Autism spectrum disorders—A genetics review

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Abstract: Autism is an etiologically and clinically heterogeneous group of disorders, diagnosed solely by the complex behavioral phenotype. On the basis of the high-heritability index, geneticists are confident that autism will be the first behavioral disorder for which the genetic basis can be well established. Although it was initially assumed that major genome-wide and candidate gene association studies would lead most directly to common autism genes, progress has been slow. Rather, most discoveries have come from studies of known genetic disorders associated with the behavioral phenotype. New technology, especially array chromosomal genomic hybridization, has both increased the identification of putative autism genes and raised to approximately 25%, the percentage of children for whom an autism-related genetic change can be identified. Incorporating clinical geneticists into the diagnostic and autism research arenas is vital to the field. Interpreting this new technology and deciphering autism's genetic montage require the skill set of the clinical geneticist including knowing how to acquire and interpret family pedigrees, how to analyze complex morphologic, neurologic, and medical phenotypes, sorting out heterogeneity, developing rational genetic models, and designing studies. The current emphasis on deciphering autism spectrum disorders has accelerated the field of neuroscience and demonstrated the necessity of multidisciplinary research that must include clinical geneticists both in the clinics and in the design and implementation of basic, clinical, and translational research. ***Genet Med* 2011:13(4):278–294.**

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Autism is an etiologically and clinically heterogeneous group of disorders, collectively referred to as the "autism spectrum disorders" (ASDs).[1] First described in the early 1940s almost simultaneously by psychiatrists Kanner[2] in the United States and Asperger[3] in Austria, autism remained mostly unnoticed outside of psychiatry through the 1980s. More than mental retardation and gender disorders, autism suffered from the extreme environmental fixation of the 1960s. Mothers were blamed for their children's illness and branded as "refrigerator mothers" being told that their children failed to develop social relationships because of their coldness; many children were placed in horrid institutions using aversive treatments. Not surprisingly, families developed a marked and deserved distrust of physicians and medicine in general, which still seems to influence responses to medical recommendations.

By the late 1980s, twin studies that compared concordance for autism in identical and fraternal twins showed, that contrary to previous dogma, autism was a highly genetic disorder with heritability indices of 0.85–0.92.[4] Smalley et al.[5] reviewed this work and reported an average concordance for identical twins of 64% vs. 9% for fraternal twins. Family studies, most notably the University of California, Los Angeles-University of Utah study reported significant sib recurrence risks in large Utah families; the latter sib recurrence risk was 8.6%, and for families with two or more affected children, the recurrence risk approached 35%.[6] The third indicator that autism is genetically determined comes from finding that a comprehensive genetics evaluation can reveal a chromosomal or Mendelian cause or at least predisposition in 15% to 40% of children who fit ASD behavioral diagnostic criteria.[7]

Understanding the genetics of autism has proven difficult. Although the etiologic and clinical heterogeneity is universally recognized, in practice, many studies still fail to take this into account. When considered as a single entity, autism does not fit known inheritance patterns, and although this could just be a product of the unrecognized etiologic heterogeneity, a number of tantalizing pieces of data indicate that the geneticist will need to incorporate new and emerging data from neuroscience, as

well as genetics to fully understand the causes of autism. In this review, I will summarize what we know at this time about the genetics of autism and suggest some of the reasons medical geneticists need to be part of the team involved in both the diagnosis and research into causes of the ASDs.

PREVALENCE

The growing number of children being diagnosed with autism has raised enormous concern from parents, physicians, and scientists fearing that some environmental toxins have emerged to cause an autism epidemic. Before 1990, the general population prevalence for autism was considered steady at 4–5/10,000 (1/2,000–1/2,500).[8] However, during the 1990s, studies of preschool children in Japan, England, and Sweden reported increasing prevalence rates for classical autism of 21–31/10,000 (1/476–1/323).[9,10] By 2005, Chakrabarti and Fombonne[11] reported a prevalence rate of 22/10,000 (1/455) for classic autism and 59/10,000 (1/169) for all pervasive developmental disorders (PDDs) in children younger than 6 years. Similar advances were reported in the United States, starting with the Center for Disease Control case-finding study in Brick Township, New Jersey, which reported prevalence at 40/10,000 (1/250) for classic autism and 67/10,000 (1/149) for all PDDs.[12] Recent studies in the United States reported the diagnosis of an ASD in 1/91 3- to 17-year olds[13] and 1/110 8-year-old children.[14]

Virtually all epidemiologic analyses, at this time, indicate that this apparent "autism epidemic" does not reflect a true increase in the incidence of ASD but rather is attributable to increased awareness by both the public and professionals, leading to more complete case finding together with broadening of the diagnostic criteria.[15–19] Studies finding the greatest increase in ASDs also observed lower rates of mental retardation. Only 30% of children with PDDs ascertained by Chakrabarti and Fombonne[11] were mentally retarded compared with 70% of children in earlier studies. This is consistent with the scenario that higher-functioning children with less language impairment and fewer aggressive behaviors had not been counted in older epidemiologic surveys. Nevertheless, ongoing research in this area is required. The recent update from an ongoing epidemiologic surveillance of autism indicated that the rise of autism in California shows no sign of plateauing. They concluded that earlier age at diagnosis and inclusion of milder cases accounted for more than two thirds of the increase but stated that the extent to which the continued rise might represent a true increase in the occurrence of autism remains unclear.[20]

CLINICAL FEATURES

ASDs develop before the age of 3 years. Infants typically do not care to be held or cuddled and do not reach out to be picked up. Often they are "colicky" and hard to console, typically quieting more readily when left alone. They may avoid and fail to initiate eye contact or stare into space. Despite early signs, children with ASD often do not come to medical attention until after the second year when language delays are obvious.

For most children, the onset of ASD symptoms is gradual; however, approximately 30% have an obvious "regressive" onset. These children begin to speak and then, either gradually or precipitously, lose language and become distant. Within a matter of days, the child may refuse to make eye contact and stop responding to his/her name. Repetitive movements may develop immediately or not until the child is 3 or 4 years of age. Although it has been debated whether these children are well and then become damaged by some exogenous exposure, the best evidence, including retrospective analysis of first birthday videotapes and neuropathologic studies, suggests their regressive course is genetically determined.[21–25]

Approximately 25% of children who fit the diagnostic criteria for ASD at the age of 2 or 3 years subsequently begin to talk and communicate and by age 6 or 7 years blend to varying degrees into the regular school population. Even for this group, social impairments generally continue. For the remaining 75%, most have some improvement with age but continue to require parent, school, and societal support. Excellent reviews of outcomes are provided by Seltzer et al., Howlin et al., Howlin et al., and Farley et al.[26–29] Some studies indicate that fewer than 5% of children with autism completely recover[30]; however, relaxation of the diagnostic criteria to include less impaired children seems to be increasing that number. A 20-year follow-up of adults between ages 22 and 46 years diagnosed with autism and average or near-average cognitive abilities in the 1980s found that half the individuals functioned quite well and half were employed in full-time or part-time paying jobs; however, only 12% lived independently and 56% lived with their parents.[29] Development of programs aimed at optimizing the transition to adulthood has recently become a major aim, as children diagnosed in the 1990s begin to age out of pediatric services.

CORE AUTISM SYMPTOMS

Autism is defined completely on the basis of its three core behavioral symptoms: impairment in social interactions, communication, and the propensity for repetitive behaviors. Impairment in social interaction isolates individuals with autism from those around them. Children with classic autism are unable to "read" other people, ignoring them and often strenuously avoiding eye contact. Typically, they do not comfort others or seek comfort and do not share interests with others, such as bringing toys or pictures to their parents. Rather, they use their parents as objects and may climb on them to get to a desired object, pull the parent by the hand, or place the parent's hand on the object, as if the child were using a tool. In clinic, the child who is content to turn pages of a magazine or spin the wheels of a car may become agitated when a simple examination is attempted. The lack of functional or spontaneous make-believe play is characteristic. Toys are lined up, sorted, twirled, or hurled but are not used for imaginative games or imitation of day-to-day activities, such as feeding the baby or washing the dishes. When play emerges later, it is stylized and not spontaneous. Children with autism fail to develop friendships with peers and siblings. In school, they often stand and watch other children from a distance. Some children respond to social overtures but take little social initiative, whereas others seek interaction but have little sense of how to proceed toward normal friendships.

Reciprocal communication, through speech, gestures, or facial expressions, is impaired. Characteristically, young children fail to use eye gaze or pointing to communicate and direct their parent's attention. Early language is limited and when present is characterized by substantially reduced rates of social interaction and establishing joint attention but with more typical rates of requesting. Deficits in pragmatic skills are present throughout life and affect both language and social interaction. The young child seems unable to grasp the concept that speech can be used to name objects, to request a toy, or to engage others. In contrast to the child with nonspecific mental retardation or a primary developmental language disorder who usually has better receptive than expressive language, the child with autism has impaired receptive language. When children with autism learn to

talk, they display stereotypic speech that may involve echolalia, pronoun reversal, and unusual inflections and intonations. Unlike typically developing children who begin talking using one-word utterances, children with autism may begin talking in "chunks" composed of commercials, movies, or others' speech. These chunks often convey idiosyncratic meanings, and the child with autism has no understanding of the conventional meaning of the individual words. Pragmatic difficulties including difficulties sustaining a conversation, turn taking, and allowing the conversational partners to introduce their topics, usually continue despite improvement in expressive speech.[22]

Repetitive and stereotypic behaviors can be noted during the first few months. Infants may stare or rock. Toddlers may have motor "stereotypies" such as movements of fingers, twirling strings, flicking pages of books, or licking. Repetitive whole body movements may include spinning and running back and forth. The repetitive behaviors often have a visual component such as holding the fingers to the side of the face and watching them with a sideways glance. Sometimes the movements become more complex with an individualized sequence of patting, rubbing, or twirling. These stereotypies may last for hours. Although the cause of the repetitive movements is unclear, they seem to have a calming effect and may, especially in the older child, surface in times of stress. This repetitiveness is reflected in a rigid need for sameness in daily routines. Children with autism can develop elaborate rituals in which the order of events, the exact words, and the arrangement of objects must be followed. Failure of parents/caretakers to follow the prescribed order of events results in inconsolable outbursts.

Children with autism also are prone to display a number of symptoms and behaviors that, though not diagnostic, may negatively impact daily living and health. Commonly in the second year of life, children develop meltdowns, aggressive and sometimes self-injurious behaviors brought on by some change in routine, an offending touch, being asked to do something they do not want to do, or for no apparent reason. Extreme hypersensitivity to certain sounds such as the vacuum cleaner may cause great discomfort, causing the child to hold his hands over his ears. The feel of certain clothes or of being touched may be unbearable; conversely, truly painful stimuli, such as a burn or laceration, are ignored. Odd behaviors around foods and their presentation, such as accepting a limited number of foods or only eating french fries that come from McDonalds® occur commonly and have a negative impact on nutrition. Being "in their own world" often leads to a total disregard for danger that results in a high risk of early death, most commonly from drowning.

DIAGNOSTIC CRITERIA AND TOOLS

The behavioral criteria listed in the 1994 American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, 4th edition, (DSM-IV)[31] remain the standard for making an autism diagnosis in the United States. The 2000 update DSM-IV-TR made some changes in the accompanying text but did not change the diagnostic criteria.[32] Currently, three subgroups (autistic disorder [AD], Asperger syndrome [AS], and PDD-not otherwise specified [PDD-NOS]) are recognized. Initially, Rett syndrome, OMIM# 312750, and childhood disintegrative disorder were included as PDDs. Discovery of *MECP2* mutations as the cause of Rett syndrome, uncertainty about the nosology of childhood disintegrative disorder, plus a growing understanding of the continuity within the autism diagnoses led to adoption of the term ASDs as the favored umbrella designation for AD, AS, and PDD-NOS.[33] It is expected that the DSM-5, due out in 2012 will further simplify the nosology by combining the three subtypes into ASD, with a variety of modifiers such as the degree of mental impairment, neurologic symptoms and, when available, etiology.[34]

To diagnose autistic disorder, i.e. classical autism, one must precisely enumerate the autism symptoms and their age of occurrence using either the DSM-IV criteria or a number of symptom checklists, structured parent interviews, or observation measures, all of which are based on the DSM-IV Diagnostic Criteria Autistic Disorder.[35–37] The DSM-IV criteria for AD listed in Table 1 are probably the easiest to use.

Asperger syndrome (AS)[3,38] is distinguished from AD by relatively normal language development including timing,

Table 1 DSM-IV diagnostic criteria for autistic disorder

I. A total of six (or more) items from A, B, and C, with at least two from A, and one each from B and C

A. Qualitative impairment in social interaction, as manifested by at least two of the following:

1. Marked impairment in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction
2. Failure to develop peer relationships appropriate to developmental level
3. A lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g., by a lack of showing, bringing, or pointing out objects of interest)
4. Lack of social or emotional reciprocity

B. Qualitative impairments in communication as manifested by at least one of the following:

1. Delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime)
2. In individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others
3. Stereotyped and repetitive use of language or idiosyncratic language
4. Lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level

C. Restricted repetitive and stereotyped patterns of behavior, interests, and activities, as manifested by at least one of the following:

1. Encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus
2. Apparently inflexible adherence to specific, nonfunctional routines or rituals
3. Stereotyped and repetitive motor mannerisms (e.g., hand or finger flapping or twisting or complex whole-body movements)
4. Persistent preoccupation with parts of objects

II. Delays or abnormal functioning in at least one of the following areas, with onset before age 3 yr: (1) social interaction, (2) language as used in social communication, or (3) symbolic or imaginative play

The diagnostic code is 299.00.

grammar, and vocabulary (Section B) but requires all other DSM-IV diagnostic criteria. Individuals with AS are generally loners, are uncomfortable in groups, are unable to empathize with others, maintain a sameness in routine, follow strict rules, and have an encompassing preoccupation with one domain, such as the weather or computers. They generally do not chat and follow a literal interpretation of speech with poor understanding of idioms or jokes. Speech may be pedantic or repetitive with odd intonations. Intelligence quotient (IQ) must be within the normal range to qualify for the diagnosis. Clumsiness is common. Whether AS is the expression of the high end of the autism spectrum or is a discrete genetic entity is unclear.

Although commonly used, the AS diagnosis is problematic with poor concordance between the various diagnostic instruments.[39] The Autism Spectrum Screening Questionnaire,[40] the Asperger Syndrome Diagnostic Interview,[41] the Australian Scale for Asperger's Syndrome,[42] and the Childhood Asperger Syndrome Test[43] are also available. It is expected that the DSM-5 will drop the term AS, which is a concern to many people who identify strongly with the AS diagnosis and do not want to be lumped diagnostically into a general ASD category.

PDD-NOS is applied to children with autistic symptoms who do not meet full criteria in all three diagnostic domains. This heterogeneous group includes children with milder symptoms in all three diagnostic categories (A, B, and C) and those meeting full criteria for autism in two of the three domains. Sometimes PDD-NOS is used as an initial or tentative diagnosis for younger children or before diagnostic evaluations are completed. Its rather pejorative connotation makes it unpopular with families and many physicians.

An additional ASD designation, with no official standing, is broader autism phenotype that may be used to designate siblings or other family members with some autism symptoms.[44–46] This terminology was originally adopted by researchers to classify family members considered more likely to carry mutations in putative autism genes. The designation is of little use clinically but does reflect the growing awareness of the broad phenotypic spectrum of ASDs.

A number of diagnostic checklists are commonly used in clinics and schools, including the Childhood Autism Rating Scale (CARS)[47] that consists of 15 questions scored by the parent and the tester. It is a reliable, well-verified measure that is relatively fast and easy to administer and, thus, is most often used. A score of 30–35 indicates mild autism and 36 or higher moderate-tosevere autism. Two others are the Aberrant Behavior Checklist (ABC)[48] and the Gilliam Autism Rating Scale (GARS).[49]

In North America, research criteria for the diagnosis of autism depend primarily on the Autism Diagnostic Interview-Revised,[36] which is a detailed parent interview, and the somewhat shorter Autism Diagnostic Observation Schedule (ADOS).[37] Both scales follow the DSM-IV criteria and were developed in an attempt to sort autism by its behavioral symptoms to permit identification of homogeneous populations. These scales are not widely used in clinical practice because of the time and expense to administer, although the shorter ADOS is becoming more widely used outside of research settings.

Universal autism screening by primary health care providers is recommended starting at a year of age and repeated through 3 years of age. The Checklist for Autism in Toddlers-Modified[50] is the most commonly used screening tool. This 23-item checklist, designed for primary care providers to identify at-risk toddlers at the 18-month visit, can be filled out by parents in the waiting room and is available in Spanish and English.[35] The Infant/Toddler Checklist from the Communication and Symbolic Behavior Scales Developmental Profile[51] is recommended by the American Academy of Pediatrics to identify at-risk children younger than 18 months.[52] A positive score on one of these tools is not diagnostic for an ASD but prompts referral to a diagnostic clinic. The push for universal screening comes from multiple research studies which have shown that early intensive educational, language, and behavioral therapy are strong indicators of better outcomes.[53–55] Also, a number of states including Missouri[56] and California[35] have developed diagnostic and screening guidelines that are available online or in print free of charge.

NEUROLOGIC AND MEDICAL SYMPTOMS

Specific neurologic and medical symptoms occur commonly in children with autism, often with profound effects on quality of life for families and long-term outcomes. Seizures develop in approximately 25% of children with autism, and the rate of electroencephalographic abnormalities is increased even when there is no history of seizures.[57,58] Although individuals with autism plus seizures are more apt to have moderate to severe mental retardation, motor deficits, and poorer adaptive, behavioral, and social outcomes,[59,60] seizures by themselves are not a sensitive outcome predictor. Another particularly disruptive problem is insomnia and abnormal sleep patterns which are reported in approximately 60% of children with autism; this is similar to that reported in other neurologically based syndromes but falls outside the range for typically developing children.[61] Although most children with autism develop early motor milestones on time, a number of specific motor impairments including prolonged toe walking, hypotonia, general clumsiness, poor hand writing, and inability to ride a two wheel bicycle are commonly seen. As a group, motor difficulties have not been adequately studied; however, it seems that these symptoms are relatively discrete and not directly correlated with general neurologic disability or IQ. Clumsiness, for instance, is a common characteristic of AS.[62] Gastrointestinal symptoms, including constipation, diarrhea, bloating, belching, abdominal pain, reflux, vomiting, and flatulence are reported in approximately 45% of children with autism. A recent consensus report and review indicate that the frequency and character of the gastrointestinal problems are not qualitatively different from what is seen in typically developing children and respond to appropriate medical management.[63] Obesity is a common complication of unknown cause. Medication side effects, inactive lifestyle, and difficulty withholding food from children with aggression may be implicated. A recently recognized complication that may develop in as many as 15% of young adults with autism is a catatonia syndrome characterized by a marked deterioration in movement with slowness and freezing in mid movement, vocalizations, and regression of self-care skills.[64]

The Autism Treatment Network (ATN)[65] is a network of treatment and research centers dedicated to improving medical care for children and adolescents with autism by establishing standards of clinical care based on research and shared clinical practice. Analysis of data provided by the ATN registry is providing important information on the frequency and character of medical problems in children with autism and is developing autism-specific diagnosis and treatment algorithms with the goal of enhancing overall well being for children with autism.

GENETICS OF AUTISM

Clarifying the "genetics" of autism has proven more challenging than anticipated in the early 1990s, when it was as-

sumed that amassing a DNA collection from approximately 300 autism families and performing genome-wide single nucleotide polymorphism-based association studies would quickly reveal the genes that predispose children to develop autism. Unfortunately, this was not the case; the major genome-wide and candidate gene association studies, which are used to test for common variants contributing to risk, did not identify consistent genomic areas of interest.[66] Although, with the recent availability of large DNA repositories and less expensive genotyping, some independently replicated findings from genome-wide association studies are beginning to appear.[67,68] The greatest progress toward identifying genetic causes of autism has come from identifying known genetic mutations and disorders that can predispose to development of autism. Using standard medical genetic evaluation techniques, a genetic cause can be identified in 20–25% of children on the autism spectrum. This number has increased with the use of array comparative genomic hybridization (aCGH) also called chromosomal microarrays (CMAs). A small number of cases can be traced to specific teratogenic exposures.[69] For the remaining 75–80%, the causes remain unknown. All these percentages are approximate as there is a significant bias of ascertainment, based on the initial referral. Medical Genetics clinics find the highest percentage of identifiable disorders, whereas dedicated autism clinics find the lowest.[7,70] This can be explained by the fact that children with autism and significant dysmorphology are more apt to have an identifiable genetic etiology and are more apt to be referred to a medical genetics clinic.

Identified genetic causes of autism can be classified as the cytogenetically visible chromosomal abnormalities (~5%), copy number variants (CNVs) (i.e., submicroscopic deletions and duplications) (10–20%), and single-gene disorders (~5%).

CHROMOSOMAL DISORDERS AND CNVS

Cytogenetic abnormalities visible with high-resolution karyotype analysis are found in approximately 5% of children with ASD. Another 3–5% has identifiable chromosomal abnormalities using fluorescence in situ hybridization (FISH) techniques. As expected, unbalanced chromosome abnormalities are found predominantly in children with autism who are dysmorphic.[70,71] Cytogenetic abnormalities have been identified on almost every chromosome, although only a few occur with a frequency suggesting the location of a specific autism gene.[72–74] A curated database of chromosome abnormalities reported in individuals with autism is available at http://projects.tcag.ca/autism/.[75]

Maternally derived 15q duplications of the imprinted Prader Willi/Angelman region are the most commonly observed chromosome abnormalities in autism, detected in 1–3%. Cytogenetically visible duplications most often take the form of a de novo supernumerary isodicentric 15q chromosome but can occasionally result from segregation of a maternal chromosome translocation. Identification of interstitial 15q duplications usually requires interphase FISH or aCGH. Similar to the deletions found in most cases of Angelman, OMIM# 105830, and Prader-Willi syndrome, OMIM# 176270, these duplications seem to be mediated by unequal homologous recombination involving low copy repeats (LCR) that are clustered in the region.[76] The maternally derived15q11-q13 interstitial duplication is a highly penetrant cause of autism, and the phenotype correlates with the number of 15q copies. A maternal duplication of 15q, resulting in trisomy for that region, causes subtle effects on the physical phenotype, whereas children with four copies of 15q including those with a supernumerary isodicentric 15 are typically more impaired and may exhibit hypotonia, seizures, microcephaly, and severe developmental delay.[77–79] The paternally derived duplication has little or no phenotypic effect, indicating genomic imprinting of this region.[79]

Children with Down syndrome, OMIM# 190685, and 45,X Turner syndrome, OMIM# 300082, have autism more often than expected. In one Down syndrome study, the incidence of autism was 7%.[80] There are also an excess of girls with Turner syndrome who fit autism diagnostic criteria.[81] The explanation for the Down syndrome association is unclear as the social phenotypes of the two disorders are dissimilar. Girls and women with Turner syndrome, on the other hand, generally have poorer social cognition skills.[82] Although autism has been reported in other sex chromosome disorders (47,XXX, 47,XXY, and 47,XYY), there does not seem to be a significant association.

aCGH/CMA is rapidly replacing high-resolution chromosomes as the initial test of choice to evaluate children with autism. CGH arrays have identified clinically relevant de novo genomic imbalances in 7–20% of individuals with autism of unknown cause.[75,83–89] As expected, the yield is higher in those with "syndromic" autism.

- Using a 1 Mb genome-wide array, Jacquemont et al.[71] identified clinically relevant CNVs in 27.5% (8/29) of individuals with autism and dysmorphology, who previously had a normal karyotype as determined by routine cytogenetic studies.
- Sebat et al.[83] found, using an oligonucleotide array, de novo copy number changes in 10% of children from simplex families (i.e., autism in a single family member) and 2% from multiplex families (i.e., autism in multiple family members) compared with 1% in controls.
- Using a dense genome-wide single nucleotide polymorphism array, Marshall et al.[89] found unbalanced CNVs in 44% of 427 unrelated families with autism that were not present in control families. Many of these CNVs were inherited, and only 7% were de novo in persons with autism of unknown cause.
- A whole-genome CNV study using 550,000 single nucleotide polymorphism markers on a cohort of 859 individuals with ASD and 1,409 healthy children of European ancestry revealed several pathogenic genomic changes in genes encoding neuronal cell-adhesion molecules (*NRXN1, CNTN4, NLGN1*, and *ASTN2*) and in genes involved in the ubiquitin pathways (*UBE3A*, *PARK2, RFWD2*, and *FBXO40*).[90]
- The International Standard Cytogenomic Array Consortium conducted a literature review of 33 studies, including 21,698 patients tested by aCGH and provided an evidence-based summary of clinical cytogenetic testing comparing CMA with G-banded karyotyping with respect to technical advantages and limitations, diagnostic yield for various types of chromosomal aberrations, and issues that affect test interpretation. They concluded that CMA offers a much higher diagnostic yield (15–20%) for genetic testing of individuals with unexplained developmental delay (DD)/intellectual disability (ID), ASD, or multiple congenital anomalies than a G-banded karyotype (approximately 3%, excluding Down syndrome and other recognizable chromosomal syndromes), primarily because of its higher sensitivity for submicroscopic deletions and duplications.[87]
- A cohort of 933 patients with autism received clinical genetic testing including G-banded karyotype, fragile X

testing, and CMA. Karyotype yielded abnormal results in 2.2%. CMA identified deletions or duplication in 18.2%; for 7%, the variants were considered pathogenetically significant.[88]

The most common autism-related CNVs are the 15q11.2-11.3 duplications, similar to duplications revealed by FISH, and reciprocal 16p11.2 microdeletions and duplications. The 16p11.2 microdeletions and microduplications of approximately 555 kb are located at a hot spot of genomic instability caused by duplicated blocks of DNA, which lead to unequal crossing over during meiosis. The 7q11.23 duplication of the Williams syndrome region is also found.[91,92] Taken together, these CNVs seem to confer susceptibility to ASD in up to 1% of patients with ASD.[75,86]

No consistent physical or behavioral features identify individuals with 16p11.2 CNVs, but clinical data are limited. Fernandez et al.[93] reported phenotypic analysis of five autistic probands with 16p11.2 CNVs (three deletions and two duplications). Of these subjects, three probands and two deletion positive mothers were dysmorphic, the rest were not. One dysmorphic male also had a diaphragmatic hernia. Two deletions and one duplication were de novo and the other two inherited. From samples submitted for clinical aCGH analysis, Shinawi et al.[94] identified 16p11.2 deletions and duplications in 0.6% and reported detailed molecular and phenotypic characterization for 17 deletions and 10 duplication subjects. Characterization revealed that speech/language delay and cognitive impairment occurred most commonly followed by motor delays, seizures, behavior problems, dysmorphology or congenital anomalies, and autism.[94] Patients with the 16p11.2 deletion were more apt to be macrocephalic ($P < 0.002$) and 20% had autism, whereas 60% of patients with the duplication had microcephaly and attention deficient hyperactivity disorder (ADHD). Two patients had normal cognitive and behavioral phenotypes, one with the deletion and one with the duplication. The authors suggest the autism plus macrocephaly observed in deletion patients and ADHD plus microcephaly in duplication patients may indicate a diametric model for these genomic sister disorders.

A number of other CNVs have been described in a few autism families, and although none seem to be particularly common, they highlight the potential of array-based techniques to point autism researchers toward specific autism genes within the CNVs. For instance, haploinsufficiency of *CHRNA7* may be causative for the majority of neurodevelopmental phenotypes identified with the 15q13.3 microdeletion syndrome[95–99] at the distal end of the Prader Willi syndrome/Angelman region.[100] Another example is the identification of de novo copy number variations in the *SHANK2* synaptic scaffolding gene in two unrelated individuals with autism and mental retardation.[101]

There are significant limitations to our current understanding of CNVs as causes of autism. First, penetrance is extremely variable. Both deletions and duplications may be inherited or occur de novo.[93,102,103] In addition, unaffected parents and family members may carry the same CNV as the ASD proband. Microdeletions are more likely to be penetrant and associated with nonspecific major or minor dysmorphism than the duplications, although this is not a constant. Second, microdeletions and duplications cannot be lumped together as their phenotypic consequences are generally different. Finally and perhaps most importantly, patients with a spectrum of other neurocognitive phenotypes, including mental retardation/DD, seizures, ADHD, dyslexia, schizophrenia, and bipolar disorder have been described with the same CNVs.[104–106] This association of many disorders with the same genetic defect indicates that although specific CNVs may predispose to a variety of neurologic disorders, the specifics of the final phenotype will depend on the individual's genetic background.[107] These findings support a second hit model to explain the causality of non-syndromic neurodevelopmental disorders including autism, DDs, neuropsychiatric disease, seizures, epilepsy and others.[108] In multiplex autism families, Itasara et al.[109] found affected probands were significantly enriched for de novo CNVs compared with their unaffected siblings, suggesting that many de novo CNV mutations contribute a subtle, but significant risk for autism. Girirajan et al.[108] observed that children with developmental delay were more apt to carry both an inherited 16p12 deletions plus a second large (>500 kb) CNV. This supports the disease model in which the presence of 16p12 by itself results in predisposition to disease and when in combination with other risk-conferring variants can explain the variable expressivity and clinical heterogeneity of many genomic disorders. Some consider this a second hit explanation. Future studies that correlate specific CNVs with clinical characteristics, that assess complete family histories, and that discover the responsible genes, their pathways, and effects on brain development will undoubtedly resolve much of the current perplexity. For now, explaining the significance of a CNV can be difficult[94,110,111] and generally requires interpretation by a clinical geneticist.

SINGLE-GENE DISORDERS

Autism or autistic features are commonly identified in a large number of single-gene disorders. Autism in these cases is often referred to as "syndromic autism." That terminology, however, is misleading because by definition autism is a syndromic disorder with variable expression and penetrance. A more accurate designation would be "autism of known etiology."

FRAGILE X SYNDROME

Approximately 1–3% of children ascertained on the basis of autism diagnosis can be shown to have fragile X syndrome, OMIM# 300624, with expansion of the CGG trinucleotide repeat in the *FMR1* gene to full mutation size of 200 or more repeats. Moreover, a considerable number of children being evaluated for autism have been found to have *FMR1* premutations (55–200 CGG repeats).[72,112–116] Farzin et al.[114] studied 14 boys with premutations ascertained through an autism clinic and found 71% met ASD diagnostic criteria. In the authors' experience, 10 of 488 (2%) persons ascertained through a dedicated autism clinic had either an *FMR1* full mutation or premutation. Of the five children with a full mutation, only one was diagnosed with AD; four did not meet criteria for an ASD diagnosis. For the five premutation carriers, four were diagnosed with AD and one with PDD-NOS. From the other perspective, we know that at least half of children with fragile X syndrome exhibit some autistic behaviors, such as avoidance of eye contact, language delays, repetitive behaviors, sleep disturbances, tantrums, self-injurious behaviors, hyperactivity, impulsiveness, inattention, and sound sensitivities. In one study of 63 males with fragile X syndrome, 30% met criteria for AD and 30% criteria for PDD-NOS.[117] There is undoubtedly a major behavioral overlap between fragile X and autism, and these data underscore the importance of performing *FMR1* molecular genetic testing in all children being evaluated for an ASD, to optimize diagnostic accuracy and genetic counseling. From a research perspective, this overlap is beginning to enhance our understanding of the underlying molecular/ge-

netic mechanisms of these two disorders. Molecular studies indicate that the *FMR1* gene may cause the autism phenotype by two mechanisms, RNA toxicity to the neurons and gene silencing, which affects neuronal connectivity.[118–120]

PTEN MACROCEPHALY SYNDROME

A particularly intriguing association is autism as a *PTEN* (phosphatase and tensin homolog) macrocephaly syndrome. The *PTEN* gene was initially described as a tumor suppressor gene associated with a broad group of disorders referred to as *PTEN* hamartoma tumor syndrome, which includes Cowden syndrome, OMIM# 158350, Bannayan-Riley-Ruvlacaba syndrome, OMIM# 153480, Proteus syndrome, OMIM# 176920, and Lhermitte-Duclos disease, OMIM# 158350.[121–123] More recently, heterozygous *PTEN* gene mutations have been identified in a subset of individuals with autism and macrocephaly,[124–126] and *PTEN* haploinsufficiency has been recognized to play a role in brain development, including neuronal survival and synaptic plasticity. How *PTEN* causes autism is unclear, although an intriguing recent report indicates that the effect may be related to repression of the phosphoinositide 3-kinase pathway. Page et al.[127] found that haploinsufficiency for *PTEN* and the serotonin transporter gene *SLC6A4* act synergistically to increase brain size and decrease sociability in a mouse model. As we try to identify autism-associated metabolic pathways, it is intriguing that in addition to *PTEN*, mutations in other repressors of phosphoinositide 3-kinase pathway have also been associated with ASD, including the tuberous sclerosis complex genes *TSC1* and *TSC2*[128] and neurofibromin 1.[129]

The frequency of *PTEN* mutations as a cause of ASD is unclear; results from studies of children ascertained through autism and macrocephaly range from 1%[125] to 8.3%[126] to 17%.[124] Both de novo and familial *PTEN* mutations have been identified in this population. It may be significant that more of the children studied by Buxbaum et al.,[125] who found *PTEN* mutations in 1%, were from multiplex families, whereas the children studied by Butler et al.,[124] who found *PTEN* mutations in 17% were from simplex families.

Children with ASD who are found to have a *PTEN* mutation generally have extreme macrocephaly ranging from +3.7 to +9.6 SD (average: +5.4 SD).[125] In addition, mutation of *PTEN* is not specific for autism: Varga et al.[126] found children with macrocephaly and mental retardation but not autism had a similar chance of having a *PTEN* mutation. Because *PTEN* germline mutations are associated with the phenotypically broad *PTEN* hamartoma tumor syndrome, it is recommended that children with *PTEN* mutations and their families be evaluated by a medical geneticist for clinical signs of any of the related disorders. Moreover, because these disorders carry a risk of cancer, including cancer of the breast, thyroid, endometrium, and kidney, these individuals need to be involved in a tumor surveillance program.

RETT SYNDROME

Rett syndrome is one of the original DSM-IV designated PDDs and the only one for which a specific genetic etiology has been identified.[130] Ninety-six percent of individuals with classic Rett syndrome have mutations in the X-linked *MECP2* gene.[131] Overall, *MECP2* mutations have been reported in approximately 1% of children diagnosed with autism.[74,131] The phenotype of *MECP2*-confirmed Rett syndrome overlaps considerably with autism of unknown cause; children with both often have a period of normal development followed by loss of language with stereotypic hand movements. However, Rett syndrome can usually be distinguished clinically based on a decreasing rate of head growth, progressive gait disturbance, and hand wringing in early childhood. Initially, the distinction may be difficult as illustrated by a study of two Rett syndrome databases that found 17.6% (55/313) of girls with *MECP2*-confirmed Rett syndrome had been given an early diagnosis of autism.[132] A retrospective analysis of the phenotype of girls initially diagnosed with autism showed that they had significantly milder, later developing Rett syndrome symptoms and were more likely to remain ambulatory and to retain some functional hand use. They were also more likely to have mutations *p.R306C* or *p.T158M*. On the basis of these data, Young et al.[132] recommend that all girls diagnosed with autism be monitored carefully for evolving signs of Rett syndrome including deceleration in head growth. This approach seems prudent and more appropriate than doing *MECP2* molecular genetic testing in all girls diagnosed with apparently classic autism.

Evidence of variable expression of the protein MECP2 in the brains of individuals with both autism and Rett syndrome and evidence that MECP2 deficiency can reduce expression of the genes *UBE3A* and *GABRB3* implicated in autism indicate some causal relationship between the two disorders.[133,134] Intensive study of the role of MECP2 in maintaining neuronal function and evidence of symptom reversal of neuronal symptoms in mice after reactivation of the silenced *MECP2* gene is projected to have implications for autism treatment.[135]

TUBEROUS SCLEROSIS COMPLEX

Although 25–50% of mentally retarded individuals with tuberous sclerosis complex (TSC), OMIM# 191100, fulfill autism diagnostic criteria, only 1.1–1.3% of individuals initially diagnosed with ASD has TSC.[136–139] Early-onset infantile spasms and temporal lobe tubers on magnetic resonance imaging (MRI) examination increase the chance that children with *TSC2* mutations will also develop autism.[140]

In a prospective study of children with TSC evaluated using the ADOS, 66% met criteria for autism or ASD at age 18 months of age, 54% at 24 months of age, 46% at 36 months of age, and 50% at 60 months of age. The children with both TSC and autism were more cognitively impaired than those with TSC only.[141]

An evaluation for signs of TSC including skin lesions (hypopigmented macules, shagreen patches, and adenoma sebaceum) and a family history consistent with autosomal dominant inheritance of TSC signs (seizures, skin lesions, and mental retardation) are generally sufficient to indicate or rule out the diagnosis of TSC in children with autism. Molecular genetic testing for the two causative genes, *TSC1* and *TSC2*, is clinically available. Recurrence risks for families with a child with autism due to TSC may be significantly higher than for families with autism of unknown cause.

TIMOTHY SYNDROME

Timothy syndrome, OMIM# 601005, an autosomal dominant disorder of calcium channels caused by a mutation in the *CACNA1C* gene at 12p13.3 is characterized by severe QT prolongation, syndactyly, cardiac defects, dysmorphic faces, developmental delays, and autistic symptoms.[142] Hope et al.[143] reported a *CACNA1F* mutation at Xp11.23 causing congenital stationary night blindness with autism, mental retardation, and seizures in hemizygous males. Subsequently, we evaluated a young boy with congenital stationary night blindness, classic

AD, mental retardation, and mild dysmorphology who also has a *CACNA1F* mutation. In addition, deleterious mutations in *CACNA1H* were identified in six of 461 individuals with autism and in none of 480 ethnically-matched controls.[144] Timothy syndrome, autism with congenital sensory night blindness and mutations in the *CACNA1H* gene indicate that alterations in ion channel function may produce autism.

JOUBERT SYNDROME

This autosomal recessive disorder is characterized by partial or complete agenesis of the cerebellar vermis, seen as the "molar tooth sign" on MRI, abnormal breathing, abnormal eye movement, cognitive impairment, and behavioral problems. A subset of Joubert syndrome, OMIM# 213300, seems related to the *AHI1* gene, encoding the "jouberin" protein.[145] In one study, 4 of 11 children with Joubert syndrome met diagnostic criteria for an ASD.[146] However, Takahashi et al.[147] delineated behavioral and genetic differences between autism and Joubert syndrome implying that these are etiologically distinct disorders. In a report of monozygotic (MZ) twins with Joubert syndrome,[127] the twin with the more severe cerebellar abnormality had autism, suggesting that some disorders may have the potential to cause the autism phenotype when other brain regions or circuits are affected.

METABOLIC CONDITIONS

Mitochondrial disorders

Although mitochondrial respiratory chain disorders have only been reported on rare occasions in autism, elevated plasma concentrations of lactate have been noted frequently.[148,149] A population-based study of 69 children with autism reported an elevated plasma lactate concentration in 20% (14/69); 11 of the 14 underwent muscle biopsy and five had a deficiency of one or more respiratory chain complexes, most frequently Complexes I, IV, and V, confirming mitochondrial disease.[150] Thus 4.5% of 120 children with ASD were determined to have a definite mitochondrial disease. If confirmed, this would be the largest etiologic autism subgroup. Identifying a mitochondrial disorder is more likely in autistic children with atypical features such as hypotonia, fatigue with activity, epilepsy, failure to thrive, intermittent episodes of regression and regression following fever than in children without these findings. Weissman et al.[151] analyzed data from 25 persons with a mitochondrial disorder and an initial diagnosis of autism and found they could all be distinguished from autism of unknown cause on the basis of an abnormal neurologic examination and/or an elevated plasma lactate concentration. However, mitochondrial dysfunction has also been reported in persons with ASD without additional neurologic features.[152,153] A comprehensive current review by Hass is recommended.[154]

Phenylketonuria

Comorbidity of ASD and phenylketonuria (PKU), OMIM# 261600, was consistently described in the literature before universal PKU newborn screening in the United States. The autism was usually complicated by the severe mental retardation. A recent systematic study found that none of 62 persons with PKU who were diagnosed and treated early met diagnostic criteria for autism, whereas 5.7% (2/35) of individuals with late diagnosis of PKU fulfilled the diagnostic criteria for ASD.[155]

Adenylosuccinate lyase deficiency

This rare autosomal disorder of de novo purine synthesis results in the accumulation of succinylpurines in body fluids. In about half of the affected individuals, the variable clinical manifestations include developmental delay, seizures, and autism symptoms including failure to make eye contact, repetitive behavior, agitation, temper tantrums, and aggression.[156] In one study, 1 of 420 children with PDD was found to have adenylosuccinate lyase deficiency, OMIM# 103050.[157]

Creatine deficiency syndromes

Three inborn errors of creatine metabolism have been described with autism symptoms. Two are creatine biosynthetic disorders, guanidinoacetate methyltransferase deficiency, OMIM# 601240, and L-arginine:glycine amidinotransferase deficiency, OMIM# 602360, and the third is the X-linked SLC6A8 creatine transporter deficiency, OMIM# 300036. Mental retardation and seizures are common to all three. Approximately 80% of individuals with guanidinoacetate methyltransferase deficiency have a behavior disorder that can include autistic behaviors and self-mutilation; approximately 45% have pyramidal/extrapyramidal findings. Onset is between 3 months and 3 years of age. Only five individuals with L-arginine:glycine amidinotransferase deficiency have been reported. The phenotype of SLC6A8 deficiency in affected males ranges from mild mental retardation and speech delay to severe mental retardation, seizures, and behavioral disorder, with onset between ages 2 and 66 years. Learning and behavior problems have been reported in approximately 50% of the SLC6A8 heterozygous female. Recent sequencing of the *SLC6A8* gene in 100 males with ASD did not detect deleterious mutations,[158] suggesting the prevalence of creatine deficiency syndromes in ASD is low.

Smith-Lemli-Opitz syndrome

This autosomal recessive multiple congenital anomaly and mental retardation syndrome, caused by a deficiency of 7-dehydrocholesterol reductase, can be associated with autism and other behavioral characteristics such as repeated self-injury, sensory hyper reactivity, temperature dysregulation, and sleep disturbance.[159,160] Rates of autistic behavior reported in individuals with Smith-Lemli-Opitz syndrome, OMIM# 270400, range from 50 to 86%.[161] One study found that three fourths of the children with Smith-Lemli-Opitz syndrome fit criteria for an ASD; half were diagnosed with AD and half with PDD-NOS.[162] No correlation was found between the abnormal metabolites and the presence or severity of autistic symptoms.

Other single-gene disorders

Autism or autistic features have been reported in children with many other single-gene disorders. Most are associated with severe mental retardation and significant dysmorphology, and the children are rarely referred with an initial question of autism. For common disorders such as neurofibromatosis 1, it is unclear whether this is a chance occurrence of two common childhood disorders or indicates a true association, perhaps related to macrocephaly common to both disorders. The list includes the following:

- Cohen syndrome, OMIM# 216550[163]
- Cole Hughes macrocephaly syndrome, OMIM# 605309[164]
- San Filippo syndrome, OMIM# 252900[165]
- Cornelia de Lange syndrome, OMIM# 122470[166]
- Angelman syndrome[167,168]
- Williams syndrome, OMIM# 194050[169] and its reciprocal 7 q11.23 microduplication syndrome[91,92]
- 17p11.2p11.2 duplication syndrome, OMIM# 610883[170]

- 22q11.1 deletion syndrome[171]
- WAGR (Wilms tumor, aniridia, genitourinary anomalies, and mental retardation), OMIM# 194072[172]
- Duchenne muscular dystrophy, OMIM# 310200[173]
- Sotos syndrome, OMIM# 117550[174]
- Neurofibromatosis 1, OMIM# 162200[136,175,176]

DEVELOPMENTAL SYNDROMES OF UNDETERMINED ETIOLOGY

Moebius syndrome or sequence

Defined by unilateral or bilateral palsy of the sixth and seventh cranial nerves, Moebius syndrome, OMIM# 157900, is characterized by facial paralysis with inability to smile and fully abduct the eyes. It is often associated with abnormal tearing, seizures, hearing loss, and limb anomalies. Approximately 30% of children with Moebius syndrome develop ASD.[177–179] A recent study confirmed the observations of Johansson et al.[177] that ASD occurs more frequently in individuals with Moebius syndrome with concurrent mental retardation.[180] Presumably caused by an early disruption of embryonic blood supply leading to brainstem disruption, Moebius syndrome has been compared with thalidomide embryopathy that also damages the sixth and seventh cranial nerves and causes autism.

Landau-Kleffner syndrome

A small subset of children with ASD and late regression has Landau-Kleffner syndrome, OMIM# 245570. These children develop gradual or sudden isolated language regression associated with seizures (epileptic aphasia) and/or severe electroencephalogram (EEG) abnormality in deep sleep.[181] In general, both the seizures and language impairment improve with normalization of EEG abnormalities.[58]

ENVIRONMENTAL CAUSES

The search for environmental causes of autism has been motivated by the increased autism prevalence over the last 20 years and the incomplete concordance for autism in MZ twins.

In utero exposures, including valproic acid, thalidomide, and misoprostol (an abortifactant commonly used in South America) are recognized causes of autism. The Liverpool and Manchester Neurodevelopment Group recently reported a long-term study of 632 children exposed to antiepileptic drugs during gestation and found that children exposed to valproate in utero were seven times more likely to develop autism than those not exposed to antiepileptic drugs. None of the families had a known family history of autism. They recommend that women taking valproate be informed of the risk for autism in children exposed during gestation.[182]

Other factors that have been considered as causes of autism include expanded use of assisted reproductive technologies[183] and tocolytic drugs such terbutaline.[184]

Childhood immunizations given around the time regressive-onset autism is recognized have been a major focus of concern, especially from autism parent groups. Thimerosal, the organic mercury-based preservative used in a number of childhood vaccines in the United States up until 2001 and the measles-mumps-rubella vaccine, which never contained mercury, have been the major targets. Although parental concern is still significant, multiple studies and lines of scientific evidence have identified no support for a relationship between immunizations and autism.[18,185–188] The original studies by Wakefield et al.[189,190] suggesting an association between immunizations and autism have been disproved, the work was retracted by *The Lancet*.[191] In 2010 the British General Medical Council revoked Mr. Wakefield's license to practice medicine. One of the tragedies resulting from fear of an autism epidemic was the decreased use of childhood immunizations leading to outbreaks of measles and childhood deaths.[192,193]

GENES INVOLVED IN AUTISM

In addition to clinically identifying known genetic disorders which may predispose to the development of autism, intense efforts have been directed to identifying genes that specifically cause or increase the risk of developing autism. The methods used include both large genome-wide association studies and investigation of candidate genes. The Genetic Association Database[194] provides online access to human genetic association studies performed on autism and other complex disorders. Authoritative reviews of the current status of candidate genes and loci are available.[195–204] SFARIGENE is a new web-based searchable list of candidate genes associated with ASD.[204] The candidate genes are richly annotated for their relevance to autism, along with an in-depth, up-to-date view of their molecular function extracted from the current scientific literature.

Table 2 that lists known and putative autism genes is unquestionably incomplete, as new candidate genes are being reported at an unprecedented rate. The genes are organized by pathogenesis to highlight the progress made in the functional assessment of autism candidate genes and pathways. Some genes are included because of their compelling initial descriptions that still await confirmation.

A number of these genes are becoming clinically relevant. Of particular recent interest are the synaptic cell adhesion and associated molecules, including neurexin 1, neuroligin 3 and 4, and *SHANK3*, which implicate glutamatergic synapse abnormalities in ASDs. Mutations in the X-linked neuroligin-3 (*NLGN3*) and neuroligin-4 (*NLGN4X* and *NLGN4Y*) genes[196,205] have been identified in brothers with autism.[67,206] Laumonnier et al.[207] identified a two base-pair deletion in *NLGN4* in 12 affected members of a French family with X-linked mental retardation, some of whom were also autistic. Jamain et al.[206] identified a C-to-T transition in the *NLGN3* gene, in two brothers, one with autism and the other with AS. The mutation was inherited from the mother and was absent in 200 controls. It should be noted that a number of studies failed to find mutations in either *NLGN3* or *NLGN4* in probands with autism,[74] and these mutations have been associated with other psychiatric disorders and language disabilities. Individuals with ASD and mutations in *NLGN4* and *NLGN3* have typically been nondysmorphic and some have lost of social and verbal milestones at the onset of disease. Molecular genetic testing of *NLGN4* and *NLGN3* should be considered in families with suspected X-linked inheritance of autism.

The *SHANK3* gene, which codes for a synaptic protein that binds directly to neuroligins, seems crucial for the development of language and social cognition. *SHANK3* mutations and small cytogenetic rearrangements have been implicated with an ASD phenotype.[208,209] In addition, *SHANK3* mutations have been found in a variety of disorders including ADHD and language deficits, as well as in unaffected family members, suggesting they may cause disease by acting synergistically with other susceptibility genes. Deletion analysis is clinically available by aCGH.

CAUSAL THEORIES

Initially, multifactorial inheritance was assumed to account for autism's heritability, based on the overall 4:1 male-to-

Table 2 Known and putative autism genes (organized by pathogenesis)

Protein name (function)	Gene symbol/locus	Test availability
Neuronal cell adhesion and/or synapse function		
Neuroligin 3 (synapse formation and function)	*NLGN3X* Xq28	Clinical
Neuroligin 4 (synapse formation and function)	*NLGN4X* Xp22.33	Clinical
Neurexin 1 (transsynaptic binding partner for neuroligins)	*NRXN1* 2p16.3	Research
SH3 and multiple ankyrin repeat domains (organizes post synaptic density and binds neuroligins)	*SHANK3* 22q13	Research
Contactin-associated protein-like 2 (synaptic binding partner for contactin molecules involved in neuronal migration)	*CNTNAP2* 7q36	Research
Contactin 4 and Contactin 3 (neuronally expressed adhesion molecules)	*CNTN4* and *CNTN3* 6p26-p25	Research
Protocadherin 10 (a cadherin-related neuronal receptor: may play a role in the establishment and function of specific cell-cell connections; essential for normal forebrain axon outgrowth)	*PCDH10* 4q28	Research
Neuronal cell adhesion molecule	*NRCAM* 7q31	Research
Neuronal activity regulation		
Methyl CpG-binding protein 1 (CAN methylation-dependent transcriptional repressor)	*MECP2* Xq28	Clinical
Ubiquitin protein ligase E3A	*UBE3A* 15q11-q13	Clinical
Deleted in autism	*DIA1* (c3orf58) 3q	Research
Ataxin 2-binding protein 1	*A2BP1* 16p13	Research
Neurodevelopmental genes		
Engrailed 2 (homeobox gene involved in midbrain and cerebellum development)	*EN2* 7q36	Research
Homeobox A1 (involved in hindbrain development)	*HOXA1* 17p15.3	Clinical
Homeobox B1 (involved in hindbrain development)	*HOXB1* 17q21-q22	Research
Reelin (signaling protein involved in neuron migration)	*RELN* 7q22	Research
WENT2 (signaling proteins involved in embryonic patterning, cell proliferation, and cell determination)	*WNT2* 7q31	Research
FOXP2 (transcription factor involved in embryogenesis and neural functioning)	*FOXP2* 7q31	Research
ARX homeobox gene	*ARX* Xp22.13	Clinical
Patched domain containing 1 gene	*PTCHD1* Xp22.11	Research
Sodium channel		
Sodium channel, voltage-gated, type VII	*SCN7A* 2q	Research
Na+/H+ exchanger isoform 9	*SLC9A9* (*NHE9*) 3q24	Research
Calcium channel		
Calcium channel, voltage-dependent, L type, alpha 1C subunit (Timothy syndrome)	*CACNA1C* 12p13.3	Clinical
Calcium channel, voltage-dependent, alpha 1H subunit	*CACNA1H* 16p13.3	Research
Calcium channel, voltage-dependent, L type, alpha 1F subunit	*CACNA1F* Xp11.23	Clinical
Neurotransmitter genes		
GABA receptor subunits (major inhibitory transmitter receptors in the brain)	*GABRB3, GABRA5, GABRG3* 15q11.2-q12	Research
Serotonin transporter	*SLC6A4* 17q11.1-q12	Clinical
Mitochondrial		
Mitochondrial aspartate/glutamate transporter (mitochondrial function and maintaining ATP levels)	*SLC25A12* 2q24	Research
Other genes		
Oxytocin receptor	*OXTR* 3p26.2	Research
Laminin beta 1	*LAMB1* 7q31.1	Research
RING finger protein 8 (ubiquitin ligase and transcriptional coactivator)	*RNF8* 6p21.3	Research

Adapted from GeneReviews, http://www.genetest.org. Copyright, University of Washington, Seattle 1997–2010.

female sex ratio and 4% recurrence risk, both of which are similar to what is found in classical multifactorial disorders such as pyloric stenosis and cleft lip and palate.[41,210] However, family studies indicate that autism does not fit the multifactorial threshold model, which predicts that the more frequently affected sex has a lower recurrence risk, and that the less often affected sex is more severely affected. Pickles et al.[211] found that the proportion of relatives with mild autism phenotypes was not increased when the proband was female (less frequent sex). In twin studies, Constantino and Todd[212] found no evidence for the existence of sex-specific genetic influences and concluded that the lower prevalence (and severity) of autistic traits in girls seem to arise from discrepant phenotypic manifestations of genetic and environmental influences that are common to both sexes. In studies limited to children with essential autism, Miles et al.[213] also found that the risk of developing autism in subsequent sibs did not correlate with the sex of the proband. Sibs of male and female probands had an equal chance of developing autism. Both the risk of developing autism and the severity correlated with the gender of the subsequent sib. Females (less frequent sex) were less likely to develop autism, and those who did had less severe symptoms. This indicates that females are in some way protected from developing autism.

This lack of fit to the classic multifactorial threshold model, however, does not eliminate the likelihood that there are many genes and many genetic pathways involved in autism. Rather, it implies that autism is a heterogeneous disorder and that difficulty recognizing heterogeneity within a broad behavioral phenotype has been a major impediment to understanding the genetics of the disorder. For the geneticist, identifying biologically discrete features or phenotypes, which occur in only a portion of individuals with the general diagnosis, is a crucial first step in delineating discrete disorders. However, because autism is a behavioral disorder with few unique physical and laboratory features, this has proven much more difficult than for the prototypic genetic disorders.[214,215]

There is an emphasis on the identification of phenotypic features (biomarkers or endophenotypes) to facilitate the recognition of subgroups that will enable prediction of outcomes, recurrence risks, and treatment choices.[69,216] For autism, the most informative broad separation has been the separation of "complex" from "essential" autism.[70] Complex autism is defined by the presence of generalized dysmorphology and/or microcephaly, features that indicate some alteration of early morphogenesis. Approximately 20–30% of children ascertained on the basis of an autism diagnosis have complex autism. Complex autism is associated with a poorer prognosis, a lower male-to-female ratio, and a lower sibling recurrence risk than essential autism. Approximately 25% of children with complex autism can be diagnosed with an autism associated syndrome or chromosome disorder using currently available diagnostic tests.[70] Children with essential autism, defined by absence of generalized dysmorphology and/or microcephaly are more likely to be male, have a higher sibling recurrence risk, and have a greater family history of autism and autism-related disorders such as alcoholism and depression than children with complex autism. Testing is less apt to discover a syndrome or chromosome diagnosis. Differences in the sex ratio, recurrence risk, and family history of autism and related disorders provide the best evidence that the separation of complex from essential autism is etiologically valid. Individuals with essential autism are twice as likely to be male (6.5:1 vs. 3.2:1), are more than twice as likely to have a family history of autism (20% vs. 9%), and have a sib recurrence risk of 4–6% compared with no recurrences in the 46 families of children with complex autism. Also, sibs of children with essential autism are twice as likely to have mild autistic traits as those with complex autism (12% vs. 6%).

The distinction between complex and essential autism represents the first pass at dissecting the etiologic heterogeneity within the autism diagnosis. Although each group remains heterogeneous, the functional and genetic differences indicate that essential autism and complex autism are inherently different. Complex autism captures the subgroup of children whose physical phenotype indicates a different embryological process. Analysis of specific morphologic abnormalities is expected to direct research toward specific developmental pathways. And removing complex autism probands from populations used for linkage and sib pair analyses is expected to improve the power of the analyses.

Table 3 lists additional phenotypic variables that may help delineate discrete autism subgroups. In many ways, the transition of autism from an inclusive behaviorally defined disorder to an etiological-based nosology mirrors the history of mental retardation, which, beginning in the 1960s, was divided into subgroups based on phenotypic differences. A number of current autism heritability theories follow this reasoning. Geschwind and Levitt[218] proposed that there are significant numbers of highly penetrant autism-causing genes that occur in small groups of families. The hope is that by identifying these rare alleles, we can elucidate their pathways and pathogenetic mechanisms and apply that information to the analysis of autism generally. An extension of this proposal posits that genetically there are two classes of autism; the most common of which is genetic but not heritable.[219] Those cases are due to new mutations, a hypothesis suggested by reports of increased parental age in autism[220,221] and the identification of de novo CNVs. The expectation is that studies of simplex families will identify a host of de novo genetic changes. A second class of mutations is hypothesized to be familial and account for most sibling recurrences. One analysis of familial cases from the Autism Genetic Resource Exchange data set suggested that the heritable genes were often carried by the mother who although unaffected had affected sons.[222] They concluded that this mechanism would account both for the high male prevalence and the families with multiple affected children. However, studies by Constantino and Todd[212,223] suggest that this explanation of recurrence is unlikely. Constantino and Todd[223] demonstrated that in most families, autistic traits as measured by Social Responsiveness Scale (SRS) are continuously distributed and moderately to highly heritable. First-degree relatives of autism probands including clinically unaffected siblings, mothers, and fathers have SRS scores midway between the affected child and the norm in the general population. In addition, 7 to 15-year-old twins selected only on the basis of their twin status were found to have highly correlated SRS scores; significantly higher correlations were found in MZ than dizygotic twins.[212] The Constantino data are consistent with family history studies, which reveal that family members of autistic probands have an increased likelihood of exhibiting autistic symptoms with a wide range of severity, often below the threshold for a diagnosis of an ASD. Social aloofness, language delays, repetitive or stereotypic activities or interests, and psychiatric symptoms and disorders are reported more often in clinically unaffected siblings, parents, and second-degree relatives.[44,136,211,224,225] This suggests that for most cases, autism is both genetic and heritable with many "small-effect" inherited changes with as-yet-undetermined interactions.

EVALUATION STRATEGY

In addition to a behavioral assessment to establish the autism diagnosis and cognitive testing to help establish the best edu-

Table 3 Phenotypic variables that may define discrete autism (ASD) subgroups

Phenotypic variables	Consistently present in ASD populations (% of ASD)
Morphology and growth	
Generalized dysmorphology	Yes (15–20)
Macrocephaly	Yes (~35)
Microcephaly	Yes (~5–15)
Brain malformations	Yes (~20)
Medical/neurologic	
Seizures	Yes (~25)
EEG abn	Yes (~50)
Sleep disorder	Yes (~65)
Savant skills	Yes (~5)
Clinical course	
Age of onset	Yes
Regressive onset	Yes (~30)
Adolescent/adult catatonic regression	Yes (~17)
Significant family history of related disorders	
ASD	Yes (~25)
Alcoholism	Yes (~30)
ADHD	Probably (~70)
Affective disorders	Probably
Bipolar/major affective disorder	Yes (~30)
Functionally defined variables	
IQ	Yes
Adaptive behaviors	Maybe
Outcome measures	Probably (poorly defined)
Response to therapy	Yes
Core autism symptoms	
Social functioning	No (100)
Communication	No (100)
Repetitive, stereotypic behaviors and/or preoccupations, obsessions	Possibly, but must be defined precisely

EEG, electroencephalogram; IQ, intelligence quotient.

cational programs, the evaluation of all individuals with autism should include a medical evaluation to identify medical issues that affect the development and behavior of nonverbal children and a clinical genetics evaluation to elucidate diagnostic possibilities. Recent reports from the American Academy of Pediatrics,[52] the American College of Medical Genetics,[7] and in Gene Reviews[69] provide practical approaches to the evaluation of children with ASD. The ATN registry is currently collecting data on the sensitivity and specificity of specific diagnostic tests.[65] In the mean time, the medical geneticist should approach the evaluation of the child with autism similar to that of any child presenting for diagnostic evaluation, i.e., beginning with a records review, a careful family history, the clinical examination, and consideration of the differential diagnosis.

FAMILY HISTORY

A three-generation pedigree should be analyzed with attention to behavioral and neurologic diagnoses. Family studies reveal significant clustering of neuropsychiatric disorders including depression, manic depression, obsessive compulsive disorder, social phobia, anxiety disorders, alcoholism, substance abuse, seizures, and motor tics, in addition to ASD phenotypes in relatives of autism probands.[44,224–226] Family histories of alcoholism and other addictive disorders, often not focused on in the medical genetics clinic, should be ascertained. Lobascher et al.[227] reported a greater incidence of alcoholism (35%), psychiatric illness (35%), and mental retardation (26%) in the parents of autistic children. DeLong and Dwyer[228] reported that 55% of their 51 autism families had a first- or second-degree relative with alcoholism although the overall incidence rate of alcoholism among all 929 first- and second-degree relatives was only 6.5%. In a study of 36 autism families, Smalley et al.[229] compared the lifetime rates of psychopathology based on direct SADS-LA interviews of parents and adult siblings of autism probands and found that 47% (17/36) of the autism families had a first-degree relative with substance abuse, including alcoholism, versus none in the 21 control families. In addition, 22% of first-degree relatives reported substance abuse compared with none in the controls ($P = 0.002$). They also found increased rates of depression (32.3% vs. 11.1%; $P = 0.013$) and social phobia (20.2% vs. 2.4%; $P = 0.016$).[229] In 2003, we reported pedigree analyses of 167 autism families finding that 39% of autism-ascertained families could be classified as having probable genetic alcoholism, based on either alcoholism in a parent or a cluster of alcoholism in at least one branch of the family.[225] The high-alcoholism families had an elevated percentage of affected relatives in all categories, with 17% of mothers, 52% of fathers, 14% of maternal grandmothers, 41% of maternal grandfathers, 21% of paternal grandmothers and 45% of paternal grandfathers reported as alcoholic. The remaining 102 families reported scattered individuals with alcoholism in unrelated branches of the family (<1% of females and <10% males). Evaluation of the autism probands from the high- and low-alcoholism groups differed in two important autism phenotypes, macrocephaly and type of autism onset. Children from the high-alcoholism families were 2.8 times less likely to be macrocephalic (14.7% vs. 40.6%) ($P = 0.0006$). This significant inverse relationship between high-alcoholism family histories and macrocephaly suggests that whatever genes predispose to both autism and macrocephaly are different and operate independently from gene(s) that predispose to alcoholism and autism. The second difference between the autism probands from high- versus low-alcoholism families was the clinical course of their AD. Children from high-alcoholism families were 1.5 times more apt to present with a regressive onset (52.5% vs. 35.8%) ($P = 0.04$). The genetic overlap between autism, alcoholism, and other neuropsychiatric disorders indicates that there are autism subgroups that are genetically mediated, highly penetrant, and share biochemical and genetic aberrations.

CLINICAL EXAMINATION

Height, weight, and occipital-frontal circumference measurements identify microcephaly and growth retardation suggestive of chromosome and monogenic syndromes, whereas macrocephaly, which is present in approximately 35% of children with autism, leads to consideration of the molecularly defined macrocephaly syndromes, especially fragile X and *PTEN* hamartoma tumor syndrome. A skin examination (including Woods lamp) will detect most cases of tuberous sclerosis complex or neurofibromatosis type 1. The dysmorphology examination that distinguishes between essential and complex autism provides the best information on which to base the diagnostic plan. In addition to the gold standard dysmorphology examination performed by the medical geneticist, a brief Autism Dysmorphology Measure has been developed for use by nondysmorphologists who evaluate children with autism.[217] By assessing 12 physical features (height, hair growth patterns, ears, face, nose size, philtrum, mouth and lips, fingers and thumbs, hand size, feet and toes) and use of a scoring algorithm children with generalized dysmorphology can be distinguished from those with essential autism with 81% sensitivity and 99% specificity.[217] The great majority of known genetic causes of autism are identified in children with generalized dysmorphology.

DIAGNOSTIC LABORATORY TESTING

At the time of the initial evaluation, it is recommended that children with autism have blood drawn for aCGH and *FMR1* molecular genetic testing for both full mutations and premutations. Metabolic evaluation, including quantitative plasma amino acids, urine organic acids, purines, creatine, and guanidinoacetate in urine and serum concentration of lactate, pyruvate, creatine kinase, and uric acid and complete blood count, is of limited benefit for the majority of individuals with autism. However, because diagnosing and treating a metabolic disorder can significantly alter prognosis, a selective and targeted metabolic work up is recommended based on history and physical examination. The evaluation should include a review of the results of the child's newborn screening tests.

NEUROLOGIC STUDIES

The utility of obtaining a routine sleep-deprived EEG and brain MRI is debated. Certainly, clinical signs of seizures or developmental regression should prompt an EEG. It is clear that a significant number of children with autism have EEG abnormalities, and the likelihood of observing an abnormality increases with the duration of the EEG. The brain MRI is indicated when the history and physical examination or neurologic examination suggests a localized lesion, tuberous sclerosis complex, Joubert syndrome, or an early environmental insult. Obtaining an MRI in children with autism generally requires sedation or anesthesia by an anesthesiologist, and it is recommended that centers who routinely evaluate children with autism develop an autism-specific EEG and MRI protocol with attention to decreasing sensory stimulation.

GENETIC COUNSELING

Genetic counseling is best done by a medical geneticist after a careful diagnostic evaluation. For those families where a specific etiology has been identified, the risk of recurrence in siblings generally depends on the etiologic diagnosis. For autism of unknown cause, the sibling risk varies across studies but is generally considered to range from 5 to 10% for autism and 10–15% for milder symptoms, including language, social, and psychiatric disorders.[70,230–233] For families with two or more affected children, the recurrence risk approaches 35%.[234] No recurrence risk data are available for families who have one autistic child plus another child or relative with mild autistic symptoms. Therefore, the amount of weight to put on mild autistic symptoms in siblings, parents, and other relatives when estimating recurrence risk for families is unknown.

Separation of essential and complex autism helps in the estimate of recurrence risks for autism of undetermined cause. In one study, brothers of a proband with essential autism had a 7% risk for autism and an additional 7% risk for milder autism spectrum symptoms; sisters had a 1% risk for autism. Their risk for milder ASD is unknown.[69,70] Complex autism, defined by generalized dysmorphology or microcephaly, when no known etiology has been found carries a lower sibling risk. The empiric risks are 1% for autism and an additional 2% for milder autism spectrum symptoms.

CONCLUSIONS

The genetics of autism is intriguing partially because we are beginning to understand what controls human behavior. However, for the clinical geneticist who can interpret family, developmental, and medical histories and truly understands penetrance, expressivity, physical variation, and heterogeneity, the study of autism and all its unanswered questions must have immense intellectual appeal. A case in point is the ongoing debate over whether autism, as a common disease, conforms to the common variant (CD/CV) or to the rare variant (CD/RV) hypothesis. The CD/CV hypothesis expects that a few common allelic variants will account for the genetic variance in disease susceptibility; whereas the CN/RV hypothesis posits that numerous rare mutations may cause the disorder, with the most extreme being that each mutation is only found once in the population.[234] The recent success in mining autism genes identified from CNVs and from isolated families with rare causes of their autism supports the CD/RV hypothesis, whereas family studies of Constantino and others suggest that there is a role for common variants in the determination of autism-related behaviors. Although it seems clear to the clinical geneticist that both hypotheses must be correct for some autism subgroups, it will take additional research to clarify the underlying genetics, especially for children with essential autism. Designing and interpreting this research will benefit from having more clinical geneticists join research teams and bring their understanding of phenotype analysis, their ability to obtain and interpret family pedigrees and their experience deciphering the nosology of complex phenotypes.

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