental agents: it may be that autistic regression reflects the postnatal manifestation of a disease mechanism that has a genetic origin, as it is the case of genetic neurodegenerative disorders with onset in childhood (e.g., spinal muscular atrophies, neurofibromatosis type 1, neuronal ceroid lipofuscinoses, Rett disorder, and others).

A Complex Neurodevelopmental Model for a Complex Disease

An important idea that should be borne in mind, and one that appears to be neglected by several researchers, is that brain development is not as deterministic as one may predict from the robustness of neurodevelopmental processes. Recent progress in the experimental and theoretical analysis of complex genetic networks shows how reliable developmental programs can be obtained from unreliable components (*see* e.g., ref. *21*). One recent exciting concept in biology is the discovery of stochasticity ("randomness") in living organisms, due to the small number of macromolecules involved in certain biological processes. It has been observed that the transcription rates of a specific gene can be variable both within a cell as well as between cells, even under identical boundary conditions. Furthermore, the size of variability of gene expression is strongest at intermediate transcription rates (*see* e.g., refs. *22,23* for a review). This so-called "noise" in genetic circuits allows for adaptive capabilities of living organisms in an environment that is constantly changing. The "other side of the coin" is that even genetic polymorphisms leading to small variations in gene expression could have strong influences on the outcome of developmental processes. An example of a disorder originating from the interaction between genetic mechanisms and "randomness" are mutations in forkhead factor HFH-4, a gene necessary for development of motile cilia in different epithelia; such mutations impair the flow of a morphogenetic signal that determines the left–right body axis: these mutations result in random determination of the left–right axis, so that 50% (not 100%) of the individuals carrying the mutation display situs inversus *(24).*

A possible pathogenetic model of AD, that takes into account the points outlined above, is depicted in Fig. 1.

Evidence for Altered Neurodevelopment in Autism

Converging evidence for altered brain development in AD stems from three lines of investigation: studies of brain volume and brain growth, functional neuroimaging, and neuropathology. Several clinical reports and postmortem studies (reviewed in ref. *25*), including a recent report by Hardan et al. *(26)* have suggested an increased frequency of macrocephaly (occipito-frontal head circumference above the 98th percentile for age, 2 standard deviations above the mean) and of megalencephaly (brain volume above the 98th percentile for age) in AD. Even if megalencephaly may not be specific for autism, it represents an interesting finding that points to a neurodevelopmental origin. It also would be logical to study subjects with macrocephaly as a potentially more homogenous subgroup, which may have relatively unique risk factors.

In principle, increased brain volume may be due to: 1) increased gray matter volume; 2) increased white matter volume; 3) increased ventricular volume; or 4) a combination of the three. MRI and neuropathological studies are not consistent with a selective increase of ventricular volume as a cause of increased brain size in autism. It is important to be able to distinguish among the three remaining possibilities, because this could give a lead about the underlying cause. An increase in gray matter volume would suggest alterations in the generation of neuronal or glial precursors, in sponta-

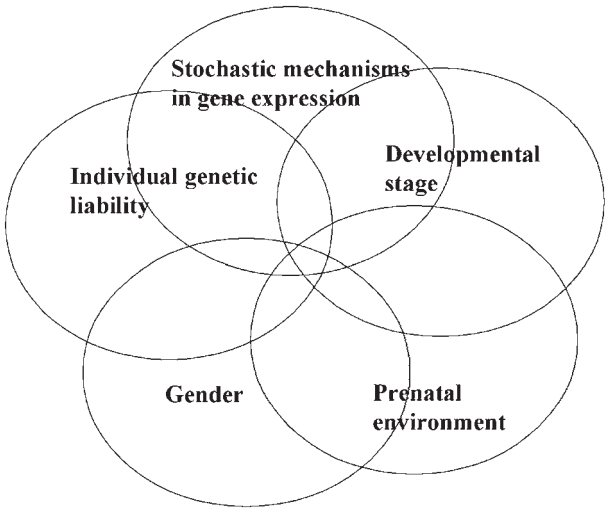

Fig. 1. Multiple interacting factors contribute to the pathogenesis of autistic disorder.

neous cell death, or in pruning of nerve terminals during neurodevelopment, whereas an increase in white matter volume may indicate that an abnormally high number of connections between neurons are generated, or that elimination of inappropriate connections is impaired. Furthermore, it is important to know whether megalencephaly is due to a proportional increase in volume of all brain structures, or if the size of some structures may be increased out of proportion, while other brain structures are relatively smaller. For example, several MRI studies have found statistically reduced sizes of hippocampus/area dentata and amygdala, particularly after correcting for total brain volume *(27,28).* An MRI study of the basal ganglia has found increased volume of the caudate nuclei in AD; caudate enlargement, though, was proportional to increased total brain volume *(29).* Decreased corpus callosum volume after adjustement for total brain volume has been reported; the posterior part of the corpus callosum was particularly affected *(30).* The finding of a decreased size of the corpus callosum in AD is counterintuitive when considering that brain volume appears to be increased (*see* above). One possible explanation is that in AD there is an increased number of neurons within the cerebral cortex, and that most of these "supernumerary" neurons project only to the ipsilateral, but not to the contralateral hemisphere. Interestingly, a recent report analyzing the columnar organization of the brain of autistic patients found abnormalities in the number and size of minicolumns: cell columns in the brain of autistic patients were more numerous and associated with a decreased neuropil volume, a finding that is consistent with the hypothesis of an increased total number of minicolumns that make less connections than normal *(31).*

Most studies on the size of specific brain areas have focused on comparisons between subjects with AD and normal controls. The study of Chan et al. *(32)* represents a remarkable change of paradigm, since these investigators have analyzed differences in gray matter concentration between autistic subjects and individuals affected by language impairment, using the technique of voxel-based morphometry. Interestingly, these investigators found decreased gray matter concentration in the occipitotemporal and occipitoparietal areas (which are related to nonverbal communication skills) in AD boys in comparison to children with language impairment, while the gray matter concentration in areas of the temporal lobe related to speech was significantly

higher in AD children. These morphometric findings lend support to the hypothesis that the language impairment in AD, if present, has a different nature (and a different neural substrate) than language defects due to impairment of language-specific circuits in the temporal lobe. Subjects affected with AD do not *use* language for communicative purposes, even if their language circuits may be normal.

One highly debated issue, that is not yet solved, is whether there are specific cerebellar abnormalities in AD. Investigators have focused from early on upon the cerebellum to find cues for specific alterations in AD. Courchesne and colleagues have reported decreased volume of cerebellar vermal lobules VI-VII *(33).* Their findings have not been replicated by other investigators (*see* ref. *34* for a review). The consensus among most investigators is that cerebellar abnormalities in AD are only expression of a more generally distributed disturbance of neural systems, involving the neocerebellum as well as other forebrain areas. A recent analysis of the increase in surface area of the cerebral and cerebellar cortices in phylogeny is consistent with the hypothesis that the cerebral cortex and cerebellum have evolved in parallel, suggesting strong functional connections between both structures *(35).* Furthermore, there is increasing evidence that developmental defects of the brainstem can lead to AD *(36,37)* (*see* below).

Dynamic considerations on the speed of brain growth may help to pinpoint the pathogenetic process. In a study of brain growth by Hashimoto et al. *(38),* the intercepts of the regression lines fitted to cerebellum and brainstem volumes were significantly lower in autistic patients than in controls, which is again consistent with an early dysgenetic event in AD. A recent study by Courchesne et al., 2001 *(39)* is consistent with abnormal regulation of brain growth in autism, resulting in early overgrowth followed by abnormally slowed growth. The concept of abnormal regulation of brain growth could help to explain many conflicting reports about increased or decreased volume of specific brain regions in AD, if one takes into account that mechanisms controlling global brain growth (i.e., genes controlling cell proliferation and apoptosis) may interact with other genes controlling the size and structure of specific brain areas (locally expressed genes). This hypothesis is compatible with a genetic model of multilocus-epistatic interactions described below.

As mentioned above, tuberous sclerosis is one of the few known medical conditions that is often associated with AD, particularly in cases where tubers are located in the temporal lobes. Interestingly, the presence of AD in tuberous sclerosis patients is associated with various indices of epileptic activity in the temporal lobes, beginning in early life *(40).* Furthermore, another condition associated with epileptic activity in childhood, acquired epileptic aphasia or Landau-Kleffner syndrome, is sometimes associated with autistic behavior *(41).* Finally, a number of cases of AD are associated with a multifocal pattern of epileptiform discharges *(42).* These observations suggest that there is a critical period during early stages of brain maturation during which abnormal electrical activity, particularly in the temporal lobes, perturb the development of neural substrates of "social intelligence," thus leading to AD.

Measurements of resting glucose utilization in AD brains has revealed a global increase in glucose utilization *(43,44).* One recent interesting study *(45)* measured total brain glucose utilization and 5-HT synthesis capacity with alfa-methyl-tryptophan (AMT) in children with tuberous sclerosis, who were divided into three subgroups: 1) autistic; 2) nonautistic, mentally retarded; and 3) with normal intelligence. The data revealed increased glucose metabolism in the deep cerebellar nuclei and increased AMT uptake in the caudate nuclei, correlated with stereotypies and impaired social interactions. The data on increased AMT uptake in the caudate nuclei are consistent with previous observations by Chugani et al. *(46),* who have shown that 5-HT synthesis capacity is high in childhood, and decreases toward adult levels after puberty, while this developmental process is

disrupted in AD. The high 5-HT synthesis capacity found in the infantile brain is consistent with the observations of elevated 5-HT concentrations in developing sensory regions of the cerebral cortex and in thalamic sensory nuclei in rodents *(47,48)* and in primates *(49).* Also, 5-HT1_A receptor expression shows a similar time-course, being highly expressed in the neonatal brain (particularly in the cerebellum) and then declining to undetectable values in adults (*see* ref. *50* for a review). Since the human cerebral cortex is known to go through a period of active synapse elimination during childhood *(51),* persistently high levels of 5-HT synthesis capacity could indicate a defect in synapse elimination in autistic brains, and hence the persistence of an immature connectivity state in the autistic brains.

The most striking finding of early morphometric analyses by Bauman and Kemper on postmortem brains from autistic subjects *(52)* was a decreased number of cerebellar Purkinje cells and also, to a lesser degree, of granule cells in the absence of gliosis or evidence of anoxic insults. In addition, neurons in the inferior olive were preserved. A subsequent study by Bailey et al. *(53)* confirmed the finding of Purkinje cell loss in AD. More recent observations also showed some degree of Purkinje cell atrophy in AD *(54).* Reduced Purkinje cell size (but not decreased cell density) is also observed in the cerebellar vermis of elderly schizophrenic patients *(55).* Thus, decreased Purkinje cell *density* is emerging as a relatively specific finding in AD. Reduced cell density, in the absence of alterations in the inferior olive, suggests the operation of an early dysgenetic event. Additional findings in the study of Bailey et al. *(56)* were evidence for cortical dysgenesis (increased cortical thickness and cell packing density) and ectopic neurons in the cortical white matter. These data, taken together, support the hypothesis of an early dysgenetic event that affects several neural systems in AD.

A glial cell reaction may be present in AD brains, as indicated by increased levels of glial fibrillay acid protein (GFAP) in the cerebrospinal fluid of AD patients *(57),* possibly indicating that an ongoing pathogenetic mechanism is present in the brain of autistic patients.

Another line of evidence for alteration of neurodevelopmental processes in AD stems from a recent investigation showing that the concentrations of vasointestinal peptide (VIP), Calcitonin Gene Related Peptide (CGRP), Brain-Derived Neurotrophic factor (BDNF), and Neurotrophin 4/5 (NT4/5) were increased in neonatal blood of children who later developed autism or mental retardation, in comparison to children with cerebral palsy and to normal children *(58).* These observations are interesting, because neuropeptides and neurotrophins are involved in cell growth and differentiation in the nervous system, and also because some neuropeptides are able to cross the blood–brain barrier *(59).* However, it is not possible at this point to decide whether increased perinatal neuropeptide concentrations play a causative role in AD, or whether they simply represent an epiphenomenon of the disease. One way to test these alternative hypotheses would be to check the relationship between concentration of neurotrophins/neuropeptides and phenotypic traits that can be quantified, for example the rates of increase in brain volume, in view of the hypothesis that increased concentration of neurotrophins could be the cause of the increased speed of growth of brain volume that has been reported in AD *(38,39).*

Genetics of Autism

It is by now clear, mainly on the basis of twin concordance studies, that AD is one of the most "genetic" psychiatric disorders (*see* Table 1). In particular, the strong increase of concordance in autism from dizygotic (DZ) to monozygotic (MZ) twins is usually considered to be an index of strong heritability. With rare exceptions (e.g., *60*), AD does not follow a simple Mendelian pattern of inheritance. Many genetic-linkage studies in multiplex families, using markers distributed over the whole genome to detect significantly increased allele sharing between affected sib-pairs, have

Table 1
Estimates of Heritability of Autism and Other Developmental Disorders Based on Twin Concordance Studies

	Autism[1]	Severe language delay[2]	Schizophrenia[3]
Heritabil.	91–93%	73%	83%

[1] Bailey A. et al. *Psychol. Med.* 25:63–78 (1995) based on 25 affected MZ pairs; 20 DZ pairs.

[2] Dale P.S. et al. *Nature Neurosci.* 1:324–328 (1998) based on individuals within the lowest 5% percentile of vocabulary acqusition.

[3] Cannon T.D. et al. *Arch. Gen. Psychiatry* 55:67–74 (1998) based on 2495 MZ and 5378 DZ pairs.

detected loci showing positive linkage with AD in a large number of chromosomal regions, distributed over most of the human chromosomes *(20,61).* The relevance of some of these loci for AD is further underscored by the fact that they are contained in regions encompassed by chromosomal abnormalities associated with AD; this is particularly true for chromosome 15 (the region of the Angelman and Prader-Willi syndromes) and for chromosome 7 *(20,62).* Interestingly, the chromosomal abnormalities on chromosome 15 associated with AD have indicated a parent-of-origin effect (in most cases, a maternal effect) which is consistent with imprinting of the relevant loci.

The currently accepted genetic model of AD assumes several genes that interact with one another to produce the clinical phenotype, i.e. a multilocus model with epistasis (*see* ref. *20* for a review). According to this model, no single gene is necessary and sufficient to cause the disease. The multilocus-epistatic model is different from the traditional concept of genetic heterogeneity, where mutations in different genes lead to an identical, or similar phenotype *(63).* In the case of genetic heterogeneity, each gene is sufficient to cause the phenotype. A well-established example of genetic heterogeneity, that is relevant for the understanding of AD, is tuberous sclerosis, where mutations in two different genes lead to the same phenotype; the defective genes (TSC1 and TSC2) are located on different chromosomes and their gene products (hamartin and tuberin, respectively) have been shown to physically associate in vivo and may function as part of the same protein complex *(64).*

It should further be noted that the signals detected by the linkage studies in multiplex families may not indicate mutations in the strict sense (i.e., alterations of the gene structure that lead to absence of the gene product, or to a loss of control of transcription of the gene) but may represent common polymorphisms that are found also in the normal population, affecting the efficiency of the expression or the function of the gene in a subtle manner, and leading to increased susceptibility for AD. In this sense, AD may be quite similar to sporadic Alzheimer's disease, where the ε4 allele of ApoE leads to a substantial increase in risk of Alzheimer, but does not automatically lead to the disease (as demonstrated by aged individuals who are homozygous for the ε4 allele and do not develop Alzheimer's disease). Interestingly, the multilocus-epistatic model is compatible with the Quantitative Trait Locus (QTL) model for differences in complex traits among individuals, proposed by Plomin and colleagues (*see* e.g., ref. *65*). According to this perspective, complex phenotypic traits, e.g. reading ability, are distributed along a continuous, bell-shaped curve. Some gene variants, which can be found also in the normal population, increase the risk for a particular disability, and are found with higher frequency among the subjects that are affected by the disability. The three areas of impairment in AD, social ability, use of language for communication purposes, and restricted spectrum of interests, can be regarded as quantitative traits. From a research perspective, these considerations could imply that QTL strategies that are widely utilized to identify genes that are relevant for specific behaviors in mice, may be successfully applied to primate models of social disability (*see* ref. *66* and below).

The genetic model should also consider the involvement of modifier genes, i.e., genes that

are not involved in the pathogenesis of the disease, but modulate its clinical expression, for example by selectively enhancing particular traits (e.g., stereotypies) without influencing others. A good example of modifying gene has recently been described in bipolar disorder, where catechol-*O*-methyltransferase alleles have been shown to modulate the cycling speed of the disorder *(67).*

Candidate Genes in AD

Moving from chromosomal regions to candidate genes has proven to be a formidable challenge. This is not surprising, given the complex nature of AD. Indeed, the large number of chromosomal regions in linkage with AD throughout the genome, and their limited overlap in distinct samples *(20,61)* lets us foresee significant difficulties in replicating positive association findings in the absence of hypothesis-driven studies performed on patients recruited according to a more precise definition of the phenotype.

Many candidate genes for AD have been scrutinized up to the present. It can be safely stated that none of them has yet reached the status of being widely accepted as a major susceptibility gene. If the multilocus-epistatic model outlined above is correct (and there is every reason to believe that it is), one should expect that genes that have shown positive association in one study may give negative association in another, and vice versa. A complete survey of all genes that have been investigated as candidates for AD is outside the scope of this review. We will focus on candidate genes that fulfill four requirements: 1) there is evidence for association with AD in at least one genetic study; 2) the gene variants conferring susceptibility for AD are associated with alterations of gene function; 3) the role of the candidate gene in neurodevelopment is well established; 4) its manipulation in experimental animals leads to neurodevelopmental and behavioral alterations that are to some degree consistent with findings in AD.

The Serotonin Transporter (HTT, SLC6A4)

Starting from the first report of elevated 5-HT whole-blood levels in autism *(68)* the serotonin transporter of the plasma membrane *(HTT)* in autism has been the subject of intensive investigations, a fact that is not surprising given the key role of 5-HT in social behavior, sleep, aggression, anxiety, and affective regulation, as well as differentiation of the cerebral cortex. In addition, selective inhibitors of 5-HT uptake (SSRIs) ameliorate some aspects of autistic behavior, and inhibition of 5-HT synthesis by tryptophan depletion exacerbates autistic behavior, suggesting that the efficiency of serotoninergic synaptic transmission may be reduced in autism (reviewed in ref. *69*).

Hyperserotoninemia is present in 25–45% of patients across studies *(70).* Elevated 5-HT blood levels may be a marker for familial autism *(71).* However, there is a general heritability of serotoninemia independent of diagnosis *(72).* Another striking finding is that 5-HT blood levels are highest in childhood and decrease after puberty *(73),* and both autistic and nonautistic children show higher 5-HT blood levels than their parents *(74).* Hence, 5-HT blood levels appear to follow a similar developmental time-course as brain 5-HT synthesis capacity *(46).* It is now known that more than 99% of 5-HT in blood is contained within platelets, leading to a high temporal stability of 5-HT blood levels in individuals *(75).* Recently, heritability of whole-blood 5-HT levels was shown to be extremely high (0.99) *(76).* Studies of the platelet 5-HTT have revealed decreased affinity *(77)* and increased B_{max} *(78)* for ^{3}H-paroxetine in AD. The underlying alterations of the 5-HTT are not known, and could be related to many different mechanisms, including genetic polymorphisms of the *HTT* gene, leading to different transcription rates of the transporter *(79),* or posttranslational modifications of the transporter. Investigations on the 5-HTT in AD bear much interest, because the transporter expressed in platelets and in serotoninergic neurons are encoded by the same gene. It should be

borne in mind, however, that transcriptional regulation and posttranslational modifications of the transporter may not be identical in platelets and in serotoninergic neurons.

The insertion/deletion polymorphism in the promoter of the 5-HT transporter gene (5-HTTLPR) has emerged as a promising candidate gene in AD (reviewed in ref. *62*). This promoter polymorphism is interesting because expression of the shorter allele leads to a decrease in serotonin transporter expression in lymphoblastoid cell lines *(79).* There is conflicting evidence about association of the shorter or the longer allele with autism *(80,81).* Aside the issue of involvement in AD, two recent reports suggest that the 5-HTTLPR does not play a major role in determining 5-HT blood levels in normal subjects or hyperserotoninemia in autistic patients *(74,82,83).* Other polymorphisms of the SLC6A4 gene besides the 5-HTTLPR may be associated with AD *(84).*

Elevated extracellular levels of 5-HT in mice impairs barrel pattern formation in the somatosensory cortex *(85,86).* Whisker barrels are clusters of neurons that are specialized in the processing of sensory information from individual whiskers. They represent an extreme example of columnar organization of the cerebral cortex. These data are interesting in view of the alterations of columnar organization in the autistic brain discussed above *(31).*

GABA Receptors

There is a large body of evidence that the neurotransmitter GABA is involved in early developmental processes of the cerebral cortex. At early stages of corticogenesis, neuroblasts are electrically coupled in clusters of 15–90 cells showing large responses to GABA application *(87).* Furthermore, Cajal-Retzius cells in the marginal zone of the developing cortical plate receive an extensive GABAergic input, and the GABAergic response is blocked by $GABA_A$ receptor antagonists *(88).* Also, GABA can either promote or arrest the motility of migrating cortical neurons via $GABA_A$ receptor-mediated depolarization *(89),* and migrating cortical neurons express $GABA_B$ receptors, release GABA, their migration is stimulated by exogenous GABA and inhibited by $GABA_B$ receptor antagonists *(90).*

A screening with ligands for different neurotransmitter receptor subtypes on postmortem brains of autistic patients revealed a decrease in $GABA_A$ receptor expression in the hippocampus *(91).* In addition, GABAergic abnormalities in AD are suggested by the observed decrease of glutamic acid decarboxylase 65 and 67 kDa isoforms in AD brains *(92).* A cluster of $GABA_A$-receptor-associated genes is located on chromosome 15q11–13, a region of the genome where cytogenetic abnormalities associated with AD most often occur. Several studies have found evidence of genetic association with markers in close proximity to the $GABA_A$ receptor gene cluster in AD *(93–96).*

Wnt-2

The wingless-type MMTV integration site family member 2 *(WNT2)* has been proposed as a susceptibility gene for AD *(97).* Wnt genes encode secreted growth factor-like proteins that are involved in growth regulation, differentiation, and tumorigenesis. Two families with mutations in the Wnt-2 coding sequence have been identified so far; in addition, a SNP in 3'UTR of the mRNA shows linkage in a subgroup of families defined by the presence of severe language abnormalities *(97).* Wnt-2 appears to be expressed in the adult human thalamus, although at low levels *(97),* but its involvement in brain development has not been demonstrated yet. In fact, targeted disruption of the Wnt2 gene results in placentation defects, smaller birth weights, and increased perinatal lethality, but surviving mice do not show any apparent anatomical or physiological defects, and show a normal behavior *(98).* On the other side mice lacking *Dvl1,* a member of a gene family that plays an essential role in the Wnt-signaling pathways, demonstrate reduced social interaction and abnormal sensorimotor gating *(99).* Since these abnormalities could result from interruption of

Wnt-signaling pathways different from Wnt-2, the evidence that Wnt-2 confers susceptibility for AD should be considered as preliminary.

HoxA1

Starting from the observation of near-complete absence of the facial nucleus and superior olive, along with shortening of the brainstem in an autistic patient *(36),* and of similar deficits in HoxA1 gene knockout mice *(100,101),* Rodier and colleagues sequenced the coding region of the HoxA1 gene from patients with autism spectrum disorders. They found a single base substitution changing one histidine residue to arginine in a series of histidine repeats; this substitution was much more frequent in the AD cases than in control cases *(37).* What is striking in the report by Ingram et al. *(37)* is the high number of patients with ear defects, defects of the facial skeleton, and defects in cranial nerves. Hence, it seems likely that HoxA1 variants are responsible for a subtype of AD secondary to brainstem defects.

Reelin

In a recent case-control and intrafamilial association study we have shown that individuals inheriting alleles of the Reelin gene (RELN) that contain (11 GGC repeats in the 5′-UTR of the RELN mRNA ("long alleles") have an increased risk of AD *(102).* RELN is located in 7q22, within a region that has shown linkage with autism in several genome-wide scans based on multiplex families. Until now, two other groups were able to replicate the preferential transmission of long alleles to affected offspring *(103,104),* while another group could not replicate the findings *(105).* RELN was also found in the duplicated region of a patient affected by Tourette syndrome *(106).* Furthermore, autistic patients and their first-degree relatives show significantly reduced plasma levels of full-length Reelin and its low molecular weight isoforms *(107).*

Reelin is a large protein of the extracellular matrix that plays a key role in layer formation during early stages of development of the cerebral and cerebellar cortices *(108).* The *reeler* mutation is a spontaneous recessive mutation in mice that leads, in the homozygous state, to absence of Reelin and to severe disorganization of cortical, hippocampal, and cerebellar development. Heterozygous *reeler* mice (rl/+), known to display Reelin levels that are reduced by 50% in comparison to wild-type mice *(109),* do not show gross developmental abnormalities of the CNS, but do show a progressive loss of Purkinje cells in the cerebellum during the first postnatal weeks (16% loss at 3 mo of age in rl/+ mice compared to their +/+ littermates). Surprisingly, this loss of Purkinje cells is seen only in male rl/+, but not in female rl/+ mice *(110),* implying that the Reelin gene exerts its effects in a gender-specific way. Converging lines of evidence suggest that Reelin may act also at later stages of development, i.e. during synaptogenesis and possibly also in synapse elimination and synaptic plasticity *(109,111–113).*

We recently have discovered that Reelin is a serine protease and that its likely targets are extracellular matrix proteins like fibronectin and laminin *(114).* Serine proteases and other serine hydrolases are selectively and irreversibly inhibited by low doses of organophosphate compounds (OP), compounds routinely used as pesticides in agriculture and as insecticides in the household. Reelin could thus represent an important target for OP neurotoxicity during development. A recent epidemiological study, involving measurement of OP metabolites in postpartum meconium, supports the link between prenatal exposure to anticholinesterase OP and postnatal neurocognitive deficits; the most frequently detected OP metabolites in meconium were diethylphosphate (DEP) and diethylthiophosphate (DETP), which are inactive metabolites of Diazinon and Chlorpyrifos, two widely used anticholinesterase pesticides in the US *(115).* Prenatal experimental infection of mice with the human influenza virus has been shown to cause a decrease in the number of Reelin-positive Cajal-Retzius cells in the

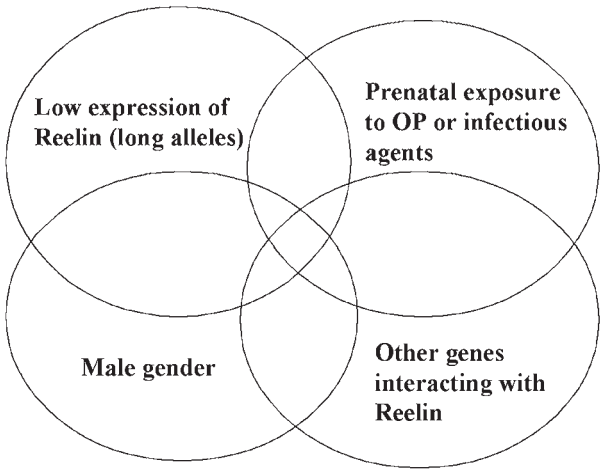

Fig. 2. A model for Reelin involvement in autistic disorder.

neonatal cortex, and a decreased thickness of the cerebral cortex and hippocampus *(116).*

Many genes that could interact with Reelin are located on 7q close to RELN, e.g., the genes encoding the laminin α1 and β4 subunits, LAMB1 and LAMB4, and the OP-inactivating paraoxonase PON1, thus possibly explaining the high density of positive linkage signals on chromosome 7q and the frequency of autistic traits in patients with chromosomal aberrations in this region *(20).*

Based on the above considerations, we are proposing a pathogenetic model for a subgroup of patients with autism, whereby subjects with genotype-dependent low Reelin production ("long" GGC alleles, male sex) compared to subjects with "normal" Reelin levels, may be at a higher risk of developing subtle neurodevelopmental abnormalities leading to autism, when exposed to external agents causing further impairment in Reelin function during critical periods in neurodevelopment (Fig. 2).

Genes Associated With the Oxcytocin-Signaling Pathway

Oxytocin, the hormone that stimulates uterine contraction and milk ejection from the mammary gland, is also known to be implicated in the central mediation of attachment behavior *(117).* Mountain and prairie voles, two species of rodents that show striking differences in social behavior and parental care of offspring, display striking differences in the pattern of distribution of oxytocin-binding sites in the brain. These differences appear to be due to differential expression of the oxytocin receptor gene, rather than to posttranslational mechanisms. Hypothetical mechanisms that could explain these large differences in oxytocin-receptor-gene expression include sequence variations of the gene promoter, differential expression of transcription factors, and also epigenetic factors *(118).* Consistent with these findings, mice with targeted deletion of the oxytocin gene appear to have selective deficits in social memory, since they fail to recognize familiar conspecifics after repeated social encounters, although their olfactory system and nonsocial memory functions appear to be intact (*see* ref. *119* for a review). Recent results suggest that children with AD display alterations of oxytocin peptide forms in plasma *(120),* suggesting alterations in oxytocin processing in autism. The human oxytocin gene is located in 20p13; chromosomal abnormalities of 20p associated with autism have been sporadically reported *(121).* Alterations in oxytocin processing could be due to

altered levels of prohormone convertases, PC2 (located on 20p11.1–11.2, close to the oxytocin gene) and PC5 (9q21.3). In genetic syndromes involving chromosome 15q, such as the Prader-Willi syndrome, there is a decrease in the number and volume of oxytocin neurons *(122).* Furthermore, PC2 activity is dependent on a polypeptide, 7B2, that is also located on 15q in the Prader-Willi region. Clearly, the genes associated with oxytocin signaling deserve further investigations.

The Cholinergic System in AD

The role of acetylcholine in morphogenesis is well established, and prenatal exposure to neurotoxins that affect cholinergic neurotransmission may seriously compromise brain development and have long-lasting functional consequences after birth (*see* ref. *123* for a review). Investigators have only recently begun to focus onto the cholinergic system in AD. Perry et al. *(124)* compared AD brains with brains of mentally retarded individuals and brains of normal-matched individuals, and found abnormalities of cholinergic markers in the cerebral cortex and basal forebrain of AD brains, compared to age-matched normal controls. Parietal and frontal cortices showed a significant increase of ^{3}H-epibatidine binding, a high-affinity probe of the neuronal nicotinic acetylcholine receptor. In the basal forebrain of AD patients, there was a threefold increase in BDNF levels (while NGF levels were similar to controls) and an increase in choline acetyltransferase and acetylcholinesterase activity that did not reach statistical significance (however, this may be due to the low statistical power of the study). A second study *(125)* found evidence of nicotinic-receptor abnormalities in the cerebellum, involving decreased ^{3}H-epibatidine binding, and a threefold increase in alfa-bungarotoxin binding. Although it is not yet clear whether these findings reflect alterations of cholinergic neurotransmission, these studies should encourage genetic research on the cholinergic system in AD.

What Are the Causes for the Higher Prevalence of Autism in Males?

The causes of the skewed sex ratio in AD, and of its striking variability, are presently unknown. The observation that the sex ratio in autism does not appear to be familial was initially taken as evidence against X linkage *(126).* While older genome-wide linkage studies failed to detect any positive signal on the X or Y chromosomes, recent genome-wide scans did detect positive-linkage signals on the X chromosome *(127,128)*. Indeed, mutations in two X–linked genes (NLGN3 and NLGN4) associated with autism have recently been identified in two Swedish families *(128a)*. Furthermore, the presence of broad autistic traits in Turner's syndrome patients with maternal inheritance of their X chromosome supports the existence of an imprinted locus on Xq or centromeric Xp relevant to social behavior. If expressed only in X chromosomes of paternal origin, the lack of expression at this locus in males could significantly contribute to their enhanced autism liability *(129).* Alternatively, males could be more frequently affected because of a higher risk for an insult occurring during prenatal or early postnatal life, or because of a nontransmissible genetic event *(126).* Recently, elevated testosterone levels *in utero* have been suggested as a risk factor for AD *(130,131),* an hypothesis that is attractive because of the well-known effects of sex hormones, particularly testosterone, on early brain development.

Another possibility that should be considered is that female sex may protect from the expression of a mutation in the heterozygous state, a phenomenon that has been observed in mouse mutants such as in the heterozygous *reeler* (rl/+) *(110)* and *staggerer* (Rora+/Rora^{sg}) mutants *(132).* The mechanism underlying this phenomenon is not yet known and represents an interesting target of investigation. In our opinion, genes that show linkage with AD and are differentially expressed in male and female individuals will represent particularly strong candidates for AD.

Animal Models of Autism?

Animal models are likely to help unravel the route from gene effects to the autism phenotype if two conditions prove themselves to be true: 1) genetic effects act on components of AD, rather than on the whole syndrome as such *(133);* 2) biochemical and neuropathological traits that are characteristic of autism will be better characterized than they are at present.

The special power of genetic mouse models lies in the ability to manipulate both genotype and environment *(134).* It is conceivable that, once "candidate genes" for AD are identified, AD-associated variants can be introduced into mice (e.g., by "knock-in" techniques, if spontaneous mouse mutants are not available), and their epistatic interactions can be investigated by crossing single mutants.

Mouse genetic research related to AD should not be driven by the goal of generating an "autistic mouse," mainly for two reasons: 1) the spectrum of social, cognitive, and language abilities of mice is obviously much more restricted than the one of humans; 2) many different mutations can lead to the same behavioral phenotype in the mouse, and none of them may be relevant for the human pathogenetic mechanism. Instead, the greatest benefit of mouse models will probably be to unravel the function of variants of a particular candidate gene for AD in the context of the whole organism, particularly during development of its interactions with other genes (epistatic interactions), and of the interplay between genotype and pre/postnatal environmental factors. Examples of the power of this strategy are already available from the field of Alzheimer research: transgenic mice that have been engineered to overexpress a human amyloid precursor protein mutation and a mutant presenilin 1 gene (double transgenics) develop visible β-amyloid deposits at earlier stages than the single mutants (reviewed in ref. *135*).

As it was described in a previous section, prenatal exposure of rats to valproic acid *(136)* or to human influenza virus *(116)* have been shown to produce anatomical defects that resemble in some aspects the neuropathology found in AD. The time-point of exposure to these agents is likely to be crucial, because of different brain structures have different critical periods for development. However, it is unlikely that environmental factors alone, in the absence of a genetic liability, lead to development of AD. The potential interest of mountain and prairie voles for the understanding of neurobiological mechanisms of social behavior has been outlined above. Of additional potential interest for autism research are Fmr1 knockout mice. These mice show phenotypic features that are surprisingly similar to the fragile X syndrome, i.e. macroorchidism, learning deficits, and hyperactivity *(137).* Interestingly, Fmr1 knockout mice show increased synaptic spine density and length in comparison to wild-type littermates *(138)* and increased cerebral glucose utilization *(139).*

Interesting lines of research for AD may also arise from animal models that are presently investigated in a different context. For example, birdsong has been intensively investigated as an animal model of speech acquisition, since it represents a unique example of interplay between genetic program and environment, allowing the investigation of developmental regulation of neural plasticity *(140).* It is well known that auditory feedback is critical throughout the period of song learning: at any point prior to song crystallization, elimination of the auditory feedback not only arrests learning, but can also lead to loss or deterioration of previously learned song patterns *(141).* It would not be surprising if genes that regulate synaptic plasticity in birdsong would turn out to be candidate genes for AD.

Finally, for the reasons outlined above, primate models deserve closer attention. Bilateral lesions of the amygdala in primates, particularly if performed in neonatal animals, selectively impair social fear, leaving fear of inanimate objects unchanged *(142,143).* However, it is doubtful that lesion models are ade-

quate for AD, since they cause profound alterations of brain architecture that are not usually seen in AD. Perhaps application of pharmacological agents to perturb electrical activity in selected brain regions during critical periods of development could represent a better model. Disorders of socialization that might occur spontaneously in monkeys could be an interesting model *(66).* Perhaps QTL approaches could be applied, in order to define the chromosomal regions that are relevant for the social trait. Obviously, this approach requires more time than mouse genetic research. Another fruitful strategy would be to study the behavioral role in primates of gene variants that are found to increase genetic liability for AD. Obviously, this will be much easier if these variants were naturally present in primates.

Concluding Remarks (Including Some Epistemological Considerations)

AD is a complex neuropsychiatric disease where multiple genetic and environmental factors may interact, resulting in a clinical continuum. The genetic component is best described by a multilocus model that takes into account epistatic interactions between several susceptibility genes. In the past 10 years enormous progress has been made in identifying chromosomal regions in linkage with AD, but no single gene has emerged yet as a major factor of liability. However, it is our opinion that the understanding of AD will not come solely from genetic studies, but by integrating genetic data with neurodevelopmental mechanisms, and with knowledge about the integrated function of neural circuits during specific critical periods of development. Genes have pleiotropic effects, and their actions strongly depend on the spatial and temporal context wherein they are expressed, and on the previous developmental history of the organism. Conversely, neuronal activity can affect gene expression in a complex manner.

Early dysgenetic events responsible for AD are unlikely to affect specific neural systems, but may rather affect several neural systems at once. This does not imply that all neural systems necessarily display the same sensitivity to these pathogenetic event(s). Indeed, depending on the particular combination of genetic liability, environmental factors, and developmental time period, different neural systems could be affected. This model could explain the variable pattern of anatomical and functional abnormalities described in AD. Furthermore, the early dysgenetic events could lead to alterations of synaptic activity during critical periods of postnatal development of the brain: this could explain the phenomenon of developmental regression.

The role of environmental factors needs further investigation, to elucidate not only which factors may be involved in autism pathogenesis, but also to clarify whether environmental factors act merely as precipitating agents, always requiring an underlying genetic liability, or whether they represent in most patients an essential component of a pathogenetic process where genetic liability alone does not lead to the full-blown autism phenotype.

"Noise" in regulatory gene networks could be a third, fundamental player in the pathogenesis of AD. The effect of "random" fluctuations of gene expression in early development should be actively investigated.

Perhaps one important step forward in thinking about AD (and about psychiatric disorders in general) is to acknowledge the "multilayered" reality of this type of diseases: at the outer level there is the clinical syndrome, then follows the pathologic–anatomic level, and then the molecular–genetic level. One can safely say that, at least at present, we are far from achieving an exact mapping between these different levels. The question is whether this is simply the consequence of insufficient knowledge, or whether this reflects some very fundamental property of the relationship between molecules, brain circuits, and behavior. If we are to consider living beings as complex systems with several levels of organization, it can be convinc-

ingly argued that the "lower levels" of organization (genome, brain circuits) only represent "boundary conditions" for the higher levels (behavior), but do not strictly determine behavior. Therefore behavior cannot be reduced to brain circuits or molecules (*see* e.g., ref. *144*). Perhaps the complex and multifaceted nature of AD is simply a consequence of the irreducible complexity of human behavior itself.

Acknowledgments

We are very grateful to Dr. Maria Dora Morgante for her patience and efficiency; Drs. Ed Cook, Susan Folstein, and Robert Plomin for their helpful comments on the manuscript; and Ramona Marino for editorial assistance.

Work by the authors has been supported by grants from the National Alliance for Autism Research (NAAR), *Consiglio Nazionale delle Ricerche (CNR), Fondazione Telethon,* and *Fondation Jerome Lejeune pour les Maladies de l'Intelligence.*

This paper is dedicated to the many parents of autistic children who have constantly stimulated and inspired us.

References

1. American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders, 4th Edition.
2. Kanner, L. (1943) Autistic disturbances of affective contact. *Nervous Child* **2,** 217–250.
3. Asperger, H. (1944) Die "autistischen Psychopathen" im Kindesalter. *Archiv für Psychiatrie und Nervenkrankheiten* **117,** 76–136.
4. Frith, U. (1991) Autism and Asperger syndrome, Cambridge University Press, Cambridge, UK.
5. Wing, L. (1981) Asperger's syndrome: a clinical account. *Psychol. Med.* **11,** 115–129.
6. Lord, C. and Schopler, E. (1988) in Diagnosis and Assessment in Autism (Schopler, E. and Mesibov, G., eds.), pp. 167–181, Plenum Press, New York.
7. Bienvenu, T., Poirier, K., Friocourt, G., et al. (2002) ARX, a novel Prd-class-homeobox gene highly expressed in the telencephalon, is mutated in X-linked mental retardation. *Hum. Mol. Genet.* **11,** 981–991.
8. Chakrabarti, S. and Fombonne, E. (2001) Pervasive developmental disorders in preschool children. *J. Am. Med. Associat.* **285,** 3093–3099.
9. Fombonne, E. (2001) Is there an epidemic of autism? *Pediatrics* **107,** 411–412.
10. Madsen, K. M., Hviid, A., Vestergaard, M., Schendel, D., Wohlfahrt, J., Thorsen, P., Olsen, J., and Melbye, M. (2002) A population-based study of measles, mumps, and rubella vaccination and autism. *New Engl. J. Med.* **347,** 1477–1482.
11. Miles, J. H., Hadden, L. L., Takahashi, T. N., and Hillman, R. E. (2000) Head circumference is an independent clinical finding associated with autism. *Am. J. Med. Genet.* **95,** 339–350.
12. Wing, L. (1997) The autistic spectrum. *Lancet* **350,** 1761–1766.
13. Miles, J. H. and Hillman, R. E. (2000) Value of a clinical morphology examination in autism. *Am. J. Med. Genet.* **91,** 245–253.
14. Smalley, S. L. (1997) Genetic influences in childhood-onset psychiatric disorders: autism and attention-deficit/hyperactivity disorder. *Am. J. Hum. Genet.* **60,** 1276–1282.
15. Davidovitch, M., Glick, L., Holtzman, G., Tirosh, E., and Safir, M. P. (2000) Developmental regression in autism: maternal perception. *J. Autism Dev. Disord.* **30,** 113–119.
16. Rapin, I. (1995) Autistic regression and disintegrative disorder: how important the role of epilepsy? *Semin. Pediatr. Neurol.* **2,** 278–285.
17. Shinnar, S., Rapin, I., Arnold, S., Tuchman, R. F., Shulman, L., Ballaban-Gil, K., Maw, M., Deuel, R. K., and Volkmar, F. R. (2001) Language regression in childhood. *Pediatr. Neurol.* **24,** 183–189.
18. Rapin, I. and Katzman, R. (1998) Neurobiology of autism. *Ann. Neurol.* **43,** 7–14.
19. Wakefield, A. J., Murch, S. H., Anthony, A., et al. (1998) Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. *Lancet* **351,** 637–641.
20. Folstein, S. E. and Rosen-Sheidley, B. (2001) Genetics of autism: complex aetiology for a heterogeneous disorder. *Nat. Rev. Genet.* **2,** 943–955.
21. McAdams, H. H. and Arkin, A. (1999) It's a noisy business! Genetic regulation at the nanomolar scale. *Trends Genetics* **15,** 65–69.

22. McAdams, H. H. and Arkin, A. (1997) Stochastic mechanisms in gene expression. *Proc. Natl. Acad. Sci. USA* **94,** 814–819.
23. Fedoroff, N. and Fontana, W. (2002) Genetic networks. Small numbers of big molecules. *Science* **297,** 1129–1131.
24. Brody, S. L., Yan, X. H., Wuerffel, M. K., Song, S. K., and Shapiro, S. D. (2000) Ciliogenesis and left-right axis defects in forkhead factor HFH-4-null mice. *Am. J. Respir. Cell. Mol. Biol.* **23,** 45–51.
25. Ghaziuddin, M., Zaccagnini, J., Tsai, L., and Elardo, S. (1999) Is megalencephaly specific to autism? *J. Intell. Disab. Res.* **43,** 279–282.
26. Hardan, A. Y., Minshew, N., Mallikarjuhn, M., and Keshavan, M. S. (2001) Brain volume in autism. *J. Child. Neurol.* **16,** 421–424.
27. Aylward, E. H., Minshew, N. J., Goldstein, G., Honeycutt, N. A., Augustine, A. M., Yates, K. O., Barta, P. E., and Pearlson, G. D. (1999) MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. *Neurology* **53,** 53.
28. Saitoh, O., Karns, C. M., and Courchesne, E. (2001) Development of the hippocampal formation from 2 to 42 years: MRI evidence of smaller area dentata in autism. *Brain* **124,** 1317–1324.
29. Sears, L. L., Vest, C., Mohamed, S., Bailey, J., Ranson, B. J., and Piven, J. (1999) An MRI study of the basal ganglia in autism. *Prog. Neuro-Psychopharmacol and Biol. Psychiat.* **23,** 613–624.
30. Piven, J., Bailey, B. S., Ranson, B. J., and Arndt, S. (1997) An MRI study fo the corpus callosum in autism. *Am. J. Psychiatry* **154,** 1051–1056.
31. Casanova, M. F., Buxhoeveden, D. P., Switala, A. E., and Roy, E. (2002) Minicolumnar pathology in autism. *Neurology* **58,** 428–432.
32. Chan, S., Kwong, K., Hodge, S., McGrath, L., Steele, S., Tager-Flusberg, H., and Harris, G. J. (2002) A voxel-based morphometric study in autism and language impairment. *Ann. Neurol.* in press.
33. Courchesne, E., Young-Courchesne, R., Press, G. A., Hesselink, J. R., and Jernigan, T. L. (1988) Hypoplasia of cerebellar vermal lobules VI and VII in autism. *New Engl. J. Med.* **318,** 1349–1354.
34. Deb, S. and Thompson, B. (1998) Neuroimaging in autism. *Br. J. Psychiat.* **173,** 299–302.
35. Sultan, F. (2002) Analysis of mammalian brain architecture. *Nature* **415,** 133–134.
36. Rodier, P. M., Ingram, J. L., Tisdale, B., Nelson, S. and Romano, J. (1996) Embryological origin for autism: developmental anomalies of the cranial nerve motor nuclei. *J. Comp. Neurol.* **370,** 247–261.
37. Ingram, J. L., Stodgell, C. J., Hyman, S. L., Figlewicz, D. A., Weitkamp, L. R., and Rodier, P. M. (2000) Discovery of allelic variants of HOXA1 and HOXB1: genetic susceptibility to autism spectrum disorders. *Teratology* **62,** 393–405.
38. Hashimoto, T., Tayama, M., Murakawa, K., Yoshimoto, T., Miyazaki, M., Harada, M., and Kuroda, Y. (1995) Development of the brainstem and cerebellum in autistic patients. *J. Autism Develop. Dis.* **25,** 1–18.
39. Courchesne, E., Karns, C. M., Davis, H. R., et al. (2001) Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology* **57,** 245–254.
40. Bolton, P. F. and Griffiths, P. D. (1997) Association of tuberous sclerosis of temporal lobes with autism and atypical autism. *Lancet* **349,** 392–395.
41. Ballaban-Gil, K. and Tuchman, R. (2000) Epilepsy and epileptiform EEG: association with autism and language disorders. *Ment. Retard. Dev. Disabil. Res. Rev.* **6,** 300–308.
42. Lewine, J. D., Andrews, R., Chez, M., et al. (1999) Magnetoencephalographic patters of epileptiform activity in children with regressive autism spectrum disorders. *Pediatrics* **104,** 405–418.
43. Rumsey, J. M., Duara, R., Grady, C., Rapoport, J. L., Margolin, R. A., Rapoport, S. I., and Cutler, N. R. (1985) Brain metabolism in autism. Resting cerebral glucose utilization rates as measured with positron emission tomography. *Arch. Gen. Psychiatry* **42,** 448–455.
44. Horwitz, B., Rumsey, J. M., Grady, C. L., and Rapoport, S. I. (1988) The cerebral metabolic landscape in autism. Intercorrelations of regional glucose utilization. *Arch. Neurol.* **45,** 749–755.
45. Asano, E., Chugani, D. C., Muzik, O., Behen, M., Janisse, J., Rothermel, R., Mangner, T. J., Chakraborty, P. K., and Chugani, H. T. (2001) Autism in tuberous sclerosis complex is related to both cortical and subcortical dysfunction. *Neurology* **57,** 1269–1277.
46. Chugani, D. C., Muzik, O., Behen, M., Rothermel, R., Janisse, J. J., and Chugani, H. T. (1999) Developmental changes in brain serotonin

synthesis capacity in autistic and nonautistic children. *Ann. Neurol.* **45,** 287–295.
47. Bennett-Clarke, C. A., Chiaia, N. L., Crissman, R. S., and Rhoades, R. W. (1991) The source of the transient serotoninergic input to the developing visual and somatosensory cortices in rat. *Neuroscience* **43,** 163–183.
48. Lebrand, C., Cases, O., Adelbrecht, C., Doye, A., Alvarez, C., El Mestikawy, S., Seif, I., and Gaspar, P. (1996) Transient uptake and storage of serotonin in developing thalamic neurons. *Neuron* **17,** 823–835.
49. Goldman-Rakic, P. S. and Brown, R. M. (1982) Postnatal development of monoamine content and synthesis in the cerebral cortex of rhesus monkeys. *Dev. Brain Res.* **4,** 339–349.
50. Chugani, D. C. (2002) Role of altered brain serotonin mechanisms in autism. *Mol. Psychiatry* **7 Suppl 2,** 16–17.
51. Huttenlocher, P. R. and Dabholkar, A. S. (1997) Regional differences in synaptogenesis in human cerebral cortex. *J. Comp. Neurol.* **387,** 167–178.
52. Kemper, T. L. and Bauman, M. L. (1993) The contribution of neuropathologic studies to the understanding of autism. *Neurol. Clin.* **11,** 175–187.
53. Bailey, A., Luthert, P., Dean, A., Harding, B., Janota, I., Montgomery, M., Rutter, M. and and Lantos, P. (1998) A clinicopathological study of autism. *Brain* **121,** 889–905.
54. Fatemi, S. H., Halt, A. R., Realmuto, G. R., Earle, J., Kist, D. A., Thuras, P. and Merz, A. (2002) Purkinje cell size reduced in cerebellum of patients with autism. *Cell Mol. Neurobiol.* **22,** 171–175.
55. Tran, K. D., Smutzer, G. S., Doty, R. L. and Arnold, S. E. (1998) Reduced Purkinje cell size in the cerebellar vermis of elderly patients with schizophrenia. *Am. J. Psychiatry* **155,** 1288–12890.
56. Bailey, A., Luthert, P., Dean, A., Harding, B., Janota, I., Montgomery, M., Rutter, M. and Lantos, P. (1998) A clinicopathological study of autism. *Brain* **121,** 889–905.
57. Ahlsen, G., Rosengren, L., Belfrage, M., Palm, A., Haglid, K., Hamberger, A., and Gillberg, C. (1993) Glial fibrillary acid protein in the cerebrospinal fluid of children with autism and other neuropyschiatric disorders. *Biol. Psychiatry* **33,** 734–743.
58. Nelson, K. B., Grether, J. K., Croen, L. A., Dambrosia, J. M., Dickens, B. F., Jelliffe, L. L., Hansen, R. L., and Phillips, T. M. (2001) Neuropeptides and neurotrophins in neonatal blood of children with autism or mental retardation. *Ann. Neurol.* **49,** 587–606.
59. Cheng, Y., Tao, Y., Black, I. B., and DiCicco-Bloom, E. (2001) A single peripheral injection of basic fibroblast growth factor (bFGF) stimulates granule cell production and increases cerebellar growth in newborn rats . *J. Neurobiol.* **46,** 220–229.
60. Ritvo, E. R., Spence, M. A., Freeman, B. J., Mason-Brothers, A., Mo, A., and Marazita, M. L. (1985) Evidence for autosomal recessive inheritance in 46 families with multiple incidences of autism. Evidence for autosomal recessive inheritance in 46 families with multiple incidences of autism. *Am. J. Psychiatry* **142,** 187–192.
61. Lamb, J. A., Moore, J., Bailey, A., and Monaco, A. P. (2000) Autism: recent molecular genetic advances. *Hum. Mol. Genet.* **9,** 861–868.
62. Cook, E. H. (2001) Genetics of autism. *Child Adolesc. Psychiatr. Clin. N. Am.* **10,** 333–350.
63. Wolf, U. (1997) Identical mutations and phenotypic variation. *Hum. Genet.* **100,** 305–321.
64. Van Slegtenhorst, M., Nellist, M., Nagelkerken, B., et al. (1998) Interaction between hamartin and tuberin, the TSC1 and TSC2 gene products. *Hum. Mol. Genet.* **7,** 1053–1057.
65. Plomin, R. (1999) Genetics and general cognitive ability. *Nature* **402,** C25–C29.
66. Caviness, V. S. (2001) research strategies in autism: a story with two sides. *Curr. Opin. Neurol.* **14,** 141–143.
67. Kirov, G., Murphy, K. C., Arranz, M. J., et al. (1998) Low activity allele of catechol-O-methyltransferase gene associated with rapid cycling bipolar disorder. *Mol. Psychiatry* **3,** 342–345.
68. Schain, R. J. and and Friedman, D. X. (1961) Studies on 5-hydroxyindole metabolism in autistic and other mentally retared children. *J. Pediatr.* **58,** 315–320.
69. Posey, D. J. and McDougle, C. J. (2001) The pathophysiology and treatment of autism. *Curr. Psychiatry Rep.* **3,** 101–108.
70. Anderson, G. M., Horne, W. C., Chatterjee, D., and Cohen, D. J. (1990) The hyperserotoninemia of autism. *Ann. NY. Acad. Sci.* **600,** 331–340.
71. Piven, J., Tsai, G. C., Nehme, E., Coyle, J. T., Chase, G. A., and Folstein, S. E. (1991) Platelet serotonin, a possible marker for familial autism. *J. Autism Dev. Disord.* **21,** 51–59.

72. Cook, E. H. and Leventhal, B. L. (1996) The serotonin system in autism. *Curr. Opin. Pediatr.* **8,** 348–354.
73. McBride, P. A., Anderson, G. M., Hertzig, M. E., Snow, M. E., Thompson, S. M., Khait, V. D., Shapiro, T. and Cohen, D. J. (1998) Effects of diagnosis, race, and puberty on platelet serotonin levels in autism and mental retardation. *J. Am. Acad. Child Adolesc. Psychiatry* **37,** 767–776.
74. Persico, A. M., Pascucci, T., Puglisi-Allegra, S., et al. (2002) Serotonin transporter gene promoter variants do not explain the hyperserotoninemia in autistic children. *Mol. Psychiat.* **7,** 795–800.
75. Anderson, G. M., Freedman, D. X., Cohen, D. J., et al. (1987) Whole blood serotonin in autistic and normal subjects. *J. Child Psychol. Psychiatry* **28,** 885–900.
76. Ober, C., Abney, M., and McPeek, M. (2001) The genetic dissection of complex traits in a founder population. *Am. J. Hum. Gene.* **69,** 1068–1079.
77. Croonenberghs, J., Delmeire, L., Verkerk, R., et al. (2000) Peripheral markers of serotonergic and noradrenergic function in post- pubertal, caucasian males with autistic disorder. *Neuropsychopharmacology* **22,** 275–283.
78. Marazziti, D., Muratori, F., Cesari, A., Masala, I., Baroni, S., Giannaccini, G., Dell'Osso, L., Cosenza, A., Pfanner, P., and Cassano, G. B. (2000) Increased density of the platelet serotonin transporter in autism. *Pharmacopsychiatry* **33,** 165–168.
79. Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H., and Murphy, D. L. (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* **274,** 1527–1531.
80. Cook, E. H. J., Courchesne, R., Lord, C., Cox, N. J., Yan, S., Lincoln, A., Haas, R., Courchesne, E., and Leventhal, B. L. (1997) Evidence of linkage between the serotonin transporter and autistic disorder. *Mol. Psychiatry* **2,** 247–250.
81. Klauck, S. M., Poustka, F., Benner, A., Lesch, K. P., and Poutska, A. (1997) Serotonin transporter (5-HTT) gene variants associated with autism? *Hum. Mol. Genet.* **6,** 2233–2238.
82. Betancur, C., Corbex, M., Spielewoy, C., et al. (2002) Serotonin transporter gene polymorphisms and hyperserotonemia in autistic disorder. *Mol. Psychiatry* **7,** 67–71.
83. Anderson, G. M., Gutknecht, L., Cohen, D. J., Brailly-Tabard, S., Cohen, J. H., Ferrari, P., Roubertoux, P. L., and Tordjman, S. (2002) Serotonin transporter promoter variants in autism: functional effects and relationship to platelet hyperserotonemia. *Mol. Psychiatry* **7,** 831–836.
84. Kim, S. J., Cox, N., Courchesne, R., Lord, C., Corsello, C., Akshoomoff, N., Guter, S., Leventhal, B. L., Courchesne, E., and Cook Jr., E. H. (2002) Transmission disequilibrium mapping at the serotonin transporter gene (SLC6A4) region in autistic disorder. *Mol. Psychiatry* **7,** 278–288.
85. Cases, O., Vitalis, T., Seif, I., De Maeyer, E., Sotelo, C., and Gaspar, P. (1996) Lack of barrels in the somatosensory cortex of monoamine oxidase A- deficient mice: role of a serotonin excess during the critical period. *Neuron* **16,** 297–307.
86. Persico, A. M., Mengual, E., Moessner, R., et al. (2001) Barrel pattern formation requires serotonin uptake by thalamocortical afferents, and not vesicular monoamine release. *J. Neurosci.* **21,** 6862–6873.
87. Lo Turco, J. J. and Kriegstein, A. R. (1991) Clusters of coupled neuroblasts in embryonic neocortex. *Science* **252,** 563–566.
88. Radnikow, G., Feldmeyer, D., and Lubke, J. (2002) Axonal projection, input and output synapses, and synaptic physiology of Cajal-Retzius cells in the developing rat neocortex. *J. Neurosci.* **22,** 6908–6919.
89. Behar, T. N., Schaffner, A. E., Scott, C. A., O'Connel, C., and Barker, J. L. (1998) Differential Response of Cortical Plate and Ventricular Zone Cells to GABA as a Migration Stimulus. *J. Neurosci.* **18,** 6378–6387.
90. Behar, T. N., Smith, S. V., Kennedy, R. T., McKenzie, J. M., Maric, I., and Barker, J. L. (2001) GABA(B) receptors mediate motility signals for migrating embryonic cortical cells. *Cereb. Cortex* **11,** 744–753.
91. Blatt, G. J., Fitzgerald, C. M., Guptill, J. T., Booker, A. B., Kemper, T. L., and Bauman, M. L. (2001) Density and distribution of hippocampal neurotransmitter receptors in autism: an autoradiographic study. *J. Autism. Dev. Disord.* **31,** 537–543.
92. Fatemi, S. H., Halt, A. R., Stary, J. M., Kanodia, R., Schulz, S. C., and Realmuto, G. R. (2002) Glutamic acid decarboxylase 65 and 67 kDa

proteins are reduced in autistic parietal and cerebellar cortices. *Biol. Psychiatry* **52,** 805–810.
93. Cook, E. H. J., Courchesne, R. Y., Cox, N. J., Lord, C., Gonen, D., Guter, S. J., Lincoln, A., Nix, K., Haas, R., Leventhal, B. L., and Courchesne, E. (1998) Linkage-disequilibrium mapping of autistic disorder, with 15q11-13 markers. *Am. J. Hum. Genet.* **62,** 1077–1083.
94. Martin, E. R., Menold, M. M., Wolpert, C. M., et al. (2000) Analysis of linkage disequilibrium in gamma-aminobutyric acid receptor subunit genes in autistic disorder. *Am. J. Med. Genet.* **96,** 43–48.
95. Menold, M. M., Shao, Y., Wolpert, C. M., et al. (2001) Association analysis of chromosome 15 GABAA receptor subunit genes in autistic disorder. *J. Neurogenet.* **15,** 254–249.
96. Buxbaum, J. D., Silverman, J. M., Smith, C. J., Greenberg, D. A., Kilifarski, M., Reichert, J., Cook, E. H. J., Fang, Y., Song, C. Y., and Vitale, R. (2002) Association between a GABRB3 polymorphism and autism. *Mol. Psychiatry* **7,** 311–316.
97. Wassink, T. H., Piven, J., Vieland, V. J., Huang, J., Swiderski, R. E., Pietila, J., Braun, T., Beck, G., Folstein, S. E., Haines, J. L., and Sheffield, V. C. (2001) Evidence supporting WNT2 as an autism susceptibility gene. *Am. J. Med. Genet.* **105,** 406–413.
98. Monkley, S. J., Delaney, S. J., Pennisi, D. J., Christiansen, J. H., and Wainwright, B. J. (1996) Targeted disruption of the Wnt2 gene results in placentation defects. *Development* **122,** 3343–3353.
99. Lijam, N., Paylor, R., McDonald, M. P., Crawley, J. N., Deng, C. X., Herrup, K., Stevens, K. E., Maccaferri, G., McBain, C. J., Sussman, D. J., and Wynshaw-Boris, A. (1997) Social interaction and sensorimotor gating abnormalities in mice lacking Dv11. *Cell* **90,** 895–905.
100. Carpenter, E. M., Goddard, J. M., Chisaka, O., Manley, N. R., and Capecchi, M. R. (1993) Loss of Hox-A1 (Hox-1.6) function results in the reorganization of the murine hindbrain. *Development* **118,** 1063–1075.
101. Mark, M., Lufkin, T., Vonesch, J. L., Ruberte, E., Olivo, J. C., Dolle, P., Gorry, P., Lumsden, A., and Chambon, P. (1993) Two rhombomeres are altered in Hoxa-1 mutant mice. *Development* **119,** 319–338.
102. Persico, A. M., D'Agruma, L., Maiorano, N., et al. (2001) Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. *Mol. Psychiatry.* **6,** 150–159.
103. Pericak-Vance, M. A., personal communication.
104. Zhang, H., Liu, X., Zhang, C., Mundo, E., Macciardi, F., Grayson, D. R., Guidotti, A. R., and Holden, J. J. R. (2002) Reelin gene alleles and susceptibility fo autism spectrum disorders. *Mol. Psychiatry* **7,** 1012–1017.
105. Krebs, M. O., Betancur, C., Leroy, S., Bourdel, M. C., Gillberg, C., Leboyer, M., and The Paris Autism Research International Sibpair (PARIS) study. (2002) Absence of association between a polymorphic GGC repeat in the 5′ untranslated region of the reelin gene and autism. *Mol. Psychiat.* **7,** 801–804.
106. Petek, E., Windpassinger, C., Vincent, J. B., Cheung, J., Boright, A. P., Scherer, S. W., Kroisel, P. M., and Wagner, K. (2001) Disruption of a novel gene (IMMP2L) by a breakpoint in 7q31 associated with Tourette syndrome. *Am. J. Hum. Genet.* **68,** 848–858.
107. Fatemi, S. H., Stary, J. M., and E. A., E. (2002) Reduced blood levels of reelin as a vulnerability factor in pathophysiology fo autistic disorder. *Cell Mol. Neurobiol.* **22,** 139–152.
108. D'Arcangelo, G., Miao, G. G., Chen, S. C., Soares, H. D., Morgan, J. I., and Curran, T. (1995) A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* **374,** 719–723.
109. Tueting, P., Costa, E., Dwivedi, Y., Guidotti, A., Impagnatiello, F., Manev, R., and Pesold, C. (1999) The phenotypic characteristics of heterozygous reeler mouse. *Neuroreport* **10,** 1329–1334.
110. Hadj-Sahraoui, N., Frederic, F., Delhaye-Bouchaud, N., and Mariani, J. (1996) Gender effect on Purkinje cell loss in the cerebellum of the heterozygous reeler mouse. *J. Neurogenet.* **11,** 45–58.
111. Rice, D. S., Nusinowitz, S., Azimi, A. M., Martinez, A., Soriano, E., and Curran, T. (2001) The Reelin pathway modulates the structure and function of retinal synaptic circuit. *Neuron* **31,** 929–941.
112. Weeber, E. J., Beffert, U., Jones, C., Christian, J. M., Förster, E., Sweatt, J. D., and Herz, J. (2002) Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. *J. Biol. Chem.* **277,** 39,944–39,952.
113. Quattrocchi, C. C., Huang, C., Niu, S., Sheldon, M., Benhayon, D., Cartwright Jr., J., Mosier, D. R., Keller, F., and D'Arcangelo, G. (2003) Reelin promotes peripheral synapse elimination and maturation. *Science,* in press.

114. Quattrocchi, C. C., Wannenes, F., Persico, A. M., Ciafré, S. A., D'Arcangelo, G., Farace, M. G., and Keller, F. (2002) Reelin is a serine protease of the extracellular matrix. *J. Biol. Chem.* **277,** 303–309.
115. Whyatt, R. M. and Barr, D. A. (2001) Measurement of organophosphate metabolites in postpartum meconium as a potential biomarker of prenatal exposure: a validation study. *Environm. Health Persp.* **109,** 417–420.
116. Fatemi, S. H., Emamian, E. S., Kist, D., Sidwell, R. W., Nakajima, K., Akhter, P., Shier, A., Sheikh, S., and Bailey, K. (1999) Defective corticogenesis and reduction in Reelin immunoreactivity in cortex and hippocampus of prenatally infected neonatal mice. *Mol. Psychiatry* **4,** 145–154.
117. Insel, T. R. (1997) A neurobiological basis of social attachment. *Am. J. Psychiatry* **154,** 726–735.
118. Young, L. J., Huot, B., Nilsen, R., Wang, Z., and Insel, T. R. (1996) Species differences in central oxytocin gene expression: comparative analysis of promoter sequences. *J. Neuroendocrinol.* **8,** 777–783.
119. Winslow, J. T. and Insel, T. R. (2002) The social deficits of the oxytocin knockout mouse. *Neuropeptides* **36,** 221–229.
120. Green, L., Fein, D., Modahl, C., Feinstein, C., Waterhouse, L., and Morris, M. (2001) Oxytocin and autistic disorder: alterations in peptide forms. *Biol. Psychiatry* **50,** 609–613.
121. Schroer, R. J., Phelan, M. C., Michaelis, R. C., et al. (1998) Autism and maternally derived aberrations of chromosome 15q. *Am. J. Med. Genet.* **76,** 327–336.
122. Swaab, D. F., Purba, J. S., and Hofman, M. A. (1995) Alterations in the hypothalamic paraventricular nucleus and its oxytocin neurons (putative satiety cells) in Prader-Willi syndrome: a study of five cases. *J. Clin. Endocrinol. Metab.* **80,** 573–579.
123. Lauder, J. M. and Schambra, U. B. (1999) Morphogenetic roles of acetylcholine. *Environ. Health Perspect.* **107 (Suppl 1),** 65–69.
124. Perry, E. K., Lee, M. L., Martin-Ruiz, C. M., Court, J. A., Volsen, S. G., Merrit, J., Folly, E., Iversen, P. E., Bauman, M. L., Perry, R. H., and Wenk, G. L. (2001) Cholinergic activity in autism: abnormalities in the cerebral cortex and basal forebrain. *Am. J. Psychiatry.* **158,** 1058–1066.
125. Lee, M., Martin-Ruiz, C., Graham, A., Court, J., Jaros, E., Perry, R., Iversen, P., Bauman, M., and Perry, E. (2002) Nicotinic receptor abnormalities in the cerebellar cortex in autism. *Brain* **125,** 1483–1495.
126. Jones, M. B., Szatmari, P., and Piven, J. (1996) Nonfamiliality of sex ratio in autism. *Am. J. Med. Genet.* **67,** 499–500.
127. Shao, Y., Wolpert, C. M., Raiford, K. L., et al. (2002) Genomic screen and follow-up analysis for autistic disorder. *Am. J. Hum. Genet.* **114,** 99–105.
128. Liu, W. S., Pesold, C., Rodriguez, M. A., Carboni, G., Auta, J., Lacor, P., Larson, J., Condie, B. G., Guidotti, A., and Costa, E. (2001) Dwnregulation of dentritic spine and glutamic acid decarboxylase 67 expression in the reelin haploinsufficientheterozygous reeler mouse. *Proc. Natl. Acad. Sci. USA* **98,** 3477–3482.
128a. Jamain, S., Quach, H., Betancur, C., Rastam M., Colineaux, C., Gillberg, I. C., et al. (2003) Paris Autism Research International *Sibpair* Study. Related Articles, Links Abstract Mutations of the X-linked genes encoding neuroligins *NLGN3* and *NLGN4* are associated with autism. *Nat. Genet.* **34,** 27–29.
129. Skuse, D. H., James, R. S., Bishop, D. V., Coppin, B., Dalton, P., Aamodt- Leeper, G., Bacarese-Hamilton, M., Creswell, C., McGurk, R., and Jacobs, P. A. (1997) Evidence from Turner's syndrome of an imprinted X-linked locus affecting cognitive function. *Nature* **387,** 705–708.
130. Manning, J. T., Baron-Cohen, S., Wheelwright, S., and Sanders, G. (2001) The 2nd to 4th digit ratio and autism. *Dev. Med. Child Neurol.* **43,** 160–164.
131. Baron-Cohen, S. (2002) The extreme male theory of autism. *Trends Cogn. Sci.* **6,** 248–254.
132. Doulazmi, M., Frederic, F., Lemaigre-Dubreuil, Y., Hadj-Sahraoui, N., Delhaye-Bouchaud, N., and Mariani, J. (1999) Cerebellar Purkinje cell loss during life span of the heterozygous staggerer mouse (Rora(+)/Rora(sg)) is gender-related. *J. Comp. Neurol.* **411,** 267–273.
133. Rutter, M. (2000) Genetic studies of autism: from the 1970s into the Millennium. *J. Abn. Child Psychol.* **28,** 3–14.
134. Plomin, R. and and McGuffin, P. (2003) Psychopathology in the postgenomic era. *Ann. Rev. Psychol.* **54,** 205–228.
135. Janus, C. and Westaway, D. (2001) Transgenic mouse models of Alzheimer's disease. *Physiology and Behavior* **73,** 873–886.
136. Ingram, J. L., Peckham, S. M., Tisdale, B., and Rodier, P. M. (2000) Prenatal exposure of rats

to valproic acid reproduces the cerebellar anomalies associated with autism. *Neurotoxicol. Teratol* **22,** 319–324.
137. [No authors listed] (1994) Fmr1 knockout mice: a model to study fragile X mental retardation. The Dutch-Belgian Fragile X Consortium. *Cell* **78,** 23–33.
138. Nimchinsky, E. A., Oberlander, A. M., and Svoboda, K. (2001) Abnormal development of dendritic spines in FMR1 knock-out mice. *J. Neurosci.* **21,** 5139–5146.
139. Qin, M., Kang, J. and Smith, C. B. (2002) Increased rates of cerebral glucose metabolism in a mouse model of fragile X mental retardation. *Proc. Natl. Acad. Sci. USA* **99,** 15,758–15,763.
140. Brainard, M. S. and Doupe, A. J. (2002) What songbirds teach us about lernaning. *Nature* **417,** 351–358.
141. Leonardo, A. and Konishi, M. (1999) Decrystallization of adult birdsong by perturbation of auditory feedback. *Nature* **399,** 466–470.
142. Bachevalier, J. (1996) Brief report: medial temporal lobe and autism: a putative animal model in primates. *J. Autism Dev. Disord.* **26,** 217–220.
143. Prather, M. D., Lavenex, P., Mauldin-Jourdain, M. L., Mason, W. A., Capitanio, J. P., Mendoza, S. P., and Amaral, D. G. (2001) Increased social fear and decreased fear of objects in monkeys with neonatal amygdala lesions. *Neuroscience* **106,** 653–658.
144. Polanyi, M. (1968) Life's irreducible structure. Live mechanisms and information in DNA are boundary conditions with a sequence of boundaries above them. *Science* **160,** 1308–1312.