



Initial Medical Evaluation of a Child Diagnosed With an Autism Spectrum Disorder

Confirmation of an autism spectrum disorder (ASD) diagnosis is usually made by a pediatric subspecialist or ideally a team of ASD or developmental specialists. Although several strategies may be used, all depend on confirmation of *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* criteria. The Autism Diagnostic Interview-Revised and Autism Diagnostic Observation Schedule are used in most research settings to confirm presence of these criteria, but the time and expertise necessary are not feasible in many clinical settings. Specialists use a series of evaluation instruments including direct observation of the child to assess social and language skills and presence of atypical ASD behaviors. A thorough history (including health, developmental, behavioral, and family histories), physical examination, and some evaluation of the child's overall level of functioning (cognitive, adaptive, and motor skills) are also very important. Hearing and visual impairment need to be considered if not previously documented. Finally, one should also assess the family's resources and coping strategies.

Once diagnosis of an ASD is confirmed by a specialist or team of specialists, the pediatrician plays an important role in medical evaluation. Although medical investigation includes a search for conditions that are known to play an etiologic role in ASDs (eg, fragile X syndrome), it may also be directed at determining the presence of coexisting medical conditions (eg, seizures, hearing loss). Some managed care systems may require the primary care pediatrician to order or approve laboratory tests. In some regions the diagnosing professional is a psychologist and the child is referred to the primary care practitioner for etiologic evaluation. Often a specialist such as a developmental pediatrician, neurologist, or geneticist will direct medical laboratory evaluation or provide consultation.

The clinician should obtain a detailed history (including a 3-generation pedigree) and conduct a thorough physical examination (including a Wood lamp evaluation of the skin to rule out tuberous sclerosis) to determine if there are any clinical indications for specific laboratory investigations. Current research supports a targeted approach to diagnostic laboratory testing. *Note:* Recommendations for laboratory workup may change over time as new technologies become available.

Studies to Consider for All Patients

- **Audiologic evaluation.** Consider for all children with language delay, including those with ASDs, even if children had normal hearing at birth or passed newborn screening. School screening may suffice in older children who can cooperate.
- Genetic diagnostic testing
 - **Microarray.** Offer to all patients.
 - Microarray panels include developmentally significant regions of the genome and common genetic syndromes caused by gene mutations.
 - **DNA study for fragile X syndrome.** Offer to all patients.
- Overall yield of positive findings from genetic testing is 10% to 20% in children with ASDs (as of 2011). Higher rates of positive findings occur in children with ASDs who also have global delay or intellectual disabilities, seizures, or dysmorphic physical features.
- Families can be counseled that genetic testing may identify a reason for their child's developmental disability, help them understand recurrence risk, and if a specific disorder is identified, alert them to potential health conditions in the affected child or other family members.

Studies to Consider for Select Patients

Additional laboratory investigations should be considered when specific indications are identified by history or physical examination. Evaluation for specific genetic, metabolic, or other neurologic disorders may be facilitated by referral to a specialist. Some examples include

- Additional genetic studies
 - **Additional testing for Angelman syndrome.** Consider in patients with ASDs and seizure disorders presenting before 5 years of age who have a normal microarray result (not all cases result from gene deletion—see page 4 for details). Features of classical Angelman syndrome include intellectual disability, happy affect, and hand waving/clapping. Because of the complexity of genetic testing for Angelman syndrome, consultation with a genetic specialist is recommended to guide testing.

- *Karyotype*. Indicated for patients where a balanced translocation is suspected (microarray will not detect these lesions), karyotype may be specifically indicated if there is a history of more than 2 miscarriages or if a specific chromosomal syndrome such as Down syndrome is suspected clinically.
- *MECP2 gene mutation and sequence analysis*. This analysis is for girls with microcephaly or deceleration of head growth and other features of Rett syndrome, or who present with stereotypical hand-wringing movements and developmental regression. *MECP2* gene mutations are extremely rare in males but may be considered in boys who present with clinical features of Rett syndrome or severe developmental regression. Given the complexity, testing in male patients should be done with consultation from a genetic specialist.
- *PTEN gene mutation and sequence analysis*. Consider for patients with macrocephaly (ie, head circumference greater than 2 standard deviations above the mean). Patients with *PTEN* mutations have a predisposition to certain malignancies and may present in several different ways (see “Bannayan-Riley-Ruvalcaba syndrome” on page 4 for more details). Male patients may present with penile macules. Patients and family members of patients with these mutations need ongoing monitoring for colon polyps and cancers of the thyroid, breast, and endometrium.
- *Metabolic laboratory testing*. Children with cyclic vomiting, hypotonia, lethargy (especially when associated with mild illnesses), poor growth, unusual odors, multiple organ involvement, ataxia or other movement disorder, or evidence of a storage disease (eg, coarse features) should be tested. Testing should include lactate, pyruvate, carnitine, acylcarnitine profile, liver and renal function, amino acids including testing for phenylketonuria, and urine organic acids.
- *Serum lead and ferritin levels*. Children with pica, especially living in at-risk environments, should be monitored with ongoing blood lead levels as long as pica persists. Serum ferritin may also be indicated to monitor iron stores. Although lead toxicity does not cause ASDs, it can have a detrimental effect on learning and cognition that may indirectly intensify symptoms.

Other Medical Workup

Other medical investigations may be indicated based on history and physical examination.

- *Electroencephalogram (EEG)*. Children with a history of seizures, acute developmental regression, unexplained behavioral change, or suspicion of subclinical seizures should receive EEGs. Screening EEGs on all children with ASDs are not currently recommended.

- *Magnetic resonance imaging (MRI)*. Consider in children with acute regression, microcephaly, midline facial defects, neurocutaneous lesions (with or without Wood lamp), or abnormalities on neurologic examination. Isolated, stable macrocephaly is not, in itself, an indication for an MRI.

Note: There is no evidence that hair analysis, micronutrient levels, intestinal permeability studies, stool analyses, urinary peptides, or mercury levels are helpful. Although functional neuroimaging studies are important research tools in understanding brain areas that function atypically in various processing tasks, they are not clinically indicated in the etiologic workup of a child with an ASD.

Children with ASDs can manifest a variety of coexisting disorders such as seizures, gastrointestinal issues, and sleep problems. These conditions may also cause an acute change in behavior. (See additional components in this toolkit on these topics.)

Glossary

Copy number variation (CNV). Term used to describe variability in DNA, which can now be appreciated given the great sensitivity of microarray. There are some regions of the genome that display a high degree of CNV, and changes on microarray in these regions are not as significant as DNA abnormalities detected by microarray in regions of the genome that are not as variable. If a parent is found to have the same CNV as the child but a normal phenotype, this CNV is usually considered to be a benign familial variant. However, if neither parent has a CNV which is unique to the child, it is more likely to be significant. A number of CNVs are now known to be associated with ASDs, with new ones frequently found. Genes that are currently associated with ASDs are catalogued in ASD CNV databases such as the MindSpec autism database, AutDB (www.mindspec.org/autdb.html), and the University of Kansas Autism Genetic Database (<http://wren.bcf.ku.edu>).

Cytogenetic testing. The study of human chromosome structure. Chromosome structure is visible only during mitosis and is most commonly evaluated in blood lymphocytes or skin fibroblasts. Certain staining techniques are used to produce a pattern of banding, which can help distinguish one chromosome from another; G-banding is most commonly used. In addition to being distinguished by banding patterns, the 22 pairs of chromosomes called autosomes are also distinguished by size, with chromosome 1 the longest and chromosome 22 the shortest. The 23rd pair of chromosomes are called the sex chromosomes, with females showing an XX pattern and males an XY pattern. When chromosomes are laid out in a characteristic pattern, this is called a *karyotype*. A *high-resolution chromosome analysis* can detect more subtle abnormalities by looking at chromosomes that are more elongated during the phase of mitosis called prometaphase.

However, this technology cannot detect subtle chromosomal abnormalities smaller than 5 million base pairs.

Fluorescent in situ hybridization (FISH). Technology in which a fluorescent dye is attached to a DNA probe, which adheres to a section of DNA with a specific pattern. Fluorescent in situ hybridization probes exist for multiple regions on all the chromosomes and can be used to detect the origin of a small extra piece of DNA such as a marker chromosome or rapidly screen for chromosome numerical abnormalities such as trisomy 13, 18, or 21, or abnormal sex chromosomes. Fluorescent in situ hybridization probes also exist for the ends of chromosomes (sub-telomeric probes) and can be used to rapidly confirm diagnosis of a suspected microdeletion syndrome, such as velocardiofacial syndrome (22q11 deletion) or Prader-Willi syndrome (15q deletion). Fluorescent in situ hybridization is often used to confirm more rare DNA changes detected with microarray, so that parental FISH of the same region can be used to determine if the abnormality is inherited or de novo (see “Copy number variation (CNV)”).

Metabolic testing. This testing would be indicated if the clinician is suspicious about the possibility of an inborn error of metabolism such as a mitochondrial or neurodegenerative disorder, or a specific metabolic disorder such as Smith-Lemli-Opitz syndrome (see page 4). Testing done when one suspects a mitochondrial disorder includes the following: lactate, pyruvate, carnitine, acylcarnitine profile, liver and renal function, amino acids, and urine organic acids. Testing done when a neurodegenerative disorder is suspected depends on the clinical phenotype; for example, if the child has organomegaly, testing for storage diseases such as a white blood cell lysosomal screen would be in order, while if the child has white matter changes on MRI, specialized testing for leukodystrophies would be indicated.

Certain metabolic disorders are associated with ASDs and characteristic features. An example is Smith-Lemli-Opitz syndrome, which may present with very mild physical features and ASDs. Specialized metabolic testing is required to make this diagnosis. Whenever considering these rare disorders, consultation with a geneticist is indicated.

Methylation testing. Specialized DNA testing that looks for a differential pattern of methyl groups attached to DNA, which can distinguish maternally versus paternally inherited alleles. Abnormal methylation patterns are detected in diseases whose associated genes are located in imprinted regions of the genome. In the genetic mechanism known as *imprinting*, certain genes are only expressed from the maternal allele, while others are only expressed from the paternal allele. Thus, a deletion might have different effects depending on where it is located. The classic example of this involves Prader-Willi

syndrome, usually caused by a deletion of paternal chromosome 15q, versus Angelman syndrome, associated with a deletion in the same region of 15q but from the maternal allele. Prader-Willi syndrome is associated with mild intellectual disabilities but not usually with autism, while Angelman syndrome is associated with more severe disabilities and often is associated with ASDs.

Microarray (aka, chromosomal microarray, comparative genomic array, comparative genomic hybridization, oligonucleotide microarray). An array-based genomic copy number analysis. This is the newest and most sensitive of DNA testing modalities and has replaced karyotype as the first-line test of choice in children with developmental disabilities and ASDs. Microarray compares the amount of DNA in the patient to a normal control and can detect very subtle copy number variations from normal, including gains and losses of genetic material. Because this is a relatively new test compared with karyotype, it is performed in many different laboratories across the country, using a variety of different platforms, which have all become more sensitive over time. A recent recommendation from the American College of Medical Genetics states that microarray platforms must have coverage to detect all areas of imbalance with a resolution of greater than 400 kb throughout the genome. Most microarray platforms currently in use have the sensitivity to detect DNA abnormalities of 200 base pairs or greater in size.

Although microarray is a relatively expensive test, because it is so much more sensitive than karyotype, it is more likely to detect an abnormality, and it is cheaper than doing a karyotype plus FISH testing, which was the norm in the past.

Molecular (DNA) testing. The direct analysis of a gene whose mutation is associated with a particular disorder. In some diagnoses, the DNA change causing the disorder is too small to be detected by analyses such as karyotype or even microarray and must be confirmed with specific testing of the gene itself. There are different types of DNA changes that can cause a variety of disorders including *insertions*, *deletions*, and *point mutations*. Duchenne muscular dystrophy, a condition more common in males, is associated with a deletion of the dystrophin gene on the X chromosome. Rett syndrome is associated with mutations of the *MECP2* gene, also located on the X chromosome, but this condition is seen mostly in females, as it is felt generally be lethal in males. Other conditions, such as fragile X syndrome (see page 4) are associated with a different mechanism of DNA mutation, namely unstable expansion of trinucleotide repeats.

Common Genetic Disorders Associated With Autism Spectrum Disorders

Angelman syndrome. Caused by abnormalities involving the PWS/AS region on chromosome 15q. A patient may have Angelman syndrome because of a deletion of the maternally inherited allele in this region, uniparental disomy, an imprinting error, or a mutation in the *UBE3A* gene. Confirming the diagnosis of Angelman syndrome may require several different testing modalities because of these different mechanisms, including FISH or microarray detection of a deletion of 15q, methylation testing to detect uniparental disomy or an imprinting error, or DNA analysis of the *UBE3A* gene.

Patients with Angelman syndrome usually have severe cognitive disabilities, seizures, and progressive lower extremity spasticity causing an abnormal gait. Older patients have mild prognathism and may have paroxysmal laughter.

Bannayan-Riley-Ruvalcaba syndrome. A disorder characterized by autism and macrocephaly (head circumference >98%), it is caused by a mutation in the *PTEN* gene. Patients may also present with lipomatous growths, penile freckling, and macrosomia. Mutations in the *PTEN* gene are inherited as autosomal-dominant traits and cause an adult cancer predisposition syndrome called Cowden syndrome, which is associated with normal intelligence but development of certain malignancies including breast, thyroid, and colon cancer. Children with Bannayan-Riley-Ruvalcaba syndrome have increased risk of the same malignancies. Detecting this mutation in a child with an ASD changes medical management for the patient, as cancer screening is mandatory, as well as careful genetic counseling and testing in parents.

Down syndrome. Caused by trisomy 21, Down syndrome is the most common chromosome abnormality in humans, occurring with an incidence of 1 per 691 live births. There is an association between Down syndrome and ASD, with up to 1 in 10 patients with Down syndrome displaying an ASD behavioral profile. Reasons for this association are not known at this time, although there are at least 2 genes on chromosome 21 that have been implicated in ASD. Sophisticated microarray technology may shed some light on this in the future.

Fragile X syndrome. An X-linked disorder with intellectual disability that is caused by mutations in the *FMR1* gene, consisting of expansion of CGG trinucleotide repeats. A normal number of repeats is less than 50. A person with 50 to 200 repeats is considered a pre-mutation carrier. If the expansion of CGG trinucleotide repeats is greater than 200 and there is hypermethylation of the CGG expansion and adjacent CpG island, the person has a full mutation. Diagnosis of fragile X syndrome is made by DNA molecular testing of the *FMR1* gene.

Because this is an X-linked gene, males are more affected than females, but there can be symptoms in females to a milder degree. Males present with developmental delay and often have macrocephaly, long facies, joint hypermobility, and testicular enlargement in postpubertal patients. A mild phenotype may also be present in pre-mutation carriers. Females with pre-mutations have an increased rate of premature ovarian failure, while adult males with a pre-mutation may develop a Parkinson-like neurologic disorder called fragile X tremor/ataxia syndrome.

Mitochondrial disorders. Complex disorders of energy metabolism that may be caused by mutations in mitochondrial DNA, which are maternally inherited, or by mutations of autosomal genes usually inherited as recessive disorders. Most children with mitochondrial disorders present with hypotonia, developmental delay, seizures, and feeding problems with failure to thrive during infancy. Intercurrent illness may precipitate severe worsening of symptoms. These disorders are usually progressive with a neurodegenerative course. Mitochondrial disorders are often difficult to confirm and may require a muscle biopsy. Mutations in mitochondrial DNA can be detected through special DNA testing. Head MRI may also point to a mitochondrial disorder, as may abnormalities on electroretinogram.

Rett syndrome. A disorder caused by a mutation in the *MECP2* gene located on the X chromosome, it is usually a lethal mutation in males, such that this diagnosis is almost exclusively seen in females. Patients usually present with a period of normal development followed by loss of milestones, development of seizures, and characteristic stereotypical movements of the hands. The head stops growing, resulting in microcephaly. Patients otherwise have no dysmorphic features and are often quite attractive children. Diagnosis is made by molecular analysis (DNA testing) of the *MECP2* gene.

Smith-Lemli-Opitz syndrome. An autosomal-recessive disorder of cholesterol biosynthesis. In its more severe form, it is associated with multiple congenital anomalies, characteristic dysmorphic features, and severe intellectual disabilities. More than half of patients with Smith-Lemli-Opitz syndrome also have ASDs. However, it may present in milder form with just ASDs and a fairly normal phenotype with the exception of 2-3 toe syndactyly. Diagnosis is made by detecting an elevated level of the cholesterol precursor 7-dehydrocholesterol in serum, which is only performed in certain specialized laboratories. It is important not to miss this diagnosis—recurrence risk in future pregnancies is 25%.

Resources

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The recommendations in this publication do not indicate an exclusive course of treatment or serve as a standard of medical care. Variations, taking into account individual circumstances, may be appropriate. Original document included as part of *Autism: Caring for Children With Autism Spectrum Disorders: A Resource Toolkit for Clinicians*, 2nd Edition. Copyright © 2013 American Academy of Pediatrics. All Rights Reserved. The American Academy of Pediatrics does not review or endorse any modifications made to this document and in no event shall the AAP be liable for any such changes.

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