Approach to the Genetic Evaluation of the Child with Autism

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Autism is defined as a behavioral disorder, characterized by the triad of impaired social skills, delayed speech, and areas of intense focus.[1] In the past autism was thought to be relatively uncommon, with incidence figures of 1 in 2500 cited by various articles written in the 1980s.[2] Recently, however, the incidence of autism has increased, with figures as high as 1 in 110 published.[3] Two possible reasons, which may not necessarily be mutually exclusive, are increased exposure to environmental toxins and a broader definition of autism, so that it now comprises a spectrum (autism spectrum disorders [ASDs]) which also includes, for example, Asperger syndrome and pervasive developmental disorder.[2]

Autism is a heterogeneous entity that clearly has a substantial genetic component to its cause. This aspect is shown by a high concordance rate in monozygous twins, with approximately a 90% concordance for both twins having one of the ASDs. However, it is not uncommon for one twin to have autism and the other to have Asperger syndrome. As a result, the heritability is one of the highest cited for a psychiatric disorder, with a figure of 90% usually cited.[4] However, in fewer than half of the cases of autism can a cause be found, so the molecular basis of autism remains mostly unknown. Several epidemiologic studies have been done in an attempt to achieve a better understanding of potential mechanisms that might lead to autism. For example, Schendel and colleagues[5] examined the frequency of congenital anomalies in children with autism as well as the frequency of autism in children with congenital anomalies. The investigators found that children with a diagnosis of autism had an approximately twofold increased frequency of congenital anomalies (6% compared with a frequency of 3% in controls). In most of these children the anomaly was

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isolated, and in no case was a syndrome diagnosed. Similarly, the frequency of autism among children with congenital anomalies was double that of a control population, with frequencies of 0.43% and 0.22%, respectively. The autism frequency was not the same across all types of anomalies, but was greater in those with either a brain and/or eye anomaly.

Although it may be tempting to suggest that there are shared prenatal environmental factors responsible for the occurrence of both the anomaly and the autism, the investigators stated that "the pattern was indicative of neither a single etiology or pathogenetic mechanism nor a specific insult." However, they did stress that congenital anomalies could serve as indicators of central nervous system dysfunction, and thus the link to development of autism.

Another noteworthy association that has been described is the finding of a correlation between a family history of autoimmune disorders and ASDs.[6] This group found that among a group of 3325 children with a diagnosis of autism, with 1089 of those having infantile autism, there was a significantly greater frequency of maternal rheumatoid arthritis, maternal celiac disease, and both maternal and paternal type 1 diabetes (but only in the parents of those with infantile autism). One possible mechanism is via a genetic link to HLA system genes, some of which have been implicated in causing infantile autism.[7]

An association with increased paternal age has also been described by several groups.[8–10] This link has generally been attributed to increased mutation load in the sperm of older males; however, one group that studied families with more than one affected child[11] found that the often described male to female ratio of 4:1 diminished with increasing paternal age, so that among offspring born to men younger than 30 years, the sex ratio was 6.2:1 whereas among offspring born to men older than 45 years, the ratio was 1.2:1. Various mechanisms proposed to explain these findings included de novo copy number variant (CNV), new mutation, or chromosome anomalies, particularly involving the X chromosome.

Other studies have found a greater frequency of parental psychiatric disorders,[12] with such frequency approaching a twofold increase. Schizophrenia in both parents, and depression or personality disorders in mothers were more common in this study. The investigators noted that other studies had also found an association between parental psychiatric disorders and childhood autism, but differences existed among the various studies. Nonetheless, there is likely enough evidence to suggest that there are common genetic mechanisms that predispose to various psychiatric disorders.

More recent studies have attempted to identify the specific genes involved in predisposition to autism. One of the tools used by these studies is exome sequencing, in which all the coding regions of the human genome are simultaneously sequenced, searching for mutations that might be causative or contributory to the cause of an individual's disorder. O'Roak and colleagues,[13] using exome sequencing in 20 individuals with ASDs, found 21 de novo mutations, with 11 causing alterations in the gene product (protein). Therefore, in the not too distant future this technique will be applied clinically in an attempt to identify the causes of a particular child's autism.

Until that time, what should be done to evaluate the child for an identifiable genetic cause? Several studies have found that in a small subgroup of children with autism or ASD, there is an underlying genetic or chromosomal abnormality, with figures ranging between 10% and 41% for that proportion. **Table 1** summarizes some of these studies. It should be noted that some of these studies presented only genetic causes, whereas others attempted to identify all causes. The populations examined were also varied; some data were obtained from a group of children referred to a genetics clinic, whereas other data were from autism units or were recruited for the study.

Table 1
Results of investigative studies in children with autism

	Schaefer and Lutz[14]	Kosinovsky et al[15]	Roesser[16]	Benvenuto et al[17]	Shen et al[18]	Herman et al[19]	Battaglia and Carey[20]	Boddaert et al[21]
Source of patients	Genetics clinic	Autism centers	Autism centers	None (review article)	Recruited patients	Genetics clinic	Autism centers	Autism centers
Total N	32	132	207	NA	852	71	85	77 (8 unused)
Physical exam	2	—	13	3%–4%	—	—	—	—
Metabolic evaluation	0	0	—	—	—	0	0	—
Audiogram	1	—	—	—	—	—	1	—
Rubella titers	0	—	—	—	—	—	—	—
Karyotype	2	—	4	—	19	2	1	—
Fragile X	2	2	1	2%–5%	2	0	1	—
MRI	1	—	—	—	—	—	2	33/69
EEG	0	1	—	—	—	—	1	—
MECP2	2	—	0	—	—	3	—	—
22q11 FISH	0	—	0	—	—	—	—	—
15 interphase FISH	1	—	0	1%–2%	—	0	2	—
17p FISH	1	—	—	—	—	—	—	—
Uric acid	1	—	—	—	—	0	—	—
Microarray		—	—	1%–2%	154 CNV, 59 pathogenic	1	—	—
PTEN		—	—	—	—	2	—	—
Total	13/32 (41%)	1.5%	9%	7%–13%	9%–20%	11%	10.5%	48%

Dashes in cells indicate testing was not done.

Abbreviations: CNV, copy number variant; EEG, electroencephalography; FISH, fluorescence in situ hybridization; MRI, magnetic resonance imaging; NA, data not available; PTEN, phosphatase and tensin homolog.

Nevertheless, these data serve as starting points for providing guidelines for the evaluation of the child with autism.

What are these genetic conditions? In general, they can be subdivided into metabolic, mitochondrial, chromosomal, and monogenic (ie, caused by mutation in a single gene). An understanding of what these conditions are is useful in understanding the recommendations of the various specialty groups. Because there are good reviews of the various conditions associated with autism,[22,23] this article tries to answer the question, "what conditions should be considered in the child who does not appear to have a syndromic cause as the reason for the ASD phenotype?"

METABOLIC DISORDERS

Metabolic disorders are generally not considered to be a significant cause of autism, but because many are amenable to some form of treatment, it is important to recognize a metabolic disorder when it is present. The following is a brief review of some of these disorders that can be a cause of ASD.

Phenylketonuria

Phenylketonuria (PKU) is one of the more common disorders of amino acid metabolism, caused by homozygous or compound heterozygous mutation of the phenylalanine hydroxylase gene. PKU is one of the disorders for which newborn screening is done in the United States and several other countries; however, screening for PKU does not occur universally. In addition, if the screening is done too early (eg, first 12 hours of life), false-negative results can occur. Therefore, the physician needs to still consider PKU in the differential diagnosis in certain circumstances.

The phenotype of untreated PKU individuals includes cognitive impairment, seizures, microcephaly, decreased pigmentation, and a musty odor.[24] Among individuals with autism, PKU has been reported to occur more frequently than expected by chance[25]; however, the absolute frequency is still expected to be low, as demonstrated by the finding that autism in untreated PKU patients only occurs 5% of the time.[26] As already noted, if there is suspicion that the child may have PKU as the cause of the autism phenotype, diagnosis can easily be achieved by measurement of serum levels of phenylalanine.

Disorders of Purine Metabolism

Only one condition in this group is associated with the development of ASD, that being adenylosuccinate lyase (ADSL) deficiency. Phenotypic manifestations in this autosomal recessive condition include seizures, cognitive impairment, and hypotonia.[27] The frequency of autism as a component manifestation is unknown, although Jaeken and Van den Berghe[28] described autistic features in 3 of 8 children with this condition. Diagnosis is achieved by measurement of succinyladenosine and succinylamino imidazole carboxamide in cerebrospinal fluid, serum, or urine.

However, a second condition in this category deserves mention. Adenosine deaminase (ADA) deficiency can lead to severe combined immunodeficiency and, if untreated, early death.[27] Most untreated children die soon after birth, but in those who were treated by bone marrow transplantation, Rogers and colleagues[29] found behavioral abnormalities such as hyperactivity/attention-deficit disorder, aggressive behavior, and social problems. Autism was not described as occurring more frequently. This finding is relevant in that there are reports of children with autism having reduced levels of ADA in their sera,[30] leading some groups to search for mutations or polymorphisms in one ADA allele. Two Italian studies[31,32] did report

a significantly increased relative risk for autism in patients with heterozygosity for an ADA allele (ADA2) associated with reduced catalytic activity, and suggested this variant might be associated with an increased risk of autism. Hettinger and colleagues[30] in a North American population did replicate these findings, so it was suggested that the ADA polymorphism may play a more significant role in Italian populations than in United States populations. In summary, ADA2, a variant form of the ADA gene, may be associated with an increased predisposition to develop autism, but having two mutations (pathogenic changes) causes a condition which is not associated with an increased risk of autism.

Succinic Semialdehyde Dehydrogenase Deficiency

This condition is a relatively rare (although the true prevalence is unknown) neurometabolic, autosomal recessive disorder that can be diagnosed by determination of succinic semialdehyde dehydrogenase (SSADH) enzymatic activity in leukocytes. The finding of elevated levels of 4-hydroxybutyric acid on urine organic acid screens often raises the suspicion that this is the underlying disorder. This condition is characterized by cognitive impairment, hypotonia of childhood onset, ataxia, and seizures. Behavioral disturbances include hyperkinesis, aggression, self-injury, and sleep disturbances.[33] Autistic features were described in 12% (4/33) of older individuals. Therefore, despite the unknown prevalence, it is likely that SSADH deficiency and autism affects fewer than 1 in several million. However, it is important to keep in mind that therapeutic decision making could be affected by knowledge that a patient has SSADH deficiency. For example, based on its pharmacologic properties, finasteride could prove to be a useful adjunctive therapy in those with SSADH deficiency.[34]

Disorders of Creatine Transport and Metabolism

Three different disorders have been identified as falling into this category. Deficiencies of arginine:glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT) affect creatine metabolism, whereas deficiency of creatine transporter 1 (CT1) enzyme affects its transport into brain tissue. As a group, these conditions are termed the creatine deficiency syndromes (CDS).[35] AGAT and GAMT deficiencies are thought to be extremely rare, whereas CT1 is thought to account for as much as 1% to 4% of cases of X-linked cognitive impairment. Clinical manifestations in all include developmental delay, cognitive impairment, autistic manifestations, seizures, and hypotonia. Those with CT1 deficiency may also have the additional manifestations of midface hypoplasia and short stature.[36] In a review of CDS, Schulze[37] noted that two-thirds of those with a CDS had autism. Diagnosis of these conditions can be achieved by detection (or lack thereof) of the creatine peak on magnetic resonance spectroscopy, and confirmed by measurement of plasma and urine levels of creatine and guanidinoacetate. The pattern of results often points to the diagnosis (eg, in AGAT deficiency, plasma creatine is low/normal, whereas plasma and urine guanidinoacetate is low; in GAMT deficiency, plasma creatine is low, whereas plasma and urine guanidinoacetate are elevated).[36] It has been questioned whether children with autism should be screened for one of these disorders.[35] These conditions are admittedly rare; however, because therapeutic interventions can improve manifestations and, if given early enough, can prevent cognitive impairment and neurologic symptoms in some of these conditions,[36,38] consideration should be given to pursuing testing in children with consistent phenotypic manifestations. Unfortunately, treatment is less successful in the more common CT1.[38]

Cerebral Folate Deficiency

This group of conditions is defined as any one of the neurologic entities character- ized by low cerebrospinal fluid concentration of 5-methlyhydrofolate in the presence of normal serum folate levels. Cerebral folate deficiency comprises a heterogeneous group of conditions for which the molecular basis is incompletely understood, although disorders/genes identified to date include dihydrofolate reductase, α- 5,10-methylene-tetrahydrofolate reductase, 3-phosphyglycerate dehydrogenase, or dihydropteridine reductase deficiencies; as well as Rett, Aicardi-Goutieres, or mito- chondrial syndromes.[39] Despite this heterogeneity, it has been suggested by some[40] but not others[41] that a clinical phenotype exists. This phenotype includes early normal development until approximately 4 to 6 months, with subsequent devel- opmental delay, agitation, sleep disturbances, deceleration of head growth, ataxia, hypotonia, and seizures.[40] However, Mangold and colleagues[41] did not find supportive evidence for this assertion, and cautioned against viewing this group of conditions as a distinct syndrome.

The diagnosis is achieved by measurement of cerebrospinal fluid 5-methyltetrahy- drofolate (5MTHF), and finding reduced levels. One group[41] recently described 103 patients with cerebral folate deficiency, although the frequency of autism in this cohort was not noted. Ramaekers and colleagues[42] described autism in 4 of 28 patients, whereas Moretti and colleagues[43] reported autism in 5 of 7 patients. Therefore, it is clear that autism is not a consistent finding in what is likely a rare group of disorders. Nonetheless, in a child with autistic manifestations with some of the other findings of this group of disorders, it may be worthwhile to pursue this diagnosis because there is some evidence that treatment with folinic acid may lead to improvement of some of the symptoms.[39]

Smith-Lemli-Opitz Syndrome

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder of cholesterol metabolism characterized by a distinctive phenotype of physical, cognitive, and behavioral manifestations. The clinical findings include microcephaly, ptosis, ante- verted nares, micrognathia, and syndactyly between toes 2 and 3 (present in more than 90% of cases). Genital anomalies in males are also common. All have cognitive impairment, and at least 75% have autistic features.[44] The frequency of SLOS in the population is approximately 1 in 30,000 (but may be as high as 1 in 20,000) so a reason- able estimate for the prevalence of those with SLOS and autism is that 1 in 40,000, or 1 in 267 of those with autism will have SLOS. Therefore, screening all with autism for SLOS will have a very low yield; this was also found by Tierney and colleagues,[45] who did not find metabolic evidence for SLOS in those with autism, and suggested that the frequency of unrecognized SLOS among individuals with autism is less than 0.2%. However, targeted testing of those in whom there is clinical suspicion is worth- while. Diagnosis is accomplished by measurement of 7-dehydrocholesterol in serum. Benefits of diagnosis include the potential for amelioration of symptoms by treating with cholesterol; however, a recent prospective trial failed to identify a true effect on behavior.

Although Tierney and colleagues[45] did not identify individuals with SLOS in their study, they did find that abnormally low levels of cholesterol were found in almost 20% of children with autism, further supporting a role for reduced lipid levels in chil- dren with autism. This finding has potential for identification of possible interven- tional or preventive therapies[46] as well as for understanding additional causes of autism.

MITOCHONDRIAL DISORDERS

A study done in 2005 found that 7% of children with ASDs had evidence of a mitochondrial disorder, as demonstrated initially by elevated serum lactate, and subsequently by the demonstration in some of those of a mitochondrial respiratory chain disorder (n = 5/69). All 5 children were found to have moderate to severe cognitive impairment, and an autism diagnosis of severe autism.[47] A subsequent study evaluated 25 children with autism and confirmed mitochondrial disease, and pointed out that there were clinical characteristics that helped distinguish this group from nonmitochondrial autism. These children were more likely to have atypical developmental problems (eg, greatly delayed age at walking) and patterns of regression, and virtually all had nonneurologic disorders. One of the most common of these was gastrointestinal dysfunction, including cases with pancreatic and/or liver malfunction. Other nonneurologic anomalies included cardiac, hematologic, and growth involvement.[48] A good review of mitochondrial autism is provided by Haas,[49] and the reader is referred to that article for more detailed discussion on the topic. In summary, it is likely that a mitochondrial disorder is a rare, if ever, cause of autism without associated findings; however, a child with evidence of mitochondrial autism merits further evaluation. To that end, Frye and Rossignol[50] have suggested a protocol for determining the likelihood of mitochondrial disease in a child with ASD, with the so-called Morava protocol at least a starting point for considering the need for mitochondrial evaluation (which can often be extensive and costly). Although there are few, if any, proven therapies, there is some promise that in the future better interventions will become available.[51]

CHROMOSOME ANOMALIES

Several years ago, the only means of investigating a child for the presence of a chromosomal disorder was to do a karyotype, which identified an anomaly in a few percent of those with ASDs.[52] Recently developed techniques, termed chromosomal microarrays (CMAs), have been used to detect smaller deletions or duplications than could be found by karyotyping, and the yield in children with ASD has risen to 7% to 10%. In the vernacular, this is the proportion of children with a CNV. Among the numerous pathogenic CNVs that have been reported, several are found significantly more often in individuals with an ASD. These CNVs are briefly summarized below.

Dup 7q11.23

Williams syndrome is a common microdeletion syndrome characterized by (among other things) a characteristic facial phenotype, outgoing, sociable personality (often described as hypersocial), and mild cognitive impairment. More recently, microduplications of this region have been found in individuals with autistic-like features. Cognitive impairment may also be a component manifestation, although this is not a constant finding. There is said to also be a characteristic facial phenotype, although manifestations may be subtle. This phenotype includes prominent forehead, straight eyebrows, thin upper lip, and broad nose in older children.[53] It is noteworthy that haploinsufficiency of one of the genes in this region, Gtf2i, leads to increased social interaction in mice.[54] It is therefore not surprising that increased dosage of this gene could lead to decreased sociability.

Dup or Del 16p11.2

This anomaly is estimated to occur in up to 1% of all children with ASD. The phenotype in those with deletion or duplication may be dysmorphic, with deep-set appearing eyes, midface hypoplasia, and smooth philtrum as consistent findings. However,

some individuals are described as nondysmorphic, thus an unremarkable phenotype does not exclude 16p11.2 CNV as the cause of ASD. The frequency of autism in 16p duplication is 75%, whereas it is 98% in those with deletions.[55]

Del 17q12

One study found this microdeletion in 24 of 23,271 patients with an ASD or schizophrenia, for a prevalence of approximately 0.1% of those tested. The phenotype is slightly dysmorphic, with macrocephaly, arched eyebrows, down-slanting palpebral fissures, epicanthal folds, and malar flattening the most common features. Although most had cognitive impairment, it was not a consistent finding. Most also had various neuropsychiatric comorbidities, including phobias, depression, mood lability, and bipolar disorder.[56] This deletion has also been found in individuals with schizophrenia, thus perhaps providing a reason for the observation that schizophrenia is more common in the parents of children with ASD described before. A similar observation has been made for duplication 16p11.2 (more common in those with schizophrenia).[57]

Del or dup 15q13

Individuals with deletions or duplications in this region have been reported to have autism or autistic manifestations. A recent study identified that 0.6% of children referred for microarray analysis for various indications (including developmental delay, cognitive impairment, learning disability, or ASD) were found to have CNVs at this locus.[58] The physical phenotype was described as nonspecific, and in a second study, some of these children were found to have inherited this CNV from a clinically unaffected parent.[59]

Many of these individuals have additional behavioral manifestations, including hyperactivity, aggression, and impulsive behavior. Other studies have also suggested a link with schizophrenia,[60] thus genes in this and previously described regions are likely responsible for alterations in brain function, which can lead to various behavioral, cognitive, and psychiatric manifestations.

Del 22q13

This underdiagnosed condition is likely secondary to the unremarkable phenotype. The described phenotype includes long eyelashes, bulbous nose, full cheeks, pointed chin, and large or unusual ears. The hands appear relatively large, and nails are dysplastic.[61] However, the recommendation for considering this diagnosis only includes hypotonia, absent speech, and global developmental delay. Most children with this condition are said to have autistic-like features. One of the relevant genes in this region is *SHANK3*, in which mutations have been found in autistic individuals.[62]

Therefore, as these pathogenic CNVs (microdeletions and duplications) are described in children with ASD, this will help to identify relevant genes for causing the ASD phenotype. CMAs are available at several laboratories throughout the United States, although in the author's experience insurance companies may not be willing to pay for this testing. It should also be emphasized that if a CNV is done, a karyotype should not be ordered at the same time, nor should specific fluorescence in situ hybridization probes be ordered. Based on the findings, the laboratory will direct the ordering physician as to which additional steps should be taken to clarify the result.

MONOGENIC DISORDERS

A few conditions that are associated with an increased frequency of ASD are described here. This list is not meant to be comprehensive (see Ref.[22] for a full list). However, the focus of this section is the conditions that may present with ASD but

not be as readily apparent, at least physically. Therefore, conditions such as Cornelia de Lange, CHARGE, or Angelman syndromes, for example, are not discussed because the clinical diagnosis would likely precede the ASD diagnosis.

Neurofibromatosis Type 1

Neurofibromatosis type 1 (NF1) is an autosomal dominant condition that is relatively common, with an estimated frequency of 1 in 3000 to 1 in 4000.[63] A study done several years ago, likely using a narrower definition of ASD (using a frequency of 1 in 6000) found NF1 in 0.3% of patients with a diagnosis of autism. It is unknown whether using a broader definition of autism would increase or decrease that figure. Nonetheless, it is not unreasonable to search for manifestations of NF1 in a child with an ASD diagnosis.

The NF1 diagnosis is usually made on a clinical basis, using diagnostic criteria developed by the National Institutes of Health.[64] These criteria include 2 or more of the following manifestations:

1. Six or more café-au-lait macules
2. Two or more neurofibromas or 1 plexiform neurofibroma
3. Axillary or inguinal freckling
4. Optic glioma
5. Two or more Lisch nodules
6. Sphenoid dysplasia or tibial pseudarthrosis
7. First-degree relative (parent or sibling) with confirmed NF1.

All but the youngest children can be confidently diagnosed using these criteria; DeBella and colleagues[65] found that whereas only 54% of nonfamilial cases met diagnostic criteria by 1 year of age, almost all (97%) did so by 8 years of age. Molecular testing is available in ambiguous cases, but given that the average age of diagnosis for autism is 3.1 years,[66] the child with autism secondary to NF1 should have enough criteria for clinical suspicion, if not diagnosis. Therefore, use of a molecular test should be approached thoughtfully.

Tuberous Sclerosis

Tuberous sclerosis (TS) is another autosomal dominant condition in which autism is found more often. TS is caused by two different genes, so the condition is sometimes referred to as TS1 and TS2, depending on the gene involved. The frequency of TS in the population is 1 in 5800, with up to 45% of those having symptoms of ASD.[67,68] Conversely, the frequency of TS among children with ASD is 1.2%.[69] As in NF1, there are established diagnostic criteria[70] based on the presence of major and minor criteria. If a thorough evaluation is done on suspected cases, penetrance is thought to be 100%. Molecular testing is available at a limited number of laboratories, and detection rate is 80% (thus even with strong clinical suspicion, the diagnosis is unable to be confirmed in 20% of cases). Most individuals have TS2 mutations (60% vs 19%), so testing in a tiered fashion may be indicated. Although this is an expensive test (costing a few thousand dollars), it may be even more expensive to make the diagnosis clinically because such would involve magnetic resonance imaging, cardiac echocardiography, renal ultrasonography, and ophthalmologic examination.

Myotonic Dystrophy

Myotonic dystrophy is an autosomal dominant disorder that has a prevalence of approximately 1 in 20,000.[71] The cause is expansion of a trinucleotide repeat within the DM1 gene. The classic form has an age of onset of 10 to 30 years, and is

characterized by the development of myotonia and relatively expressionless face. Additional manifestations that develop over time may include weakness (particularly distally), and cardiac, endocrine, and gastrointestinal dysfunction. The frequency of ASD in a group of children with myotonic dystrophy was reported as 49%, with age of onset of the myotonic dystrophy correlating with the presence and severity of ASD. In addition, 86% of this group had moderate to severe cognitive impairment.[72] This figure suggests that although children in this group who also have ASD are more likely to have cognitive impairment, this is not a consistent finding.[72,73] Although in the past it was necessary for electromyography and/or muscle biopsy to be done to make the diagnosis, molecular testing is 100% accurate and costs less than $300. Although myotonic dystrophy accounts for less than 0.2% of cases of ASD, in a child with evidence of myotonia, particularly when there is a positive family history that is supportive of this diagnosis, testing for myotonic dystrophy is a reasonable option.

Fragile X

Fragile X syndrome is another trinucleotide repeat disorder inherited as an X-linked trait. The population prevalence is estimated at approximately 1 in 5000.[74] The frequency among boys with ASD is estimated at 2.7%,[69] which is comparable with the frequency among children with developmental delay.[75,76] Autism is present in approximately 25% of those with fragile X[77]; pervasive developmental disorder is present in an additional 30%.[78] It has also been shown that among males with a pre-mutation (55–200 repeats, frequency 1/813 males and 1/259 females), autism is more common[78,79]; a second study found that 13% of premutation carrier males and 1% of premutation carrier females had ASD.[80] Testing is widely available for a few hundred dollars, and may be worth doing despite the anticipated relatively low yield.

Several single genes have also been identified as having a role to some extent in the cause of ASD. Here again, see Scherer and Dawson[81] for a more detailed review.

As a result of these and other studies, the American Academy of Neurology, the American Academy of Pediatrics, and the American College of Medical Genetics (ACMG) have developed guidelines for the investigation of autism (with the ACMG guidelines focusing on the genetic causes) (**Table 2**). Testing for some of these conditions has already been discussed in the context of the particular condition. It should not be understood from these guidelines that testing should be done on every child, nor should it be exhaustive. In a recent article on the topic of genetic testing in autism, Rapin[84] discussed that there are several reasons for testing, including:

1. Providing medical therapy for children with treatable manifestations
2. Providing information or recurrence risks for subsequent pregnancies or for other family members
3. Defining a specific etiology, even if therapy is not available.

Bockenhauer and colleagues[85] provide a similar discussion in their article on genetic testing in renal disease; they also examine reasons for genetic testing, and list as reasons:

1. Confirmation of diagnosis
2. Precise genetic counseling
3. Better understanding of pathophysiology
4. Supporting clinical management.

Genetic tests can be of different types, and include diagnostic, presymptomatic (testing done to determine if an individual has a gene for a disorder before the

Table 2
Recommendations of select professional societies

Evaluations	American Association of Pediatrics[82]	American Association of Neurology[83]	American College of Medical Genetics[a,4]
Physical exam	Yes, all children		First tier
Metabolic evaluations	Yes, if symptoms	Yes, if symptoms	First tier, but only if symptoms
Audiogram	Yes, all children	Yes	Preevaluation for autism
Rubella titers	NA	NA	First tier
Karyotype		If cognitive impairment	First tier
Fragile X	If cognitive impairment	If cognitive impairment	First tier
MRI	If tuberous sclerosis suspected	Not recommended	Third tier
EEG	History of seizures	Only if suspect seizures	Preevaluation
MECP2	Female with regression	NA	Females, second tier
Uric acid			Third tier
Microarray	If cognitive impairment	If cognitive impairment	Second tier
Mitochondrial evaluation	NA	Not recommended	First tier (lactate, pyruvate, acylcarnitines)
PTEN	NA	NA	If OFC >2.5 SD above mean, second tier
Fibroblast karyotype	NA	NA	If pigmentary anomaly, Second tier

Abbreviations: NA, not addressed; OFC, occipitofrontal circumference.
 [a] Recommendations were allotted to tiers.

individual is showing any symptoms of that disease), or predispositional (testing to identify genetic changes that could increase the liability of an individual developing a particular disorder). When considering whether to order a genetic test, one needs to know some things about the test, including:

1. Sensitivity: how many individuals will be accurately diagnosed?
2. Specificity: how many gene changes are harmless (false positives)?
3. Ease of interpretation: will the specific genetic change assist with the prognosis? For example, there are several examples of mutations in the same gene causing different phenotypes
4. Cost and availability: most genetic tests are only done at a few laboratories, and are often expensive. Some insurance companies may not agree to pay for genetic testing, unless the results have an impact on management or treatment.[86]

In addition to testing for one specific disorder, some laboratories now offer panels of tests (in some cases, the so-called autism panel). The ordering physician needs to know whether these panels are for genetic changes of major effect (eg, monogenic conditions) or for changes that could be considered predispositional. Regardless, it is one thing to be able to tell a family that the child with ASD has a premutation in the fragile X gene, and quite another to inform the family that there are changes in one or more genes that are susceptibility loci for ASD.

At present newer tests are being developed for clinical use, which will likely be used fairly extensively in testing individuals for not only ASD but also a plethora of other conditions, be they syndromic or not. One such test is exome sequencing, which will be able to provide information on sequence variations in every coding region of an individual's genome. Although the test currently costs several thousand dollars, it is anticipated that such costs could come down to as much as $500, which is less than it currently costs to sequence one gene. Physicians will need to be able to explain to families what genetic testing will provide in terms of information and patient care.

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