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OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

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*Pediatrics* 2004;113:e472-e486

DOI: 10.1542/peds.113.5.e472

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<http://www.pediatrics.org/cgi/content/full/113/5/e472>

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## The Genetics of Autism

Rebecca Muhle, BA\*; Stephanie V. Trentacoste, BA\*; and Isabelle Rapin, MD†

**ABSTRACT.** Autism is a complex, behaviorally defined, static disorder of the immature brain that is of great concern to the practicing pediatrician because of an astonishing 556% reported increase in pediatric prevalence between 1991 and 1997, to a prevalence higher than that of spina bifida, cancer, or Down syndrome. This jump is probably attributable to heightened awareness and changing diagnostic criteria rather than to new environmental influences. Autism is not a disease but a syndrome with multiple nongenetic and genetic causes. By autism (the autistic spectrum disorders [ASDs]), we mean the wide spectrum of developmental disorders characterized by impairments in 3 behavioral domains: 1) social interaction; 2) language, communication, and imaginative play; and 3) range of interests and activities. Autism corresponds in this article to pervasive developmental disorder (PDD) of the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition and International Classification of Diseases, Tenth Revision*. Except for Rett syndrome—attributable in most affected individuals to mutations of the methyl-CpG-binding protein 2 (*MeCP2*) gene—the other PDD subtypes (autistic disorder, Asperger disorder, disintegrative disorder, and PDD Not Otherwise Specified [PDD-NOS]) are not linked to any particular genetic or nongenetic cause. Review of 2 major textbooks on autism and of papers published between 1961 and 2003 yields convincing evidence for multiple interacting genetic factors as the main causative determinants of autism. Epidemiologic studies indicate that environmental factors such as toxic exposures, teratogens, perinatal insults, and prenatal infections such as rubella and cytomegalovirus account for few cases. These studies fail to confirm that immunizations with the measles-mumps-rubella vaccine are responsible for the surge in autism. Epilepsy, the medical condition most highly associated with autism, has equally complex genetic/nongenetic (but mostly unknown) causes. Autism is frequent in tuberous sclerosis complex and fragile X syndrome, but these 2 disorders account for but a small minority of cases. Currently, diagnosable medical conditions, cytogenetic abnormalities, and single-gene defects (eg, tuberous sclerosis complex, fragile X syndrome, and other rare diseases) together account for <10% of cases. There is convincing evidence that “idiopathic” autism is a heritable disorder. Epidemiologic studies report an ASD prevalence of ~3 to 6/1000, with a male to female ratio of 3:1. This skewed ratio remains unexplained: despite the con-

tribution of a few well characterized X-linked disorders, male-to-male transmission in a number of families rules out X-linkage as the prevailing mode of inheritance. The recurrence rate in siblings of affected children is ~2% to 8%, much higher than the prevalence rate in the general population but much lower than in single-gene diseases. Twin studies reported 60% concordance for classic autism in monozygotic (MZ) twins versus 0 in dizygotic (DZ) twins, the higher MZ concordance attesting to genetic inheritance as the predominant causative agent. Reevaluation for a broader autistic phenotype that included communication and social disorders increased concordance remarkably from 60% to 92% in MZ twins and from 0% to 10% in DZ pairs. This suggests that interactions between multiple genes cause “idiopathic” autism but that epigenetic factors and exposure to environmental modifiers may contribute to variable expression of autism-related traits. The identity and number of genes involved remain unknown. The wide phenotypic variability of the ASDs likely reflects the interaction of multiple genes within an individual’s genome and the existence of distinct genes and gene combinations among those affected. There are 3 main approaches to identifying genetic loci, chromosomal regions likely to contain relevant genes: 1) whole genome screens, searching for linkage of autism to shared genetic markers in populations of multiplex families (families with >1 affected family member); 2) cytogenetic studies that may guide molecular studies by pointing to relevant inherited or de novo chromosomal abnormalities in affected individuals and their families; and 3) evaluation of candidate genes known to affect brain development in these significantly linked regions or, alternatively, linkage of candidate genes selected a priori because of their presumptive contribution to the pathogenesis of autism. Data from whole-genome screens in multiplex families suggest interactions of at least 10 genes in the causation of autism. Thus far, a putative speech and language region at 7q31-q33 seems most strongly linked to autism, with linkages to multiple other loci under investigation. Cytogenetic abnormalities at the 15q11-q13 locus are fairly frequent in people with autism, and a “chromosome 15 phenotype” was described in individuals with chromosome 15 duplications. Among other candidate genes are the *FOXP2*, *RAY1/ST7*, *IMMP2L*, and *RELN* genes at 7q22-q33 and the *GABA<sub>A</sub>* receptor subunit and *UBE3A* genes on chromosome 15q11-q13. Variant alleles of the serotonin transporter gene (*5-HTT*) on 17q11-q12 are more frequent in individuals with autism than in nonautistic populations. In addition, animal models and linkage data from genome screens implicate the oxytocin receptor at 3p25-p26. Most pediatricians will have 1 or more children with this disorder in their practices. They must diagnose ASD expeditiously because early intervention increases its effectiveness. Children with dysmorphic features, congenital anomalies, mental retardation, or family members with developmental disorders are those most likely to benefit from extensive medical testing and genetic consultation. The yield of testing is much less in

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Received for publication Aug 27, 2002; accepted Dec 1, 2003.

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high-functioning children with a normal appearance and IQ and moderate social and language impairments. Genetic counseling justifies testing, but until autism genes are identified and their functions are understood, prenatal diagnosis will exist only for the rare cases ascribable to single-gene defects or overt chromosomal abnormalities. Parents who wish to have more children must be told of their increased statistical risk. It is crucial for pediatricians to try to involve families with multiple affected members in formal research projects, as family studies are key to unraveling the causes and pathogenesis of autism. Parents need to understand that they and their affected children are the only available sources for identifying and studying the elusive genes responsible for autism. Future clinically useful insights and potential medications depend on identifying these genes and elucidating the influences of their products on brain development and physiology. *Pediatrics* 2004;113:e472-e486. URL: <http://www.pediatrics.org/cgi/content/full/113/5/e472>; autism, genetic, chromosome, review.

ABBREVIATIONS. ASD, autistic spectrum disorder; PDD, pervasive developmental disorder; MMR, measles-mumps-rubella; DSM-IV, *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition*; ICD-10, *International Classification of Diseases Tenth Revision*; TSC, tuberous sclerosis complex; FXS, fragile X syndrome; AS, Angelman syndrome; PWS, Prader-Willi syndrome; MZ, monozygotic; DZ, dizygotic; LD, linkage disequilibrium; GABA,  $\gamma$ -amino butyric acid; IMGSAC, International Molecular Genetic Study of Autism Consortium; MLS, multipoint logarithm of the odds score; DBH, dopamine  $\beta$  hydroxylase; Hox, homeobox; OT, oxytocin.

Autism, also known as autistic spectrum disorder (ASD) or pervasive developmental disorder (PDD), is of great concern to the practicing pediatrician. The US Department of Developmental Services reported a 556% increase in the prevalence of autism from 1991 to 1997,<sup>1</sup> a rate that is higher than the prevalence rates reported for other pediatric disorders such as spina bifida, cancer, and Down syndrome.<sup>2</sup> Likely explanations for this astonishing increase include the inclusion of broader criteria for the diagnosis of ASD and physicians' increased awareness of ASD symptoms.<sup>3</sup> Although the media have focused attention on the measles-mumps-rubella (MMR) vaccine and, more recently, mercury poisoning as potential causes of autism, epidemiologic studies to date have shown no correlative associations.<sup>4,5</sup> Greater public awareness of autism has led to increased funding for autism research, yet the cause of ASD remains largely unknown because of the complex behavioral phenotypes and multigenic etiology of this disorder.<sup>6</sup>

According to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (DSM-IV-TR)<sup>7</sup> and *International Classification of Diseases, Tenth Revision* (ICD-10)<sup>8</sup> classifications, autism is characterized by impairments in 3 behavioral domains: 1) social interaction; 2) language, communication, and imaginative play; and 3) range of interests and activities.<sup>7</sup> Assignment to 1 of 5 subtypes is based on the number and distribution of endorsed behavioral descriptors in each of the domains, as well as on the age at onset. The 5 DSM-IV PDD subtypes are 1) autistic disorder (classic autism), 2)

Asperger disorder (language development at the expected age, no mental retardation), 3) disintegrative disorder (behavioral, cognitive, and language regression between ages 2 and 10 years after entirely normal early development, including language), 4) PDD not otherwise specified (individuals who have autistic features and do not fit any of the other subtypes), and 5) Rett disorder (a genetic disorder of postnatal brain development, caused by a single-gene defect predominantly affecting girls).

The highly variable cognitive manifestations of the ASDs range from a nonverbal child with severe mental retardation and self-injury<sup>9</sup> to a high-functioning college student with an above-average IQ despite impaired language use and inadequate social skills.<sup>10</sup> Mental retardation thus is not a defining criterion for autism (albeit certain cognitive abilities are characteristically affected), but the mean distribution of IQs is lower than average,<sup>11</sup> and the likelihood of retardation increases with more widespread brain dysfunction.<sup>12</sup> Mental retardation is itself a behaviorally defined disorder of complex human abilities with many genetic and nongenetic causes. The more severe the retardation, the more likely the underlying brain dysfunction will affect the widely distributed networks responsible for sociability, language, and cognitive flexibility.

Like mental retardation, autism is a behaviorally defined syndrome with a wide variety of both genetic and nongenetic causes. With the exception of Rett syndrome, which is caused in the majority of cases by de novo mutations or microdeletions of the methyl-CpG-binding protein 2 (*MeCP2*) gene on Xq28,<sup>13</sup> there is no current evidence that the other DSM-IV subtypes of autism are linked to any particular genetic or nongenetic disorder. Therefore, when we refer in this article to autism, we are referring to the entire spectrum of behaviorally defined autism with the exception of Rett syndrome. Current evidence indicates that multiple genetic factors are the causative determinants of the majority of cases of autism.<sup>14</sup>

## METHODS

We performed a comprehensive search of Medline using the terms "autism," "autistic," "gene," "genome," "genomic," "genetic," "chromosome," "chromosomal," and "loci" in various combinations. These queries returned >500 citations. We reviewed papers published between 1961 and 2003, focusing on scientific articles published between 1995 and 2003. After study of these papers, we performed additional searches to examine specific topics (eg, "autism, oxytocin") not included in the initial set. We also reviewed 2 current definitive textbooks concerned with autism: Cohen and Volkmar<sup>15</sup> and Gillberg and Coleman.<sup>9</sup>

## RESULTS

### Defined Nongenetic and Genetic Medical Conditions Associated With Autism

Autism has been linked to a wide variety of prenatal and postnatal insults but predominantly in individual case reports or short series. In the aggregate, they account for only a small percentage of cases.<sup>9,16</sup> Obstetric complications (eg, an increased incidence of uterine bleeding) have often been blamed for autism<sup>17</sup> despite that many studies show no significant

causal relationship.<sup>18,19</sup> Intrauterine exposure to the teratogenic drugs thalidomide and valproate have been implicated as the cause of autism in a few affected children.<sup>20,21</sup> Mean levels of some of the neuropeptides substance P, vasoactive intestinal peptide, pituitary adenylate cyclase-activating polypeptide, calcitonin gene-related peptide, and neurotrophin nerve growth factor, the concentration of all of which is under genetic control, were elevated in the cord blood of children who later received a diagnosis of autism or mental retardation<sup>22</sup>; they were normal in nonautistic children with cerebral palsy, which generally results from an abnormal intrauterine environment or peri-/postnatal insult rather than a genetic condition. Maternal factors have also been examined as potential causes of autism; antibodies in the sera of a mother of 2 children, one with autism and another with severe language impairment, were shown to bind to the cerebellar cells of developing fetal mice.<sup>23</sup> There is no evidence in population surveys of any association between autism and immigrant status, socioeconomic status, or ethnicity.<sup>16</sup>

Various epidemiologic studies have reported that cerebral palsy, defined as a static motor deficit of brain origin present from early life, is present in 2.1% to 2.9% of individuals with autism and mental retardation.<sup>24-27</sup> Congenital rubella infection, initially found to be highly associated with autism,<sup>28</sup> is present in only 0.75% of recent autistic populations,<sup>24</sup> thanks to the near eradication of rubella after the introduction of quasi-universal immunization in Western countries. Other pre- and postnatal infections by organisms such as *Haemophilus influenzae* and cytomegalovirus can cause autism when they significantly damage the immature brain.<sup>9</sup>

In a review of several epidemiologic studies of autism, Fombonne<sup>24</sup> found no association between autism and inflammatory bowel disease or with a live MMR vaccination. This contradicts an earlier publication by Wakefield et al.<sup>29</sup> Large surveys that have examined the prevalence of autism before and after the initiation of widespread MMR vaccination have also failed to corroborate an association with autism<sup>4,5</sup> but have not reassured a skeptical public of the safety of the vaccine.<sup>30</sup> Some investigators postulate that it is the mercury-based preservative thimerosal in vaccines, rather than the vaccines themselves, that poses a risk to the developing infant.<sup>31</sup> This theory has also met with significant criticism.<sup>32</sup>

Epilepsy has the highest association with autism, reported in up to a third of individuals with an ASD by adulthood.<sup>25-27,33-35</sup> The epilepsy may be subclinical, yielding an electroencephalogram that is epileptiform but without clinical seizures, and is particularly frequent in disintegrative disorder.<sup>36</sup> Like autism, epilepsy is a disorder of the brain with multiple genetic and nongenetic causes and a broad range of phenotypes. Infantile spasms are particularly likely to result in autism with nondevelopment of language and mental retardation, especially when the epileptiform activity involves both temporal lobes.<sup>37</sup> An occasional nonverbal child with mental

retardation, autism, and epilepsy has exhibited early bilateral hippocampal sclerosis.<sup>38,39</sup>

Behavioral symptoms of autism are frequent in tuberous sclerosis complex (TSC) and fragile X syndrome (FXS), but these 2 disorders nevertheless account for only a minority of the total cases of autism.<sup>40,41</sup> Given the high rate of epilepsy in children with TSC and the association between autism and epilepsy, it is perhaps not surprising that as many as 25% of patients with TSC have autism.<sup>42,43</sup> An autosomally dominant neurocutaneous disorder, TSC arises from genetic mutations of either *TSC1* on 9q or *TSC2* on 16p and is characterized by ash-leaf depigmented or other cutaneous manifestations and hamartomatous lesions in multiple organs. In the brain, these lesions are termed tubers, and they are thought to cause the epilepsy seen in more than three quarters of children with TSC.<sup>44,45</sup> Furthermore, it is the haphazard distribution of these tubers, together with other metabolic changes, that influences the phenotype of TSC, giving rise in some individuals to autism or epilepsy (often infantile spasms).<sup>37</sup> In the population of patients with autism, numerous studies have quoted TSC rates of 1.1% to 1.3%,<sup>25-27,46</sup> rates that, although low, are 30% higher than the prevalence of TSC in the general population.

FXS is an X-linked genetic disorder that is significantly associated with autism and that is denoted by unusual facial features, macro-orchidism in adulthood, and cognitive impairment of variable severity. It is caused by an increased number of trinucleotide (CGG) repeats in the gene coding for the fragile X mental retardation protein. Approximately 30% of individuals with FXS are on the autistic spectrum.<sup>47,48</sup> There is disagreement, however, over the degree of FXS prevalence in patients with autism. Some early studies reported little or no association between FXS and autism,<sup>24,49</sup> whereas others found a high association<sup>50</sup> (see<sup>41</sup> for additional review). More recent epidemiologic studies have documented rates of FXS between 7% and 8% in populations with autism.<sup>26,33,51,52</sup> The discrepancies regarding the prevalence of FXS among individuals with autism may reflect the limited reliability of the cytogenetic tests used in the past compared with the more sensitive molecular tests currently used; as such, the number of girls who receive a diagnosis of FXS has increased.<sup>6</sup>

Genetic mutations that give rise to a number of additional diagnosable diseases may also be associated with autism. Neurofibromatosis, a common autosomal dominant disorder with neurologic and cutaneous manifestations, is much less frequently associated with autism than is TSC or FXS.<sup>53</sup> Angelman syndrome (AS) and Prader-Willi syndrome (PWS) usually result from genetic deletions or uniparental disomy (inheritance of both chromosomes from 1 parent) of the chromosome 15q11-q13 locus.<sup>54,55</sup> with abnormal imprinting or genetic mutations found in up to 5.1% of PWS cases and up to 15% of AS cases.<sup>55</sup> Loss of paternally derived genes results in PWS, whereas AS, more commonly associated with autism than PWS,<sup>56,57</sup> can result from the loss or mutation of the maternally derived ubiquitin



protein ligase gene *UBE3A* or the *ATP10C* gene.<sup>58–60</sup> An unexpectedly large proportion of boys with Duchenne muscular dystrophy are on the autistic spectrum.<sup>61</sup> Many other rare single-gene defects have been associated with autism in case studies, including those found in Sotos syndrome,<sup>62</sup> Williams syndrome,<sup>63</sup> hypomelanosis of Ito,<sup>64</sup> Cowden syndrome,<sup>65</sup> and Moebius syndrome.<sup>66,67</sup> We refer the reader to *The Biology of the Autistic Syndromes* by Gillberg and Coleman (p. 136–184)<sup>9</sup> for a more complete listing of rare genetic conditions that are responsible for autism in occasional individuals.

Finally, autism may also occur in the context of abnormal cellular metabolism, such as mitochondrial disease or dysfunction.<sup>68,69</sup> Untreated phenylketonuria is a well-documented metabolic cause of autism<sup>70–72</sup>; however, whether this is attributable to the resulting severe mental retardation or to the specific deficit in the dopamine pathway is uncertain.<sup>73</sup> Some clinic-based studies report high levels of uric acid secretion in up to one quarter of patients with autism and amelioration of certain symptoms with antihyperuricosuric metabolic therapy.<sup>74</sup> This represents a significant proportion of these clinical samples, but it has not been widely replicated and the genes that are responsible for this type of “purine autism” remain to be identified.

Although the links between autism and these diagnosable conditions are often convincing, we emphasize that the total number of individuals who are on the autistic spectrum and have known genetic or nongenetic conditions is only a small percentage of the whole<sup>9,14,24</sup> and that an association with autism is not universal in any 1 of the diagnosable medical or genetic conditions mentioned. In population-based studies of children with autism, they account for a small minority, probably <10%, of individuals with autism.<sup>16,25,27,75</sup> The vast majority of individuals with autism do not have any 1 of these infrequent nongenetic or rare genetic causes, yet family studies indicate that genetics play the major causative role in most individuals with “idiopathic” autism.<sup>6,76,77</sup>

#### Inherited Autism of Unknown Cause: Family Studies

Epidemiologic studies of autism report a prevalence of 5–10 cases of classic autism per 10 000 (some 3–6 per 1000 if the entire spectrum of autism is included) with a male to female ratio of 3:1.<sup>3,9,11</sup> The preponderance of males suggests an X-linked disorder, and recent genome-wide screens by 2 separate groups have found evidence of linkage to the X chromosome,<sup>78,79</sup> but the data are inconsistent. Cases of male-to-male transmission of autism in multiplex families, however, rule out X-linkage as the predominant mode of inheritance in these families.<sup>80,81</sup> Similarly, analysis of Y haplotypes in patients with autism showed no significant associations,<sup>82</sup> although Y chromosome abnormalities have been documented in case reports.<sup>83</sup>

There is strong and convincing evidence from 2 main sources that autism without a diagnosable cause is a heritable disorder. First, the rate of recurrence in siblings of affected individuals is 2% to 8%, much greater than the prevalence rate in the general

population.<sup>9,27,46</sup> Second, early twin studies in the United Kingdom and Scandinavia reported that monozygotic (MZ) twins had a rate of concordance >60% for classic autism, with no concordance found between dizygotic (DZ) twins.<sup>76,77</sup> The higher rate of MZ concordance provides compelling evidence for the strong influence of genetics in the cause of autism, influence that extends well beyond the aforementioned associated genetic disorders. Furthermore, when the unaffected twin discrepant for autism was reevaluated for broader autistic phenotypes, including communication skills and social disorders, the concordance among the UK twins rose remarkably, from 60% to 92% in MZ twins and from 0% to 10% in DZ pairs.<sup>76,84</sup> The existence of a susceptible genetic background is also suggested by the preponderance of traits such as obsessive-compulsive disorder, communication disorders, and social phobias in nonautistic family members of patients with autism.<sup>85–87</sup> These crucial observations suggest that the interactions of multiple genes cause autism and that there is variable expression of autism-related traits.

Surprising disparity in some MZ twin pairs who share 100% of their genes and are concordant for diagnosis indicates that other factors can modify these phenotypes. For example, 2 MZ twin girls with Joubert syndrome were concordant for most of its classic manifestations and underlying brain malformation but were dramatically discordant for autism: only the more severely affected twin, with a much more extensive cerebellar anomaly, had autism. This informative case study illustrates the range of possible phenotypes expressed by an identical genetic background.<sup>88</sup> Because each MZ twin was exposed to a variety of pre-, peri-, and postnatal environmental modifiers, differences in their phenotypes suggests that as-yet-undefined environmental factors were encountered by only 1 of them and that the multifactorial influence of a susceptible genetic background and random environmental stresses may be necessary for full expression of the disorder. Alternatively, 1 of the discrepant MZ twins may have sustained a random epigenetic mutation in early embryonic life that altered the expression of the genetic trait.

Despite the evidence from twin and family studies, the identity and number of genes involved are not yet known. Data from whole-genome screens in multiplex families (families with more than a single affected family member) strongly indicate that 10 or more genes interact to cause autism.<sup>89,90</sup> Cytogenetic abnormalities in individuals with autism have been found on virtually every chromosome.<sup>83</sup> Autism, therefore, seems to be multigenic in that similar autistic phenotypes may arise from different genes or gene combinations in different families. An example of single-mutation genetic heterogeneity is tuberous sclerosis caused by *TSC1* on chromosome 9q in some families and *TSC2* on 16p in others. In addition, autistic disorders are polygenic; that is, several synergistically acting genes in an affected individual's genome may be required to produce the full autistic phenotype. Thus, in individuals with autism, certain sets of genes acting in concert have lowered a theo-

retical threshold to allow the development of autism either by themselves or, in some cases, given the right set of environmental or immunologic modifiers.<sup>91</sup> Family members with other related developmental disorders (but not diagnosable autism) can be presumed to have inherited some of the susceptibility genes found in the affected family member or to have the same set of susceptibility genes but without exposure to the same environmental "trigger factors" for autism.

### The Search for Candidate Genes

A number of approaches are being used to elucidate the association between specific genes and autism (Fig 1). Whereas genome screens search for common genetic markers in populations of multiplex families with autism, cytogenetic studies search for inherited or spontaneous genetic abnormalities on an individual basis. Additional investigations, referred to as linkage disequilibrium (LD) studies, are performed to narrow the search region identified by cytogenetic analysis or genome screen or to examine linkage to a specific gene. LD refers to the inheritance of a particular allele more frequently in the affected family members than would be expected by chance and is assessed using DNA sequences called microsatellite markers. The statistically significant finding of 1 or more markers to a greater extent in the affected population denotes the inheritance of a susceptibility allele. Finally, hypothesis-driven research is a fundamentally different approach in that several candidate genes are chosen a priori for additional study on the basis of a plausible pathogenetic model of autism. The ultimate goal of all of these techniques is to identify heritable genetic mutations in candidate genes that predispose an individual to autism or to traits associated with autism. Candidate genes that are involved in the cause of autism are genes whose product is known to play a role in brain development or to be associated with brain structures, neurotransmitters, or neuromodulators implicated in autism on the basis of previous research findings.

Once candidate genes have been identified, affected individuals and age-, gender-, and ethnically matched control subjects are tested for the presence

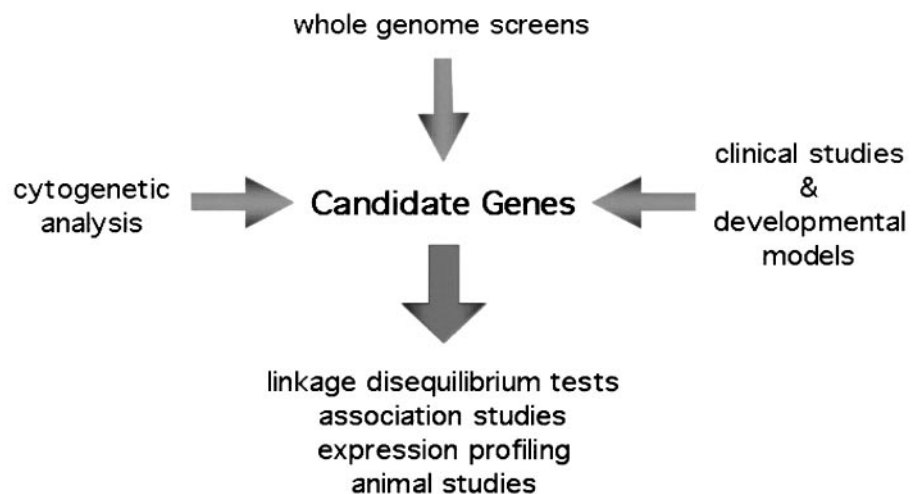
of mutations in the gene sequence or relative levels of expressed protein. Association studies use polymerase chain reaction to amplify putative candidate genes and search for mutations to determine whether a polymorphism (a change in the typical genetic sequence that may or may not be expressed as a functional mutation) within a gene shows a significant association with the disease. RNA hybridization and protein blots can be performed to identify the relative levels of the gene product. In addition, the creation of animal models through targeted gene disruption or mutation provides a complementary approach to unraveling the pathophysiology of the disorder.

### Cytogenetics and Chromosomes 15q and 7q

Cytogenetic assays have long been used to uncover chromosomal defects in patients with autism, and a number of cytogenetic abnormalities besides fragile X have been described.<sup>9</sup> Although <10% of cases of autism are associated with chromosomal abnormalities,<sup>92,93</sup> high-resolution cytogenetic scans in families with affected individuals help to locate specific genes or chromosomal regions (loci) potentially associated with the ASDs. Using various stains, the chromosomes of patients with autism are analyzed for visible breakpoints, translocations, duplications, and deletions. These regions are then scrutinized for the presence of genes that potentially are involved in the pathogenesis of ASD.

Cytogenetic abnormalities found at the 15q11-q13 locus are reported most frequently in patients with autism, up to 1% to 4%.<sup>83,93-96</sup> Various population studies and case reports have described duplications,<sup>93,96-99</sup> deletions,<sup>93,95</sup> and inversions<sup>100,101</sup> at this locus. Duplications can occur as interstitial tandem repeats (such that multiple copies of this locus are present in the chromosome) or as a supernumerary isodicentric chromosome 15 (an extra chromosome 15 with 1 or 2 copies of the chromosome 15q11-q13 region), leading to trisomy or tetrasomy of genes at the 15q11-q13 locus.<sup>54</sup> Inherited duplications are of maternal origin,<sup>69,93,97,102,103</sup> and seem to cause autism by creating an overabundance of product from the nonimprinted (and therefore not silenced) mater-

**Fig. 1.** The search for candidate genes. Investigators use various experimental techniques and pathophysiologic models of autism to identify candidate genes. The relevance of these genes to autism pathogenesis is determined by the use of experimental methods to assess the biological activity, expression, and allelic associations in populations with autism and their families.



nally derived genes. Several investigators have described a "chromosome 15 phenotype" in individuals with chromosome 15 duplications, characterized to variable degrees by ataxia, language delay, epilepsy, mental retardation, and facial dysmorphism.<sup>101,103</sup> The manifestations of this phenotype overlap with the autistic phenotype, giving credence to the involvement in ASD pathogenesis of a gene or genes in this region.

The cytogenetic abnormalities of chromosome 15q11-q13 point to several gene targets for additional study. The  $\gamma$ -amino butyric acid (GABA<sub>A</sub>) receptor gene cluster (which contains genes for 3 of the receptor's subunits: GABRB3, GABRA5, and GABRG3) is strongly implicated in the pathogenesis of autism, given its involvement in the inhibition of excitatory neural pathways and its expression in early development.<sup>104</sup> Mice deficient in GABRB3 have epilepsy and electroencephalographic abnormalities, as well as learning and memory deficits reminiscent of ASD.<sup>105,106</sup> LD studies have also pointed to the involvement of the GABA<sub>A</sub> cluster. Two groups independently found LD with marker 155CA-2 near GABRB3,<sup>96,107</sup> but attempts to replicate these findings in other populations have failed.<sup>95,108-111</sup> Other groups have found LD to other markers near the GABRB3 gene<sup>108,112</sup> or near the GABRG3 gene.<sup>111</sup> Another gene at the 15q11-q13 locus is the maternally derived AS gene *UBE3A*.<sup>113,114</sup> The expression of *UBE3A* is predominantly in the human brain, and it is regulated by complex mechanisms involving imprinting and possibly silencing by antisense RNA transcribed from the paternal chromosome.<sup>95,98,115</sup> In a screen of an autistic population using markers spanning a known translocation region at 15q11-q13, investigators found LD with a marker at the 5' end of the *UBE3A* gene, providing additional support for a link between the AS gene and autism.<sup>95</sup> Reports that individuals who harbor an abnormal chromosome 15q11-q13 do not always develop an ASD,<sup>116</sup> however, suggest that mutation of these genes is not sufficient to cause autism and again points to the requirement for multiple susceptibility genes on different chromosomes.

Chromosomal translocations have also implicated the q22-q33 region of chromosome 7.<sup>83,117-119</sup> The protein reelin (*RELN*), which localizes to a site of chromosomal translocation at 7q22, is a large secreted glycoprotein potentially involved in neural migration during development.<sup>120</sup> It is of particular interest given that it binds to neuronal receptors and that the pathology of autism can include migration cell defects.<sup>121</sup> Alterations in *RELN* protein affect cortical and cerebellar development, and the cerebellar neuronal abnormalities are among the more robust pathologic findings in autism.<sup>122</sup> Persico et al<sup>123</sup> reported an association between individuals with autistic disorder and a long trinucleotide repeat polymorphism in the 5' region of the *RELN* gene, and Western blot analysis of postmortem cerebellar cortices from 5 individuals with autism demonstrated a 44% reduction in *RELN* protein levels as compared with 8 nonautistic control subjects.<sup>124</sup>

Numerous other genes are under investigation at

the 7q22-q33 locus. A recent report analyzed the chromosome 7 breakpoints of 3 patients with autism. These breakpoints localized to 3 different regions and affected the genes *FOXP2*, neuronal pentraxin 2 (*NPTX2*), and a noncoding RNA transcript labeled TCAG\_4133353.<sup>119</sup> *NPTX2* is thought to be involved in excitatory synaptogenesis and localizes to chromosome 7q22.1. Both *FOXP2* and TCAG\_4133353 are mutated in patients with speech and language disorders, and therefore the 7q31-q33 region is designated a putative language and speech locus.<sup>125,126</sup> Disorders of language and communication are a core feature of the autistic phenotype,<sup>7</sup> and studies show that family members of individuals with autism have higher rates of communication and social difficulties than control subjects.<sup>125,127</sup> The *FOXP2* gene mutation was identified in a genetic analysis of a large nonautistic British family with developmental language and speech disorders.<sup>128</sup> Although the presenting family members do not have autism and the relevance of the *FOXP2* gene to autism is disputed,<sup>129</sup> the subsequent finding of a breakpoint in the *FOXP2* gene in a patient with autism is an important result confirming the presumed involvement of *FOXP2*. Other genes in the 7q31-q33 region include *IMMP2L*, identified as the site of a chromosomal breakpoint in a patient with Tourette's syndrome and autism,<sup>130</sup> and *RAY1/ST7*, which was interrupted by a translocation breakpoint in a boy with autism.<sup>131</sup> Researchers are currently performing association studies on these and other genes to validate these findings.

#### Whole-Genome Searches

Cytogenetic techniques, although valuable in case studies to delineate probable regions of interest, cannot identify specific genes in affected individuals with a normal karyotype. Investigators apply genome-wide screening technology to uncover specific chromosomal regions that affected individuals inherit more often than predicted by chance. These studies entail multiplex family screening using microsatellite markers. The DNA analysis of the affected family members and their first-degree affected/unaffected relatives identifies loci that co-segregate with the particular condition, a phenomenon termed linkage. Linkage of a putative autism susceptibility gene with a microsatellite marker results in decreased recombination at that locus during meiosis because of their proximity to each other. As the markers have known chromosomal locations, they allow investigators to extrapolate the position of the postulated autism genes to create a genetic map. Researchers validate the linkage by repeating the screens using markers at a higher density. A physical map can then be created by DNA isolation and sequencing to identify candidate genes (for additional information, see<sup>132-134</sup>). Fortunately, the Human Genome Project has already sequenced many regions of the genome, thereby making sequencing unnecessary in some cases and allowing rapid identification and investigation of candidate genes.<sup>135</sup>

In the first published genome-wide screen for autism-associated genes, the International Molecular



Genetic Study of Autism Consortium (IMGSAC) obtained DNA samples from 99 multiplex autistic families and looked for evidence of linkage to 354 different polymorphic microsatellite markers.<sup>136</sup> IMGSAC identified 6 regions of interest (Table 1) with a multipoint logarithm of the odds score (MLS)  $\geq 1.0$ . (The MLS ratio assesses the likelihood that the marker and the autism locus are indeed linked, or are unlinked if the presumed linkage data are insignificant.) According to Lander and Kruglyak,<sup>137</sup> an MLS score between 3.0 and 3.6 is highly significant for genetic linkage, whereas scores  $< 3.0$  are weak associations. None of the MLS scores obtained by IMGSAC in the initial study reached this threshold. However, analysis of a more epidemiologically homogeneous population subset that included only UK families uncovered a significant MLS score of 3.55 at the chromosome 7q locus. Table 1 shows the results of a later study that reexamined the sample group with an additional 69 multiplex families. This follow-up study verified linkage findings on chromosomes 7q and 16p and found additional sites of linkage on chromosomes 2q and 17q.<sup>138</sup> Therefore, although linkage data can seem weakly significant in a single study, the examination of more homogeneous populations and the inclusion of a larger number of study subjects can increase the significance of the initial findings. Of course, replication in independent samples is essential to validate these data.<sup>132,134</sup>

IMGSAC performed an additional study in 2001 to evaluate the chromosome 7 locus more closely.<sup>139</sup> They screened 170 multiplex families (91 from the original study plus 79 additional families) using a higher density of markers targeting the 40-cM region identified in the previous study. Multipoint linkage analysis showed linkage with a high MLS of 3.37 to

a specific marker at 7q31-q33, and researchers postulated the existence of an autism susceptibility locus, termed AUTS1, in affected family members. Beyer et al<sup>140</sup> constructed a physical map of this region, mapping 23 genes to the site, and Scherer et al<sup>119</sup> recently published the annotated sequence of the entire chromosome, thereby providing specific sequence data for subsequent candidate gene investigations.

Given that the ASDs display significant clinical heterogeneity, analysis of particular behavioral phenotypes exhibited by probands (the affected presenting family members) and their relatives might expose susceptibility alleles involved in the pathogenesis of these specific autism-related traits that would be otherwise weak in a screen of a phenotypically heterogeneous population of multiplex families. Researchers with the Collaborative Linkage Study of Autism hypothesized that inclusion of multiplex families selected for a specific autistic phenotype would uncover the genetic basis of these particular behavioral deficits. By selecting autistic probands with both impaired and delayed acquisition of language and speech production as well as a family history of difficult or late development of language or reading, they found increased linkage to the putative speech and language locus on chromosome 7q.<sup>141</sup> The MLS for linkage to the 7q31-q33 locus rose from 1.4 in the mixed sample to 2.2 in this impaired-language subtype of autism, whereas the linkage score at this locus in a group of probands who did not exhibit language disorders decreased from 1.4 to 0.1. The Collaborative Linkage Study of Autism also demonstrated linkage to chromosome 13q in the group selected for language difficulty. Studies by others have shown evidence of increased linkage to chromosome 2q in other populations with language difficulty.<sup>142,143</sup> A similar approach was used to show increased linkage to the chromosome 15q11-q13 locus in probands and families with repetitive movement disorders or stereotypies.<sup>144</sup> This result is particularly exciting, given that although chromosome 15q11-q13 cytogenetic abnormalities are highly associated with autism, genome screens to date have reported only weak linkages.<sup>78,145</sup>

Additional targeted studies have corroborated linkage to the autism susceptibility locus AUTS1 on 7q31-q33,<sup>117,141,146</sup> and linkage to this locus is the most highly replicated finding in the genome scans performed to date (Table 2). Although the MLS scores have been variable (0.83–3.2), the importance of this region is reinforced by the documented translocations in patients with autism. The AUTS1 locus contains several potential genes, including the aforementioned *FOXP2*, *RAY1/ST7*, and *IMMP2L*, as well as the glutamate receptor *GRM8*,<sup>147</sup> *CADPS2*,<sup>148</sup> and *WNT2*.<sup>149</sup> The *WNT2* gene codes for an evolutionarily conserved glycoprotein that is part of a developmentally important signaling pathway.<sup>150</sup> Mice harboring a *WNT2* protein signaling defect display reduced social interaction and aberrant behaviors reminiscent of autism.<sup>151</sup> Researchers have found 2 different *WNT2* mutations in multiplex families with autistic disorder.<sup>149</sup> Additional tests revealed that a

**TABLE 1.** Linkage Data From Sequential Studies by the IMGSAC Group\*

Chromosome	IMGSAC, 1998 (136)		IMGSAC, 2001 (138)	
	Location (cM)	MLS	Location (cM)	MLS
1			225.21	0.58
2	103	0.65	255.59	0.6
			24.3	0.39
			111.43	1.6
4	4.8	1.55	206.39	3.74
			0	0.71
			140.57	0.44
7	144.7	2.53	230.15	0.43
			119.6	3.2
10	51.9	1.36	53.66	1.08
			64.29	1.43
13	85	0.59		
15			34.1	0.76
			40.46	0.65
16	17.3	1.51	16.7	1.59
			21.8	2.12
			23.1	2.93
			45.37	2.34
19	48.2	0.99	63.02	0.13
22	5	1.39	41.43	0.33

\* The theoretical genetic distance between recombination events is expressed in morgans (M), which represent the distance between 2 loci such that on average 1 crossing over will occur per meiosis. Linkage studies use the centimorgan (1 cM = 0.01 M), which on average contains 1 million base pairs.



**TABLE 2.** Genetic Sites of Putative Autism Susceptibility Loci, as Determined by Genomic Screens\*

Chromosome Locus	Location (cM)	Highest LOD Score (ref)	Studies Demonstrating Additional Linkage Data	Candidate Genes (Partial Listing)
1p	149	2.63 (153)	89, 142, 221	
2q32	200	3.74 (138)	78, 142	<i>DLX1/DLX2</i> (HOX genes), secretin receptor ( <i>SCRT</i> ), <i>Cd28/ccla4</i> (involved in celiac disease)
3p25-p26	190	2.88 (153)	78	OT receptor
5q	45	2.55 (79)	142	
6q21	120	2.23 (145)		Glutamate receptor ( <i>GRIK2/GLUR6</i> )
7q22	111	3.2 (138)	79	Reelin ( <i>RELN</i> ), neuropentraxin 2 ( <i>NPTX2</i> ), <i>HOXA1</i>
7q31-q33	144	2.53 (136)	78, 89, 139, 142, 153, 222	<i>FOXP2</i> , <i>IMMPL2</i> , <i>RAY1/ST7</i> , <i>WNT2</i> , <i>PEG/MEST</i>
13q	55	3.0 (222)		
15q11-q13	43	1.1 (145)	78, 138	<i>GABA<sub>A</sub></i> receptor ( <i>GABRB3</i> ), ubiquitin protein ligase ( <i>UBE3A</i> )
16p13	23.1	2.93 (138)	79, 136	NMDA receptor, tuberous sclerosis complex ( <i>TSC2</i> )
17	45	2.34 (138)		
19p	52	2.46 (79)	78, 136, 142, 145	
X	82	2.67 (79)	78	

NMDA indicates N-methyl-D-aspartate.

\* The highest reported linkage (reference in italics) is listed followed by the reference numbers of any additional studies documenting linkage scores >1.0 to the same site. Sites with no reported linkage score >2.0 were not included (with the exception of chromosome 15q11-q13).

variable DNA sequence adjacent to the *WNT2* gene increased the risk of autism by 50% in proband sibling pairs and trios. It is unclear, however, whether the mutation affects the expression of other genes in the locus or whether the mutation will be found in a wider autistic population. Indeed, a subsequent report did not find an association between *WNT2* and autism.<sup>152</sup>

Besides FXS and Rett syndrome, the X chromosome has been putatively implicated as a cause of autism, but only recently have genome studies published data in support of its involvement. Genome screens by 2 separate groups have found linkage to the Xq13-q21 region that contains the neuroligin genes.<sup>78,79,153</sup> Neuroligins are cell-adhesion molecules potentially involved in synaptogenesis.<sup>154</sup> Most recently, a group in France has identified mutations of the neuroligins *NLGN3* (at Xq13) and *NLGN4* (at Xp22.3) in a screen of 158 multiplex ASD families. Two families exhibited maternal transmission of a mutated neuroligin allele to affected male offspring: a de novo truncation of *NLGN4* and a mutation compromising the functional structure of *NLGN3*.<sup>155</sup> Evidence of an association between a new X-linked form of mental retardation and mutations of the angiotensin II receptor gene (*AGTR2*) on Xq22-q23 is relevant given that 2 of 9 subjects with mental retardation also had autism.<sup>156</sup> The importance of these data is corroborated by previous case study findings of deletions at the Xp22.3 locus in individuals with autism<sup>157</sup> and the high rate of mental retardation in patients with autism.<sup>16</sup> The Rett syndrome-associated gene *MeCP2* is located at the Xq28 locus, but studies have not yet shown that it plays a role in the pathogenesis of "idiopathic" autism.<sup>150,151</sup>

Other linkages to potential autism susceptibility loci have been identified on all but 7 chromosomes (Table 2). Although some linkages may not survive the study of larger cohorts, the number of loci identified to date supports the multigenic and polygenic theories of autism inheritance.

### Hypothesis Driven Studies: The Search for Candidate Genes

Cytogenetic assays and whole-genome screens are techniques for identifying relevant genes without reliance on an a priori hypothesis of autism pathophysiology. As just discussed, the hope is that these empirical studies may highlight genes involved with, for example, language impairment, neurotransmitter defects, or metabolic abnormalities in autism that would otherwise be overlooked. In contrast, hypothesis-driven studies predict the involvement of certain candidate genes on the basis of clinical and empirical evidence. A researcher might see an alleviation of ASD symptoms with certain pharmacologic interventions and then look for differences in the genes that regulate the corresponding endogenous metabolites in affected patients as compared with control subjects. Association studies are crucial in this type of research, as they examine polymorphisms in candidate genes selected without previous evidence from cytogenetic or genome analysis but because there is empirical evidence that the gene product(s) may be implicated in the pathogenesis of the disorder. Serotonin reuptake inhibitors, dopamine antagonists, and some adrenergic drugs have favorable effects on the behavioral symptoms of autism<sup>158</sup>; therefore, the genes that code for the receptors or neurotransmitters of these substances are targets for these types of genetic studies.

Serotonin is pivotal during development and if altered may contribute to structural brain abnormalities and to the core behavioral characteristics of autism.<sup>159,160</sup> Studies have long shown a 30% to 50% increase in platelet serotonin levels in some individuals with autism,<sup>161</sup> but investigators have not yet found the physiologic basis for this well-documented phenomenon. The serotonin transporter gene (*5-HTT*) has been examined in several different populations. Whereas Cook et al<sup>162</sup> found preferential inheritance of a short promoter variant of the *5-HTT* gene in affected individuals, others reported that a

long promoter variant of the *5-HTT* transporter was inherited more frequently by affected family members.<sup>163,164</sup> Still another group found that neither long nor short promoter alleles were preferentially inherited by individuals with autism but that the short promoter variant was associated with a clinical phenotype of increased severity.<sup>165</sup> These data are contradicted by reports of little or no association between autism and the serotonin transporter promoter variants in other autistic populations.<sup>166–170</sup>

Dopamine-blocking agents, such as Haldol, are the oldest and most effective drugs for treating the core symptoms of autism, although their potentially irreversible motor and other side effects drastically limit their use.<sup>158</sup> There is evidence of abnormal dopaminergic activity in the low medial prefrontal cortex of children with autism,<sup>171</sup> as well as elevated levels of catecholamines in the blood, urine, and cerebrospinal fluid of some children with autism.<sup>172,173</sup> Genetic studies have examined the dopamine receptors D2, D3, and D5; the tyrosine hydroxylase gene; and the dopamine  $\beta$  hydroxylase gene (among others) but with few results.<sup>172,174</sup> One patient with autism exhibited a missense mutation in the *DRD5* gene, but the relevance to the wider clinical population is unknown.<sup>175</sup>

The dopamine  $\beta$  hydroxylase (*DBH*) gene, which maps to chromosome 9q34, encodes a protein that catalyzes the conversion of dopamine to norepinephrine, a key player during embryonic neural development. In a study of multiplex autistic families, researchers found no increased concordance for *DBH* alleles among affected siblings. However, they found that reductions in the level of maternal dopamine hydroxylase significantly increased the risks of autism in her offspring. Mothers of multiple children with autism had a higher frequency of *DBH* alleles containing a 19-bp deletion (*DBH*–) when compared with matched control subjects.<sup>176</sup> The attributable risk of autism (ie, the rate of disease in exposed individuals that can be attributed to a *DBH*– allele) was 42%, suggesting a strong correlation between autism and homozygous *DBH*– mothers. The deletion was associated with decreased maternal enzyme activity, which in turn causes decreased levels of norepinephrine and increases levels of dopamine in utero. Reduced *DBH* activity in these women, however, may yet reflect another underlying genetic disorder, which causes the observed reduction in *DBH* activity but may cause a predisposition to autism in the offspring through different, undetermined mechanisms.<sup>177</sup>

Specific chemical insults in utero can lead to long-lasting physiologic imbalances of neurotransmitters, and the diagnosis of an ASD in such patients reinforces the neurotransmitter imbalance model of autism. Mice exposed on embryonic day 9 to valproate or thalidomide, documented causative agents of autism, display increased concentrations of serotonin in plasma and the hippocampus and greater levels of dopamine in the frontal cortex than controls at 4 weeks of age.<sup>178</sup> Thalidomide exposure on days 20 to 24 postconception in humans causes autism as well as specific abnormalities in ear and limb develop-

ment that pinpoint the time of injury to the closure of the neural tube.<sup>179</sup> The physical abnormalities of the brain include an absence of cranial motor nuclei and shortening of the brainstem, which are very similar to the congenital malformations caused by deletions of homeobox (*Hox*) genes.<sup>180</sup> *Hox* genes regulate hindbrain development, differentiation of the urogenital system, and appendicular skeletal growth. They include the genes *HOXA1* on chromosome 7p15, *HOXB1* on chromosome 17q, and *HOXD1* on chromosome 2q31.<sup>179,181</sup> Abnormalities of the *HOXA1* gene may give rise to genetic forms of the Moebius syndrome,<sup>180</sup> which is highly associated with autism.<sup>182</sup> One group found aberrant forms of *HOXA1* and *HOXB1* in a survey of autistic families,<sup>179</sup> but this was contradicted by additional studies.<sup>183–185</sup> This does not rule out the involvement of other *Hox* genes as causes of autism, however. Manning et al<sup>186</sup> reported a lower ratio of second to fourth digit length in families with autism, possibly reflecting derangement of prenatal testosterone levels as a result of mutations in *HOXA13* or *HOXD13*. Furthermore, the *Hox* genes *DLX1* and *DLX2* lie at chromosome 2q32,<sup>187</sup> which is a site of significant linkage in genomic screens.<sup>138</sup>

In addition to serotonin and dopamine, recent evidence suggests that the neurotransmitter acetylcholine may be associated with autism. Chemical and histochemical studies showed a reduction in the number of the neuronal  $\alpha$ -4 nicotinic acetylcholine receptor subunits in postmortem parietal neocortex and cerebellum of individuals with autism when compared with normal control subjects and individuals with mental retardation without autism.<sup>188,189</sup> This receptor is linked to chromosome 20q13.2-q13.3,<sup>190</sup> a locus thus far unexplored in autism genetics but linked to several epilepsy syndromes and schizophrenia.<sup>191</sup>

Recently, researchers have begun to examine the glutamatergic system in the pathogenesis of autism. Several lines of evidence suggest the involvement of glutamate receptors: 1) symptoms of hypoglutamatergia mimic the behavioral phenotypes of autism<sup>192</sup>; 2) serotonin receptor 2A (*5-HT2A*) agonists cause behavior similar to autism, perhaps via expression of *5HT2A* on glutamatergic-inhibiting GABAergic neurons<sup>193</sup>; 3) association studies have implicated the involvement of *GABA<sub>A</sub>* receptors on 15q11-q13 that in turn modulate glutamatergic function<sup>107</sup>; and 4) excessive glutamatergic activity is associated with epileptiform activity, which is highly associated with autism.<sup>194</sup> Although these theories are putative and even contradictory, several studies have reinforced the involvement of the glutamate system. Upregulated expression of the glutamate transporter gene was found in postmortem studies of autistic brain tissue<sup>195</sup> and in the striatum of a dopamine-depleted mouse model of autistic behavior.<sup>196</sup> The inotropic glutamate receptor 6 (*GluR6*) gene on chromosome 6q21 was associated significantly with autism by LD and multipoint linkage analysis, and a surveyed autistic population possessed a single amino acid substitution in *GluR6* to a greater degree than a control population.<sup>197</sup> Finally, the metabotropic glutamate

receptor *GRM8* in the chromosome 7q31-q33 autism susceptibility locus has exhibited LD with autism.<sup>147</sup> These data highlight the need for additional investigations into the relationship between the glutamate system and autism.

The potential relevance of endogenous opiates to autism comes from animal models that indicate its influence on sociability. Administration of exogenous morphine agonists to rats enhances social play, whereas treatment with antagonists reduces it.<sup>198</sup> Imaging studies of the rats' brains showed an increase in opiate peptide release during social play,<sup>199</sup> and prenatal exposure to morphine elevated the level of social play and grooming in juvenile pups.<sup>200</sup> The relevance of these opiate studies to autism is not clear, however; although impaired sociability is a core symptom of autism,<sup>201</sup> it is often associated with a high threshold for pain, which suggests an abnormally high (not low) level of endogenous opiates. Indeed, Willemsen-Swinkels et al<sup>202</sup> found that plasma  $\beta$ -endorphin levels were elevated in individuals who have autism and exhibit severe self-injurious behavior, and the widely used opiate antagonist naltrexone may have some limited utility for treating the self-injurious behaviors associated with autism.<sup>203</sup> Evidence of a genetically based opiate deficiency or overexpression in individuals with autism is currently lacking, however.

The neuromodulator oxytocin (OT) is also potentially relevant to the impaired sociability of autism. OT is a nonapeptide that affects human parturition and lactation. Investigators have determined that OT levels affect social behavior in rats, mice, and prairie voles.<sup>204-206</sup> Postulating the involvement of OT in the pathophysiology of autism, Modahl et al<sup>207</sup> found significantly lower overall plasma OT levels in children with autism versus age-matched control subjects. Subsequently, the ratio of the inactive OT precursor (OT-X) to active OT peptide was found to be significantly higher in children with autism than in control subjects.<sup>208</sup> These findings point to additional candidate genes for investigation, including the prohormone convertases PC2 and PC5 that convert OT precursor to OT, the OT peptide variants themselves, and the OT receptor. Two recent genome-wide screens have found significant linkage in autism to the chromosome 3p25-p26 locus containing the OT receptor gene.<sup>78,153</sup> Although intriguing, no genome scan performed to date has shown evidence of linkage to the OT gene locus itself on chromosome 20p13.

## DISCUSSION

In light of the high prevalence of children with an ASD, pediatricians are likely to have 1 or more children with this disorder in their practices. Awareness of the symptoms and causes of autism therefore is relevant to the pediatrician in several ways. First, the spectrum of causes and presentations of the ASDs are confusing and complicate diagnosis, yet physicians must recognize autism expeditiously.<sup>209</sup> Research has shown that early diagnosis and intervention significantly improve a child's long-term outcome.<sup>210-212</sup> Parental reports of early social or

language deficits, delays, or regressions should be addressed promptly and thoroughly, and pediatricians should not delay investigation of abnormal development because they want to avoid placing additional stress on the family.<sup>2</sup> There are various screening tests for autistic behaviors, such as the Checklist for Autism in Toddlers<sup>213</sup> and the Pervasive Developmental Disorder Screening Test,<sup>214</sup> but there is no definitive medical or biological test for autism. Few children with autism have diagnosable diseases such as TSC, FXS, Rett syndrome, or AS.<sup>9,15</sup> These specific causes and others must be investigated when the family history or examination suggests them, but in most individuals the cause of the autism remains unidentifiable at present.<sup>210</sup>

Although physicians must diagnose ASD promptly in their patients to provide proper treatment, we emphasize that tests for the many but rare genetic conditions reported in association with autism are stressful, costly, and often unavailable outside a research project. DNA studies are expensive and have a very low yield unless the family history, medical history, presence of mental retardation, or dysmorphic or other findings on examination suggest a diagnosable condition. The benefit of testing for a high-functioning child with a normal appearance and IQ and moderate social and language impairment is minimal.<sup>215</sup> Testing may be useful for genetic counseling but rarely leads to a meaningful change in the affected child's management. Children with abnormal features on physical examination are 10-fold more likely than those without them to have a diagnosable genetic condition.<sup>33,216</sup> Findings such as micro- or macrocephaly, abnormal finger digit ratios, and posteriorly rotated ears are associated with various developmental abnormalities of the brain and mental retardation.<sup>46,186,209,217,218</sup> Because the yield of specific diagnoses is highest in children with cognitive impairment or congenital anomalies, we recommend, for routine clinical care, limiting extensive testing to those with a suspicious family or medical history, mental retardation, or dysmorphology<sup>46,209,215,219,220</sup> and to families who wish to have additional children, as different genetic disorders have different recurrence risks.<sup>219</sup>

It is therefore important for pediatricians to be able to educate families regarding recurrence risks. A survey conducted at the New England Medical Center found that parents are confused about the causes of autism but would like prenatal testing and diagnosis.<sup>30</sup> It is necessary to inform parents of the known causes of autism and that, whereas prenatal diagnosis is possible in the case of defined disorders such as FXS, there is no prenatal test to identify "idiopathic" autism. Given the recurrence rate of 2% to 8% in siblings of affected children and that the initial diagnosis of autism is made between 1 and 4 years of age, it is especially important to offer parents information about their recurrence risks before they conceive another child.<sup>219</sup> Physicians must also be attentive to the psychological concerns of the family and be prepared to inform the parents of individuals with autism about available state and federal services.<sup>212</sup>

For research purposes, exhaustive causative inves-



tigations in families who have given informed consent are required to exclude known associated conditions that might cloud the interpretation of the data. It is crucial for pediatricians to try to involve families with multiple affected members in such formal projects, as family studies are key to unraveling the causes of autism. Many families must be screened to untangle the subtle genetic differences from the environmental influences that contribute to its complex causation, and studies can be validated only by the replication of results in multiple different populations.<sup>132,134</sup> These studies are required to identify the underlying genetic mutations associated with autistic phenotypes that target potential candidate genes. With an understanding of the many genetic causes of autism, prenatal screening and counseling may one day become available for affected families as more autism-causing conditions become diagnosable.

### CONCLUSION

Although many genes and proteins have been implicated as causes of autism, too little is known about their functions or their role in brain development to generate a parsimonious hypothesis about the brain dysfunctions that underlie autism. Evidence from multiplex families with the broader autism phenotypes, together with twin studies, indicates that single-gene defects are rare even within families. This is a general feature of many genetically influenced complex disorders such as obesity or diabetes because, first, different mutations in a given gene in different families do not have the same consequences for gene inactivation, and, second, phenotypic variability within a family may be a random stochastic event or result from interactions among different genes in different members of the same family. Furthermore, brain development and complex behaviors are multidetermined, with genes turning cascades of proteins on or off while they influence one another. A specific mutation, deletion, or unique set of genetic polymorphisms may determine one's susceptibility to autism, yet even then environmental triggers may modify the phenotypic expression of the disorder.<sup>88</sup>

Despite the profusion of investigations into the genetics of autism, few significant genetic linkages to autism have been identified. Even when strong genetic linkage is suggested, its significance remains undetermined until the functions of the gene product have been defined and its influence on brain development and physiology have been elucidated. Clinical researchers must then attempt to devise effective treatment regimens from this information, a task that is hardly trivial. Therefore, linkage is but the very first step toward understanding the contribution of a gene to the pathophysiology of autism. Perhaps future strategies using high-throughput microarray screening and animal models will assist in the study of genetic mutations and brain lesions in the behavioral phenotypes of autism. The value of these studies will become apparent only with time. Autism is fascinating given its wide array of behavioral manifestations and variable severities, yet it is this very nature that makes understanding its complex causes

so difficult. It invites much additional work in an exciting yet daunting area of research.

### ACKNOWLEDGMENTS

We thank Dr Laurie Ozelius for reviewing an earlier draft and 3 anonymous reviewers for helpful suggestions.

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*Pediatrics* 2004;113:e472-e486

DOI: 10.1542/peds.113.5.e472

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