Health Evidence Review Commission's Genetics Advisory Panel

October 18, 2023
2:00 PM - 4:00 PM
Online Meeting

Join online meeting here
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Section 1.0
Call to Order
AGENDA
HERC’s Genetics Advisory Panel (GAP)
October 18, 2023
2:00 – 4:00 pm
Online meeting

(All agenda items are subject to change and times listed are approximate)

<table>
<thead>
<tr>
<th>#</th>
<th>Time</th>
<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2:00 PM</td>
<td>Call to Order &amp; Introductions</td>
</tr>
<tr>
<td>2</td>
<td>2:05 PM</td>
<td>Staff report</td>
</tr>
<tr>
<td>3</td>
<td>2:10 PM</td>
<td>1) 2024 CPT codes related to genetic testing</td>
</tr>
<tr>
<td>4</td>
<td>2:45 PM</td>
<td>1) Genetic testing for developmental disabilities, intellectual disability and autism spectrum disorders</td>
</tr>
<tr>
<td>6</td>
<td>3:50 PM</td>
<td>Other business</td>
</tr>
<tr>
<td>7</td>
<td>3:55 PM</td>
<td>Public comment on topics not on agenda above</td>
</tr>
<tr>
<td>8</td>
<td>4:00 PM</td>
<td>Adjournment</td>
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Note: Public testimony will be taken on each topic per HERC policy at the time at which that topic is discussed. Public testimony not related to a topic on the agenda will be taken at the end of the meeting.
The meeting was called to order at 2:00 PM. Roll was called. This is an advisory panel to the Health Evidence Review Commission (HERC). All documents discussed during this meeting were materials prepared by the HERC Medical Director for deliberation by the Value-based Benefits Subcommittee at its 9/29/21 meeting. Given the advisory nature of this meeting, a quorum was not necessary as no votes were taken. The highlights from the 2021 GAP meeting were reviewed and no changes were suggested.

1) Routine NCCN reference update for genetics-related guidelines: no discussion on this item.

2) 2023 genetic-related CPT codes
   a. CPT 81418 drug metabolism genomic sequence analysis: Carl Stevens felt that the staff recommendation was appropriate.

   Public comment: Devki Nagar from Myriad Genetics testified regarding CPT 81418. Myriad is supportive of the addition of this code to the Diagnostic File. Myriad would like to have GAP consider covering pharmacologic guidelines based on the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines. CPIC is an international body that uses rigorous evidence to recommend pharmacogenetic testing. CMS adopted use of CPIC guidelines for their coverage. Multigene testing is more useful than single gene testing. Medicare issued an LCD in 2020 specifying that pharmacogenetic tests are medically necessary for patients on medications with known gene-drug interactions that are called out by the FDA or CPIC guidelines.

   Stevens asked Nagar what drug classes would be covered and asked about Genesight. Nagar said that some Genesight components would align and is covered under Medicare. indicated Plavix. Stevens said Genesight is not covered now. Nagar said 81418 is not specific to Genesight but Genesight could fall in that code if it met the other criteria. She said she included an attachment from Medicare which lists the medications and genes.

   Staff was directed to research CPIC guidelines to see if these would be useful.

Smits said that the CPIC guidelines are available at: https://cpicpgx.org/guidelines/. CPIC guidelines include recommendations for genetic testing of P450 enzyme mutations prior to use of various proton pump inhibitors, clopidogrel, voriconazole, phenytoin, warfarin,
atomoxetine, ondansetron, tropisetron, tamoxifen, SSRIs, tricyclic antidepressants, opioids, and tacrolimus. In general, the reviews appeared to be current (within the past 5 yrs), evidence based, and funded by impartial bodies (for example, the NIH). Some authors had conflicts of interest. Staff conclusion was that these reviews are evidence based, but the recommendations went far beyond current standard of care. Staff recommendation is to continue to use FDA guidelines and monitor CPIC and other evidence-based sources going forward.

b. CPT 81441 Inherited bone marrow failure syndromes sequence analysis panel: panel members agreed with staff recommendation with no discussion

3) TPMT gene and enzyme activity testing: Stevens supported moving both gene and phenotype testing to the Diagnostic Procedures file. There was minimal discussion.

4) Next generation sequencing: Stevens noted there is a high volume of these tests requested. FoundationOne [CPT 0037U] is a major test in this area. He approves these tests despite the type or stage of cancer. Caris [Molecular Intelligence, CPT 81479] is another major testing group in this area. Stevens/CareOregon prices these codes similar to CPT 81455. He recommended EbGS look at minimum or maximum number of genes, type of cancer, and state of cancer when they begin to address the cancer biomarkers topic.

The group felt that the staff recommended guideline wording that included “at least 5 genes” should be removed as that number is not based on evidence. Stevens was concerned about having a guideline for these tests at all, as the guideline review would be very time consuming if the reviewer had to constantly refer to the NCCN guidelines. He recommended making these tests Diagnostic with no guideline. Panel members agreed that the tests should have no guideline. There was discussion about how often then tests should be repeated. This was felt to be dependent on the tumor behavior. There was another suggestion that this testing could be limited to once per patient.

Staff was directed to discuss this topic with a medical oncologist or an ad hoc group of medical oncologists and pathologist to inform the question of scoping the EbGS biomarker review. The advisory panel agreed with staff recommended 2023 CPT code placement, without the new guideline for cancer biomarker panels.

5) Other topics: Carl Stevens requested that Decipher Prostate RP (CPT 81542) be considered for coverage. This is now an NCCN recommended test with a strong recommendation. Dr. Stevens requested that this code be removed from GN173 and added to coverage. Staff will research this prior to the November VBBS/HERC meeting.

The meeting adjourned at 3:20 pm.
Section 2.0
New Codes
2024 CPT Code Review: Tumor Testing

Issue:
Among the 2024 CPT codes are 6 new codes for next generation sequencing (NGS) of tumor tissue. The HERC discussed NGS at their September 2023 meeting and adopted a new diagnostic guideline to apply to all NGS testing of malignant tissue.

Multiple CPT codes for “targeted genomic sequence analysis panel” (for example, CPT 81450-81456) are on the Diagnostic Procedures File. FoundationOne CDx (CPT 0037U) was added to the Diagnostic Procedures file at the September 2023 meeting.

One additional PLA (proprietary analysis code) was added in the 2024 code cycle for Quest Labs NGS lab. Dr. Carl Stevens (formerly of CareOregon) recommended coverage for this code as it is a commonly used test by local oncologists.

New codes:

<table>
<thead>
<tr>
<th>CPT code</th>
<th>Code description</th>
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<tbody>
<tr>
<td>81457</td>
<td>Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis, microsatellite instability</td>
</tr>
<tr>
<td>81458</td>
<td>Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis, copy number variants and microsatellite instability</td>
</tr>
<tr>
<td>81459</td>
<td>Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis or combined DNA and RNA analysis, copy number variants, microsatellite instability, tumor mutation burden, and rearrangements</td>
</tr>
<tr>
<td>81462</td>
<td>Solid organ neoplasm, genomic sequence analysis panel, cell-free nucleic acid (eg, plasma), interrogation for sequence variants; DNA analysis or combined DNA and RNA analysis, copy number variants and rearrangements</td>
</tr>
<tr>
<td>81463</td>
<td>Solid organ neoplasm, genomic sequence analysis panel, cell-free nucleic acid (eg, plasma), interrogation for sequence variants; DNA analysis, copy number variants, and microsatellite instability</td>
</tr>
<tr>
<td>81464</td>
<td>Solid organ neoplasm, genomic sequence analysis panel, cell-free nucleic acid (eg, plasma), interrogation for sequence variants; DNA analysis or combined DNA and RNA analysis, copy number variants, microsatellite instability, tumor mutation burden, and re</td>
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New guideline adopted September 2023, effective 1/2/2024

**DIAGNOSTIC GUIDELINE DX, NEXT GENERATION SEQUENCING OF MALIGNANCES**

Next Generation Sequencing (NGS, for example CPT 81479, 81455, 0037U) is covered when all of the following requirements are met:

1) The patient has
   a. Either recurrent, relapsed, refractory, metastatic, or advanced stage III or IV cancer; AND
   b. Has not been previously tested using the same NGS test for the same primary diagnosis of cancer, unless the criteria in 4) below are met; AND
2024 CPT Code Review: Tumor Testing

c. Decided to seek further cancer treatment (for example, therapeutic chemotherapy) and has adequate performance status (ECOG 0-2) to undergo such treatment; AND

2) The diagnostic laboratory test using NGS must have:
   a. Clinical Laboratory Improvement Amendments (CLIA)-certification; AND
   b. The test is being used as a companion diagnostic test in accordance with Food & Drug Administration (FDA)-approved therapeutic labeling; AND
   c. Results provided to the treating physician for management of the patient using a report template to specify treatment options; AND

3) A single CPT or HCPCS code is covered for each multigene panel performed on tumor tissue. Additional codes for individual genes and for molecular pathology procedures CPT 81400-81408 are excluded from coverage when the multigene panel is covered under the appropriate CPT or HCPCS code.

4) Repeat NGS testing may be required in the setting of patients who have clinically progressed per standardized professional guidelines after therapy. Coverage in this situation is limited to 3 times per primary malignancy unless there is indication for additional testing after individualized review of medical necessity.

GAP input:

HERC staff recommendations:

1) Place the following CPT codes on the Diagnostic Procedures File subject to the new NGS guideline
   a. 81547 Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis, microsatellite instability
   b. 81548 Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis, copy number variants and microsatellite instability
   c. 81549 Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis or combined DNA and RNA analysis, copy number variants, microsatellite instability, tumor mutation burden, and rearrangements
   d. 81462 Solid organ neoplasm, genomic sequence analysis panel, cell-free nucleic acid (eg, plasma), interrogation for sequence variants; DNA analysis or combined DNA and RNA analysis, copy number variants and rearrangements
   e. 81463 Solid organ neoplasm, genomic sequence analysis panel, cell-free nucleic acid (eg, plasma), interrogation for sequence variants; DNA analysis, copy number variants, and microsatellite instability
   f. 81464 Solid organ neoplasm, genomic sequence analysis panel, cell-free nucleic acid (eg, plasma), interrogation for sequence variants; DNA analysis or combined DNA and RNA analysis, copy number variants, microsatellite instability, tumor mutation burden, and rearrangements

2) Place 0379U (Solid Tumor Expanded Panel, Quest Diagnostics®) on the Diagnostic Procedures File
Section 3.0
New Discussion Items

Coverage Question: Should the non-prenatal genetic testing guideline section on diagnostic evaluation for intellectual disability, developmental delay, and Autism Spectrum Disorder be updated with additional genetic tests?

Specific questions:
1) Should PTEN testing be added to the autism spectrum disorder section of the non-prenatal genetic testing guideline?
2) Should large panel testing for fragile X be covered?
3) Should whole exome sequencing (WES) be added to the developmental delay section of the non-prenatal genetic testing guideline?

Question source: Holly Jo Hodges, CCO medical director; P&T staff

Background: Several questions have been raised regarding genetic testing for intellectual and developmental delay and autism spectrum disorder. Dr. Hodges had a question about adding PTEN testing. P&T requested clarification around covered tests for Rhett’s syndrome due to a new medication for this condition. In investigating P&T’s question, HERC staff found that large panel tests with the genes for Rett’s syndrome which include fragile X testing are not included for coverage, while other fragile X testing is covered.

Previous HSC/HERC reviews:
Coverage of X linked intellectual disability genetic panels (81470, 81471) was last discussed in 2014 as new codes. At that time, GAP recommended non-coverage. They noted that “The labs consulted on this question report a pick up rate for significant mutations is quite low, <5%.”

PTEN has never been discussed as a test for autism spectrum disorder.

Current Prioritized List/Coverage status:
Testing for Rett syndrome: CPT 81302-81304 (MECP2 [methyl CpG binding protein 2] (eg, Rett syndrome) gene analysis) are Diagnostic

CPT 81229 (Cytogenomic [genome-wide] microarray analysis) is Diagnostic

Fragile X testing: CPT 81243-81244 (FMR1 [fragile X mental syndrome] 1) are Diagnostic. CPT 81171, 81172 (AFF2 [AF4/FMR2 family, member 2 [FMR2]] (eg, fragile X intellectual disability 2 [FRAXE]) gene analysis) are diagnostic. Testing is governed by Diagnostic Guideline D1 and D17 (prenatal and non-prenatal genetic testing guidelines).

Fragile X panel testing: CPT 81470 (X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); genomic sequence analysis panel, must include sequencing of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3, and SLC16A2) and 81471 (X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); duplication/deletion gene analysis, must include analysis of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3, and SLC16A2) are on line 662/GN173

PTEN testing: CPT 81321-81323 (PTEN (phosphatase and tensin homolog)) are Diagnostic but are limited to hereditary cancer screening by diagnostic guideline D25

DHCR7 testing: CPT 81405 (Molecular pathology procedure, Level 6) is Diagnostic

NF1 testing: CPT 81409 (Molecular pathology procedure, Level 9) and CPT 81479 (Unlisted molecular pathology procedure) are both Diagnostic

Tuberous sclerosis: (TSC1, TSC2): CPT 81406, 81405 are Diagnostic

Whole exome sequencing: CPT 81415-81416 are Diagnostic with limitations in Diagnostic Guideline D1

GUIDELINE NOTE 173, INTERVENTIONS THAT ARE UNPROVEN, HAVE NO CLINICALLY IMPORTANT BENEFIT OR HAVE HARMs THAT OUTWEIGHT BENEFITS FOR CERTAIN CONDITIONS Line 662

The following Interventions are prioritized on Line 662 CONDITIONS FOR WHICH CERTAIN INTERVENTIONS ARE UNPROVEN, HAVE NO CLINICALLY IMPORTANT BENEFIT OR HAVE HARMs THAT OUTWEIGHT BENEFITS:

<table>
<thead>
<tr>
<th>Procedure Code</th>
<th>Intervention Description</th>
<th>Rationale</th>
<th>Last Review</th>
</tr>
</thead>
<tbody>
<tr>
<td>81470, 81471</td>
<td>X-linked intellectual disability (XLID) genomic sequence panels</td>
<td>Insufficient evidence of effectiveness</td>
<td>November, 2014</td>
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DIAGNOSTIC GUIDELINE D1, NON-PRENATAL GENETIC TESTING GUIDELINE [excerpt: see Appendix A for full guideline]

A) Genetic tests are covered as diagnostic, unless they are listed below in section E1 as excluded or have other restrictions listed in this guideline. To be covered, initial screening (e.g. physical exam, medical history, family history, laboratory studies, imaging studies) must indicate that the chance of genetic abnormality is > 10% and results would do at least one of the following:
   1) Change treatment,
   2) Change health monitoring,
   3) Provide prognosis, or
   4) Provide information needed for genetic counseling for patient; or patient’s parents, siblings, or children.

D) Related to diagnostic evaluation of individuals with intellectual disability (defined as a full scale or verbal IQ < 70 in an individual > age 5), developmental delay (defined as a cognitive index <70

on a standardized test appropriate for children < 5 years of age), Autism Spectrum Disorder, or multiple congenital anomalies:

1) CPT 81228, 81229 and 81349, Cytogenomic constitutional microarray analysis: Cover for diagnostic evaluation of individuals with intellectual disability/developmental delay; multiple congenital anomalies; or, Autism Spectrum Disorder accompanied by at least one of the following: dysmorphic features including macro or microcephaly, congenital anomalies, or intellectual disability/developmental delay in addition to those required to diagnose Autism Spectrum Disorder.

2) CPT 81243, 81244, 81171, 81172 Fragile X genetic testing is covered for individuals with intellectual disability/developmental delay. Although the yield of Fragile X is 3.5-10%, this is included because of additional reproductive implications.

3) A visit with the appropriate specialist (often genetics, developmental pediatrics, or child neurology), including physical exam, medical history, and family history is covered. Physical exam, medical history, and family history by the appropriate specialist, prior to any genetic testing is often the most cost-effective strategy and is encouraged.

Expert guidelines:
1) **Hyman 2020**, AAP guideline on identification, evaluation and management of children with autism spectrum disorder
   a. Recommended genetic testing
      i. Chromosomal microarray testing
      ii. Fragile X analysis
      iii. MECP2 (Rett syndrome) testing for girls
      iv. If the above do not reveal an etiology, refer to genetics and consider whole exome sequencing (WES)
   b. Other genetic testing to consider
      i. A macrocephalic boy with ASD should have PTEN testing
      ii. Syndromic appearing child: Smith-Lemi-Optiz syndrome (DHCR7)
      iii. Skin findings concerning for neurofibromatosis 1: NF1 testing
      iv. Physical exam findings concerning for tuberous sclerosis: TSC1, TSC2
2) **Spector 2021**, ACMG technical standard for fragile X testing
   a. Next generation sequencing (NGS)
      i. Testing for FMR1 repeats is included in expanded carrier testing using NGS for multiple genes. Inherent limitations of short read NGS technology include difficulties sequencing across GC-rich regions, ineffective mapping of repetitive elements, and in the case of capture-based technology, PCR amplification bias of smaller alleles compared to larger full-mutation FMR1 alleles. To combat these constraints, multiple algorithms have been designed to identify clinically relevant repeat expansions from short read sequence data. However, these attempts demonstrated poor sensitivity and specificity performance in detection of FMR1 expanded alleles. Currently, short read NGS technology cannot reliably detect expanded FMR1 alleles and should not be used to rule out or confirm any FMR1-related disorders.

Other payer policies:
1) United Health Care: covers CPT 81470 and 81471 for fragile X panel testing
2) Aetna covers FMR1 gene testing but not fragile X panel testing
3) Regence BCBS: covers CPT 81470 and 81471 for fragile X panel testing

GAP input:

HERC staff summary:
Most genetic testing for developmental or intellectual disability and autism spectrum disorder are already covered. A section should be added to the non-prenatal genetic testing guideline to include PTEN testing for macrocephalic boys with ASD. The other genetic tests in the AAP guideline currently have no guideline limitations and would be indicated for children with syndromic exam findings.

Panel testing for fragile X is specifically not recommended by ACMG due to the technology not being able to reliably detect expanded FMRI alleles. HERC staff recommends continued non-coverage of these panels.

HERC staff recommendations:
1) Update the date of last review for fragile X panel testing in GN173 as shown below
2) Modify section D of Diagnostic Guideline D1 as shown below

GUIDE NOTE 173, INTERVENTIONS THAT ARE UNPROVEN, HAVE NO CLINICALLY IMPORTANT BENEFIT OR HAVE HARMS THAT OUTWEIGH BENEFITS FOR CERTAIN CONDITIONS

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DIAGNOSTIC GUIDELINE D1, NON-PRENATAL GENETIC TESTING GUIDELINE [excerpt with suggested edits, see Appendix A for the full guideline note]
D) Related to diagnostic evaluation of individuals with intellectual disability (defined as a full scale or verbal IQ < 70 in an individual > age 5), developmental delay (defined as a cognitive index <70 on a standardized test appropriate for children < 5 years of age), Autism Spectrum Disorder, or multiple congenital anomalies:

1) CPT 81228, 81229 and 81349, Cytogenomic constitutional microarray analysis: Cover for diagnostic evaluation of individuals with intellectual disability/developmental delay; multiple congenital anomalies; or, Autism Spectrum Disorder accompanied by at least one of the following: dysmorphic features including macro or microcephaly, congenital anomalies, or intellectual disability/developmental delay in addition to those required to diagnose Autism Spectrum Disorder.

2) CPT 81243, 81244, 81171, 81172 Fragile X genetic testing is covered for individuals with intellectual disability/developmental delay. Although the yield of Fragile X is 3.5-10%, this is included because of additional reproductive implications.

3) Additional testing that might be appropriate based on physical exam findings include Rett syndrome testing (CPT 81302-81304) and PTEN testing (CPT 81321-81323). Whole exome sequencing (81415-81416) may be considered when all of the testing above is non-diagnostic and after a genetic counseling/geneticist consultation.

4) A visit with the appropriate specialist (often genetics, developmental pediatrics, or child neurology), including physical exam, medical history, and family history is covered. Physical exam, medical history, and family history by the appropriate specialist, prior to any genetic testing is often the most cost-effective strategy and is encouraged.

Appendix A

Genetic testing guidelines

DIAGNOSTIC GUIDELINE D1, NON-PRENATAL GENETIC TESTING GUIDELINE

E) Genetic tests are covered as diagnostic, unless they are listed below in section E1 as excluded or have other restrictions listed in this guideline. To be covered, initial screening (e.g. physical exam, medical history, family history, laboratory studies, imaging studies) must indicate that the chance of genetic abnormality is > 10% and results would do at least one of the following:
   1) Change treatment,
   2) Change health monitoring,
   3) Provide prognosis, or
   4) Provide information needed for genetic counseling for patient; or patient’s parents, siblings, or children

F) Pretest and posttest genetic counseling is required for presymptomatic and predisposition genetic testing. Pretest and posttest genetic evaluation (which includes genetic counseling) is covered when provided by a suitable trained health professional with expertise and experience in genetics.
   1) “Suitably trained” is defined as board certified or active candidate status from the American Board of Medical Genetics, American Board of Genetic Counseling, or Genetic Nursing Credentialing Commission.

G) A more expensive genetic test (generally one with a wider scope or more detailed testing) is not covered if a cheaper (smaller scope) test is available and has, in this clinical context, a substantially similar sensitivity. For example, do not cover CFTR gene sequencing as the first test in a person of Northern European Caucasian ancestry because the gene panels are less expensive and provide substantially similar sensitivity in that context.

H) Related to diagnostic evaluation of individuals with intellectual disability (defined as a full scale or verbal IQ < 70 in an individual > age 5), developmental delay (defined as a cognitive index <70 on a standardized test appropriate for children < 5 years of age), Autism Spectrum Disorder, or multiple congenital anomalies:
   1) CPT 81228, 81229 and 81349, Cytogenomic constitutional microarray analysis: Cover for diagnostic evaluation of individuals with intellectual disability/developmental delay; multiple congenital anomalies; or, Autism Spectrum Disorder accompanied by at least one of the following: dysmorphic features including macro or microcephaly, congenital anomalies, or intellectual disability/developmental delay in addition to those required to diagnose Autism Spectrum Disorder.
   2) CPT 81243, 81244, 81171,81172 Fragile X genetic testing is covered for individuals with intellectual disability/developmental delay. Although the yield of Fragile X is 3.5-10%, this is included because of additional reproductive implications.
   3) A visit with the appropriate specialist (often genetics, developmental pediatrics, or child neurology), including physical exam, medical history, and family history is covered. Physical exam, medical history, and family history by the appropriate specialist, prior to any genetic testing is often the most cost-effective strategy and is encouraged.

I) Related to preconception testing/carrier screening:
   1) The following tests are covered for a pregnant patient or patient contemplating pregnancy as well as the male reproductive partner:
      a) Screening for genetic carrier status with the minimum testing recommended by the American College of Obstetrics and Gynecology:

i) Screening for cystic fibrosis carrier status (CPT 81220-81224)
ii) Screening for fragile X status (CPT 81243, 81244, 81171, 81172)
iii) Screening for spinal muscular atrophy (CPT 81329)
iv) Screening for Canavan disease (CPT 81200), familial dysautonomia (CPT 81260), and Tay-Sachs carrier status (CPT 81255). Ashkenazi Jewish carrier panel testing (CPT 81412) is covered if the panel would replace and would be of similar or lower cost than individual gene testing including CF carrier testing.
v) Screening for hemoglobinopathies (CPT 83020, 83021)

b) Expanded carrier screening (CPT 81443): A genetic counseling/geneticist consultation must be offered prior to ordering test and after test results are reported. Expanded carrier testing is ONLY covered when all of the following are met:
i) the panel includes only genes with a carrier frequency of ≥ 1 in 200 or greater per ACMG Guideline (2021), AND
ii) the included genes have well-defined phenotype, AND
iii) the included genes result in conditions have a detrimental effect on quality of life OR cause cognitive or physical impairment OR require surgical or medical intervention, AND
iv) the included genes result in conditions have an onset early in life, AND
v) the included genes result in conditions that must be diagnosable prenatally to inform antenatal interventions and/or changes in delivery management and/or education of parents about special needs after birth.

J) Related to other tests with specific CPT codes:
1) Certain genetic tests have not been found to have proven clinical benefit. These tests are listed in Guideline Note 173 INTERVENTIONS THAT ARE UNPROVEN, HAVE NO CLINICALLY IMPORTANT BENEFIT OR HAVE HARMs THAT OUTWEIGH BENEFITS FOR CERTAIN CONDITIONS.
2) The following tests are covered only if they meet the criteria in section A above AND the specified situations:
a) CPT 81205, BCKDHB (branched-chain keto acid dehydrogenase E1, beta polypeptide) (eg, Maple syrup urine disease) gene analysis, common variants (eg, R183P, G278S, E422X): Cover only when the newborn screening test is abnormal and serum amino acids are normal
b) Diagnostic testing for cystic fibrosis (CF)
i) CFTR, cystic fibrosis transmembrane conductance regulator tests. CPT 81220, 81221, 81222, 81223: For infants with a positive newborn screen for cystic fibrosis or who are symptomatic for cystic fibrosis, or for clients that have previously been diagnosed with cystic fibrosis but have not had genetic testing, CFTR gene analysis of a panel containing at least the mutations recommended by the American College of Medical Genetics* (CPT 81220) is covered. If two mutations are not identified, CFTR full gene sequencing (CPT 81223) is covered. If two mutations are still not identified, duplication/deletion testing (CPT 81222) is covered. These tests may be ordered as reflex testing on the same specimen.
c) CPT 81224, CFTR (cystic fibrosis transmembrane conductance regulator) (eg. cystic fibrosis) gene analysis; introm 8 poly-T analysis (eg. male infertility): Covered only after genetic counseling.

d) CPT 81225-81227, 81230-81231, 81418 (cytochrome P450). Covered only for determining eligibility for medication therapy if required or recommended in the FDA labeling for that medication. These tests have unproven clinical utility for decisions regarding medications when not required in the FDA labeling (e.g. psychiatric, anticoagulant, opioids).

e) CPT 81240, F2 (prothrombin, coagulation factor II) (eg, hereditary hypercoagulability) gene analysis, 20210G>A variant: Factor 2 20210G>A testing should not be covered for adults with idiopathic venous thromboembolism; for asymptomatic family members of patients with venous thromboembolism and a Factor V Leiden or Prothrombin 20210G>A mutation; or for determining the etiology of recurrent fetal loss or placental abruption.

f) CPT 81241, F5 (coagulation Factor V) (eg, hereditary hypercoagulability) gene analysis, Leiden variant: Factor V Leiden testing should not be covered for: adults with idiopathic venous thromboembolism; for asymptomatic family members of patients with venous thromboembolism and a Factor V Leiden or Prothrombin 20210G>A mutation; or for determining the etiology of recurrent fetal loss or placental abruption.

g) CPT 81247, G6PD (glucose-6-phosphate dehydrogenase) (eg, hemolytic anemia, jaundice), gene analysis; common variant(s) (eg, A, A-) should only be covered:

i) After G6PD enzyme activity testing is done and found to be normal; AND either
   (a) There is an urgent clinical reason to know if a deficiency is present, e.g. in a case of acute hemolysis; OR
   (b) In situations where the enzyme activity could be unreliable, e.g. female carrier with extreme Lyonization.

h) CPT 81248, G6PD (glucose-6-phosphate dehydrogenase) (eg, hemolytic anemia, jaundice), gene analysis; known familial variant(s) is only covered when the information is required for genetic counseling.

i) CPT 81249, G6PD (glucose-6-phosphate dehydrogenase) (eg, hemolytic anemia, jaundice), gene analysis; full gene sequence is only covered:

   i) after G6PD enzyme activity has been tested, and
   ii) the requirements under CPT 81247 above have been met, and
   iii) common variants (CPT 81247) have been tested for and not found.

j) CPT 81256, HFE (hemochromatosis) (eg, hereditary hemochromatosis) gene analysis, common variants (eg, C282Y, H63D): Covered for diagnostic testing of patients with elevated transferrin saturation or ferritin levels. Covered for predictive testing ONLY when a first degree family member has treatable iron overload from HFE.

k) CPT 81332, SERPINA1 (serpin peptidase inhibitor, clade A, alpha-1 antitrypsin, antitrypsin, member 1) (eg, alpha-1-antitrypsin deficiency), gene analysis, common variants (eg, *S and *Z): The alpha-1-antitrypsin protein level should be the first line test for a suspected diagnosis of AAT deficiency in symptomatic individuals with unexplained liver disease or obstructive lung disease that is not asthma or in a middle age individual with unexplained dyspnea. Genetic testing of the anpha-1 phenotype test is appropriate if the protein test is abnormal or borderline. The genetic test is appropriate for siblings of people with AAT deficiency regardless of the AAT protein test results.

l) CPT 81415-81416, exome testing: A genetic counseling/geneticist consultation is required prior to ordering test

m) CPT 81430-81431, Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); genomic sequence analysis panel: Testing for mutations in GJB2 and GJB6 need to be done first and be negative in non-syndromic patients prior to panel testing.

n) CPT 81440, 81460, 81465, mitochondrial genome testing: A genetic counseling/geneticist or metabolic consultation is required prior to ordering test.

o) CPT 81425-81427, whole genome sequencing: testing is only covered when
i) The testing is for a critically ill infant up to one year of age admitted to an inpatient intensive care unit (NICU/PICU) with a complex illness of unknown etiology; AND
ii) Whole genome sequencing is recommended by a medical geneticist or other physician sub-specialist, including but not limited to a neonatologist or pediatric intensivist with expertise in the conditions and/or genetic disorder for which testing is being considered.


DIAGNOSTIC GUIDELINE D17, PRENATAL GENETIC TESTING

The following types of prenatal genetic testing and genetic counseling are covered for pregnant women:

A) Genetic counseling (CPT 96040, HPCPS S0265) for high-risk women who have family history of inheritable disorder or carrier state, ultrasound abnormality, previous pregnancy with aneuploidy, or elevated risk of neural tube defect.

B) Genetic counseling (CPT 96040, HPCPS S0265) prior to consideration of chorionic villus sampling (CVS), amniocentesis, microarray testing, Fragile X, and spinal muscular atrophy screening

C) Validated questionnaire to assess genetic risk in all pregnant women

D) Screening for aneuploidy with any of six screening strategies [first trimester (nuchal translucency, beta-HCG and PAPP-A), integrated, serum integrated, stepwise sequential, contingency, and cell free fetal DNA testing] (CPT 76813, 76814, 81508, 81510, 81511, 81420, 81507, 81512, 82105, 82677, 84163)

E) Ultrasound for structural anomalies between 18 and 20 weeks gestation (CPT 76811, 76812)

F) CVS or amniocentesis (CPT 59000, 59015, 76945, 76946, 82106, 8235, 88261-88264, 88267, 88269, 88280, 88283, 88285, 88289, 88291) for a positive aneuploidy screen, maternal age >34, fetal structural anomalies, family history of inheritable chromosomal disorder or elevated risk of neural tube defect

G) Array CGH (CPT 81228, 81229, 81349) when major fetal congenital anomalies are apparent on imaging, or with normal imaging when array CGH would replace karyotyping performed with CVS or amniocentesis in (G) above

H) FISH testing (CPT 88271, 88272, 88274, 88275, 81171, 81172) only if karyotyping is not possible due a need for rapid turnaround for reasons of reproductive decision-making (i.e. at 22w4d gestation or beyond)

I) Screening for genetic carrier status with the minimum testing recommended by the American College of Obstetrics and Gynecology:
   1) Screening for cystic fibrosis carrier status (CPT 81220-81224)
   2) Screening for fragile X status (CPT 81243, 81244, 81171, 81172)

3) Screening for spinal muscular atrophy (CPT 81329)
4) Screening for Canavan disease (CPT 81200), familial dysautonomia (CPT 81260), and Tay-Sachs carrier status (CPT 81255). Ashkenazi Jewish carrier panel testing (CPT 81412) is covered if the panel would replace and would be of similar or lower cost than individual gene testing including CF carrier testing.
5) Screening for hemoglobinopathies (CPT 83020, 83021)

J) Expanded carrier screening (CPT 81443): A genetic counseling/geneticist consultation must be offered prior to ordering test and after results are reported. Expanded carrier testing is ONLY covered when all of the following are met:
1) the panel includes only genes with a carrier frequency of ≥ 1 in 200 or greater per ACMG Guideline (2021), AND
2) the included genes have well-defined phenotype, AND
3) the included genes result in conditions have a detrimental effect on quality of life OR cause cognitive or physical impairment OR require surgical or medical intervention, AND
4) the included genes result in conditions have an onset early in life, AND
5) the included genes result in conditions that must be diagnosable prenatally to inform antenatal interventions and/or changes in delivery management and/or education of parents about special needs after birth.

The following genetic screening tests are not covered:
A) Serum triple screen

The development of this guideline note was informed by a HERC coverage guidance. See https://www.oregon.gov/oha/HPA/DSI-HERC/Pages/Evidence-based-Reports.aspx

DIAGNOSTIC GUIDELINE D25, HEREDITARY CANCER GENETIC TESTING
Related to genetic testing for patients with breast/ovarian and colon/endometrial cancer or other related cancers suspected to be hereditary, or patients at increased risk to due to family history, services are provided according to the Comprehensive Cancer Network Guidelines.

A) Lynch syndrome (hereditary colorectal, endometrial and other cancers associated with Lynch syndrome) services (CPT 81288, 81292-81300, 81317-81319, 81435, 81436) and familial adenomatous polyposis (FAP) services (CPT 81201-81203) should be provided as defined by the NCCN Clinical Practice Guidelines in Oncology (Genetic/Familial High-Risk Assessment: Colorectal V1.2022 (6/8/22) www.nccn.org).

B) Breast and ovarian cancer syndrome genetic testing services (CPT 81162-81167, 81212, 81215-81217) for patients without a personal history of breast, ovarian and other associated cancers should be provided to high-risk patients as defined by the US Preventive Services Task Force or according to the NCCN Clinical Practice Guidelines in Oncology (Genetic/Familial High-Risk Assessment: Breast, Ovarian and Pancreatic V1.2023 (9/7/22) www.nccn.org).

C) Breast and ovarian cancer syndrome genetic testing services (CPT 81162-81167, 81212, 81215-81217) for women with a personal history of breast, ovarian, or other associated cancers and for men with breast or other associated cancers should be provided according to the NCCN Clinical Practice Guidelines in Oncology (Genetic/Familial High-Risk Assessment: Breast, Ovarian and Pancreatic V1.2023 (9/7/22) www.nccn.org).

D) PTEN (Cowden syndrome) services (CPT 81321-81323) should be provided as defined by the NCCN Clinical Practice Guidelines in Oncology (Genetic/Familial High-Risk Assessment: Ovarian and Pancreatic. V1.2023 (9/7/22) or Genetic/Familial High-Risk Assessment: Colorectal V1.2022 (6/8/22) www.nccn.org).

Genetic counseling should precede genetic testing for hereditary cancer whenever possible.

A) Pre and post-test genetic counseling should be covered when provided by a suitable trained health professional with expertise and experience in cancer genetics. Genetic counseling is recommended for cancer survivors when test results would affect cancer screening.
   1) “Suitably trained” is defined as board certified or active candidate status from the American Board of Medical Genetics, American Board of Genetic Counseling, or Genetic Nursing Credentialing Commission.

B) If timely pre-test genetic counseling is not possible for time-sensitive cases, appropriate genetic testing accompanied by pre- and post- test informed consent and post-test disclosure performed by a board-certified physician with experience in cancer genetics should be covered.
   1) Post-test genetic counseling should be performed as soon as is practical.

If the mutation in the family is known, only the test for that mutation is covered. For example, if a mutation for BRCA 1 has been identified in a family, a single site mutation analysis for that mutation is covered (CPT 81215), while a full sequence BRCA 1 and 2 (CPT 81163) analyses is not. There is one exception, for individuals of Ashkenazi Jewish ancestry with a known mutation in the family, the panel for Ashkenazi Jewish BRCA mutations is covered (CPT 81212).

Costs for rush genetic testing for hereditary breast/ovarian and colon/endometrial cancer is not covered.

Hereditary breast cancer-related disorders genomic sequence analysis panels (CPT 81432, 81433, 81479) are only included for patients meeting the criteria for hereditary cancer syndrome testing per NCCN guidelines.
Identification, Evaluation, and Management of Children With Autism Spectrum Disorder

Susan L. Hyman, MD, FAAP, Susan E. Levy, MD, MPH, FAAP, Scott M. Myers, MD, FAAP, COUNCIL ON CHILDREN WITH DISABILITIES, SECTION ON DEVELOPMENTAL AND BEHAVIORAL PEDIATRICS

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder with reported prevalence in the United States of 1 in 59 children (approximately 1.7%). Core deficits are identified in 2 domains: social communication/interaction and restrictive, repetitive patterns of behavior. Children and youth with ASD have service needs in behavioral, educational, health, leisure, family support, and other areas. Standardized screening for ASD at 18 and 24 months of age with ongoing developmental surveillance continues to be recommended in primary care (although it may be performed in other settings), because ASD is common, can be diagnosed as young as 18 months of age, and has evidenced-based interventions that may improve function. More accurate and culturally sensitive screening approaches are needed. Primary care providers should be familiar with the diagnostic criteria for ASD, appropriate etiologic evaluation, and co-occurring medical and behavioral conditions (such as disorders of sleep and feeding, gastrointestinal tract symptoms, obesity, seizures, attention-deficit/hyperactivity disorder, anxiety, and wandering) that affect the child’s function and quality of life. There is an increasing evidence base to support behavioral and other interventions to address specific skills and symptoms. Shared decision making calls for collaboration with families in evaluation and choice of interventions. This single clinical report updates the 2007 American Academy of Pediatrics clinical reports on the evaluation and treatment of ASD in one publication with an online table of contents and section view available through the American Academy of Pediatrics Gateway to help the reader identify topic areas within the report.

INTRODUCTION

Autism spectrum disorder (ASD) is a category of neurodevelopmental disorders characterized by social and communication impairment and

abstract

at Golisano Children's Hospital, University of Rochester, Rochester, New York; Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; and Geisinger Autism & Developmental Medicine Institute, Danville, Pennsylvania

Clinical reports from the American Academy of Pediatrics benefit from expertise and resources of liaisons and internal (AAP) and external reviewers. However, clinical reports from the American Academy of Pediatrics may not reflect the views of the liaisons or the organizations or government agencies that they represent.

Drs Hyman, Levy, and Myers all participated in development of the outline of material to be covered, generation of content, and editing of the document; and all authors approved the final manuscript as submitted.

The guidance in this report does not indicate an exclusive course of treatment or serve as a standard of medical care. Variations, taking into account individual circumstances, may be appropriate.

All clinical reports from the American Academy of Pediatrics automatically expire 5 years after publication unless reaffirmed, revised, or retired at or before that time.

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restricted or repetitive behaviors. ASD affects more than 5 million Americans, with an estimated prevalence of approximately 1.7% in children. The care needs of children with ASD are significant, affecting parents and siblings as well, and requiring substantial community resources. Direct and indirect costs of caring for children and adults with ASD in the United States in 2015 were estimated to be $268 billion, more than the cost of stroke and hypertension combined. The lifetime cost of education, health, and other service needs for an individual with ASD ranges from $1.4 to $2.4 million, depending on whether he or she has any co-occurring intellectual disabilities. To deliver timely and effective medical, behavioral, educational, and social services across the lifespan, primary care providers must understand the needs of individuals with ASD and their families. ASD is more commonly diagnosed now than in the past, and the significant health, educational, and social needs of individuals with ASD and their families constitute an area of critical need for resources, research, and professional education.

In the 12 years since the American Academy of Pediatrics (AAP) published the clinical report “Identification and Evaluation of Children With Autism Spectrum Disorders” and its companion, “Management of Children With Autism Spectrum Disorders,” reported prevalence rates of ASD in children have increased, understanding of potential risk factors has expanded, awareness of co-occurring medical conditions and genetic contribution to etiology has improved, and the body of research supporting evidence-based interventions has grown substantially. This updated clinical report builds on previous reports and guidance for care of children and youth with ASD. It also reflects changes in diagnostic criteria after publication of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) in 2013. The DSM-5 established a single category of autistic disorder, Asperger syndrome, and pervasive developmental disorder not otherwise specified in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR). With the current reported prevalence rate of 1:59 children (approximately 1.7%), all primary care providers can expect to have children and youth with ASD in their practices. As noted in earlier clinical reports, the primary care provider has critical access to the child in the context of the medical home to identify symptoms of ASD early in childhood, support the family through the process of diagnosis and intervention, address etiologic evaluations, help the family understand how to interpret the evidence supporting different interventions so they can effectively engage in shared decision-making, and manage co-occurring medical conditions that may influence outcome and affect daily function. The primary care provider can help minimize disparities in age of diagnosis of African American and Hispanic children and be alert to the potential for gender bias in symptom recognition. This updated document aims to provide primary care providers with a summary of current information in a single report that will help guide them in providing a medical home for the patient with ASD.

SECTION 1: PREVALENCE

Incidence is the onset of new diagnoses over time in a selected cohort. Without consistent longitudinal data in a specified cohort, incidence cannot be determined. Because of the heterogeneity of symptoms and severity in ASD, it may be diagnosed in children at different ages. What is reported is age at recognition of symptoms, not the actual onset. As a result, prevalence is more typically reported than incidence, reflecting rates of ASD in the population at a point in time.

The reported prevalence of children with ASD has increased over time, and primary care providers are often asked about the reasons for this increase. This increase may be attributable to several factors, including broadening in the diagnostic criteria with ongoing revisions of the Diagnostic and Statistical Manual of Mental Disorders (DSM), the more inclusive definition of pervasive developmental disorder with the adoption of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) in 1994, increased public awareness of the disorder and its symptoms, recommendations for universal screening for ASD, and increased availability of early intervention and school-based services for children with ASD. In part, the increasing numbers of children with a diagnosis of ASD may reflect diagnostic substitution, the recognition of ASD in children previously primarily diagnosed with intellectual disability or a co-occurring genetic syndrome. A true increase in the prevalence of ASD associated with other biological risk factors is also possible.

Prevalence rates in US populations are similar to those of other industrialized countries, and lower rates are reported in resource-limited countries, where epidemiological data are more difficult to collect. Data on national samples suggest that the prevalence of ASD is stabilizing. Ongoing epidemiological studies help to understand changes in the reported prevalence over time. Epidemiological data help to predict the need for services and identify potential risk factors. Surveillance methods include regional, state, and/or national registry systems;
records- or services-based analyses; surveys; and other methods, including population-based case findings.

In 2000, the US Centers for Disease Control and Prevention (CDC) established the Autism and Developmental Disabilities Monitoring (ADDM) Network as a population-based public health surveillance system to estimate the prevalence of ASD in children 8 years of age. ADDM reports published in 2014 and 2016 revealed comparable prevalence rates (approximately 1 in 60), but the report published in 2018 revealed a slightly increased rate (1 in 59). Additional data over time will help determine if rates have stabilized. The data also revealed some variation in prevalence rates across the participating states, with the highest rates in the locations where both educational and health records were available for chart abstraction and standardized application of diagnostic criteria. Regional variation in prevalence may also reflect availability of services, local provider practices for ASD screening, educational policies, school and/or community resources, and insurance mandates, among other factors. The CDC also published data on the prevalence of ASD in children who were 4 years of age in 2010. A lower prevalence rate for diagnosis (1.34%) was reported in these children (approximately 30% less than that of children 8 years of age). The lower identified prevalence and higher proportional rate of children 4 years of age with ASD and intellectual disabilities may be attributable, in part, to later diagnosis of children with ASD and average-range cognitive abilities. The National Survey of Children’s Health (2011–2012) and the National Survey of Children with Special Health Care Needs (2009–2010) were analyzed for the age the parents reported diagnosis as well as for parent-reported subjective severity. The minority of children were identified as having ASD before 3 years of age. Diagnosis later than 6 years of age was reported in one-third to half of children. Later age at diagnosis was associated with reported mild presentation.

CDC surveillance data published in 2014 revealed that white, non-Hispanic children were approximately 20% more likely to be identified with ASD before the case review than were non-Hispanic African American children and were about 50% more likely to be identified with ASD than were Hispanic children. Recent prevalence data reveal increasing rates of ASD in Hispanic and African American children. This may reflect more widespread awareness of the symptoms among parents, schools, and health care providers and improved rates of screening in health supervision care. Studies examining the effects of race and ethnicity on age at diagnosis are conflicting, but earlier diagnosis of ASD is associated with higher socioeconomic status and access to services. African American and Hispanic children diagnosed with ASD by age 4 years were more likely to have coexisting intellectual disability than were white, non-Hispanic children, suggesting that some African American and Hispanic children with ASD and average to above-average intelligence may not have been identified.

SECTION 2: CLINICAL SYMPTOMS

Despite advances in understanding the neurobiology and genetics of ASD, the diagnosis of ASD continues to be based on identifying and reporting behaviorally defined clinical symptoms. The challenges in determining accurate prevalence rates, in part, relate to the need for consistency in clinical diagnosis of a very heterogeneous disorder. In 2013, the DSM-5 consolidated the diagnosis of ASD into a single category and emphasized the importance of identifying coexisting developmental and behavioral disorders and symptoms. In the years since the 2007 AAP clinical reports on ASD, both professional education and public awareness have promoted recognition of symptoms that might lead to early identification of ASD, use of standardized screening approaches, and management of associated medical and behavioral features of ASD from infancy through adolescence.

Core Symptoms

Although symptoms of ASD are neurologically based, they manifest as behavioral characteristics that present differently depending on age, language level, and cognitive abilities. Core symptoms cluster in 2 domains (social communication/interaction and restricted, repetitive patterns of behavior), as described in the DSM-5. Atypical development in several functional areas contribute to symptoms of ASD. Abnormalities in understanding the intent of others, diminished interactive eye contact, and atypical use and understanding of gesture presage atypical development of social communication and pretend play as well as interest in other children. Symptoms of ASD are further shaped by deficits in imitation and of processing information across sensory modalities, such as vision (gesture) and hearing (language). Repetitive behaviors and perseveration may be primary compulsions but may also be related to atypical processing of sensory information or may reflect a desire to instill predictability when an individual does not understand the intent of others. The CDC “Learn the Signs. Act Early” Web site provides free resources to help families recognize developmental concerns, including autism (https://www.cdc.gov/ncbddd/actearly/), and Autism Navigator (www.autismnavigator.com) has a video glossary of early symptoms in toddlers.
Approximately one-quarter of children with ASD will be reported to have a regression in language or social skills, most typically between 18 and 24 months of age. The reason for this loss of previously acquired milestones is not yet known. Although medical evaluation of loss of milestones is indicated, a history of regression are not yet well understood. Current theories include synaptic “over pruning" in response to genetic factors.

**Diagnostic Criteria: DSM-5**

The DSM has been central in establishing criteria for diagnosing mental and behavioral disorders. The diagnosis of infantile autism was introduced in the *Diagnostic and Statistical Manual of Mental Disorders, Third Edition* nearly 30 years after the first edition of the DSM was published in 1952. The initial descriptions were narrow and referred to individuals with profound impairment. Publication of the DSM-IV in 1994 expanded the diagnosis to a spectrum of symptoms called pervasive developmental disorders (PDDs), which included the diagnoses of autistic disorder, Asperger disorder, pervasive developmental disorder not otherwise specified (PDD-NOS), childhood disintegrative disorder, and Rett disorder. The PDDs included individuals with lower- and higher-functioning cognitive skills. PDD-NOS was a diagnostic category requiring some, but not all, of the core symptoms necessary for other diagnoses in this category. Subsequent research has demonstrated that the subgroupings within PDD were not reproducible across research sites by using the same diagnostic data and were not stable over time. The overlap between DSM-IV-defined subgroups paired with inconsistency in their application across research sites supports the decision to consolidate the subgroups into 1 diagnostic category, ASD, in the DSM-5. The DSM-IV divided the symptoms of ASD into 3 areas: qualitative impairment of social reciprocity, qualitative impairment of communication, and restricted and repetitive behaviors. In the DSM-5, core symptoms were divided into 2 domains (social communication and social interaction and restrictive, repetitive patterns of behaviors). To fulfill diagnostic criteria for ASD by using the DSM-5, all 3 symptoms of social affective difference need to be present in addition to 2 of 4 symptoms related to restrictive and repetitive behaviors. Examples in Table 1 are illustrative but not exhaustive. The recognition of symptoms of ASD related to sensory processing led to the inclusion of sensory symptoms, such as hyper- or hyporeactivity to sensory input or unusual interests in sensory aspects of the environment. Examples include apparent indifference to pain or temperature; sensitivity to sound, taste, or textures; and intense visual interest in objects or movement. The DSM-5 notes that a diagnosis may be made at older ages, when the demands of the social or school environment may result in functional impairment.

Almost all individuals with a diagnosis of autistic disorder or Asperger syndrome by using DSM-IV criteria would be diagnosed with ASD by using DSM-5 criteria. To determine if the same patients would be identified by the DSM-IV and DSM-5 criteria, the CDC ADDM Network looked at its chart abstraction data on 8-year-old children. This analysis revealed that more than 80% of children diagnosed with PDD-NOS would also be diagnosed with ASD. It is possible that the narrative in the charts that were abstracted was influenced by knowledge of the DSM-IV criteria. There is a high level of agreement of surveillance data by using DSM-IV-TR and DSM-5 criteria.

The DSM-5 criteria have been shown to appropriately identify younger children and those with mild symptoms. These children with milder cognitive and adaptive symptoms may be the ones most likely to have significant change with early intervention services.

The DSM-5 also introduced an approach to severity rating, which is summarized in Table 2. Severity rating reflects the impairment of the ASD symptoms and the resultant service needs of the individual. Severity rating is not a quantifiable score that can be used to monitor progress at this time; in clinical use, it often reflects the impact of cognitive limitations. Measures have been published that attempt to capture severity of core symptoms and allow for measurement of improvement with intervention. To date, no single measure adequately reflects the combination of medical, behavioral, and educational severity in a fashion that will help clinicians and families determine progress with intervention across multiple functional domains. Coexisting medical disorders also affect the perception of severity and the prognosis for children with a diagnosis of ASD. The DSM-5 includes course specifiers that help describe the variation in symptoms of individuals with ASD. Course specifiers include the presence or absence of intellectual impairment, language impairment, catatonia, medical conditions, and known genetic or environmental etiologic factors. Patients with Rett syndrome are no longer automatically considered to have a diagnosis of ASD according the DSM-5, although individuals with this neurogenetic disorder may also meet diagnostic criteria for ASD. Specific genetic causes of ASD should be recorded as specifiers for individuals with ASD when identified. The DSM-5 promotes
TABLE 1 DSM-5 Criteria for Autism Spectrum Disorder

<table>
<thead>
<tr>
<th>Domains</th>
<th>Criteria: Deficits</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history; must have all 3 symptoms in this domain</td>
<td>1. Social-emotional reciprocity</td>
<td>Abnormal social approach and failure of normal back-and-forth conversation; reduced sharing of interests, emotions, or affect; failure to initiate or respond to social interactions</td>
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<tr>
<td></td>
<td>2. Nonverbal communicative behaviors used for social interaction</td>
<td>Poorly integrated verbal and nonverbal communication; abnormalities in eye contact and body language or deficits in understanding and use of gestures; total lack of facial expressions and nonverbal communication</td>
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<tr>
<td></td>
<td>3. Developing, maintaining, and understanding relationships</td>
<td>Difficulties adjusting behavior to suit various social contexts; difficulties in sharing imaginative play or in making friends; absence of interest in peers</td>
</tr>
<tr>
<td>B. Restricted, repetitive patterns of behavior, interests, or activities, as manifested by at least 2 of the following, currently or by history; must have 2 of the 4 symptoms</td>
<td>1. Stereotyped or repetitive motor movements, use of objects, or speech</td>
<td>Simple motor stereotypes, lining up toys or flipping objects, echolalia, idiosyncratic phrases</td>
</tr>
<tr>
<td></td>
<td>2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns or verbal nonbehavioral behavior</td>
<td>Extreme distress at small changes, difficulties with transitions, rigid thinking patterns, greeting rituals, need to take same route or eat food every day</td>
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<tr>
<td></td>
<td>3. Highly restricted, fixated interests that are abnormal in intensity or focus</td>
<td>Strong attachment to or preoccupation with unusual objects, excessively circumscribed or perseverative interest</td>
</tr>
<tr>
<td></td>
<td>4. Hyper- or hyporeactivity to sensory input or unusual interests in sensory aspects of the environment</td>
<td>Apparent indifference to pain/temperature, adverse response to specific sounds or textures, excessive smelling or touching of objects, visual fascination with lights or movement</td>
</tr>
</tbody>
</table>

Symptoms must be present in the early developmental period (but may not become fully manifest until social demands exceed limited capacities or may be masked by learned strategies in later life). Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning. These disturbances are not better explained by intellectual disability (intellectual developmental disorder) or global developmental delay. Intellectual disability and ASD frequently co-occur; to make comorbid diagnoses of ASD and intellectual disability, social communication should be below that expected for the general developmental level. Specify whether: with or without accompanying intellectual impairment, language impairment or associated with a known medical or genetic condition or environmental factor. Add code 293.89 if catatonia is also present. Reprinted with permission from the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (copyright 2013). American Psychiatric Association. All Rights Reserved.

Social pragmatic communication disorder is a new diagnosis described within the DSM-5 that describes individuals who exhibit functionally impairing symptoms in social language use but do not have habitual or repetitive behaviors. Individuals who are affected must have deficits in using language for social purposes, impaired ability to match their communication style with the context for communication, difficulty following the conventional rules for conversation, and difficulty with idioms and understated meanings in language (Table 3). As with ASD, the symptoms cannot be better explained by another DSM-5 diagnosis. Research and experience with DSM-5 diagnoses over time will give clinicians a better sense of how ASD and social communication disorder are similar and different in terms of etiology, prognosis, and treatment. Evaluation of pragmatic (social) language use by a speech-language pathologist provides additional information to consider this diagnosis. The characteristics of social pragmatic communication disorder and how best to address symptoms require additional study.

Although the DSM-5 provides the criteria and definitions to accurately assign mental health and behavioral diagnoses, the International Classification of Diseases, 10th Revision, Clinical Modification is the standardized code set used for payment as well as for statistical tracking through electronic medical records. The International Classification of Diseases, 10th Revision, Clinical Modification continues to include the subtypes of diagnoses as defined by the DSM-IV.

The DSM-5 provides the clinician with criteria and definitions for diagnosis of ASD and should guide the clinician in the diagnosis and management of ASD.

Co-occurring Symptoms and Conditions

Co-occurring conditions are common in children with ASD and may have great effects on child and family functioning and clinical management (see also Section 5: Interventions). Examples include medical conditions such as sleep disorders and seizures; other developmental or behavioral diagnoses, such as attention-deficit/hyperactivity disorder (ADHD), anxiety, and mood disorders; and behavioral disorders, such as food refusal, self-injury, and aggression. Approximately 30% of children with...
a diagnosis of ASD will also have intellectual disability, and 30% are minimally verbal. Increasingly, researchers and clinicians recognize how co-occurring disorders help identify phenotypic differences within populations affected by ASD, which can influence prognosis and choice of interventions.

**Prognosis**

The prognosis and trajectory of development for a young child diagnosed with ASD typically cannot be predicted at the time of diagnosis. However, most children (≥80%) who are diagnosed with ASD after a comprehensive evaluation at less than 3 years have retained their diagnosis. It may be more difficult to recognize mild symptoms of ASD in children younger than 3 years of age, especially if they have average or above-average cognitive abilities. Across early childhood development, communication skills and social affective symptoms may improve, whereas repetitive behaviors may change, possibly reflecting maturation and/or intervention. In general, young children with ASD with language impairment appear to have more social difficulty than do children with ASD without language impairment. Children with ASD and intellectual disability have the most difficulty developing social competence. The prognosis for children with ASD in phenotypic and demographic subgroups (eg, girls, racial and ethnic subgroups, children with macrocephaly) needs additional study.

Approximately 9% of children who are diagnosed with ASD in early childhood may not meet diagnostic criteria for ASD by young adulthood. Youth who no longer meet criteria for ASD are more likely to have a history of higher cognitive skills at 2 years of age, to have participated in earlier intervention services, and to have demonstrated a decrease in their repetitive behaviors over time. A change in clinical diagnosis (eg, to ADHD or obsessive-compulsive disorder [OCD]) is more likely in children who were diagnosed with ASD before 30 months of age or had a diagnosis of PDD-NOS per the DSM-IV. Severity scores are most likely to improve in youth who have had the greatest increase in tested verbal IQ. Executive function difficulties

### TABLE 2 ASD Symptoms by Level of Severity

<table>
<thead>
<tr>
<th>Severity Level</th>
<th>Social Affective</th>
<th>Restricted and Repetitive Behaviors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1. “Requiring support”</td>
<td>Without supports in place, deficits in social communication cause noticeable impairments. Difficulty initiating social interactions, and clear examples of atypical or unsuccessful response to social overtures of others. May appear to have decreased interest in social interactions.</td>
<td>Inflexibility of behavior causes significant interference with functioning in one or more contexts. Difficulty switching between activities. Problems of organization and planning hamper independence.</td>
</tr>
<tr>
<td>Level 2. “Requiring substantial support”</td>
<td>Marked deficits in verbal and nonverbal social communication skills. Social impairments apparent even with supports in place. Limited initiation of social interactions and reduced or abnormal responses to social overtures from others.</td>
<td>Inflexibility of behavior, difficulty coping with change, or other restricted and repetitive behaviors appear frequently enough to be obvious to the casual observer and interfere with functioning in a variety of contexts. Distress and/or difficulty changing focus or action.</td>
</tr>
<tr>
<td>Level 3. “Requiring very substantial support”</td>
<td>Severe deficits in verbal and nonverbal social communication skills cause severe impairments in functioning, very limited initiation of social interactions, and minimal response to social overtures from others.</td>
<td>Inflexibility of behavior, extreme difficulty coping with change, or other restricted and repetitive behaviors markedly interfere with functioning in all spheres. Great distress at or difficulty with changing focus or action.</td>
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</tbody>
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### TABLE 3 DSM-5 Social (Pragmatic) Communication Disorder (DSM-5 315.39)

A. Persistent difficulties in the social use of verbal and nonverbal communication as manifested by all of the following:

1. Deficits in using communication for social purposes, such as greeting and sharing information, in a manner that is appropriate for the social context.
2. Impairment of the ability to change communication to match context or the needs of the listener, such as speaking differently in a classroom than on the playground, talking differently to a child than to an adult, and avoiding use of overly formal language.
3. Difficulties following rules for conversation and storytelling, such as taking turns in conversation, rephrasing when misunderstood, and knowing how to use verbal and nonverbal signals to regulate interaction.
4. Difficulties understanding what is not explicitly stated (eg, making inferences) and nonliteral or ambiguous meanings of language (eg, idioms, humor, metaphors, multiple meanings that depend on the context for interpretation).

B. The deficits result in functional limitations in effective communication, social participation, social relationships, academic achievement, or occupational performance, individually or in combination.

C. The onset of the symptoms is in the early developmental period (but deficits may not become fully manifest until social communication demands exceed limited capacities).

D. The symptoms are not attributable to another medical or neurologic condition or to low abilities in the domains of word structure and grammar and are not better explained by ASD, intellectual disability (intellectual developmental disorder), global developmental delay, or another mental disorder.

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are associated with poorer adaptive outcomes, independent of IQ. Measured intelligence (eg, IQ) and language ability in childhood tend to predict outcome in adulthood. However, reported quality of life in high-functioning adults with ASD was associated more with the presence of family and community supports than their symptoms related to ASD.

### Section 3: Screening and Diagnosis

The AAP recommends screening all children for symptoms of ASD through a combination of developmental surveillance at all visits and standardized autism-specific screening tests at 18 and 24 months of age in their primary care visits because children with ASD can be identified as toddlers, and early intervention can and does influence outcomes. This autism-specific screening complements the recommended general developmental screening at 9, 18, and 30 months of age. Efficient screening of all children would be aided by inclusion of valid screening tools in the electronic health record with appropriate compensation for the staff and professional time necessary to complete the administration, scoring, and counseling related to screening.

Screening tools are designed to help caregivers identify and report symptoms observed in children at high risk for ASD. The screens are based on early manifestations of symptoms of core deficits related to social communication. Some of these early symptoms that may alert the provider to the risk for ASD have been called “red flags” (Table 4).

Developmental surveillance for ASD includes asking caregivers about concerns they have about their child’s development or behavior, informal observation, and monitoring of symptoms in the context of routine health supervision. The “Learn the Signs. Act Early” parent resources developed by the CDC may help educate families about developmental and behavioral milestones (https://www.cdc.gov/ncbddd/actearly/index.html). Developmental surveillance alone is not sufficient to identify children who need further evaluation because children with ASD may not demonstrate characteristic symptoms in brief office visits, and caregivers may not volunteer social and emotional concerns unless specifically asked. Use of a standardized screening tool for ASD can help families identify potential symptoms. In a large study evaluating universal screening with the Modified Checklist for Autism in Toddlers (M-CHAT), researchers asked physicians to note whether they were concerned about ASD. Sensitivity of physician clinical concern was low (0.244; 30 of 123 cases; 95% confidence interval 0.17–0.32). The sensitivity of the M-CHAT when used as directed in this low-risk population was 0.91. Accurate early identification has been a goal of the AAP since the publication of the 2 previous autism clinical reports in 2007, with focused continuing medical education and a tool kit (AAP Autism Toolkit: https://toolkits.solutions.aap.org/toolkits.aspx). The goal of universal screening, including screening for ASD, has been supported by public health agencies and family support organizations. Rates of screening for both developmental delays and ASD in primary pediatric care have increased steadily. In the 2015 AAP survey of screening practices, almost three-quarters of pediatricians who responded reported routine ASD screening. Pediatricians increasingly report including office staff for efficient workflow, including administration and scoring of screening tests. Although time and remuneration remain as concerns, fewer pediatricians rate these as barriers. Referral for and tracking of evaluation and services remain a challenge associated with lack of office-based systems for making referrals and after screen-positive outcomes.

The authors of the 2019 AAP developmental surveillance and screening clinical report discuss strategies for billing for screening and counseling in primary care. The following sections describe tools commonly used to screen and diagnose ASD and emphasize the importance of ongoing surveillance, especially in children at high risk.

#### Screening

Results of a screening test are not diagnostic; they help the primary care provider identify children who are at risk for a diagnosis of ASD and require additional evaluation. General developmental screening tools used for screening at ages 9, 18, and 30 months identify language, social communication, and adaptive skills.

### Table 4: Red Flags: Early Symptoms of ASD

<table>
<thead>
<tr>
<th>By 12 months</th>
<th>Symptom</th>
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<tbody>
<tr>
<td>Has unusual reactions to the way things sound, smell, taste, look, or feel</td>
<td></td>
</tr>
<tr>
<td>Repeats words or phrases over and over (echolalia)</td>
<td></td>
</tr>
<tr>
<td>Gives unrelated answers to questions</td>
<td></td>
</tr>
<tr>
<td>Gets upset by minor changes</td>
<td></td>
</tr>
<tr>
<td>Has obsessive interests</td>
<td></td>
</tr>
<tr>
<td>Makes repetitive movements like flapping hands, rocking, or spinning in circles</td>
<td></td>
</tr>
<tr>
<td>Has unusual reactions to the way things sound, smell, taste, look, or feel</td>
<td></td>
</tr>
</tbody>
</table>

*Information from this table is adapted from [http://www.cdc.gov/ncbddd/autism/signs.html](http://www.cdc.gov/ncbddd/autism/signs.html).*
cognitive, and motor delays but may not be sensitive to social symptoms associated with identification of ASD. This limitation associated with general developmental screening is why ASD-specific tools are needed to capture differences in social interaction, play, and repetitive behaviors. See the AAP clinical report “Promoting Optimal Development: Identifying Infants and Young Children With Developmental Disorders Through Developmental Surveillance and Screening.”

### Screening by Age Group

#### Children Younger Than Age 18 Months

Earlier diagnosis of ASD may lead to earlier treatment. The M-CHAT is the most studied and widely used tool for screening toddlers for ASD. Additional tools are under investigation and are listed in Table 6 as promising autism screening tests. Language delay can be identified by using the Infant and Toddler Checklist (parent questionnaire) in low-risk infants and toddlers between 12 and 18 months of age. This questionnaire might be useful in identifying infant siblings of children with ASD who are at increased risk for ASD. Additional research may allow for screening of toddlers as young as 12 months by using parent-administered questionnaires such as the Communication and Symbolic Behavior Scales Development Profile and the Infant and Toddler Checklist.

Primary care providers are tasked with identifying all children who would benefit from early intervention, not just children at risk for ASD (see the AAP clinical report “Promoting Optimal Development: Identifying Infants and Young Children With Developmental Disorders Through Developmental Surveillance and Screening” for further information). It is important to identify all clinically significant delays in children with referral for appropriate diagnostic evaluation and intervention. Problems with sleep, eating, constipation, and state regulation are common in the general population but may be particularly challenging in young children with ASD. Pediatricians can help families with management of these symptoms.

#### Children Ages 18 to 30 Months

The most commonly used questionnaire-based screening tool is the M-CHAT. It has been further validated, and the scoring has been modified for ease of administration in primary care settings for children ages 16 to 30 months. The Modified Checklist for Autism in Toddlers, Revised with Follow-Up (Questions) (M-CHAT-R/F) eliminates 3 questions from the previous version. Children who score ≥8 are at high risk for ASD or another developmental disorder and should be referred immediately for diagnostic assessment. For children with scores of 3 to 7, publicly available scripted follow-up interview questions are required for the items scored as positive. Children who continue to have 3 to 7 items positive for ASD diagnosis after clarifying follow-up questions have a 47% risk of having ASD diagnosed and a 95% chance of being identified with some other developmental delay that would benefit from intervention. Children screened with the M-CHAT-R/F are identified with ASD at younger ages than predicted by national statistics. Children who do not pass ASD screening tests or who score as at risk for a diagnosis should be referred for both diagnostic assessment and intervention services. A definitive diagnosis is not necessary to institute services for documented delays that would be served through early intervention or school services. Although the M-CHAT-R/F appears to be useful for general screening of diverse populations, decreasing the disparity in early diagnosis will require adapting and validating measures and addressing cultural and linguistic barriers to screening.

Measures under development may provide rapid screening while addressing clinician concerns for compatibility with an electronic record system and open access.
### TABLE 6 Commonly Used ASD Screening Tests

| Autism Screening Tests | Description                                                                 | Age Range | Average No. Items | Administration Time | Forms Available | Psychometric Properties | Scoring Method | Cultural Considerations | Source | Key References |
|------------------------|------------------------------------------------------------------------------|-----------|-------------------|---------------------|-----------------|-------------------------|----------------|--------------------------|-------|----------------|}
<p>| M-CHAT-R/F             | Parent-completed questionnaire designed to identify children at risk for autism from the general population; follow-up clinician-administered questions and repeat questionnaire required for specificity | 18–30 mo | 20                | 5–10 min            | Yes             | Standardization sample included 18,071 children screened; 115 had positive screen results, 348 needed evaluation, 221 were evaluated, and 105 diagnosed with an ASD; validated by using the ADI-R, ADOS-G, CARS, and DSM-IV-TR; sensitivity: 0.91; specificity: 0.95 for low-risk 18- and 24-mo-old children with follow-up questionnaire and interview; 45% of children with a score ≥3 on the initial screen and ≥2 on follow-up had ASD; 95% had clinically significant developmental delay | Risk categorization for questionnaire (pass/need interview/fail); after interview (pass/fail) | Available in multiple languages; see test information for details | <a href="http://mchatscreen.com/">http://mchatscreen.com/</a> | Ref 51 |
| SCQ                    | Parent-completed questionnaire; designed to identify children at risk for ASD from the general population; based on items in the ADI-R | 4 + y     | 40                | 5–10 min            | No              | Validated by using the ADI-R and DSM-IV on 200 subjects (180 with pervasive developmental disorder, 40 without pervasive developmental disorder); for use in children with mental age of at | Risk categorization (pass/fail) | Available in multiple languages; see test information for details. | Western Psychological Corporation: <a href="http://www.wpspublish.com">www.wpspublish.com</a> | Refs 77 and 572 |</p>
<table>
<thead>
<tr>
<th>Autism Screening Tests</th>
<th>Description</th>
<th>Age Range</th>
<th>Average No. Items</th>
<th>Administration Time</th>
<th>Forms Available EHR compatible</th>
<th>Psychometric Properties</th>
<th>Scoring Method</th>
<th>Cultural Considerations</th>
<th>Source</th>
<th>Key References</th>
</tr>
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<tbody>
<tr>
<td>STAT</td>
<td>Clinician-directed, interactive, and observation measure; requires training of clinician for standardized administration; not for population screening</td>
<td>24–35 mo; &lt;24 mo (exploratory)</td>
<td>12</td>
<td>20–30 min</td>
<td>No</td>
<td>Validated by comparison with ADOS-G results in 52 children 24–35 mo Q26 with autism, Q26 with developmental delay; sensitivity: 0.85, specificity: 0.86, PPV: 0.77, NPV: 0.90, for &lt;24 mo: sensitivity: 0.95, specificity: 0.73, PPV: 0.56, NPV: 0.97; screening properties improved for children &gt;14 mo</td>
<td>12 activities to observe early social-communicative behavior; risk categorization (high risk/low risk)</td>
<td>English</td>
<td><a href="http://stat.vueinnovations.com/">http://stat.vueinnovations.com/</a></td>
<td>Refs 573 and 574</td>
</tr>
<tr>
<td>Promising autism screening tests</td>
<td>The Infant/Toddler Checklist (Communication and Symbolic Behavior Scales)</td>
<td>Parent questionnaire: screens for language delay</td>
<td>6–24 mo</td>
<td>24</td>
<td>15 min</td>
<td>No</td>
<td>PPV DD: 0.43 (6–8 mo); PPV DD: 0.79 (21–24 mo)</td>
<td>Identifies language delays (alone/with ASD); risk for ASD; risk status for social, Available in multiple languages; see test</td>
<td>Paul H. Brookes Publishing Co Inc: 800-638-3775 or www</td>
<td>Ref 59</td>
</tr>
<tr>
<td>Autism Screening Tests</td>
<td>Description</td>
<td>Age Range</td>
<td>Average No. of Items</td>
<td>Administration Time</td>
<td>Forms Available</td>
<td>Psychometric Properties</td>
<td>Scoring Method</td>
<td>Cultural Considerations</td>
<td>Source</td>
<td>Key References</td>
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<tr>
<td>Developmental Profile</td>
<td>Parent questionnaire: research edition, 47 items</td>
<td>12–36 mo</td>
<td>47</td>
<td>10–15 min</td>
<td>No</td>
<td>Sensitivity: 0.85–0.91; specificity: 0.82–0.84; PPV: 0.55–0.81; NPV: 0.88–0.98</td>
<td>investigation ongoing of subset (24 items)</td>
<td>English</td>
<td><a href="https://firstwordsproject.com/screen-my-child/">https://firstwordsproject.com/screen-my-child/</a></td>
<td>Not in peer-reviewed literature</td>
</tr>
<tr>
<td>Early Screening for Autism and Communication Disorders</td>
<td>Parent questionnaire; promising in high-risk population to identify risk in 12-mo-old infants</td>
<td>12 mo</td>
<td>63</td>
<td>10 min</td>
<td>No</td>
<td>Sensitivity, specificity, PPV not reported</td>
<td>Scores at risk; promising in high-risk (infant sibling) cohort (Rowberry et al. 575)</td>
<td>English</td>
<td><a href="https://www.med.unc.edu/ahs/pearls/first-year-inventory-fyi-development/">https://www.med.unc.edu/ahs/pearls/first-year-inventory-fyi-development/</a></td>
<td>Ref 575</td>
</tr>
<tr>
<td>First-Year Inventory Parent's Observations of Social Interactions</td>
<td>Parent questionnaire used to assess autism risk; ASD screening included on 18-, 24-, and 30-mo The Survey of Well-Being of Young Children: forms</td>
<td>16–35 mo</td>
<td>7</td>
<td>~5 min</td>
<td>Available through patient tools, epic, and CHADIS; available for free download as pdfs from <a href="http://www.theswyc.org">www.theswyc.org</a></td>
<td>Sensitivity: 83%–95%, average 88.5%; specificity: 42%–75%, average 56.9%</td>
<td>3 of 7 symptoms in at-risk range</td>
<td>Available in multiple languages; see test information for details</td>
<td>Free download from <a href="http://www.theswyc.org">www.theswyc.org</a></td>
<td>Publications and User's Manual available at <a href="http://www.theswyc.org">www.theswyc.org</a>; Refs 576 and 577</td>
</tr>
<tr>
<td>Rapid Interactive Screening Test for Autism in Toddlers 13</td>
<td>Clinician observation: administered by trained examiner</td>
<td>12–36 mo</td>
<td>9 interactive items</td>
<td>20–30 min</td>
<td>No</td>
<td>Cutoff &gt;15; sensitivity: 1; specificity: 0.84; PPV: 0.88; NPV: 0.94; needs further study in larger samples</td>
<td>9 interactive activities; total score summed, cutoff score of 15 (for that sample)</td>
<td>English</td>
<td><a href="https://umassmed.edu/AutismRITA/about-the-test/">https://umassmed.edu/AutismRITA/about-the-test/</a></td>
<td>Ref 578</td>
</tr>
</tbody>
</table>

The AAP does not approve/endorse any specific tool for screening purposes. This table is not exhaustive, and other tests may be available. ADOS-G, Autism Diagnostic Observation Schedule – Generic; CARS, Childhood Autism Rating Scale; CHADIS, Comprehensive Health and Decision Information System; EHR, electronic health record; ICD-10, International Classification of Diseases, 10th revision; IMFAR, International Meeting for Autism Research; NPV, negative predictive value; PPV, positive predictive value.
Further adaptations of the Communication Symbolic Behavior Scale for use in screening for language delays in addition to ASD have the potential to identify children at risk for both disorders (functional communication; ages 6–24 months). Use of this or other screening tools may be coupled with the online support of a video glossary of symptoms of ASD, such as that in the Autism Navigator (http://www.autismnavigator.com/). These and other online approaches to support screening strategies may be integrated into efficient patterns of practice. Results of screening conducted online, in community settings, and in preschools should be communicated to the primary care provider to ensure appropriate evaluation of etiology, co-occurring conditions, referral for diagnosis, and follow-up to ensure that intervention is accessed.49

A systematic review by the US Preventive Services Task Force (USPSTF) concluded that the literature on existing screening tools did not demonstrate sufficient specificity to justify universal screening.63 The USPSTF noted that no study has directly examined whether children with ASD detected by early screening have better outcomes than those detected by other means. However, such a study would require random assignment of large representative samples from across the country to either a screening or nonscreening condition, with follow-up of long-term outcomes and societal costs. Given that early treatment of children younger than 36 months has been shown to result in positive outcomes,43,64 such a study would be challenging to support. The USPSTF concluded that further research is indicated to evaluate the appropriate ages and populations of children who should be screened for ASD and that more accurate and culturally sensitive measures should be developed. The AAP continues to recommend screening using the most valid of current measures at 18 and 24 months of age. Pediatricians cannot assume that early intervention systems will screen participants being served for language or global delays for ASD at the recommended ages. Universal screening is recommended because symptoms of ASD can be identified in early childhood, and a diagnosis of ASD by skilled professionals is accurate in children as young as 18 months of age.65 Diagnostic stability is high for children who are diagnosed with ASD at 18 to 36 months of age.43 Early screening does not identify many children with milder symptoms and typical cognitive ability as at risk for ASD; therefore, ongoing surveillance remains necessary.16 Participation in early intervention in general is greatest among children who had screening and surveillance.66

Children Older Than 30 Months
At present, for children older than 30 months, there are no validated screening tools available for use in pediatric practice, nor are there current recommendations by the AAP for universal screening for ASD in that age group. The Social Communication Questionnaire (SCQ) (see Table 6) has been studied in different populations (eg, clinical sample, population reference sample, community sample, and convenience sample), with best results in population samples67 when using the lifetime version, and appears to have reasonable psychometric properties. However, questionnaires like the SCQ may identify symptoms that overlap with other conditions, such as ADHD, that affect function at school age.68,69 Further validation of population-based screening tools for children older than 30 months is needed before recommendations for universal screening of school-aged children can be made. At this time, ongoing surveillance in the context of primary care is recommended.

Barriers to Identifying Risk for ASD
Children with milder symptoms and/or average or above-average intelligence may not be identified with symptoms until school age, when differences in social language or personal rigidities affect function. Some children who are later diagnosed with ASD are initially believed to have precocious language, reading, or math skills, and it is not until the social demands of school that the social language symptoms become problematic. It has also been suggested that girls may have lesser intensity of symptoms and fewer externalizing behaviors. These differences may, in part, result in underdiagnosis in girls.70 Specific coexisting conditions may prevent clinicians from recognizing symptoms of ASD in early childhood. For example, 1 study revealed that children who were initially identified with ADHD in primary care were diagnosed with ASD 3 years later compared with children who did not have earlier symptoms of ADHD.69 Recognition and referral for older children with social-skill deficits would be facilitated by the development of accurate and brief screening tests for use in primary care and school settings. Population surveillance data reveal later age at diagnosis for African American and Hispanic children, suggesting that there are barriers to screening and surveillance and referral for diagnosis in groups with other unmet health needs.7 Race, ethnicity, and socioeconomic status did not affect the accuracy of routine screening tests for ASD in low-risk toddlers, suggesting that screening with appropriate supports for follow-up care can lower the age at diagnosis in diverse populations.60 Language barriers, inaccurate translations, and low parental literacy may compromise use of parent-completed questionnaires.71 Limited understanding of cultural differences experienced by the patient’s family.
and lack of trust in the health care provider may further limit identification and reporting of symptoms of autism. Screening tools need to be developed for populations of individuals whose primary language is not English and who are also sensitive to cultural barriers that may limit reporting of symptoms of ASD.

**Diagnostic Evaluation**

Once a child is determined to be at risk for a diagnosis of ASD, either by screening or surveillance, a timely referral for clinical diagnostic evaluation and early intervention or school services, depending on his or her age, is indicated. Children with developmental delay with or without an ASD diagnosis should be referred to early intervention or school services, in which cognitive and language testing may be completed. The primary care provider should discuss with the family the importance of both the assessment of developmental status and evaluation for an ASD diagnosis and assist the family in navigating through the process, including connecting them with community resources. Families with low income or language barriers may need additional attention to take the next steps.

Although most children will need to see a specialist, such as a developmental-behavioral or neurodevelopmental pediatrician, psychologist, neurologist, or psychiatrist, for a diagnostic evaluation, general pediatricians and child psychologists comfortable with application of the DSM-5 criteria can make an initial clinical diagnosis. Having a clinical diagnosis may facilitate initiation of services. At this time, there are no laboratory tests that can be used to make a diagnosis of ASD, so careful review of the child’s behavioral history and direct observation of symptoms are necessary. To meet diagnostic criteria, the symptoms must impair function. Formal assessment of language, cognitive, and adaptive abilities and sensory status is an important component of the diagnostic process.

Short clinical visits may not allow even a skilled clinician the opportunity to accurately recognize symptoms of ASD. An accurate history needs to reflect a longitudinal experience with the individual and reflect the effects of symptoms on the patient’s ability to function in family, peer, and school settings. This history is obtained by interview with the patient and caregivers, reports of behavior in other environments (such as school), and descriptions of behavior during formal testing. The history of symptoms of ASD can be supported by questionnaires such as the SCQ or Social Responsiveness Scale (SRS). None of these questionnaires is sufficient alone to make a diagnosis of ASD, but all provide a structured approach to elicit symptoms of ASD. Measures such as the Behavior Assessment System for Children, Diagnostic Interview for Social and Communication Disorders (DISCO), and the Child Behavior Checklist are used to assess children and youth for other behavioral health conditions but may also identify behavioral profiles consistent with ASD.

In some clinical and research settings, the behaviors associated with ASD are reported through the Autism Diagnostic Inventory-Revised (ADI-R), a lengthy, semistructured parent interview. It supports a knowledgeable clinician in applying diagnostic criteria of ASD. The SCQ was designed to elicit similar information to the ADI-R in an abbreviated questionnaire format. The SRS is a 65-item questionnaire that may be used to measure autistic traits on a continuum as part of a more complete evaluation of ASD.

Elevated scores may be seen with greater severity of symptoms of ASD as well as with intellectual disability, communication difficulties, and behavioral challenges.

Structured observation of symptoms of ASD during clinical evaluation is helpful to inform the diagnostic application of the DSM-5 criteria. Validated observation tools used to provide structured data to confirm the diagnosis include the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) and the Childhood Autism Rating Scale, Second Edition (CARS-2). No single observation tool is appropriate for all clinical settings. The observation tool is meant to support application of the DSM-5 criteria informed by history and other data.

The ADOS-2 was developed to elicit atypical social language and behaviors. With the ADOS-2, modules are specific for use across the age span of toddlers to adults. The ADOS-2 requires intensive training to accurately administer and score and takes 30 to 45 minutes to administer. It is often a component of both research and clinical evaluations. The information obtained from the ADOS-2 is used by the clinician in conjunction with the history of peer interactions, social relationships, and functional impairment from symptoms to determine if the DSM-5 criteria are met. The CARS-2 is another structured approach a clinician might use to support a clinical diagnosis of ASD. The clinician completes a 15-point scale that is based on history and observation. The ADOS-2, CARS-2, and SRS (Parent and Teacher) all rate children similarly in approximately half of identified cases. The integration of historical information and objective observation by a clinician trained to diagnose autism and related conditions to inform the DSM-5 diagnostic criteria is the
critical element to diagnostic evaluation.

**Evaluation of Co-occurring Developmental Conditions**

Patients with ASD may have intellectual disabilities, learning disabilities, ADHD, anxiety disorders, or speech and language disorders, among others. These conditions may influence the presentation of the symptoms of ASD. These conditions may influence the presentation of symptoms of ASD and may influence the social and functional impairment of the individual in different ways at different ages. Valid assessment of cognitive and language ability is an important component of the diagnostic evaluation. In the United States, early intervention services and school systems will evaluate children in these domains to assess educational needs. In some areas, initial evaluations are performed in clinical settings and paid for by insurance.

**Cognitive Testing**

A range of standardized measures are used to determine developmental levels of younger children and IQ in children older than 3 years. The intelligence test selected by the psychologist will depend on the age and language level of the child. Administration of a valid cognitive test is important in ascribing symptoms to ASD as part of the initial diagnosis but also helps to establish co-occurring diagnoses with ASD, such as intellectual disability. There are valid tests that can be used in children who are nonverbal. Although the prevalence of a diagnosis of ASD is increased in children with an intellectual disability,91 other children diagnosed with intellectual disability may have some symptoms of ASD without meeting diagnostic criteria for the disorder.

**Language Testing**

Inherent in the core symptoms of ASD are differences in the use of verbal and nonverbal communication for social interaction. Formal assessment of communication by a speech or language pathologist at the time of diagnosis should include the documentation of expressive and receptive language skills as well as the pragmatic or conversational use of language.92

**Adaptive Function Testing**

A caregiver report and/or teacher report of adaptive functioning complements objective cognitive testing. Determining the extent that ASD affects daily function is necessary to establish eligibility for some publicly funded programs as well as to identify and monitor developmental goals for treatment. Adaptive behaviors are typically delayed in people who have intellectual disability with ASD but can be impaired in people with ASD and an average-range IQ.93,94 Commonly used adaptive measures include the Vineland Adaptive Behavior Scales and the Adaptive Behavior Assessment System.95

**Motor Assessment**

Children with ASD are more likely to have mild delays in gross motor skills and coordination compared with children in the general population and may meet DSM-5 criteria for developmental coordination disorder in addition to ASD.96 General screening tests or adaptive measures may suggest motor delays that would benefit from formal evaluation by an occupational or physical therapist. A relationship of early motor delays and subsequent language and adaptive development in children with ASD has been proposed.97,98

**Sensory Assessment: Hearing**

Children with language delay or inattention to language should have an evaluation of their hearing as part of their initial evaluation.99 Hearing loss may co-occur with ASD and needs to be considered in children with language delays, behavior problems, or inattention. Appropriate amplification should be offered, if indicated. The clinical utility of auditory processing evaluations available in current practice remain an area of study.100,101

**Sensory Assessment: Vision**

Visual function should be considered in the initial evaluation of children who are visually inattentive, have stereotypical behaviors (such as eye poking or close visual scrutiny), or do not make eye contact. Decreased visual acuity may affect interactive gaze and require accommodations in the educational setting.102 Children with visual impairment may also demonstrate stereotyped motor behaviors.

**Sensory Assessment: Sensory Processing**

The DSM-5 includes sensory symptoms in the diagnostic criteria for ASD. The DSM-5 does not include sensory processing disorder as a discrete diagnosis. Commonly used evaluation tools (such as the Short Sensory Profile and others) quantify parent perception of sensory differences relative to smell, taste, vision, hearing, and touch.103,104 In addition to capturing what is conventionally considered as a sensory disturbance, questionnaires that are used to assess sensory symptoms also capture motor hyperactivity and hypoactivity as sensory-seeking or sensory-avoiding behaviors. These latter symptoms may reflect co-occurring ADHD. Sensory symptoms may be more evident at younger ages and may define subtypes of the disorder.105,106

**SECTION 4: ETIOLOGIC EVALUATION**

Children with a diagnosis of ASD should be assessed for potential etiology and common coexisting medical conditions. At the time of the 2007 AAP clinical reports on autism, karyotype and DNA testing for fragile X syndrome were the state-of-the-art...
etiological investigations. Soon thereafter, chromosomal microarray (CMA) was endorsed by the American College of Medical Genetics and Genomics and the American Academy of Child and Adolescent Psychiatry as the most appropriate initial test for etiologic evaluation of children with ASD.76,107–116 Despite rapid technological advances in neuroimaging and other areas, many of the recommendations for clinical evaluation published in 2007 are unchanged. This section summarizes recent advances in understanding the etiologies of ASD and how they translate into recommendations for clinical practice.

**Medical Workup of the Child With ASD**

**Genetic Testing**

Advances such as the development of CMA and next-generation sequencing technologies and the application of these technologies to well-characterized patient cohorts have led to progress in the understanding of the complex genetics of ASD and other neurodevelopmental disorders in the last decade. Identifying a genetic etiology provides clinicians with more information for families about prognosis and recurrence risk and may help to identify and treat or prevent co-occurring medical conditions, guide patients and families to condition-specific resources and supports, and avoid ordering unnecessary tests (Table 7).111–117 Most parents find this information to be useful.118 As research progresses, genetic testing may contribute to identifying effective interventions related to specific etiologies.

Etiologic investigation begins with a careful medical, developmental-behavioral, and family history and a thorough physical and neurologic examination.109 The physical examination should include assessment of growth relative to typical curves (including head circumference), dysmorphic features, organomegaly, skin manifestations of neurocutaneous disorders (eg, tuberous sclerosis and neurofibromatosis), and neurologic abnormalities.109 Genetic evaluation should be recommended and offered to all families as part of the etiologic workup. A stepwise general approach is provided in Table 8 as a practical guideline.110,120 The presence of dysmorphic features or intellectual disability is generally associated with

### TABLE 7 Potential Benefits of Establishing a Genetic Etiologic Diagnosis

- Improving accuracy of counseling provided to patients and families:
  - Prognosis or expected clinical course
  - Recurrence risk for the family and the individual affected
- Providing condition-specific family support, such as:
  - Improving psychosocial outcomes for patients and their families (eg, knowledge and sense of empowerment, parental quality of life)
- Preventing morbidity and treating medical conditions associated with the genotype, such as:
  - Conditions or anomalies likely to be present at diagnosis
  - Conditions that may develop later
- Refining treatment options, including:
  - Avoiding therapeutic interventions that may be based on unfounded etiologic theories
  - Avoiding ineffective or potentially harmful treatments
  - Providing access to emerging etiology-specific treatments
- Facilitating acquisition of needed services and access to research treatment protocols
- Avoiding additional diagnostic tests, which may be unnecessary, expensive, and/or uncomfortable


### TABLE 8 Genetic Etiologic Investigations in Patients With ASD

<table>
<thead>
<tr>
<th>Step</th>
<th>Genetic Etiologic Investigations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Consider referral for pediatric genetics evaluation</td>
</tr>
<tr>
<td>2</td>
<td>Comprehensive history (including 3-generation family history with emphasis on individuals with ASD and other developmental, behavioral and/or psychiatric, and neurologic diagnoses) Physical examination (including dysmorphology, growth parameters [including head circumference], and skin examination) If syndrome diagnosis or metabolic disorder is suspected, go back to step 1 (genetics and/or metabolism referral) and/or order the appropriate targeted testing Otherwise, proceed to step 3</td>
</tr>
<tr>
<td>3</td>
<td>Laboratory studies Discuss and offer CMA analysis Discuss and offer fragile X analysis; if family history is suggestive of sex-linked intellectual disabilities, refer to genetics for additional testing If patient is a girl, consider evaluation for Rett syndrome, MECP2 testing If these studies do not reveal the etiology, proceed to step 4</td>
</tr>
<tr>
<td>4</td>
<td>Consider referral to genetics, workup might include WES</td>
</tr>
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</table>

increased likelihood of finding a genetic abnormality. However, authors of some clinical studies have identified similar yield for genetic testing in children without these risk factors. In some cases, individuals with clinical genetic syndromes, such as fragile X syndrome, tuberous sclerosis complex, and others (such as those described in Supplemental Table 13), also meet criteria for ASD. When a specific syndrome or metabolic disorder is suspected, the clinician should proceed with the appropriate targeted testing or referral to a pediatric geneticist or neurologist. For example, a girl with significant developmental delays, deceleration in head growth velocity, and characteristic midline hand movements should prompt genetic testing for a mutation or deletion or duplication of MECP2, the gene implicated in Rett syndrome. Another specific example would be a boy with ASD with marked macrocephaly and pigmented macules on the penis, findings that would warrant sequencing and deletion or duplication analysis of the PTEN gene. Descriptions of these and other clinical syndromes associated with ASD are provided in Supplemental Table 13.

CMA is recommended if the etiology for developmental disability is not known. CMA identifies copy number variants (CNVs) at this time, which are DNA duplications or deletions that alter the function of genes (Table 8, step 2). CMA reveals a definitively pathogenic CNV in 5.4% to 14% (median 9%) of individuals with ASD in clinical samples. When CNVs of uncertain significance are included, approximately 17% to 42% of patients with ASD have findings on the CMA. Some of the variants of uncertain significance may be determined as pathogenic in the future. The most commonly identified recurrent pathogenic CNVs among individuals with ASD are provided in Supplemental Table 14.

Because fragile X syndrome increases risk for ASD, DNA testing for fragile X syndrome should be recommended for all children with ASD, but especially for boys and children with a suggestive family history of male members with intellectual disability. Physical examination might reveal the common features of a large head size, prominent jaw, large ears, ligamentous laxity, and, in male patients, large testes after puberty. The cytosine-guanine-guanine trinucleotide repeat expansion that is responsible for fragile X syndrome is not detected on CMA and must be ordered as a separate test. The current estimate is that approximately 0.45% of individuals with ASD have the full mutation for fragile X syndrome, and many of them are female. Because fragile X syndrome testing is relatively inexpensive and the condition has important genetic counseling implications, it is reasonable to consider testing both male and female patients with ASD, at least until more data become available to clarify the issue.

When the history and physical examination, CMA, and fragile X analysis do not identify an etiology, the next step at this time in the etiologic evaluation for ASD is whole-exome sequencing (WES). WES technology allows for the identification of single-nucleotide variants, including pathogenic loss-of-function mutations and missense mutations, which have been found to be associated with ASD. Examples of ASD risk genes identified or confirmed in WES studies are provided in Supplemental Table 15. As with other tests, clinicians ordering this test should be familiar with both pretest counseling and interpretation of the results. A genetic counselor is helpful in explaining the reason for testing as well as the results. Large clinical WES studies have consistently been used to identify a molecular diagnosis in 26% to 29% of individuals for whom neurodevelopmental disorders were the primary indication for testing. Authors of studies of clinical populations with ASD have reported diagnostic yields of 8% to 20%.

The yield of WES is higher when both the parents and the child who is affected are evaluated to allow for comparison of the child with parents who are unaffected. Some geographic areas may have limited availability of pediatric subspecialists (eg, in genetics or metabolism) who can guide the genetic workup, so primary care providers may be in the position to consider and direct etiologic evaluation. The complexity of genetic testing is such that most primary care providers may want to consult with a specialist to plan testing and interpret results. The clinical etiologic evaluation should be tailored to the individual patient, taking into consideration information from the history and physical examination and the values and wishes of the family. The stepwise general approach summarized in Table 8 can be used to guide this process.

It is important for families to understand that genetic tests may explain the cause of their child’s ASD or provide information about the statistical risk of ASD, but they are not diagnostic of ASD; the diagnosis of ASD is made on the basis of clinical symptoms. Unlike CMA and WES, commercially marketed tests may not have the potential to provide a molecular etiologic diagnosis. Genomic testing technology is evolving rapidly, as is our understanding of the genetic architecture of ASD, and these recommendations for testing will need to be updated as new studies are published. For example, it is anticipated that CMA and WES will soon be combined because of...
improvements in accurate identification of CNVs using sequence data and that sequencing of the exome will be replaced by sequencing of the entire genome as issues with interpretation and cost become more manageable.121,130,147–150

Parents of a child with ASD should be counseled regarding recurrence risk in subsequent offspring, and the nature of the counseling depends greatly on whether a specific genetic cause of the child’s ASD has been identified. When a specific genetic etiology has been determined, the family can be provided with information about the risk of recurrence in subsequent offspring. However, when genetic testing has not been completed or has not revealed the etiology of the child’s ASD, recurrence risk counseling is based on group averages derived from the existing literature. For a couple with 1 child with ASD of unknown cause, the current best estimate of recurrence in a subsequent child is approximately 10% (range 4%–14%).151–153. If a couple already has ≥2 children with ASD of unknown etiology (idiopathic), the chance of a subsequent child having ASD may be as high as 32% to 36%.153,154 However, the risk is not limited to ASD. Siblings of children with ASD who do not have ASD themselves may have a 20% to 25% risk for language disorders and other neurodevelopmental and psychiatric disorders.152,155,156

**Neuroimaging**

Specific clinical neuroimaging findings are not more prevalent in ASD compared with other neurodevelopmental disorders, nor do specific abnormalities correlate with clinical, etiologic, or pathophysiological aspects of ASD.120,157,158 Incidental findings are common in neuroimaging studies obtained in the workup of children diagnosed with ASD but rarely provide etiologic information or require intervention.159,160 The need for clinical MRI should be directed by a history and physical examination. MRI may be indicated in the evaluation of atypical regression, microcephaly, macrocephaly, seizures, intracranial manifestations of genetic disorders, abnormal neurologic examination, or other clinical indications.119,161,162 Imaging technology used to examine brain structure and function provides valuable insight into the neurobiology of ASD in research settings and may lead to useful clinical applications in the future.

**Metabolic Testing**

The yield of routine metabolic testing for children with ASD is low and not recommended for regular use.163–167 However, large population-based studies are lacking, so accurate prevalence and diagnostic yield estimates are not available. Metabolic workup should be informed by history, family history, symptoms, and examination and might include measurement of fasting plasma amino acid levels, urine organic acid levels, and acylcarnitine metabolite levels and other testing for specific metabolic disorders. History of atypical regressions (later than 2 years of age, motor regression, or multiple regressions), family history of early childhood death or diagnosed metabolic disorders, and physical features, such as significant hypotonia or weakness, visual and hearing impairment, and dysmorphic features, would suggest consultation with a specialist to guide evaluation for metabolic or mitochondrial disorders.109,168 Children who present with motor delay should be evaluated with creatine kinase and thyroid-stimulating hormone testing, according to AAP recommendations.4,49 Although metabolic disorders are uncommon causes of ASD, the potential impact is high because treatment may be available and the inheritance pattern may be known.109,124 Examples of metabolic conditions that may be associated with an ASD phenotype are provided in Supplemental Table 16. There is no evidence at this time for routine testing of hair, blood, or urine for environmental toxins or heavy metals outside of laboratory screening for lead exposure.169

**EEG**

Children with ASD have an increased risk for seizures, and EEG abnormalities are common in the absence of clinical seizures (see Seizures section for more information).170–175 However, EEG is not recommended as a routine baseline evaluation in the absence of clinical concern about seizures, atypical regression, or other neurologic symptoms on history or examination that would suggest an EEG is indicated.161,170,172,176 Late or atypical loss of language, as might be observed in electrical status epilepticus of sleep with loss of language, should be evaluated with an overnight EEG.161,170,172,176 Primary care clinicians should discuss the increased risk and the signs and symptoms of seizures with the families of children diagnosed with ASD, maintain a high index of clinical suspicion for seizures, and consult with a pediatric neurologist when concerned about atypical regression or the possibility of seizures.170,172,176

**The Biology of ASD**

**Genetics and ASD**

ASD is clinically and etiologically heterogeneous yet highly heritable. The rate of ASD in siblings is much higher than the rate in the general population. Twin studies demonstrate substantially higher concordance rates for symptoms of ASD in monozygotic twins than in dizygotic twins.177 A meta-analysis involving 6413 twin pairs revealed a 98% concordance in monozygotic twins, a 53% to 67% concordance in dizygotic twins, and heritability estimates from 64% to 91%.177,178
Siblings may also be at risk for symptoms related to ASD that do not meet the threshold for a diagnosis of ASD and have been described as the broader autism phenotype. These data provide strong evidence for a genetic contribution to ASD risk.

Risk for ASD also is increased in the children of both older fathers and older mothers. The increased risk with parental age may be related to germline mutations in older fathers. Mechanisms mediating the effect of advancing maternal age on ASD risk are less clear. Increased maternal and paternal age are independently associated with ASD risk, and a joint effect seems to occur as well.

Important aspects of the genetics of ASD are still poorly understood, including the role of common variants, epistasis (gene-gene interactions), and environmental modification of genotype effects. In contrast, advances such as CMA and next-generation sequencing technologies have resulted in identification of large-effect (pathogenic) rare variants that appear to be causally associated with ASD, including CNVs, which are deletions or duplications ≥1000 bp in size that alter the dosage of genes, and sequence-nucleotide variants. Pathogenic rare variants may arise de novo or be inherited as autosomal dominant, autosomal recessive, or X-linked mutations. Researchers of CMA and WES studies have established that although de novo and inherited rare variants of large effect size are collectively common, no individual pathogenic variant accounts for more than 1% of cases of ASD. Genes that contribute to ASD are involved in a variety of biological functions, with convergence on aspects of brain development and function, including synaptic structure and function, intracellular signaling, transcription regulation, and chromatin remodeling. It is important to note that no specific mutation has been identified that is unique to ASD; there is substantial genetic overlap between ASD and other neurodevelopmental disorders, including intellectual disability, epilepsy, and schizophrenia.

Genes, Environmental Exposures, and ASD

The potential environmental factors that may be related to increased reported prevalence of ASD is an area of active study that, as yet, is without firm conclusions. Environmental factors associated with ASD include in utero exposure to medications such as valproate and thalidomide. Other prenatal influences, such as short interpregnancy interval, multiple gestation, maternal obesity, gestational bleeding, gestational diabetes, advanced parental age, and infections (eg, rubella and cytomegalovirus), may be associated with increased risk for ASD. Perinatal factors, such as preterm birth, low birth weight, fetal growth restriction (ie, small for gestational age), intrapartum hypoxia, and neonatal encephalopathy, are associated with increased ASD risk. Environmental factors may present independent risk to prenatal brain development or may affect gene function in individuals with genetic predisposition. Population-level associations with ASD have been examined for organophosphates and certain other pesticides, metals, volatile organic compounds, and air pollution, particularly particulate matter and nitrogen dioxide. Research on environmental exposures may be of great importance in identifying modifiable risk factors related to ASD and other developmental disorders. It is prudent to limit exposure of children and pregnant women to known neurotoxicants.

Genes, Immunologic Exposures, and ASD

It has been proposed that children with ASD-associated CNVs may be more susceptible to environmental insult in the form of maternal immune activation. Report of maternal infection or fever during pregnancy may be associated with increased severity of ASD-related symptoms in offspring who are affected. The pathogenic role of circulating maternal antibodies directed to fetal brain tissue and the potential value of maternal antibody panels as biomarkers of ASD are currently being studied. Unless otherwise indicated (eg, history suggestive of autoimmune or immunologic disorder), no immune testing is recommended in the etiologic workup of a child with ASD.

Epigenetics

Epigenetic modifications, such as DNA methylation and posttranslational histone modification, produce heritable changes in gene expression that do not involve a change in the DNA sequence. Some genetic disorders associated with ASD (eg, Rett syndrome; CHARGE syndrome; 15q duplication; Angelman syndrome; and fragile X syndrome), involve genes that either encode epigenetic regulators or are sensitive to alterations in their epigenetic regulation. Because epigenetic modifications can be influenced by environmental factors, such as prenatal maternal exposures and postnatal experience, they represent 1 interface between genes and environment. However, epigenetic modifications are not the only mechanisms by which gene expression is regulated, and epigenetics should not be conflated with the broader category of environmental effects. Currently, the evidence that alteration of gene expression by environmental factors plays a causal role in ASD is very limited. Investigation of
the role of epigenetic and other nongenetic modifications that alter gene activity without changing the DNA sequence is an active area of etiologic research in ASD.

**Vaccines**

The scientific literature does not support an association of vaccination as an environmental factor that increases the risk for ASD. Children with ASD should be vaccinated according to the recommended schedule. Epidemiological studies do not demonstrate any association of the measles-mumps-rubella vaccine, mercury exposure by thimerosal-containing vaccines, aluminum in vaccines, or increased level of immunologic exposure attributable to a larger number of vaccines (either given at 1 time or cumulatively) with ASD. Vaccines used for children in the United States have not contained thimerosal since 2001. The authors of a 2012 Cochrane review and a 2014 quantitative meta-analysis of pooled data from cohort studies involving 1,256,407 children and case-control studies involving 9,920 children reviewed the scientific literature and came to this conclusion. Evidence implicating immunizations as a “second hit” conferring ASD risk in genetically susceptible subgroups is lacking. It has been shown that the measles-mumps-rubella vaccine is not associated with increased risk for ASD, even among children who are already at higher risk because of having an older sibling with ASD. Media coverage of vaccine issues may inflate the perception of uncertainty by equal coverage of vaccine proponents and opponents. The overwhelming weight of evidence supports vaccine safety. Communicating information about vaccine safety is a critical component of pediatric practice.

**Brain Structure and ASD: Neuropathology**

Neuropathological research has been limited by the small number of postmortem brains available for study. Developmental brain abnormalities in people with ASD are reported in the cerebral neocortex; limbic system structures, including the hippocampal formation and amygdala; basal ganglia; thalamus; brainstem; and cerebellum. These brain abnormalities include dysplasia, altered neurogenesis, and abnormal neuronal migration. The vast majority of abnormalities described originate during prenatal brain development. Findings in the cerebral cortex may include focal disruption of neuronal migration, minicolumnar abnormalities, and variations in neuronal density. A decreased number of Purkinje cells in the cerebellum is 1 of the most consistently reported neuropathologic findings associated with ASD. Although it was initially thought to be of prenatal onset, evidence now indicates that this phenomenon is more likely to be an acquired process that occurs postnatally, potentially related to seizures, medications, and/or ischemia near the time of death or factors other than ASD. No uniform neuropathology has been identified in people with ASD.

**Biomarkers**

Objectively measured biological characteristics, or biomarkers, of ASD could potentially be used to predict ASD risk, enhance screening, and permit presymptomatic detection. Their use could improve the reliability and validity of clinical diagnosis (identifying clinically meaningful subgroups that would allow for prediction of prognosis or treatment response), identify mechanisms for developing treatment, and confirm the need for a specific intervention.

**Early Brain Overgrowth**

Cross-sectional and longitudinal studies suggest that as a group, children later diagnosed with ASD may have an average or below-average head circumference at birth, with an acceleration in brain growth before 2 years of age. This rapid brain growth leads to significantly above-average head circumferences and MRI brain volumes in toddlers, followed by a plateau in brain growth, with brain volumes in adolescence and adulthood similar to those of controls. Almost 16% of young children with ASD have a head circumference greater than the 97th percentile. A preliminary study suggested that infant siblings of children with ASD who exhibited a larger head circumference at 12 months and showed more slowing of head circumference growth from 12 to 24 months had an increased chance of demonstrating symptoms of ASD. Although this finding raises the possibility that patterns of brain growth might be used for early identification, the rate of head growth did not predict which infants developed ASD in the first 3 years of life in a large prospective study of high-risk infants. It is possible that a large head size is unrelated to ASD and/or may be part of general somatic overgrowth.

**Neuroimaging Patterns Associated With ASD in Research Studies**

Although there are conflicting findings, structural MRI volumetric studies suggest that young children with ASD differ from controls in total brain volume, cortical gray and white matter volume (particularly frontal, temporal, and cingulate cortices), extraaxial cerebral spinal fluid volume, and amygdala volume. A research-level analysis also has identified asymmetries in multiple brain structures in people with ASD. Diffusion tensor imaging has been used to identify altered patterns in white matter by 6 months of age in
infants later diagnosed with ASD. Functional MRI has demonstrated differences in people with ASD relative to controls in efficiency of visual processing, executive function, language, and basic and complex social processing skills. Functional MRI in research settings demonstrate differences in the mechanisms of attention to social stimuli, modulation in response to task demands or intensity of stimuli, and executive function in people with ASD. Functional underconnectivity has also been demonstrated across a wide variety of the brain regions that support language, executive function, social cognition, emotion processing, and motor tasks, especially for long-range, frontal-posterior networks.

Electrophysiologic Testing and Measurement of Eye Tracking

Electrophysiologic research studies demonstrate differences in auditory processing (including language processing), visual processing (including face processing), somatosensory response, multisensory integration, attentional shifting, selective attention, recognition memory, and neural connectivity in people with ASD. Continuous measures of resting-state and task-related quantitative EEG are used to calculate and describe spectral power, complexity, and coherence. Although promising, the clinical utility of these measures as biomarkers requires additional study. Eye tracking has been used to determine if infants who are younger siblings of children with ASD and, therefore, at increased risk for ASD exhibit differences in fixation on faces. Preliminary evidence suggests that infants later diagnosed with ASD exhibit a decline in gaze fixation from age 2 to age 6 months.

Other Potential Biomarkers

Although some studies have attempted to differentiate people with and without ASD on the basis of differences in laboratory profiles of platelet serotonin, plasma melatonin, urine melatonin sulfate, redox status, placental trophoblast inclusions, and immune function, currently no diagnostic laboratory tests have been approved for ASD. To date, none of these potential biomarkers under study have sufficient evidence to be recommended.

Biomarkers: Future Directions

Proposed biomarkers for ASD risk include genetic and biochemical findings in blood, urine, or brain tissue; placental pathology; maternal autoantibody profiles; structural and functional MRI patterns; electrophysiological test results on EEG, including event-related potentials; responses in eye tracking; and physical parameters such as head circumference growth trajectory. Although none of these proposed biomarkers has demonstrated sufficient predictive validity for clinical use at this time, the search for biomarkers is a major research focus. Biomarker research has important ethical issues and concerns have appropriately been raised regarding premature translation of research data into commercially available tests marketed to patients and families. However, the capabilities to screen large numbers of bioactive compounds, examine the entire genome, and simultaneously analyze large data sets have accelerated research into the neurobiology of ASD and may result in the identification of valid biomarkers.

SECTION 5: INTERVENTIONS

The goals of treatment of children with ASD are to (1) minimize core deficits (social communication and interaction and restricted or repetitive behaviors and interests) and co-occurring associated impairments; (2) maximize functional independence by facilitating learning and acquisition of adaptive skills; and (3) eliminate, minimize, or prevent problem behaviors that may interfere with functional skills. Treatments should be individualized, developmentally appropriate, and intensive, with performance data relevant to treatment goals to evaluate and adjust intervention. All interventions should be based on sound theoretical constructs, rigorous methodologies, and objective scientific evidence of effectiveness. Since the publication of the 2007 AAP clinical reports on autism, a substantial published literature has examined the effectiveness of interventions. Legal mandates in education law in the United States, which include the Individuals with Disabilities Education Improvement Act of 2004 (IDEA) (Public Law 108–446) and the No Child Left Behind Act of 2001 (Public Law 107–110) and its successor, the Every Student Succeeds Act of 2015 (Public Law 114–95), require the use of practices supported by scientifically based research (IDEA and the No Child Left Behind Act of 2001) or evidence-based practices (Every Student Succeeds Act of 2015) (https://www.ed.gov/). Early intervention services under part C of IDEA provide for assessment and intervention for children younger than 3 years with developmental delays, including ASD.

Interventions for children with ASD are provided through educational practices, developmental therapies, and behavioral interventions. Treatment strategies may vary by the age and strengths and weaknesses of the child. For example, intervention for a toddler with a recent diagnosis of ASD may include behavioral and developmental approaches (individually or in the context of...
comprehensive approach) and, as he or she progresses, involvement in a specialized or typical preschool program. For older children, intervention is more likely to occur in educational settings, with integration of behavioral and developmental therapies to promote skill development. In addition to variation by age of the child, interventions differ in theoretical approach and scope (eg, focused and targeted or comprehensive), settings and/or modality of delivery (eg, individual versus group or classroom, delivered by a professional versus a trained parent, and school versus home setting), and targets of intervention.40,297 Interventions may be provided through public and/or not-for-profit agencies, schools, and early intervention services, and some may be paid for through insurance.299 Families should be involved in the selection of intervention approaches and remain an involved participant in subsequent educational and therapeutic decisions. There is regional variation in the availability of various types of therapy and providers that sometimes results in long waits for service, less-than-desired intensity, or inability to obtain a desired intervention altogether. By law, students with ASD should receive an appropriate educational program, although it may not include all of the components desired by the family. Advocacy is often necessary to obtain desired services through schools or through mechanisms paid for by insurance. It is noted that many of the interventions in common use do not have a strong evidence base. Some types of intervention may not be paid for by insurance.

Systematic reviews of the evidence base for treatment have been completed on early intensive intervention,44,300 medical treatments,301 behavioral interventions,”294,298 and evidence-based practice guidelines.292,302 Wong et al295 described 2 categories of evidence-based interventions, the comprehensive treatment model (CTM) and focused interventions. These interventions may be provided in different settings (eg, the home, classroom, naturalistic environment, or community), by different providers (eg, developmental specialist, behavioral therapist, educator, or trained parent), individually or in group settings, and by using a set curriculum or guide.

The CTM uses a central conceptual framework to address a broad array of symptoms and is designed to address specific skill(s) or symptom(s). A CTM should be replicable, intense, and designed to address multiple therapeutic goals over a period of time. Provision of services may occur in individual instruction or class settings (specialized or inclusive), should include parents, and may involve technology-assisted intervention.203

Applied behavior analysis (ABA), developmental approaches, and/or naturalistic approaches may be used in CTMs.303 Examples of CTMs include early intensive behavioral intervention, Treatment and Education of Autistic and Related Communication-Handicapped Children (TEACCH), and the Early Start Denver Model (ESDM).295,303 Focused intervention practices are designed to address a single or limited range of skills, such as increasing social communication or learning a specific task, and may be delivered over a short period of time.295,297,303 Focused intervention practices may be behavioral, developmental, and/or educational. Focused interventions may be grounded in principles of ABA, in which specific skills are taught in a stepwise progression by using principles of reinforcement or developmental theory, in which the emerging skills inherent in neurobehavioral maturation are promoted. These interventions are provided in a structured setting by an adult, in naturalistic environments with peers, or as a component of a more comprehensive approach.295 Focused interventions may be effective for promoting skill development and communication.295,297,304,305

Pediatricians may be asked to advise families on therapy choices or write prescriptions for therapies.306 It is helpful for clinicians to have an understanding of intervention terminology and of the evidence base so they can effectively communicate the rationale for medically indicated treatment recommendations with families, educators, therapists, and other service providers as well as with insurance companies, health care administrators, funding agencies, and policy makers.295

This report describes various types of interventions provided for children and youth with ASD. Additional research is needed to evaluate the effectiveness of current approaches and develop interventions that address core deficits of ASD. At the time of diagnosis, parents of young preschool children may ask their provider to help them decide what type of intervention they should elect. Two common theoretical approaches to intervention for symptoms of ASD are ABA and developmental models.296–298,307 Although these approaches have important distinctions, they also have significant overlap, and interventions increasingly are incorporating aspects of both. There is considerable regional variation in the availability of various interventions. Table 9 describes common characteristics of empirically supported interventions.296,297,308,309

Approaches to Intervention

ABA

Most evidence-based treatment models are based on principles of ABA. ABA has been defined as “the
### TABLE 9 Characteristics of Effective Interventions

<table>
<thead>
<tr>
<th>Features of Practice</th>
<th>Common Characteristics of Empirically Supported Interventions</th>
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<tbody>
<tr>
<td>Assessment and Goals</td>
<td>Systematically assess skills</td>
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<td></td>
<td>Include input of family (shared decision-making)</td>
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<td>Select individualized measurable goals and instructional procedures on the basis of objective assessment of each child</td>
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<td></td>
<td>Use assessment-based, empirically supported instructional methods to build, generalize, and maintain skills and reduce problem behaviors</td>
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<tr>
<td>Instructional Methods</td>
<td>Address core symptoms in social communication and restricted and repetitive behaviors as well as skill deficits</td>
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<td>Provide a student/teacher ratio low enough to address the child's individualized goals</td>
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<td></td>
<td>Interventions should be by providers who are properly trained and should maintain fidelity with the treatment approach selected</td>
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<td></td>
<td>Ensure that multiple providers work collaboratively</td>
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<td>Services and Supports</td>
<td>Individualize services and support</td>
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<td>Make use of the child's interests and preferences in determining reinforcement systems</td>
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<td>Incorporate preferred activities to increase engagement in activities</td>
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<td>Provide a structured learning environment that helps children anticipate transition between activities, including a predictable routine and visual activity schedules</td>
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<td>Organize workspaces to minimize distraction and promote task completion</td>
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<td>Limit access to things that may distract a student</td>
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<td></td>
<td>The environment should promote opportunities for the student to initiate communication and interact with peers</td>
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<td>Behavioral Management</td>
<td>Implement a functional behavioral analysis to identify the reasons why challenging behaviors occur and develop a behavior improvement plan based on this assessment (IDEA-mandated approach)</td>
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<td></td>
<td>Teach children more appropriate responses using the behavior improvement plan</td>
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<td>Progress</td>
<td>Systematically measure and document the individual child's progress</td>
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<tr>
<td>Family Support</td>
<td>Adjust instructional strategies as necessary to enable acquisition of target skills</td>
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<tr>
<td>Transition Planning</td>
<td>Plan for transitions in school settings and to adulthood (eg, from home-based early intervention to preschool services, preschool to elementary school, elementary school to middle school, middle school to high school, high school to work or postsecondary education, and home to community living)</td>
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The process of systematically applying interventions based upon the principles of learning theory to improve socially significant behaviors to a meaningful degree, and to demonstrate that the interventions employed are responsible for the improvement in behavior. The use of ABA methods to treat symptoms of ASD suggests that behaviors exhibited can be altered by programatically reinforcing skills related to communication and other skill acquisition. ABA treatments may target development of new skills (eg, social engagement) and/or minimize behaviors (eg, aggression) that may interfere with a child’s progress.

ABA interventions vary from highly structured adult-directed approaches (eg, discrete trial training or instruction, verbal behavior applications, and others) to interventions in natural environments that may be child led and implemented in the context of play activities or daily routines and activities and are altered on the basis of the child’s skill development (eg, pivotal response training, reciprocal imitation training, and others). ABA programs are typically designed and supervised by professionals certified in behavior analysis. The majority of states at this time have licensure for board-certified behavior analysts with provisions for payment by insurance. ABA may be prescribed or recommended by a physician or licensed psychologist. A comprehensive ABA approach for younger children, also known as early intensive behavioral intervention, is supported by a few randomized controlled trials (RCTs) and a substantial single-subject literature. When only RCTs are considered, few interventions have sufficient evidence to be endorsed either for children younger than 12 years or for adolescents. Children younger than 12 years receiving more hours per week of ABA were found to be more likely to achieve the individualized goals identified in their programs. In retrospective studies, more intense ABA therapy was associated with achieving optimal developmental outcomes. Given the heterogeneity of the ASD phenotype, the service needs of children, youth, and adults need to be individualized by using available clinical data.

In some instances, a behavioral intervention is needed to address acute serious problem behaviors that...
must be given priority, for example, because of safety issues.295 Whether a student is getting formal ABA under IDEA or not, a family can request that challenging behaviors be evaluated in the school setting by using behavioral principles through a functional behavioral assessment. The target symptoms to treat may then be divided into component parts that are addressed in a stepwise fashion (task analysis).313,317 Once the reasons for the behavior are understood, a behavior improvement plan may be implemented.

Developmental Relationship–Focused Interventions

Intervention for young children also may be derived from developmental theory, which is focused on the relationship between the caregiver’s level of responsiveness and the child’s development of social communication.296,318–320 Through interaction with others, children learn to communicate and regulate emotions and establish a foundation for increasingly complex thinking and social interaction. Therefore, developmental models designed to promote social development in children with ASD are focused on the relationship between the child with ASD and his or her caregiver through coaching to help increase responsiveness to the adult (ie, the interventionist or parent or caregiver) through imitating, expanding on, or joining into child-initiated play activities. This approach may address core symptoms of ASD, such as joint attention, imitation, and affective social engagement.296,297,321,322

Developmental models for intervention are focused on teaching adults to engage in nondirective interactive strategies to foster interaction and development of communication in the context of play. One such approach is known as DIRFloortime (The Developmental, Individual Differences, and Relationship-Based model). In 1 RCT comparing parent coaching using this approach to community intervention alone (N = 112) in children ages 2 to 5 years, parents who were taught this approach were less directive, and their children were rated as more socially responsive, although IQ and language scores were no different between groups, and half of the children in the control group improved in their affective ratings.323 A similar approach is relationship development intervention,324 and more research is needed to evaluate efficacy and community use.

Naturalistic Developmental Behavioral Intervention

Naturalistic developmental behavioral interventions (NDBIs) incorporate elements of ABA and developmental principles, such as emphasis on developmentally based learning targets and foundational social learning skills, with delivery of interventions in the context of naturally occurring social activities within natural environments. They use child-initiated teaching episodes, naturally occurring opportunities for learning, and turn-taking interactions within play routines and implement ABA-based approaches to address measurable goals.296

The most extensively studied NDBI approach is the ESDM, which prepares children to learn in naturalistic environments.295 In a multisite trial of ESDM, early age at entry to therapy and more hours of total therapy were associated with improved outcome.326 Of note, the 48 children randomly assigned to ESDM or community treatment in the original trial were studied by using event-related potentials and spectral power on EEG while viewing faces as opposed to objects and were compared with typical controls on these tasks. This is an early demonstration of improvement on a neurophysiologic measure associated with improvement on a clinical measure of social behavior through an early intervention program.327

Combined Approaches

Common factors in combined developmental and behavioral approaches include use of principles of ABA to reinforce skill building; a systematic approach with a manual for training practitioners who would use the intervention in a standard fashion; individualized treatment goals for the child and means of measuring progress; child-initiated teaching, imitation, and modeling; and adult prompting that fades over time to promote independence.296 It may be difficult to advise parents on specific programs in community settings because the way the program is conducted may differ from the research settings.328 However, it is always accurate to describe the common characteristics of empirically supported interventions and recommend that families seek interventions that incorporate these features (Table 9).

Parent-Mediated Treatment or Parent Management Training

Increasing evidence reveals that focused interventions delivered by trained parents or other caregivers can be an important part of a therapeutic program.297,329–332 More RCTs have been published on parent-mediated therapies than on other nonpharmacologic interventions. What is sometimes called parent management training is divided into 2 categories: parent support and parent-mediated interventions. Parent support interventions, which are knowledge-focused and provide indirect benefit to the child, include care coordination and psychoeducation. Parent-mediated interventions, which are technique-focused and provide direct benefit to the child, may target core symptoms of ASD or other behaviors or skills and may be built on ABA approaches in natural settings.231
Training sessions for caregivers may be delivered in the home, clinic, school, or other community settings or remotely by telehealth. An RCT involving 86 toddlers and their primary caregivers demonstrated that 10 weeks of hands-on parent training in joint attention, symbolic play, engagement and regulation (an NDBI) was superior to a parent-only psychoeducational intervention for increasing joint engagement. A parent training approach may be used to promote compliance with instruction, social communication, and other identified goals of the caregiver, such as reducing maladaptive behaviors. Including parents in the intervention process is critically important.

**Educational Interventions**

**Classroom-Based Models**

It is the expectation that school-aged children will be educated in classroom settings with supports for a broad effect on the symptoms of ASD and associated deficits. Educating students with ASD in the least restrictive environment typically requires an individualized program that is modified to meet the Individualized Education Program (IEP) goals set by the family, student, and school team. Some students who do not qualify for an IEP by educational criteria may be supported with accommodations through a Section 504 plan or with classroom-level accommodations. Many students with ASD are educated in inclusive classrooms with supports. Other school-aged children and youth benefit from disorder-specific approaches. Examples of classroom-based models include Learning Experiences and Alternative Programs for Preschoolers and their Parents (LEAP) and TEACCH.

LEAP blends principles of ABA with special and general education teaching techniques for elementary-aged pupils in inclusive settings for teaching social interaction. An RCT of 294 preschool-aged children revealed that LEAP was associated with improvement in socialization, cognition, language, and challenging behavior and that LEAP was superior to a treatment-as-usual method. TEACCH class settings are visually organized to promote engagement and learning. The TEACCH approach to skill acquisition includes assessment-based curriculum development and an emphasis on structure, including predictable organization of activities and use of visual schedules, organization of the physical environment to optimize learning and avoid frustration (eg, by minimizing distractions and/or sensory dysregulation), and adaptation and organization of materials and tasks to promote independence from adult directions or prompts. Instruction is organized in a predictable fashion and uses visual schedules with promotion of independence in activities planned into the instruction. This approach is associated with a small, but measurable, benefit in perceptual, motor, verbal, and cognitive skills in students with ASD, with less measured effect on adaptive and motor function and challenging behaviors. Rigorous studies of educational interventions for students with ASD at school age and beyond are necessary to understand the effectiveness of different models.

A comparison of the effects of LEAP and TEACCH classrooms with those of standard special education classes taught by teachers familiar with ASD revealed that the common features of these interventions may be responsible for improvements seen in all students. TEACCH was associated with more reported improvement in ASD severity for students who had greater cognitive delays. This finding may speak to the benefit of the environmental and behavioral supports. Research interventions may not be comparable with community-provided school programs. Future research is needed to address how best to provide evidence-based intervention in classroom settings.

**Education in the Least Restrictive Educational Environment**

Pediatricians have an important role in advocating for children and youth with special health care needs, including ASD, in the educational setting. Students have a right to a free and appropriate public education. Educational programs for school-aged children with ASD should promote language, academic, adaptive, and social skills development and prepare them for postsecondary education or employment. Most, but not all, students with ASD will have some individualization of their education under the guidance of an IEP determined by the school multidisciplinary team in conjunction with the family. Others may receive accommodation and/or environmental modifications under Section 504 of the Rehabilitation Act of 1973. A medical diagnosis of ASD alone does not automatically translate into eligibility for school-based services. Functional impairment that affects participation in the typical curriculum is required to qualify for supports in the educational setting and may lead to an IEP for the educational handicap of autism. Most youth with ASD and average-range intelligence will likely require academic intervention because of coexisting learning disabilities, executive function challenges, ADHD, motor processing deficits, the effects of their pragmatic language differences on reading and writing, and/or challenges in comprehension of spoken or written language. Attention to the needs of the individual student must be central to the IEP process. Social skills of students with ASD may benefit...
School-aged children and adolescents with ASD, including those with typical academic skills, should have social skills support considered in their school and perhaps in other therapeutic settings if indicated. Although families identify the need to address social skill developments in settings outside of school, the success of these types of interventions is variable. Interventions may be divided into adult-mediated (skill building with the individual child), peer-mediated (skill building with the child and typically developing classmates), and mixed approaches. Child-directed social skills interventions are often delivered individually or in small groups with other children with similar needs. Therapy may be provided in behavioral health settings to complement the social skills interventions at school.

Interventions addressing social skills may increase the child’s knowledge of the cues for social behavior and teach strategies for social problem-solving. A popular method uses the social narrative to help a child define the social context of an anticipated or experienced situation, put it in perspective, and then develop statements on how it makes the child feel and on what to do in response to the event and feelings. This coached rehearsal strategy may be included within other programmatic approaches. Implementation may use a cognitive behavioral intervention strategy in which the child identifies feelings and thoughts and learns to substitute more socially appropriate alternatives. Video- and computer-based social skill interventions may extend access to intervention once an evidence base is established. A systematic review of RCTs of social skills training for children aged 6 to 21 years revealed that interventions improved social competence and friendship quality but did not result in differences in emotional recognition and social communication. Transfer of skills to other settings was inconsistent. Because child-mediated interventions taught separately from social settings have not had consistently beneficial effects, interventions have been developed for implementation in the social settings that include peers, such as the classroom and playground. These interventions demonstrate improved playground interaction between children with and without ASD and improved identification as friends by typical peers.

Peer-mediated intervention for students with ASD have revealed improved social connectedness and reduced social isolation and provide evidence to support the use of these interventions in the classroom and playground. An evidence-based approach designed for group administration, the Program for the Education and Enrichment of Relational Skills, may improve both teacher-reported social functioning and adolescent-reported social cognition. Fewer studies are available to guide programs to promote social skills development for adults with ASD. However, the Program for the Education and Enrichment of Relational Skills group model has been demonstrated to improve social skills in young adults with ASD.

Families should be counseled to include development of social skills with discrete goals and interventions in the IEP or educational plan as well as to be cognizant of potential opportunities to promote social interaction in the natural environment and in the context of other therapies. Implementing IEP goals across the day and generalizing specific skills to promote conversation and nonverbal communication, such as providing eye contact, directing facial expressions, and using appropriate gestures, is important, independent of age, and should involve both the caregivers and professionals. More information about IEPs in
general can be found at http://www.wrightslaw.com/info/iep.index.htm.

Other Therapeutic Interventions

Speech and Language Interventions

Delayed language is an early concern for many children who are later diagnosed with ASD. The communication symptoms included in the DSM-5 criteria for ASD reflect core deficits in social communication and interaction, such as failure of back-and-forth communication, deficits in nonverbal communication (such as eye gaze and use of gesture), difficulty adjusting behavior to suit the social context, and restricted and repetitive behaviors leading to perseverative vocalization, echolalia, and preoccupation with restricted topics of interest. All children with ASD should have documentation of specific coexisting speech and language diagnoses so that appropriate intervention might be provided.

Speech-language therapy is the most commonly identified intervention provided for children with ASD.\(^\text{361}\) The strategies used by speech-language pathologists to reinforce sound repetition and word use in children with typical development are often initially used with young children with ASD. Such strategies include reinforcement of speech sounds and communicative acts, imitation of the sounds the child makes, and exaggerated imitation and slowed tempo.\(^\text{362}\) The literature offers the most support for approaches with preverbal children with ASD in which adult prompts are used for communication, prompt fading, and reinforcement of their own attempts at communication. Intervention in naturalistic settings and involvement of caregivers may help reinforce the initiation of communication and functional use of sounds, gestures, and words.

A significant minority (up to 30%) of individuals with ASD ultimately do not acquire verbal speech.\(^\text{363}\) Delayed onset of speech may be complicated by general delays in development (intellectual disabilities) or coexisting speech disorders, such as childhood apraxia of speech. Although using communicative spoken phrases before age 4 years is considered a good prognostic sign for language development in youth with ASD, emergence of phrase speech may occur to at least age 10 years, especially in children with preserved nonverbal skills and evidence of social engagement.\(^\text{364}\)

When children do not spontaneously speak, augmentative and alternative communication (AAC) may be introduced. Examples of AAC strategies include sign language, the Picture Exchange Communication System, and speech-generating devices.\(^\text{365,366}\) The use of AAC may help promote social interaction and understanding of the purpose of communication and does not delay onset of speech. Indeed, it may enhance emergence of spoken words by pairing nonverbal and verbal communication.

The Picture Exchange Communication System is used to build communication through picture identification and exchange as communication. With training, pictures can be sequenced to build on communication.\(^\text{367}\) Picture strips that sequentially explain medical procedures, for example, take advantage of this approach. Use of speech-generating devices and programs that use AAC on digital tablets also are increasing. These devices provide acoustic feedback to the child, and touch-screen tablets are relatively inexpensive and portable. Medical providers are often asked to justify the purchase of touch-screen tablets or AAC devices. It cannot be assumed that the use of AAC alone will lead to functional oral communication without a therapeutic plan.\(^\text{368}\) Current scientific evidence does not support the use of facilitated communication in which a nonverbal individual is guided to communicate.\(^\text{369,370}\) This differs from AAC, in which the individual is taught to communicate independently. Future strategies to promote communication are expected to incorporate evolving knowledge about sensory processing and connectivity of brain functions in people with ASD.

Children and youth with ASD often have deficits in pragmatic language that can affect social interaction with adults and peers and academic performance as more complex language becomes required for reading comprehension and analysis of information. In addition, literal interpretation of language and difficulty in understanding the intent of other people leads to behavioral challenges in some people with ASD and affects success in school, leisure activities, and employment. School-aged students with spoken language should have their pragmatic language assessed as part of their school-related reevaluations, with consideration of pragmatic language testing if academic problems and inattention are noted in the classroom. Interventions may include individual and group approaches that include teaching and practicing conversation. The pediatrician may refer the child for private speech-language therapy if he or she is not eligible for services in school or if increased intensity of intervention is desired. Although the impact of speech-language therapy on structural language improvement has not been adequately studied, improvement in ratings of conversational competence by parents and of classroom learning skills by teachers supports the recommendation for social skills and social language interventions for students with ASD.\(^\text{371}\)

Motor Therapies

Children with ASD may have low muscle tone or a developmental
coordination disorder. Although the ages for sitting and walking do not differ between children with ASD and children with typical development, both fine and gross motor skills may be delayed in preschool-aged children with ASD.

Attention to position in space in children with a coexisting diagnosis of ADHD may further complicate delays in coordination.

Occupational therapy services may be indicated to promote fine motor and adaptive skills, including self-care, toy use, and handwriting.

Almost two-thirds of preschool-aged children with ASD are reported to receive occupational therapy services.

Similarly, some children with ASD may have gross motor impairment on formal testing that may benefit from therapeutic intervention focused on building strength, coordination, motor planning, or skill acquisition to promote safer mobility or play. Toe walking is common among children with ASD as well as in other developmental disorders in early childhood. The etiology of toe walking in ASD is unclear, although sensory aversion and habit or perseveration have been proposed. Common interventions for toe walking may include passive stretching, orthotics, and casting. Impairment in gross motor function may affect the capacity of a child with ASD to participate in leisure activities with the family or with peers and may impair participation in sports or interactive play beyond the effect of their social skills alone. Impaired motor skills may further decrease opportunities for social skills development and active learning and may be a risk factor for overweight and obesity. For motor therapies to be provided in the educational setting, a significant delay for age that affects function in school must be identified on a valid assessment measure.

**Sensory Therapies**

In 2012, the AAP published a clinical report, “Sensory Integration Therapies for Children With Developmental and Behavioral Disorders,” providing important background information and recommendations for pediatrics. Since that publication, the DSM-5 criteria now includes sensory symptoms in the diagnostic criteria for ASD in recognition of the fact that individuals with ASD have sensory challenges that may be related to repetitive and other challenging behaviors. Indeed, sensory symptoms exhibited by young children, such as food selectivity, covering their ears for certain sounds, and visual scrutiny of aspects of objects, may be among the earliest differences families identify in their children’s development.

Sensory goals may be included in treatment objectives for students with ASD. Adult-directed approaches provided through sensory-based interventions may be included in the context of motor and behavioral therapies and in educational settings. Despite the increasing scientific understanding of the neurobiological basis for sensory symptoms in individuals with ASD, empirical interventions in common practice have modest evidence to support their general use at this time. Commonly used sensory-based interventions, including brushing of the skin, proprioceptive stimulation by using weighted vests, or kinesthetic stimulation (such as swinging or use of specialized seating, such as a therapy ball, to modulate level of arousal), are not yet supported in the peer-reviewed literature.

Proponents of sensory integration therapies distinguish them from interventions with sensory modalities because of the active engagement with the child in skill building or desensitization. This type of therapy requires a trained clinician, often an occupational therapist, to work with a child by using play and sensory activities to reinforce adaptive responses. The therapist explains the child’s behaviors and responses to caregivers in sensory terms and provides them with strategies to help the caregivers accommodate the child’s sensory needs to decrease functional impairment and tolerate environmental triggers. Advocates of these interventions claim that dysfunction in integration of sensory input contributes to inefficiencies in learning and to behavioral challenges and that therapeutic approaches to sensory integration need to be considered separately from focal sensory-based treatments.

Although sensory-based therapies are among the most commonly requested therapies by caregivers, the evidence supporting their general use remains currently limited. As with any other intervention, specific goals for sensory-based therapies should be identified, and outcomes should be monitored so that the utility for any given child can be documented.

**Medical Management of Co-occurring Conditions**

Co-occurring medical and other conditions, such as seizures, sleep disorders, gastrointestinal (GI) disorders, feeding disorders, obesity, catatonia, and others, have a significant effect on the health and quality of life for children and youth with ASD and their families. In this section, the co-existing conditions commonly observed in children and youth with ASD are described, and anticipatory guidance and management strategies that primary care providers may consider are provided.

**Seizures**

There is both an increased risk for ASD among children and youth with epilepsy and an increased risk for seizures in those with ASD. The pooled risk for ASD among children
with epilepsy is 6.3%, with almost 5 times as many in samples with the highest rates of co-occurring intellectual disabilities. The rate of seizures among people with ASD in community-based populations has been reported to range from 7% to 23%, with rates as high as 46% reported in clinically ascertained samples. It has been suggested that the risk for seizures is not increased in individuals with ASD without intellectual disability. Risk factors for the increased likelihood of seizures in people with ASD include female sex, and lower gestational age. Specific genetic disorders associated with ASD, such as tuberous sclerosis, also may contribute to seizure risk in early childhood. Onset is bimodally distributed, with most first seizures occurring in early childhood and in adolescence; 20% of first seizures occur in adults with ASD. Children with ASD and seizures tend to have more behavioral challenges, independent of cognitive skills. Screening EEGs are not recommended for patients who are asymptomatic. An overnight EEG should be considered when the clinical history suggests seizures and atypical regression. Response to conventional antiepileptic drug therapy varies greatly, with some reports suggesting an increased risk for treatment-resistant epilepsy in individuals with early onset of seizures and delayed global development.

**GI Symptoms**

GI symptoms, such as abdominal pain, constipation, diarrhea, gastroesophageal reflux, and feeding problems, are more commonly reported in children and adolescents with ASD than in those with developmental delay or typical development. A large prospective cohort study revealed differences as early as 6 to 18 months of age in stooping patterns and feeding behaviors in children who were later diagnosed with ASD. Because of language delays and atypical sensory perception or report of pain, individuals with ASD may be less likely to report specific GI discomfort and may present with agitation, sleep disruption, or other behavioral symptoms rather than GI discomfort. Characteristics of ASD that might affect GI symptoms include resistance to change (feeding and constipation), comorbid anxiety (pain, feeding, and motility disorders), and altered sensory perception (pain, feeding, and constipation). At present, there is no evidence of an association of ASD with celiac disease, specific immune dysfunction, or motility disorders (eg, gastroesophageal reflux) in children with ASD.

It would be expected that these disorders would occur at least as frequently among individuals with ASD as among individuals in the general population, and they should be considered when the child has a history of GI symptoms or a change in behavior. Ongoing research is focused on whether differences are present in immunologic function, motility, or the microbiome in individuals with ASD.

Selective eating is common in children with ASD. A limited diet may influence GI symptoms, such as constipation, and alter the intestinal microbiota. GI disorders should be considered in patients with ASD if they present with typical GI symptoms or with agitation, food refusal, or sleep disturbance. The indicated GI workup will depend on the specific symptoms. Children with ASD should be offered the same approaches to treatment of GI disorders as other children. Modifications of conventional interventions to accommodate for symptoms of ASD might include consistent behaviorally informed approaches for constipation and encopresis.

**Feeding Disorders**

Up to three-quarters of children with ASD have problems related to eating, including food selectivity based on texture, color, or temperature; rituals around food presentation; and compulsive eating of certain foods. Behavioral refusal may also present as the child holding food in the mouth, volitional gagging, and emesis. Common related problems include pica (eating of nonfood items) and rumination (self-stimulatory emesis and reswallowing of stomach contents). By age 16 months, children who are later diagnosed with ASD are observed to be more selective in their eating patterns than are other toddlers. Problems around mealtimes and food choice often persist into adolescence. The frequency of feeding challenges in children and youth with ASD may relate to the core symptoms of restrictive and repetitive behavior and differences in sensory perception related to smell, taste, and texture.

Children with developmental delays may also have delayed oral motor skill development and may demonstrate food refusal of textures that they cannot physically chew or swallow. Discomfort can lead to food refusal, so initial evaluation should include consideration of gastroesophageal reflux, dental pain, food allergies, lactose intolerance, and significant constipation. If oral-motor concerns are observed, speech or occupational therapy assessment is indicated.

Because feeding problems are so common among children with ASD, a dietary history should be obtained at health supervision visits. Physiologic needs for macronutrients and micronutrients are the same for children with ASD as for other children. As with other children in the United States, insufficient intake of fiber, vitamin D, and calcium are common. Rare cases of severe nutritional deficiencies, such as...
rickets (vitamin D),403 scurvy (vitamin C),404 and keratoconus (vitamin A)405 have been reported in children with ASD with severe food aversions. If supplements are used to correct for poor vitamin D or calcium intake, it is important to confirm that the dose is sufficient for the age and sex of the child.406 Food fortification in the United States may supply adequate amounts of vitamins and minerals for some children with selective diets, so additional multivitamins may not be necessary.407 Consultation with a registered dietitian may be helpful to be able to guide families regarding the nutritional sufficiency of their child’s diet.

The clinician can counsel families about offering children routine meals and snacks, discouraging snacking through the day, promoting self-feeding, and using basic behavioral approaches to encourage mealt ime structure and predictability with minimal distraction. Children with ASD need to be offered new foods multiple times to become familiar with them. Feeding problems that affect nutrition or family function or that are specialized, such as mouth packing, rumination, severe pica, and intense aversions, are likely to need the support of professionals with expertise in behavior management and/or oral-motor therapies (speech or occupational therapy).408,409 Food refusal may stem from discomfort, so consultation with a gastroenterologist may be helpful. Gastrostomy-tube placement and nonoral feeding should only be considered after appropriate behavioral intervention has failed.

Obesity

Children and youth with ASD have greater risk for overweight and obesity than those in the general population.410–413 People with ASD have fewer opportunities and perhaps less interest for active leisure or organized sports, have repetitive eating patterns that may include energy-dense foods, and are more likely to be prescribed medications, such as atypical neuroleptics (or antipsychotic medication) and anticonvulsants, that often contribute to excessive weight gain. Sleep disorders may further predispose them for obesity. Primary care providers should monitor a child’s age-specific BMI percentile in the context of health supervision care and address modifiable risk factors through anticipatory guidance for their patients with ASD. Programs that address healthy weight for children and youth with typical development may need to be modified for successful use for patients with ASD.114

Dental Health

Children with ASD commonly have unmet dental needs. Difficulty cooperating with hygiene and professional care are reported barriers for dental care. Even when insurance coverage is available, children with ASD have fewer visits for routine care.415 There are limited data about the prevalence of caries or gingival disease in children with ASD. As with other children, anticipatory guidance should include attention to dental hygiene and fluoride use, if appropriate, from a young age. Behavioral strategies may be helpful to prevent the need for dental care under sedation.

Pica

Children and youth with ASD may put nonfood items in their mouths long after the developmental period of early childhood, when pica is expected. Pica is reported in up to one-quarter of preschool-aged children with ASD and is documented to persist in individuals with intellectual disability.416,417 The persistence of pica may be attributable to sensory differences, perseveration or obsession, and oral exploration of the environment. Clinicians need to be aware of persistence of pica in children and youth with ASD because of the risk for toxic ingestions, risk for lead intoxication, potential for infection, and the risk for mechanical ingestions ranging from batteries to bezoars.418 Obstruction and perforation need to be considered in children with pica who have acute abdominal symptoms. Iron deficiency is associated with pica in the general population.419 Laboratory monitoring of blood lead and iron deficiency in children with pica is suggested in the context of primary care. Behavioral intervention includes reinforcing appropriate behaviors, ensuring adult supervision, and putting into place environmental safeguards for prevention.

Sleep Problems

Sleep disturbance is common in individuals with ASD and may be associated with exacerbation of problematic daytime behavior.420–427 Problems with initiating and maintaining sleep are reported for 50% to 80% of children with ASD.428 Children who are later diagnosed with ASD are reported to have had sleep problems by 30 months of age.429 Sleep problems in individuals with ASD persist; almost half of adolescents with ASD continue to have sleep symptoms.430 Adolescents are more likely to have shorter sleep duration, daytime sleepiness, and delayed sleep onset compared with younger children with ASD, who are more likely to have bedtime resistance, parasomnias, and nightwaking. Reasons for the increased frequency of sleep disturbances in children and youth with ASD may include differences in melatonin metabolism,431 developmental disruption of other neurotransmitter systems critical to sleep, and lack of social expectations, among other explanations. Genetic disorders, such as Smith-Magenis syndrome, are associated with both ASD and sleep disruption.432 Biological reasons for disrupted sleep that are not unique to
children with ASD may include restless leg syndrome, which may be associated with low iron stores, and coexisting neurologic or behavioral diagnoses, such as epilepsy, anxiety, ADHD, or mood disorders. The most common cause of both delayed sleep onset and night wakings are learned behaviors. As with other children, the evaluation of the child with ASD with delayed sleep onset, night wakings, and/or early-morning wakings should include a history of comorbid medical conditions that might disrupt sleep, such as gastroesophageal reflux, seizures, asthma, allergies, eczema, or enuresis. Snoring might suggest obstructive sleep apnea and would prompt referral for additional assessment. Children who play video games or engage in other screen time close to bedtime have later bedtimes and may have more difficulty falling asleep. Restless sleep and night wakings would suggest a need for laboratory evaluation for ferritin stores might be present. An environmental history of the household may help to determine if household noise, parental work hours, or other factors may affect sleep. The bedtime routine and response to night-waking should be reviewed to determine the behavioral approaches to consider.

Empirical support exists for the effectiveness of parent education and behavioral interventions for children with ASD and sleep disturbances. Behavioral intervention includes parents establishing bedtime routines and making clear their expectation that the child sleeps in his or her own bed. This may be difficult to establish for children with ASD, who may not appreciate the social conventions around sleep time and may have repetitive rituals and comorbid anxiety or ADHD. Despite these challenges, behavioral strategies are successful when consistently implemented.

No medication is currently approved by the US Food and Drug Administration for the treatment of insomnia in children with or without ASD. Any medication elected should be started at a low dose and monitored for adverse effects. Sleep onset may be aided by treatment with melatonin at doses from 1 to 6 mg and may be maintained with long-acting melatonin. Adverse effects are uncommon but may include nightmares. α-adrenergic agents (eg, clonidine) and antihistamines (eg, diphenhydramine) are often prescribed to help with sleep onset or to address night-waking in children, but the literature provides little support for their use.

Disordered sleep is associated with challenging daytime behaviors in children with ASD, addressing one may help with the other.

Wandering

Accidents, including drowning, are a major cause of morbidity and mortality in children and youth with developmental disabilities, including ASD. Children and youth with ASD may have decreased awareness of social convention and community rules as well as impulsivity and perseverative interests that draw them to potential dangers, such as bodies of water and busy roads. Wandering off (also called elopement) places them at risk for injury. Wandering, if present, should be included in the problem list as a coexisting diagnosis in patients with ASD. In an online study, 1218 families of children with ASD were questioned about elopement. Nearly half of children with ASD between the ages of 4 and 10 years had tried to elope. Almost half of those children were missing long enough for their parents to contact the police. Of those children, approximately two-thirds were at risk for traffic-related injury and almost one-third were reported to have had near-drowning episodes.

Data from a national survey revealed that elopement attempts in the past year were reported by approximately one-third of parents whose children had ASD with or without intellectual disability. Wandering may persist into adulthood.

In the survey by Anderson et al, parents reported that the most common perceived reasons for elopement were enjoyment of running, attempts to get to a desired location (such as a park), pursuit of an intense interest (eg, water), and escape from situations or sensory events that made them anxious. Because the risk for elopement increases with the severity of ASD and with co-occurring intellectual disabilities, many of the individuals at greatest risk have limited language and cannot tell first responders their names, addresses, or phone numbers if they get lost. Police may interpret aggression caused by fear as combative behavior.

Prevention is the most important intervention for elopement. Parents participating in a large national survey of children with special health care needs reported primarily using physical and electronic barriers to try to prevent elopement, especially in children who also had intellectual disabilities. Information on prevention and management of wandering is available for parents and clinicians (http://nationalautismassociation.org/big-red-safety-box/). Consistent, adequate adult supervision is important in all environments: school, home, and community settings. Families note that increased supervision needs result in increased family stress. Families may need to consider deadbolts, fencing, and alarm systems for safety as well as personal GPS devices and identification bracelets or other identification. Local law enforcement
agencies may support GPS tracking. Alerting neighbors and local law enforcement officials as well as securing pools in the neighborhood and creating a family emergency plan are suggested. If impulsivity and motor hyperactivity contribute to elopement, examining the utility of medication as part of an overall plan may be considered. Similarly, addressing sleep issues becomes important if the child is at risk for wandering at night. Teaching safety skills and appropriate community behaviors is critical to prevention. All children with ASD, no matter their level of cognitive skills, are at risk for wandering.449

Motor Disorders

There is increasing appreciation that individuals with ASD may have developmental coordination disorder and other neurologic problems. Tic disorders occur with an increased frequency in children with ASD.450 Distinguishing complex tics from stereotyped movements may be challenging.

Catatonia was added as a possible coexisting condition to ASD in the DSM-5. Slow initiation of movement and reported deterioration in motor performance have been treated with lorazepam, electroconvulsive therapy, and behavioral interventions, but the therapies do not have a strong literature base.451 Later loss of motor skills in adolescence should prompt evaluation by a neurologist for underlying reasons. Regression in language or social interest is reported in approximately one-quarter of children later diagnosed with ASD. It is recognized most commonly between 18 and 24 months of age. Regression later in childhood requires evaluation.

Co-occurring Behavioral Health Conditions

Co-occurring behavioral symptoms include hyperactivity or inattention, aggression, outbursts, and self-injurious behaviors. Although these behaviors are not core features of ASD, they commonly interfere with functioning in school, at home, and in the community and contribute substantially to the challenges faced by families.293,294,381,452–457 Psychiatric conditions (such as ADHD, anxiety, OCD, mood disorders, conduct disorders, or others) are identified in 70% to 90% of children and youth with ASD.458,459 Behavioral challenges have a significant effect on health and quality of life for children and adolescents with ASD and their families.460 Patients with ASD, like other children and adolescents, should be regularly screened for behavioral and/or emotional conditions, as recommended by the AAP.461 The effect of behavior on home and school functioning is often assessed as part of school testing by using parent and teacher questionnaires, such as the Behavior Assessment System for Children, Third Edition, Parent Rating Scales,79,462 or the Child Behavior Checklist.82,463,464

With change in behavioral symptoms, physical sources of discomfort and behavioral intervention should be considered.465 If behavioral interventions are insufficient to address the challenges or are unavailable at the time, medication might be considered (see Table 10 for guidance on prescribing medication).

ADHD

Changes in DSM-5 criteria have provided flexibility to diagnose other DSM-5 disorders in addition to ASD, which can help guide treatment. Approximately half of children and youth with ASD also may fulfill diagnostic criteria for ADHD.459 Pediatricians should keep in mind that some children who are later diagnosed with ASD may have been initially identified as having ADHD.466 Symptoms of ADHD may further compromise social skills function in children with ASD because of inattention to social cues and impulsivity. Standard rating scales used to assess symptoms of ADHD have not yet been validated for

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**TABLE 10 Considerations Surrounding Medication Use**

<table>
<thead>
<tr>
<th>Medication should only be considered after</th>
<th>Coexisting behavioral health disorders (eg, ADHD, mood disorders, or anxiety disorders)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Careful accounting of when the behavior started and what seems to exacerbate it</td>
<td>Associated problem behaviors or symptoms causing significant impairment and distress</td>
</tr>
<tr>
<td>A functional behavioral assessment should guide development of a treatment plan in the school setting</td>
<td>o Examples include the following: aggression, self-injurious behavior, sleep disturbance, mood lability, anxiety, hyperactivity, impulsivity, inattention</td>
</tr>
<tr>
<td>Consider whether the behavior serves as communication of distress or refusal</td>
<td>Consider referral to a behavior therapist outside of school to assess the reasons for the behavior; provide the family with strategies, and collaborate in care</td>
</tr>
<tr>
<td>Careful history and physical to look for medical factors that may cause or exacerbate challenging behaviors (eg, gastroesophageal reflux and acute sources of pain, such as otitis media, dental injury, fracture, and others)</td>
<td>Consider medication after treatable medical conditions and behavioral factors assessed and intervention does not address the symptoms of concern</td>
</tr>
</tbody>
</table>

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Include the family and patient in shared decision making that considers their goals and values443
individuals with ASD. However, they are useful in determining the clinical impact of symptoms for an individual patient and in monitoring treatment. It is important, however, to consider the differential diagnosis of inattention and hyperactivity in the context of the language impairment and perseverative focus that often accompanies ASD. Children with delayed language may appear more inattentive. If they are expected to perform activities (including schoolwork) that they are not able to understand or accomplish, a child with ASD may engage in behaviors to escape, which can be interpreted as inattention and hyperactivity. Patients with ASD may be focused on their perseverative interests and may be internally distracted, as opposed to distracted by the environment. Evaluation of the symptom of inattention or impulsivity includes assessing language and educational abilities. Appropriate educational modifications and use of language for instruction that the student can understand are critical for successful intervention. Behavioral strategies should address reinforcement of on-task behaviors, breaking down tasks into units that can be completed successfully, breaks for activity (often included in sensory activities), and adult supervision appropriate for the demands. The same medications that are used for symptoms of ADHD in children without ASD are used in similar doses for children with ASD. Routine monitoring is important because children with ASD may be at greater risk for adverse effects (Table 11). The evaluation of a child for a possible co-occurring diagnosis of ADHD also should include consideration of a co-occurring diagnosis of anxiety.

**Anxiety Disorders**

The DSM-5 classification system separates anxiety disorders into separation anxiety disorder, selective mutism, specific phobia, social phobia, panic disorder, agoraphobia, and generalized anxiety disorder as well as unspecified anxiety disorder. As many as 40% to 66% of school-aged children and adults with ASD are reported to also have anxiety disorders. Anxiety disorders are most commonly identified in children with ASD and typical cognitive and language abilities. Symptoms may be present in early childhood and manifest as behavioral challenges, such as overreactivity. Biological predisposition to both ASD and anxiety may be attributable to common genetic factors and/or altered neurophysiologic responses to stress.

Core symptoms of ASD decrease the ability of individuals with ASD to predict the actions or interpret the beliefs of others, which may lead to a constant state of heightened worry. Repetitive behaviors may, in part, serve to instill predictability, so anxiety may lead to increased stereotyped behaviors or perseverative thoughts. Evaluation of anxiety requires consideration of the language demands of the environment, academic expectations, social demands, and underlying fears or phobias. Youth with ASD may lack sufficient language or insight to describe their symptoms. Getting information from multiple sources and looking at the behavioral manifestations related to context will help to correctly identify anxiety in patients with ASD.

Strong evidence from RCTs supports the use of cognitive behavioral therapy for anxiety symptoms in school-aged children with ASD, especially those with typical-range intelligence. Anxiety may be associated with reported GI and sensory symptoms. Some individuals find that sensory redirection or sensory activities used in the context of a behavioral program are helpful to diminish feelings of anxiety. Other individuals may find symptom relief with the introduction of routine and structure if anxiety is exacerbated by uncertainty or associated with sensory under- and overreactivity. Nonpharmacologic approaches, such as neurofeedback and digitally delivered approaches to self-regulation, are being evaluated for their therapeutic potential. Medications used for anxiety in the general population may be considered as part of an overall treatment plan for children and youth with ASD (see Table 11 for psychopharmacotherapy of children with ASD and anxiety).

**Mood Disorders**

Depressive disorders are more common among children and adults with ASD than in the general population. Reported rates of coexisting depression in adults and children are highly variable, ranging from 12% to 33%. Symptoms of depression are more likely to lead to dual mental health and developmental disability diagnoses in adolescents and adults with ASD than in children. The coexistence of mood disorders and ASD may be associated with genetic and neurobiological factors as well as environmental factors related to chronic stress and difficulty with understanding social situations. Both elevated and depressed mood may present as behavioral symptoms in youth with ASD. Changes in affect, participation, sleep habits, and eating may be symptoms of an underlying mood disorder. Attempted suicide is reported to occur more frequently in people with ASD than in the general population. Risk factors include peer victimization, behavioral problems, minority race or ethnicity, male sex, lower socioeconomic status, and lower level of education. The AAP recommends screening for depression in patients older than 12 years. Until ASD-specific measures are developed, the same approaches used for all other adolescents at increased risk for depression should be considered.
### TABLE 11: Psychotropic Medication Options for Common Target Symptoms

<table>
<thead>
<tr>
<th>Target Symptoms</th>
<th>Medication Class (Examples)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperactivity</td>
<td>Psychostimulants (methylphenidate, dexamphetamine, mixed amphetamine salts, lisdexamfetamine, dextroamphetamine) with other coexisting symptoms, medication may not appear as effective</td>
<td>May be more sensitive to adverse effects</td>
</tr>
<tr>
<td>Impulsivity</td>
<td>SNRIs (atomoxetine) with other coexisting symptoms, medication may not appear as effective</td>
<td>Steps:</td>
</tr>
<tr>
<td>Inattention</td>
<td>α-2 adrenergic agonists (clonidine, guanfacine)</td>
<td>Problems persist, trial of medication management</td>
</tr>
<tr>
<td>Distractibility</td>
<td>Atypical (second generation) antipsychotics (aripiprazole, risperidone)</td>
<td>Start with a low-dose stimulant (eg, methylphenidate or mixed dextroamphetamine salts) and increase as needed and tolerated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May be most effective in children without comorbid intellectual disability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Targets symptoms of impulsivity and hyperactivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If there are adverse effects or if not effective: Consider atomoxetine, especially if also with social anxiety</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Consider α-2 agonists (eg, short- or long-acting guanfacine, clonidine)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other medications (less evidence): atypical antipsychotic medications may decrease hyperactivity; their primary use is for irritability and aggression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adverse effects:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Psychostimulants: appetite suppression and insomnia; also irritability, depressive symptoms, and social withdrawal; it does not appear to worsen repetitive behavior or oppositional behavior</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Guanfacine, clonidine: drowsiness, fatigue, and irritability; may also include appetite suppression, nausea, sleep disturbance, and decreased blood pressure and heart rate; rebound if not weaned</td>
</tr>
<tr>
<td>Irritability and severe disruptive behavior</td>
<td>Atypical (second generation) antipsychotics (aripiprazole, risperidone) with other coexisting symptoms, medication may not appear as effective</td>
<td>Medication most effective if combined with behavioral strategies addressing identified environmental causes for the behavior and developing more appropriate responses for the child</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DB/PCs strong support for 2 second-generation atypical antipsychotic medications (risperidone and aripiprazole) for reducing irritability, stereotyped or repetitive movements, self-injury, and hyperactivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Risperidone and aripiprazole are currently the only medications with FDA-approved labeling specific to irritability in ASD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adverse effects and monitoring:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Common adverse effects include wt gain and dyslipidemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monitoring: periodic assessment for extrapyramidal symptoms; measurement of wt, height, and BMI; and laboratory monitoring of glucose and lipid levels</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Metformin might be a useful treatment to help control wt gain.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other agents in this class, such as olanzapine and quetiapine, may have utility on the basis of their adverse effect profiles but do not have current FDA package insert indication for use in children with ASD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Small studies documenting beneficial effects on irritability; need larger trials; may have better adverse effect profiles than atypical antipsychotics</td>
</tr>
<tr>
<td></td>
<td>α-2 adrenergic agonists (clonidine, guanfacine) with other coexisting symptoms, medication may not appear as effective</td>
<td>Few studies focused on irritability and/or aggression; some reporting improvement in irritability; insufficient evidence to advise practice</td>
</tr>
<tr>
<td></td>
<td>SSRIs (fluvoxamine, citalopram) with other coexisting symptoms, medication may not appear as effective</td>
<td></td>
</tr>
</tbody>
</table>

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**Legend**: DB/PC = Disruptive Behavior Problem Checklist; FDA = Food and Drug Administration; BMI = body mass index.
<table>
<thead>
<tr>
<th>Target Symptoms</th>
<th>Medication Class (Examples)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repetitive behavior</td>
<td>Anticonvulsant mood stabilizers (valproic acid and divalproex sodium)</td>
<td>Small studies suggestive of improvement in irritability; need larger studies; a limited number of placebo-controlled studies either do not support or are inconclusive regarding anticonvulsant medication as a treatment of irritability in patients with ASD</td>
</tr>
<tr>
<td></td>
<td>Serotonin-norepinephrine reuptake inhibitor (venlafaxine)</td>
<td>Effect size of improvement associated with venlafaxine was small, and irritability was not the primary outcome measured</td>
</tr>
<tr>
<td></td>
<td>Atypical (second generation) antipsychotics (aripiprazole, risperidone)</td>
<td>Multiple DB/PCs documenting improvement in repetitive behavior; short-term treatment</td>
</tr>
<tr>
<td></td>
<td>Anticonvulsants (valproic acid and divalproex sodium)</td>
<td>May have improvement with topiramate as a second agent with risperidone</td>
</tr>
<tr>
<td></td>
<td>SSRI (fluoxetine, fluvoxamine)</td>
<td>Studies to date have not revealed effectiveness of SSRI medications for repetitive behaviors related to ASD, although they may diminish anxiety</td>
</tr>
<tr>
<td>Anxiety, depression</td>
<td>SSRI(s)</td>
<td>Anxiety relief has been reported in trials of citalopram and buspirone, with fluvoxamine revealing some effect in female patients with ASD; documented utility in children and youth without ASD</td>
</tr>
<tr>
<td></td>
<td>α-adrenergic (clonidine, guanfacine)</td>
<td>Hyperactivation is an adverse effect of SSRIs in children and youth with ASD that may result in stopping the medication</td>
</tr>
<tr>
<td></td>
<td>Atypical (second generation) antipsychotics</td>
<td>If a mood dysregulation disorder is identified, treatment with a mood stabilizer and/or a second-generation antipsychotic is recommended, although an SSRI may be used to treat comorbid anxiety, OCD, or depression; behavioral activation with hypomanic or manic switches has been reported</td>
</tr>
</tbody>
</table>

As in children and youth with typical development, assessment of depression and other mood disorders must include family history, history of environmental stressors, the potential for toxic ingestions, and evaluation for comorbid conditions. Interventions for depression include supportive therapy, cognitive behavioral therapy, and medication, if indicated, as coordinated interventions (see Table 11 for medication use). Antidepressant use in people with ASD has not been demonstrated to address aggression and has inconsistent effect on anxiety. Medication recommendations are based on data from the general pediatric population and expert consensus.

The DSM-5 criteria for bipolar illness include changes in activity, energy, and mood. It may be difficult to make a diagnosis in people with ASD with limited language. The co-occurrence of bipolar illness and ASD in individuals with typical intelligence ranges from 6% to 21%. Lifetime diagnosis of bipolar illness in adults with ASD is reported to be 9%.

**OCD-Related Disorders**

Although restricted and repetitive behaviors are symptoms of ASD, some individuals with ASD may also have coexisting OCD. Obsessions are recurrent, unwanted, and persistent thoughts, images, or urges that cause distress. Compulsions are repetitive behaviors or thoughts with rigid rules performed to reduce anxiety. Unlike the stereotypic behaviors of ASD, compulsions usually follow an obsession, diminish anxiety, and are not desired by the individual or perceived as pleasurable. Under the DSM-5, OCD-related disorders include hoarding disorder, excoriation (skin-picking) disorder, trichotillomania, substance- or medication-induced obsessive-compulsive and related disorder, and obsessive-compulsive and related disorder due to another medical condition. The perseverations associated with ASD may be qualitatively different and less sophisticated than the repetitive and intrusive thoughts and actions associated with OCD. Repetitive behaviors in general may help an individual with ASD regain a sense of predictability. Anxiety, phobias, and/or depression may coexist with OCD in youth with ASD.

Behavioral approaches are recommended as the first line of treatment of symptoms of OCD, depending on the language and cognitive level of the patient. Cognitive behavioral therapy, including exposure and response prevention with or without a selective serotonin reuptake inhibitor, has been demonstrated to be the most effective treatment for youth with OCD who do not have ASD. Cognitive behavioral therapy may be less effective, with fewer remissions, in youth who also have ASD (see Table 11 for medication management).

**Disruptive Behavior Disorders:**

*Aggression, Self-Injurious Behavior, and Tantrums*

Disruptive behaviors, such as aggression, self-injury, and tantrums, may complicate home and community management of individuals with ASD. Behavioral outbursts may occur in response to stressful events in the environment, in reaction to a medical condition, as functional communication, or as a symptom supporting diagnosis of a co-occurring mental health disorder. Functional behavioral analysis and implementation of behavioral strategies can be an important initial step in management. A proposed pathway for the primary care setting for management of irritability that leads to disruptive behaviors in youth with ASD is proposed by McGuire et al. Disruptive behaviors may serve as communication to escape from a demand or an undesired situation. If successful, they may become part of a behavioral pattern. New onset of severe behaviors requires consideration of potential medical reasons (see Table 12). Pharmacologic treatment should be considered if no medical etiology is identified and if the behavior is associated with irritability, is not responsive to available behavioral interventions, or is related to a co-occurring diagnosable behavioral health disorder, such as anxiety, mood

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**TABLE 12 Common Presentations of Self-Injurious Behavior and the Medical Conditions to Consider If New Onset**

<table>
<thead>
<tr>
<th>Type of Self-Injury</th>
<th>Potential Associated Conditions</th>
<th>Potential Associated Injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head banging</td>
<td>Headache, toothache, sinus infection, ear infection</td>
<td>Detached retina, abrasions, contusions</td>
</tr>
<tr>
<td>Head hitting or slapping</td>
<td>Headache, toothache, sinus infection, ear infection</td>
<td>Fracture of bones in hand, detached retina, abrasions, contusions</td>
</tr>
<tr>
<td>Eye poking</td>
<td>Vision loss, eye pain</td>
<td>Eye abrasion</td>
</tr>
<tr>
<td>Gum or tooth digging or</td>
<td>Dental pain, gingivitis</td>
<td>Gum injury, tooth autoextraction, tooth fracture</td>
</tr>
<tr>
<td>banging</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scratching and skin</td>
<td>Allergy, eczema, drug reaction, skin infection or infestation (eg, fleas, scabies)</td>
<td>Infection, scarring</td>
</tr>
<tr>
<td>picking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finger and toenail biting</td>
<td>Pain</td>
<td>Infection, nail removal, ingrown nails, paronychia</td>
</tr>
<tr>
<td>or picking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kicking or stomping</td>
<td>Restless leg syndrome, leg pain</td>
<td>Bruises, fractures</td>
</tr>
<tr>
<td>Rumination</td>
<td>Gastroesophageal reflux, eosinophilic esophagitis</td>
<td>Esophageal ulceration and bleeding, dental damage, nutritional compromise, precancerous lesions of esophagus</td>
</tr>
</tbody>
</table>
disorders, thought disorders, and/or ADHD.

It has been reported that between 8% and 68% of children with ASD demonstrate aggressive behavior, depending on how stringent the definition is. Aggressive behaviors were reported on the Child Behavior Checklist for one-quarter of children attending an ASD clinic, with similar rates from 2 to 16 years of age. Aggression was associated with hyperactivity, lower cognitive skills, sleep problems, and internalizing behaviors such as anxiety. There was no association with sex. Researchers of other studies have observed increased rates of physical aggression in children with ASD who have lower adaptive skills and frequent repetitive behavior. Management of co-occurring sleep problems and hyperactivity may be helpful in a treatment plan that includes behavioral intervention to address aggression and targeted pharmacotherapy.

Self-injurious behaviors are reported in 40% to 50% of individuals with ASD at some point across the lifespan and may occur more frequently in people with ASD who also have aggressive behaviors and sleep problems. Self-injurious behaviors in individuals with ASD may be repetitive and self-stimulatory (such as scratching, pica, or rumination). Head banging and self-hitting may occur as part of a tantrum. Like aggression and other disruptive behaviors, self-injurious behaviors may serve as communication to escape from demands or situations that the individual does not want to be in. The type of self-injurious behavior may change if the intervention of prevention or blocking is not associated with addressing the underlying reason for the behavior. Persistence of self-injurious behaviors in individuals with ASD is associated with more limited cognitive and language abilities, hyperactivity, impulsivity, repetitive behavior, and more challenges with social interaction. There is an association of self-injury with specific genetic disorders that are not associated with ASD, such as the severe self-biting of Lesch-Nyhan syndrome. Self-injurious behavior is associated with genetic disorders that are also associated with ASD, such as Cornelia de Lange syndrome, fragile X syndrome, and Smith-Magenis syndrome. In the case of aggressive, self-injurious, and disruptive behaviors, the primary care provider needs to assess the safety of the child and family in an ongoing fashion. Referral to community services and for behavioral intervention should take place if behaviors are unsafe or if the patient is not responding to the treatment plan.

**Psychopharmacologic Approaches to Management**

The use of medications to treat behavioral and psychiatric symptoms in children and youth with ASD has increased significantly since the publication of the 2007 AAP clinical reports. With a shortage of specialists, more medication management, including prescription of atypical antipsychotic medications, is taking place in the primary care setting. Large national studies of insurance claim data from Medicaid and commercial insurers reveal rates of psychopharmacology prescription for patients with ASD to be 56% to 65%. One or more psychotropic medications are prescribed for 1% of children with ASD younger than 3 years, for 10% to 11% of children aged 3 to 5 years, for 38% to 46% of children aged 6 to 11 years, and for 64% to 67% of adolescents aged 12 to 17 years. Psychotropic medication use increases with increased age, lower range of cognitive skills and/or presence of intellectual disability, and higher prevalence levels of challenging behavior or coexisting psychiatric diagnoses. Prescription of medication also appears to be affected by demographic factors, such as race, ethnicity, and geography. Reported polypharmacy rates range from 12% in a registry cohort recruited from diagnostic clinics to 29% to 35% in large studies of Medicaid claims data.

Medication may be helpful to address co-occurring symptoms or disorders. Clinicians should carefully weigh potential risks and benefits before prescribing medication for behavior and use psychotropic medications as part of a comprehensive treatment approach. The prescribing clinician should understand the indications and contraindications, dosing, potential adverse effects, drug-drug interactions, and monitoring requirements of the medications they prescribe. Table 10 provides guidance for principles of prescribing medication, and Table 11 lists pharmaceutical options for common behavioral-symptom clusters. Psychopharmacogenetic testing for genetic variants that increase the likelihood of adverse effects is an emerging area for precision medicine. Prescribers should consider CYP2D6 and CYP2C19 metabolizer status in making medication decisions for selective serotonin reuptake inhibitors (SSRIs), for example, despite limited data at present to guide practice. The limited data on the utility of psychopharmacogenetic testing at the time of this publication limits insurance coverage for many patients. Recommendations for testing are expected to rapidly change with ongoing research.

**Areas of Psychopharmacologic Research**

As the neurobiology of ASDs are better understood, novel psychopharmacologic agents might be developed that will better manage
co-occurring symptoms and/or address core deficits. Some potentially important lines of research involve medications that modulate metabolism of excitatory neurotransmitters (such as glutamate and γ-aminobutyric acid), block acetylcholinesterase and/or nicotinic acid receptors, and act as hormones that naturally promote social affiliation (such as oxytocin and vasopressin). Drug trials involve newly formulated agents as well as repurposing exiting medications used for other purposes.506–509

Better understanding of the neurobiology responsible for the symptoms of ASD will allow for the identification of targeted psychopharmacologic interventions. The use of psycho-pharmacogenomics to identify which patients might genetically be at greater likelihood of benefit or at increased risk for adverse effects from specific medications is an important area of research.510

**Integrative, Complementary, and Alternative Therapies**

Despite the advances in understanding the neurobiology of ASD, many unanswered questions remain about why ASD occurs and how best to treat it. Families often consider nutritional interventions and nonmedical therapies without a scientific evidence base to address the symptoms that conventional interventions cannot rapidly address, or there is limited access to conventional services in their community. Primary care providers are often asked about nonstandard interventions that are used in integrative practice or are promoted on the Internet, in the popular press, by other families, and by celebrities.511–516 The National Center for Complementary and Integrative Health maintains a Web site in which current information on novel therapies in popular use for people with ASD is reviewed.517 In the past decade, an increasing number of interventions based on theories of causation of ASD that are, as yet, unproven have been examined in clinical trials. Appropriately designed trials have provided evidence to support some interventions, such as the dietary supplement melatonin, and have disproven others, such as secretin.518 Many interventions, although still widely used, remain unproven.

Complementary therapies are often attractive to families because they are purported to correct putative biological causes of behavioral symptoms and may be discussed with an optimism about outcome that is often not conveyed with the recommendation for conventional therapies. Between 28% and 74% of children with ASD are given at least 1, and usually more than 1, complementary therapy.519–521 Although use of novel therapies is common among children with a range of developmental disabilities, children with ASD who are irritable or overactive or who are reported to have food allergies may be more likely to be given additional therapies.522

Complementary, alternative, and integrative therapies used for ASD can be grouped into 3 general areas: (1) natural products (including herbs, vitamins and minerals, and probiotics), (2) mind and body practices (including yoga, chiropractic, massage, acupuncture, progressive relaxation, and guided imagery), and (3) other therapies (including traditional medicine and naturopathy).517

Dietary interventions used to treat symptoms of ASD are perceived by many families as beneficial because they are natural and without adverse effects. Dietary elimination of gluten- and casein-containing foods is often implemented in an attempt to ameliorate core symptoms of ASD, not on the basis of allergy or celiac disease.523,524 The double-blind clinical trials to date have not demonstrated a treatment effect with diet.524,525 Whether a subgroup of children with GI symptoms might benefit from these or other dietary interventions requires additional study. Children may be adequately nourished on a casein-free diet with calcium and vitamin D supplementation. Nutritional counseling is recommended if a trial of this diet is elected.506 It may be that improvement in unrelated conditions may influence behavioral symptoms (eg, removal of dairy products may decrease irritability attributable to lactose intolerance).

Dietary supplements are often given to children who are selective eaters by their families to compensate for a limited diet.506 However, many children with ASD are given vitamins and minerals to treat proposed biochemical abnormalities that have been proposed to be unique to ASD. Popular dietary supplements include vitamin D,526,527 vitamin B12,528 vitamin B6 with magnesium,529 omega-3 fatty acids,530 and multivitamin preparations. The literature to date is controversial with respect to vitamin supplementation as a treatment of symptoms of ASD, and at this time, no conclusive evidence exists that people with ASD require different nutrient intake than that recommended in the Dietary Reference Intakes (https://www.ncbi.nlm.nih.gov/books/NBK225472/). The long-term risks of high-dose supplementation have not been studied.531 Although maternal folic acid status may provide biologically plausible risk for ASD, there is no evidence that supplementing with B vitamins has therapeutic benefit at this time, whether a child carries common variants in the MTHFR gene.532,533 Of dietary supplements in common use, melatonin has been demonstrated to be a safe and effective intervention for sleep in children with ASD.528
Nonbiological interventions used for symptoms of ASD are popular and have also been increasingly studied. There has been conflicting evidence regarding the effect of music therapy, yoga, massage, and equine-assisted therapy on the symptoms of ASD in children, but evidence does not support these therapies for treatment of the core deficits of ASD at this time. Evidence to date does not support the use of auditory integration training, in which an individual listens to altered sounds through headphones in an effort to change auditory or other processing. Existing studies are insufficient at this time to support dance therapy, drama therapy, and chiropractic therapy.

Medical interventions used for nonstandard purposes also are sometimes prescribed for symptoms of ASD. Clinical trials do not support the use of antifungal agents, immunotherapy, or hyperbaric oxygen treatment, and concern for safety, in addition to lack of supporting data, cautions against chelation therapy for children with ASD.

As with any intervention, families electing a novel therapy should work with their therapeutic team to identify target symptoms they hope to address and develop a monitoring system to track change. Interventions should be implemented in a stepwise fashion so that proper attribution of effect is possible and confounding factors can be identified. It is important that the medical home provider and family collaborate to select and monitor safe and effective interventions.

### SECTION 6: WORKING WITH FAMILIES

Families play a key role in effective treatment for children with ASD. Recognition that individuals who are affected and their families are partners with the professionals in all aspects of planning a personal, local, and national agenda for ASD has emerged and has shaped approaches to community services as well as research planning. Provision of patient- and family-centered care requires the clinician to educate the family about the child’s health and engage in respectful dialogue. Resources to support the clinician in talking to families about the diagnosis include a toolkit developed by the Autism Speaks Autism Treatment Network (https://www.autismspeaks.org/tool-kit/tnair-p-guide-providing-feedback-families-affected-autism).

### Impact of ASD on the Family

The impact of having a child with ASD on other family members and on society is considerable. Parents of children with ASD report more stress and increased costs than do parents who do not have a child with ASD. More than half of families report that a parent needs to cut back on work or stop working because of the care needs of the child. The largest societal costs associated with ASD are special education, residential care, and lost days of caregiver work.

Peer support for families of children with ASD is associated with less parental stress, less negative mood, and more positive perceptions. Parents who understand more about their child’s ASD can advocate for more intensive and appropriate services. Best practice includes giving families contact information for a family support group at the time of diagnosis. This support may be a local group that provides face-to-face interaction and community activities or an online community. Many families may not have the time or inclination at the time of diagnosis to communicate with other families affected by ASD but may find the support useful later when they are facing the transitions of preschool, adolescence, or adulthood. National support groups that address a wider community of children and youth with special health care needs (such as Family Voices and Parent2Parent), autism-specific national support organizations (such as Autism Speaks and the Autism Society), and local organizations are effective in helping families obtain information and feel supported. Clinicians should familiarize themselves with national and local sources of support and information so that families can be given Web sites or phone numbers at the time of diagnosis and again as indicated. State-specific information on services and Maternal and Child Health Bureau–supported programs are found online (https://mchb.hrsa.gov/maternal-child-health-initiatives/autism). It is important for providers to advocate for instructional material in other languages as well as be knowledgeable of other resources in their communities that can provide services or support to the culturally diverse groups they serve.

Comorbid conditions, such as intellectual disability and/or psychiatric disorders, add to the impact of ASD on family functioning and access to care. Although families of older children and youth typically report fewer interactions with professionals, the stress on the parent related to the ASD diagnosis persists. Primary care providers should speak with families about the stresses associated with ASD and the health of other family members and make appropriate referrals, either for supportive counseling for the caregivers or agencies that can address behavioral and respite needs of the child or to address unmet health needs in family members.

The effect on siblings also needs to be considered in the context of both anticipatory guidance and primary care. Most siblings of children with ASD do not report having a sibling with a disability to be a negative experience; however, they, too, are at risk for increased stress and subsequent emotional problems.
Siblings may have precocious involvement in the care of the child with ASD, and some resent the amount of attention and resources the child with ASD requires or the family’s inability to participate in activities in which they see their peers engaging. Proactively teaching siblings about ASD and providing them with peer support may be helpful [Autism Speaks Sibling tool kit: http://www.autismspeaks.org/sites/default/files/a_siblings_guide_to_autism.pdf]. Many areas have groups to provide education and support to siblings. It appears that positive parental attitudes and a supportive family setting are associated with better sibling adjustment as well. The pediatrician should monitor the well-being and need for behavioral health supports of siblings as well as parents.

**Medical Home**

In the AAP’s medical home model, primary care is envisioned as accessible, continuous, comprehensive, family centered, coordinated, compassionate, and culturally sensitive for all children and youth, including those with special health care needs. Children with ASD represent a population that has had difficulty accessing comprehensive coordinated services. The chronic care model provides the structure for clinicians to collaborate with patients and their families.554 Parents of children with ASD perceive care to be less comprehensive, less well coordinated, and less family centered than they desire and report that they are less satisfied with their care compared with parents of children with other special health care needs.555 Parents also perceive their providers as less well informed regarding treatments for ASD, especially complementary, alternative, and integrative therapies, than they would like them to be. Pediatricians report that they lack the knowledge to provide this support to patients with ASD556 as well as the time and resources for specialized care.555,557 Parents of children and youth with ASD would like better access to specialty care and report greater unmet medical and behavioral health care needs558 and a higher financial burden for care compared with parents of children without ASD.559

Increasing family awareness and understanding of the medical home can promote partnership of the parents and primary care provider in planning and coordinating the child’s care and advocating for their needs. National survey data reveal that family-centered and coordinated care through a medical home results in fewer unmet needs,550 including dental needs.560 Organizations, such as Family Voices and Family-to-Family Health Information Centers, can provide information and support as well as resources for guiding families in developing care notebooks for their child. Through their ongoing relationship, providers can help children understand their own diagnosis at their developmental level. Clinicians can remind their patients with ASD of their strengths, such as focus, memory, visual-spatial problem-solving, and others, as well as their personal accomplishments in building skills and mastering barriers to achieve goals. Recognition of achievement of milestones, whether it is toilet training or college graduation, should be acknowledged.

Shared decision-making promotes a collaborative process for planning care through dialogue among the individual who is affected, caregivers, and clinicians. It can be particularly useful when the evidence for an intervention is either controversial or if there is not a uniformly accepted approach.561 Shared decision-making requires clarity of the question to be answered, the options to be understood, and the family context and beliefs to be respected. It is often a process rather than a single conversation. Helping children and youth with ASD understand their diagnosis within the context of their developmental level can help them understand their symptoms and participate in decision-making.562

**Transition to Adulthood**

Planning for children with ASD to understand and participate in their own health care should begin early in adolescence, with adaptation for developmental abilities. The AAP clinical report “Supporting the Health Care Transition From Adolescence to Adulthood in the Medical Home” provides guidance on the steps necessary to address health care transitions for all patients with chronic conditions.563 Got Transition recommends 6 core elements that need to be addressed for health care transition without disruption in care, including (1) a transition policy for the practice, (2) tracking and monitoring transition, (3) assessing transition readiness for youth and/or family, (4) actively planning the details of transition, (5) transfer of care, and (6) transition completion.564 The pediatric health care provider is also in a position to advise the family about teaching their adolescent with ASD about sexuality.565 Planning for wellness requires considering young adult opportunities for exercise and leisure activities. Planning for medical transition for all aspects of health care should start around ages 12 to 14 years. Educational transition starts at the school level at age 14 years and should involve the student as much as possible.

As a child approaches legal adulthood, the family may need to consider guardianship, either full guardianship in cases in which an adult child cannot make health, financial, or other decisions because of cognitive impairment; limited guardianship in cases in which an individual can participate in decision-making; or conservatorship in cases in which the oversight extends only to...
financial decision-making. Many young adults with ASD will be capable of independent decision-making and should be prepared for transition to adulthood like other teenagers. The young adult with ASD may be eligible for Supplemental Security Income (SSI) benefits. SSI is a federal program that provides funds for the care of individuals with developmental disabilities who will not be able to support themselves independently. Because of the strict guidelines regarding cognitive and adaptive delays, some adults with ASD may not be eligible for SSI even if their disability is a barrier to employment. Families may wish to meet with a counselor who can advise them on financial planning, with attention to the needs of an adult child with developmental disability.

Students with disabilities who plan to continue their education need to be advised of the transitioning process into postsecondary education. Students with disabilities are protected under IDEA (1990; amended 1997 and 2004); Section 504 of the Rehabilitation Act of 1973; the Americans with Disabilities Act (1990); and the ADA Amendments Act of 2008. Some colleges may provide accommodations to students with developmental disabilities with proper documentation of their needs, including recent academic testing. College students with ASD may benefit from continued support around social skills development, medication monitoring, and mentoring on living independently.566

Although resources are still insufficient, attention is growing for the need to provide social skills training for youth with ASD with and without intellectual disabilities to enter the workforce in competitive employment as well as job skill development. There are insufficient group-home and supported community-living arrangements for adults with ASD to meet the demands in most communities. The clinician should initiate discussions with parents regarding their plans for where their child with ASD will progress to postsecondary school education and/or employment and their plans for where their child will live in adulthood early in adolescence so the family can plan appropriately with community agencies.

Families should work with their child’s school throughout adolescence to target the skills their child will need to master to be successful in young adult programs, the workforce, or postsecondary education. Goals for increasing skills may include academic, social, communication, leisure, and self-care goals. Families need information to be as proactive as possible in planning for health, academic, job, and residential needs in young adulthood. Additional research is needed to develop and evaluate evidence-based and effective interventions for this age group.514 The pediatric health care provider should provide anticipatory guidance to the family in the context of ongoing health supervision and communicate with identified adult providers for smooth health care transition.567

The social services and home- and community-based waiver services available to families whose children have developmental disabilities, including ASD, differ from state to state.568 The clinician should be familiar with the requirements for programs in their state that might lead to a Medicaid waiver (medical assistance as a secondary insurance for children with special health care needs), service coordination, respite care, and other financial or behavioral supports afforded a family when a child has special health care needs. The clinician may need to complete a form to verify the diagnosis and needs for eligibility. Of note, some children with ASD who have typical cognitive abilities may not qualify for many special education and social service supports. However, later on, at the time of transition to adulthood, if they experience difficulty with employment and daily-living skills, they may qualify for support services.

SECTION 7: RESEARCH AND SERVICE NEEDS

More than $1.5 billion of private and public research funding was devoted to ASD between 2008 and 2010.569 The passage of the Combating Autism Act of 2006 (Public Law 109–416) and its reauthorization in 2014 as the Autism Collaboration, Accountability, Research, Education and Support Act (CARES) Act (Public Law 113–157) continued a trend in funding to address the intervention needs of individuals diagnosed with ASD. Before this time, research funding was largely focused on the genetics and neurobiology of the disorder. However, this changed with the convening of the National Institutes of Health Interagency Autism Coordinating Committee in 2006. The committee was assembled to provide guidance to the agencies funding autism services, and the research agenda was expanded on the basis of the contributions of stakeholders, including families, individuals.

State Programs, Supports, and Laws

State laws related to education, social service, and insurance for individuals with ASD vary significantly. Although the federal government mandates early intervention for children at risk for developmental delay and a free and appropriate education for students aged 3 to 21 years who have specific educationally handicapping conditions, the implementation of educational services varies by state and locality. The law states that services need to be appropriate, not necessarily optimal. No legal mandate for adult services exists, although the agencies that provide residential services, service coordination, job training, and adult day services typically are funded through the states.
affected, and federal agencies. The committee’s 2009 strategic plan, updated in 2017, identified 7 areas for research funding: (1) early detection, (2) underlying biology, (3) genetic and environmental risk factors, (4) treatments and interventions, (5) services and implementation science, (6) lifespan services and supports, and (7) epidemiological surveillance and infrastructure. The committee recommended that multiple levels of inquiry be pursued simultaneously to inform evidence-based clinical care. These levels include the following:

- basic and translational science in the areas of genetics and epigenetics, neurobiology, and psychopharmacology to understand typical and atypical brain development and function to develop ASD-specific behavioral and pharmacologic therapies; additional research is needed to identify and understand ASD risk factors that might be mitigated to reduce ASD-related disability;

- research into the underlying neurobiology of sensory symptoms and restricted interests and repetitive behaviors to inform development of targeted interventions;

- clinical trials to test focused interventions based on the underlying biological processes involved with ASD to determine if they are appropriate for community application;

- epidemiological surveillance to gather data important for planning for current and future needs, including screening, diagnosis, and lifespan health and mental health services; and

- health services research to provide guidance for comprehensive, accessible, and culturally appropriate medical, educational, and behavioral care for children, youth, adults, and families affected by ASD.

Research in all of these areas is critical to move forward with early diagnosis, effective treatment, and evidence-based interventions at each age.

**PEDIATRIC RECOMMENDATIONS**

To provide appropriate care to all children and families affected by ASD, health, education, and public health systems need to collaborate and build integrated and adequately funded and staffed systems.

- Early identification and treatment: Pediatric providers should use screening and surveillance to provide accurate and early identification, cost-effective and timely diagnosis, prompt implementation of evidence-based interventions, and elimination of disparities to access to care for children with ASD. Clinicians should respond appropriately to family or clinical concerns and results of screening to avoid delays in diagnosis and treatment.

- Collaboration of systems of care: Children with ASD should be provided evidence-based services to address social, academic, and behavioral needs at home and school; access to appropriate pediatric and mental health care; respite services; and leisure activities.

- Planning for adolescence and transition to adult systems of care: Communities should build services to promote social skills appropriate for work and postsecondary education, access to appropriate medical and behavioral health services, job skills development, and community leisure opportunities. Pediatricians need to engage with families and youth to plan a transition to adult medical and behavioral health care. The medical home provider should support the family and youth in advocating for appropriate postsecondary work or schooling, residential supports, and activities to maintain a healthy lifestyle.

- Informed individuals and families: The pediatrician can educate youth with ASD and their families about the evidence for interventions, refer families for possible participation in clinical research when appropriate, refer families to support organizations, and prepare families to navigate transitions.

- Informed pediatric providers: To best serve patients and families affected by ASD, the clinician caring for children and youth with ASD should be familiar with issues related to diagnosis, coexisting medical and behavioral conditions, and the impact of ASD on the family to provide a medical home for these patients. Actively addressing capacity building to care for children and youth with ASD requires initiatives directed at provider education and practice quality improvement and public health, educational, and social programs to support families in their journey from diagnosis to service provision to transition to adult care.

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ABBREVIATIONS

AAC: augmentative and alternative communication
AAP: American Academy of Pediatrics
ABA: applied behavior analysis
ADDM: Autism and Developmental Disabilities Monitoring
ADHD: attention-deficit/hyperactivity disorder
ADI-R: Autism Diagnostic Inventory-Revised
ASD: autism spectrum disorder
CARS-2: Childhood Autism Rating Scale, Second Edition
CDC: Centers for Disease Control and Prevention
CMA: chromosomal microarray
CNV: copy number variant
CTM: comprehensive treatment model
DSM: Diagnostic and Statistical Manual of Mental Disorders

DSM-5: Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
DSM-IV-TR: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision
ESDM: Early Start Denver Model
G1: gastrointestinal
IDEA: Individuals with Disabilities Education Improvement Act of 2004
IEP: Individualized Education Program
LEAP: Learning Experiences and Alternative Programs for Preschoolers and their Parents
M-CHAT: Modified Checklist for Autism in Toddlers
M-CHAT-R/F: Modified Checklist for Autism in Toddlers, Revised with Follow-Up (Questions)

NDBI: naturalistic developmental behavioral intervention
OCDD: obsessive-compulsive disorder
PDD: pervasive developmental disorder
PDD-NOS: pervasive developmental disorder not otherwise specified
RCT: randomized controlled trial
SCQ: Social Communication Questionnaire
SRS: Social Responsiveness Scale
SSI: Supplemental Security Income
SSRI: selective serotonin reuptake inhibitor
STAT: Screening Tool for Autism in Toddlers and Young Children
TEACCH: Treatment and Education of Autistic and Related Communication-Handicapped Children
USPSTF: US Preventive Services Task Force
WES: whole-exome sequencing
POTENTIAL CONFLICT OF INTEREST: MeMix LLC is a company that makes an application (for phones). Dr Levy is on the advisory board for the application's development. This application is being developed to assist in nutritional and dietary management of children with autism. Dr Levy has not received any money yet from this company. This application is the focus of a National Institutes of Health R21 grant, for which Dr Levy is funded for ~2% of her salary. Once it is studied and marketed (if appropriate), Dr Levy will (possibly in the future) earn some money. Her years of relationship with the company are 2015 to the present. Dr Hyman has a relationship with Roche. Dr Hyman is the site principal investigator of a clinical trial of a novel agent being tested to promote social function in patients with autism. The University of Rochester (Dr Hyman's institution) was 1 of >40 sites and had 2 study participants in 2018. University of Rochester will be leaving the trial in 2019 (withdrawal submitted) because of staffing, and that reimbursement for staff time does not cover the cost of participation. Funding was for the staff to complete the assessments required for the clinical trial. Dr Hyman got no personal reimbursement from the company; the funding was for staff time for recruitment and assessment and clinical research center support for the trial.

COMPANION PAPER: A companion to this article can be found online at www.pediatrics.org/cgi/doi/10.1542/peds.2019-3448.

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### SUPPLEMENTAL TABLE 14 Recurrent CNVs Most Commonly Identified in Cohorts With ASD by Using CMA Analysis

<table>
<thead>
<tr>
<th>CNV Region</th>
<th>Frequency</th>
<th>Common Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>16p11.2 deletion</td>
<td>1 in 304</td>
<td>ASD, DD or ID, expressive language impairment, relative or absolute macrocephaly, overweight</td>
</tr>
<tr>
<td>16p11.2 duplication</td>
<td>1 in 396</td>
<td>ASD, schizophrenia, bipolar disorder, ADHD, relative or absolute microcephaly, underweight</td>
</tr>
<tr>
<td>15q11.2-q13 (BP2–BP3) duplication</td>
<td>1 in 494</td>
<td>ASD, DD or ID, epilepsy, hypotonia, ataxia, behavior problems</td>
</tr>
<tr>
<td>15q13.2-q13.3 (BP4–BP5) deletion</td>
<td>1 in 659</td>
<td>ASD, DD or ID, epilepsy, schizophrenia, cardiac defects</td>
</tr>
<tr>
<td>1q21.1 duplication</td>
<td>1 in 659</td>
<td>ASD, DD or ID, schizophrenia, ADHD, relative macrocephaly, hypertelorism</td>
</tr>
<tr>
<td>22q11.2 duplication</td>
<td>1 in 659</td>
<td>ASD, DD or ID, hypotonia, motor delay</td>
</tr>
<tr>
<td>18p13.11 deletion</td>
<td>1 in 791</td>
<td>ASD, DD or ID, epilepsy, schizophrenia, congenital anomalies</td>
</tr>
<tr>
<td>7q11.23 duplication</td>
<td>1 in 888</td>
<td>ASD, DD or ID, growth retardation, hypotonia</td>
</tr>
<tr>
<td>18p12.2 deletion</td>
<td>1 in 888</td>
<td>ASD, DD or ID, schizophrenia, epilepsy, growth retardation, cardiac defects, microcephaly, hypotonia</td>
</tr>
<tr>
<td>17q12 deletion</td>
<td>1 in 1978</td>
<td>ASD, DD or ID, schizophrenia, renal cysts, mature-onset diabetes of the young type 5</td>
</tr>
<tr>
<td>15q13.2–13.3 (BP4–BP5) duplication</td>
<td>1 in 1978</td>
<td>ASD, DD or ID, obesity</td>
</tr>
</tbody>
</table>

BP2 breakpoint 2; BP3 breakpoint 3; BP4 breakpoint 4; BP5 breakpoint 5; DD developmental delay; ID intellectual disability.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Physical Findings</th>
<th>Gene</th>
<th>Confirmatory Testing</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragile X syndrome</td>
<td>Long face, prominent forehead and jaw, large ears, joint laxity, macroorchidism after puberty in boys</td>
<td>FMR1 (CGG repeat expansion, abnormal methylation)</td>
<td>Targeted mutation analysis (PCR and Southern blot)</td>
<td>Genetic counseling (X-linked dominant inheritance); all mothers of individuals with an FMR1 full mutation are carriers of an FMR1 premutation or full mutation; extended family counseling is necessary; premutation carriers are at risk for fragile X–associated tremor/ataxia syndrome and FMR1-related primary ovarian insufficiency in female patients; several targeted pharmacologic therapies are under investigation</td>
</tr>
<tr>
<td>Neurofibromatosis 1</td>
<td>Multiple café-au-lait macules, axillary and inguinal freckling, iris Lisch nodules, cutaneous neurofibromas</td>
<td>NF1</td>
<td>Clinical criteria; optimized protein truncation testing, sequence analysis, and deletion or duplication analysis are available but infrequently required</td>
<td>Genetic counseling (autosomal dominant inheritance); 50% de novo, 50% inherited; associated problems requiring investigation or monitoring (optic gliomas, other CNS tumors, peripheral nerve sheath tumors, vasculopathy, hypertension, orthopedic issues, osteopenia)</td>
</tr>
<tr>
<td>PTEN hamartoma tumor syndrome (includes Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome)</td>
<td>Marked macrocephaly, skin hamartomas, pigmented macules of the glans penis</td>
<td>PTEN</td>
<td>PTEN sequence analysis, deletion or duplication analysis</td>
<td>Genetic counseling (autosomal dominant inheritance with highly variable expression); associated problems requiring investigation or monitoring (significant risk of benign and malignant tumors of the thyroid, breast, and endometrium as well as intestinal polyps, colorectal cancer, renal cell carcinoma, cutaneous melanoma, and cerebellar dysplastic gangliocytoma)</td>
</tr>
<tr>
<td>Rett syndrome</td>
<td>Deceleration of head growth velocity, acquired microcephaly, loss of purposeful hand use, prominent hand stereotypes (especially hand wringing or clapping), apraxia, hyperventilation or breath-holding, seizures</td>
<td>MECP2</td>
<td>MECP2 sequence analysis, deletion or duplication analysis</td>
<td>Genetic counseling (&gt;98% de novo, &lt;1% germline mosaicism); associated problems requiring investigation or monitoring and anticipatory guidance (failure to thrive, gastroesophageal reflux, respiratory problems, osteopenia, sudden death); targeted pharmacologic therapy under investigation</td>
</tr>
<tr>
<td>Smith-Lemli-Opitz syndrome</td>
<td>Characteristic facial features (narrow forehead, low-set ears, ptosis, epicantthal folds, short nose, anteverted nares), microcephaly, cleft palate, 2- to 3-toe syndactyly, postaxial polydactyly, hypospadias in male</td>
<td>DHCR7</td>
<td>7-dehydrocholesterol level (elevated); DHCR7 sequence analysis available</td>
<td>Genetic counseling (autosomal recessive inheritance); potential role for treatment with cholesterol</td>
</tr>
<tr>
<td>Condition</td>
<td>Physical Findings</td>
<td>Gene</td>
<td>Confirmatory Testing</td>
<td>Importance</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------------------------------------------------------------------------</td>
<td>-----------</td>
<td>------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Timothy syndrome</td>
<td>patients, prenatal and postnatal growth retardation, long QT interval, other ECG</td>
<td>CACNA1C</td>
<td>Targeted mutation analysis, sequence analysis, deletion or duplication analysis</td>
<td>Genetic counseling, autosomal dominant, usually de novo, but parental germline mosaicism has been observed; treatment related to long QTc (β-blocker, pacemaker, implantable defibrillator) and avoidance of hypoglycemia</td>
</tr>
<tr>
<td></td>
<td>abnormalities (atrioventricular block, macroscopic T-wave alternans), congenital</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>heart defects, cutaneous syndactyly, low-set ears, flat nasal bridge, thin upper</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>lip, round facies, baldness for the first 2 y of life followed by thin scalp hair;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>dental abnormalities, frequent infections because of altered immune response,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>intermittent hypoglycemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberous sclerosis</td>
<td>Hypopigmented macules, angiofibromas, shagreen patches (connective tissue nevi),</td>
<td>TSC1, TSC2</td>
<td>Clinical criteria; TSC1 and TSC2 sequencing available</td>
<td>Genetic counseling (autosomal dominant inheritance); associated problems requiring investigation or monitoring (CNS tumors, seizures, renal angiomyolipomas or cysts, cardiac rhabdomyomas and arrhythmias); potential role for targeted pharmacologic therapy (mTOR inhibitors)</td>
</tr>
<tr>
<td></td>
<td>ungual fibromas, retinal hamartomas</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CACNA1C, calcium channel, voltage-dependent, L-type, α-1c subunit; CGG, cytosine-guanine-guanine; CNS, central nervous system; DHCR7, 7-dehydrocholesterol reductase; ECG, electrocardiogram; FMR1, fragile X mental retardation 1; MECP2, methyl CpG binding protein 2; mTOR, mammalian target of rapamycin; PCR, polymerase chain reaction; PTEN, phosphatase and tensin homolog; QTc, corrected QT interval; TSC1, tuberous sclerosis 1; TSC2, tuberous sclerosis 2. Adapted with permission from Myers SM, Challman TD. Autism Spectrum Disorders. In: Voigt RG, Macias MM, Myers SM, eds. Developmental and Behavioral Pediatrics. Elk Grove Village, IL: American Academy of Pediatrics; 2011:249–291.
Table 15: Selected ASD Risk Genes Identified or Confirmed in Whole-Exome Studies

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Name</th>
<th>Broad Functional Categorization</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN2A</td>
<td>sodium channel, voltage-gated, type II, α subunit</td>
<td>Synaptic functions (e.g., ion channels, neurotransmitter receptors, cell adhesion molecules, microtubule assembly, scaffolding proteins, actin cytoskeleton)</td>
</tr>
<tr>
<td>GRIN2B</td>
<td>glutamate receptor, ionotropic, N-methyl-D-aspartate 2B</td>
<td></td>
</tr>
<tr>
<td>KATNAL2</td>
<td>katanin p90 subunit A-like 2</td>
<td></td>
</tr>
<tr>
<td>ANK2</td>
<td>ankyrin 2, neuronal</td>
<td></td>
</tr>
<tr>
<td>DSGM</td>
<td>Down syndrome cell adhesion molecule</td>
<td></td>
</tr>
<tr>
<td>NRXN1</td>
<td>neurexin 1</td>
<td></td>
</tr>
<tr>
<td>SHANK2</td>
<td>SH3 and multiple ankyrin repeat domains 2</td>
<td></td>
</tr>
<tr>
<td>SHANK3</td>
<td>SH3 and multiple ankyrin repeat domains 3</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>phosphatase and tensin homolog</td>
<td>Intracellular signaling, activity-dependent synaptic protein synthesis and degradation</td>
</tr>
<tr>
<td>SYNGAP1</td>
<td>synaptic Ras GTPase activating protein 1</td>
<td></td>
</tr>
<tr>
<td>DYRK1A</td>
<td>dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A</td>
<td></td>
</tr>
<tr>
<td>POGZ</td>
<td>pogo transposable element with ZNF domain</td>
<td></td>
</tr>
<tr>
<td>CUL3</td>
<td>cullin 3</td>
<td></td>
</tr>
<tr>
<td>CHD2</td>
<td>chromodomain helicase DNA binding protein 2</td>
<td>Transcription regulation, chromatin remodeling</td>
</tr>
<tr>
<td>CHD8</td>
<td>chromodomain helicase DNA binding protein 8</td>
<td></td>
</tr>
<tr>
<td>ADNP</td>
<td>activity-dependent neuroprotector homeobox</td>
<td></td>
</tr>
<tr>
<td>ARID1B</td>
<td>AT rich interactive domain 1B (SWI1-like)</td>
<td></td>
</tr>
<tr>
<td>ASH1L</td>
<td>ASH1 (absent, small, or homeotic)-like</td>
<td></td>
</tr>
<tr>
<td>KDM5B</td>
<td>lysine-specific demethylase 5B</td>
<td></td>
</tr>
<tr>
<td>KMT2C</td>
<td>lysine-specific methyltransferase 2C</td>
<td></td>
</tr>
<tr>
<td>SETD5</td>
<td>SET domain containing 5</td>
<td></td>
</tr>
<tr>
<td>TBR1</td>
<td>T-box, brain, 1</td>
<td></td>
</tr>
</tbody>
</table>


* Also involved in microtubule dynamics at the synapse.
SUPPLEMENTAL REFERENCES

## Supplemental Information

### SUPPLEMENTAL TABLE 14 Recurrent CNVs Most Commonly Identified in Cohorts With ASD by Using CMA Analysis

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<td>16p11.2 deletion</td>
<td>1 in 304</td>
<td>ASD, DD or ID, expressive language impairment, relative or absolute macrocephaly, overweight</td>
</tr>
<tr>
<td>16p11.2 duplication</td>
<td>1 in 396</td>
<td>ASD, schizophrenia, bipolar disorder, ADHD, relative or absolute microcephaly, underweight</td>
</tr>
<tr>
<td>15q11.2-q13 (BP2–BP3) duplication</td>
<td>1 in 494</td>
<td>ASD, DD or ID, epilepsy, hypotonia, ataxia, behavior problems</td>
</tr>
<tr>
<td>15q13.2-q13.3 (BP4–BP5) deletion</td>
<td>1 in 659</td>
<td>ASD, DD or ID, epilepsy, schizophrenia, cardiac defects</td>
</tr>
<tr>
<td>1q21.1 duplication</td>
<td>1 in 659</td>
<td>ASD, DD or ID, schizophrenia, ADHD, relative macrocephaly, hypertelorism</td>
</tr>
<tr>
<td>22q11.2 duplication</td>
<td>1 in 659</td>
<td>ASD, DD or ID, hypotonia, motor delay</td>
</tr>
<tr>
<td>16p13.11 deletion</td>
<td>1 in 791</td>
<td>ASD, DD or ID, epilepsy, schizophrenia, congenital anomalies</td>
</tr>
<tr>
<td>7q11.23 duplication</td>
<td>1 in 888</td>
<td>ASD, DD or ID, growth retardation, hypotonia</td>
</tr>
<tr>
<td>16p12.2 deletion</td>
<td>1 in 888</td>
<td>ASD, DD or ID, schizophrenia, epilepsy, growth retardation, cardiac defects, microcephaly, hypotonia</td>
</tr>
<tr>
<td>17q12 deletion</td>
<td>1 in 1978</td>
<td>ASD, DD or ID, schizophrenia, renal cysts, mature-onset diabetes of the young type 5</td>
</tr>
<tr>
<td>15q13.2–13.3 (BP4–BP5) duplication</td>
<td>1 in 1978</td>
<td>ASD, DD or ID, obesity</td>
</tr>
</tbody>
</table>

BP2 breakpoint 2; BP3 breakpoint 3; BP4 breakpoint 4; BP5 breakpoint 5; DD developmental delay; ID intellectual disability.

\(^a\) Moreno-De-Luca D et al\(^{631}\); the frequency of each CNV among 3955 probands with ASD from the Autism Genetic Resource Exchange, Autism Genome Project, and Simons Foundation Autism Research Initiative Simplex Collection cohorts.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Physical Findings</th>
<th>Gene</th>
<th>Confirmatory Testing</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragile X syndrome</td>
<td>Long face, prominent forehead and jaw, large ears, joint laxity, macroorchidism after puberty in boys</td>
<td>FMR1 (CGG repeat expansion, abnormal methylation)</td>
<td>Targeted mutation analysis (PCR and Southern blot)</td>
<td>Genetic counseling (X-linked dominant inheritance); all mothers of individuals with an FMR1 full mutation are carriers of an FMR1 premutation or full mutation; extended family counseling is necessary; premutation carriers are at risk for fragile X-associated tremor/ataxia syndrome and FMR1-related primary ovarian insufficiency in female patients; several targeted pharmacologic therapies are under investigation</td>
</tr>
<tr>
<td>Neurofibromatosis 1</td>
<td>Multiple café-au-lait macules, axillary and inguinal freckling, iris Lisch nodules, cutaneous neurofibromas</td>
<td>NF1</td>
<td>Clinical criteria; optimized protein truncation testing, sequence analysis, and deletion or duplication analysis are available but infrequently required</td>
<td>Genetic counseling (autosomal dominant inheritance); 50% de novo, 50% inherited; associated problems requiring investigation or monitoring (optic gliomas, other CNS tumors, peripheral nerve sheath tumors, vasculopathy, hypertension, orthopedic issues, osteopenia)</td>
</tr>
<tr>
<td>PTEN hamartoma tumor syndrome (includes Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome)</td>
<td>Marked macrocephaly, skin hamartomas, pigmented macules of the glans penis</td>
<td>PTEN</td>
<td>PTEN sequence analysis, deletion or duplication analysis</td>
<td>Genetic counseling (autosomal dominant inheritance with highly variable expression); associated problems requiring investigation or monitoring (significant risk of benign and malignant tumors of the thyroid, breast, and endometrium as well as intestinal polyps, colorectal cancer, renal cell carcinoma, cutaneous melanoma, and cerebellar dysplastic gangliocytoma)</td>
</tr>
<tr>
<td>Rett syndrome</td>
<td>Deceleration of head growth velocity, acquired microcephaly, loss of purposeful hand use, prominent hand stereotypies (especially hand wringing or clasping), apraxia, hyperventilation or breath-holding, seizures</td>
<td>MECP2</td>
<td>MECP2 sequence analysis, deletion or duplication analysis</td>
<td>Genetic counseling (&gt;99% de novo, &lt;1% germline mosaicism); associated problems requiring investigation or monitoring and anticipatory guidance (failure to thrive, gastroesophageal reflux, respiratory problems, osteopenia, sudden death); targeted pharmacologic therapy under investigation</td>
</tr>
<tr>
<td>Smith-Lemli-Opitz syndrome</td>
<td>Characteristic facial features (narrow forehead, low-set ears, ptosis, epicanthal folds, short nose, anteverted nares), microcephaly, cleft palate, 2- to 3-toe syndactyly, postaxial polydactyly, hypospadias in male</td>
<td>DHCR7</td>
<td>7-dehydrocholesterol level (elevated); DHCR7 sequence analysis available</td>
<td>Genetic counseling (autosomal recessive inheritance); potential role for treatment with cholesterol</td>
</tr>
<tr>
<td>Condition</td>
<td>Physical Findings</td>
<td>Gene</td>
<td>Confirmatory Testing</td>
<td>Importance</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>-----------</td>
<td>-----------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Timothy syndrome</td>
<td>patients, prenatal and postnatal growth retardation</td>
<td>CACNA1C</td>
<td>Targeted mutation analysis, sequence analysis, deletion or duplication analysis</td>
<td>Genetic counseling, autosomal dominant, usually de novo, but parental germline mosaicism has been observed; treatment related to long QTc (β-blocker, pacemaker, implantable defibrillator) and avoidance of hypoglycemia</td>
</tr>
<tr>
<td></td>
<td>Long QT interval, other ECG abnormalities (atrioventricular block, macroscopic T-wave alternans), congenital heart defects, cutaneous syndactyly, low-set ears, flat nasal bridge, thin upper lip, round facies, baldness for the first 2 y of life followed by thin scalp hair, dental abnormalities, frequent infections because of altered immune response, intermittent hypoglycemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberous sclerosis</td>
<td>Hypopigmented macules, angiofibromas, shagreen patches (connective tissue nevi), ungual fibromas, retinal hamartomas</td>
<td>TSC1, TSC2</td>
<td>Clinical criteria; TSC1 and TSC2 sequencing available</td>
<td>Genetic counseling (autosomal dominant inheritance); associated problems requiring investigation or monitoring (CNS tumors, seizures, renal angiomyolipomas or cysts, cardiac rhabdomyomas and arrhythmias); potential role for targeted pharmacologic therapy (mTOR inhibitors)</td>
</tr>
</tbody>
</table>

CACNA1C, calcium channel, voltage-dependent, L-type, α1c subunit; CGG, cytosine-guanine-guanine; CNS, central nervous system; DHCR7, 7-dehydrocholesterol reductase; ECG, electrocardiogram; FMR1, fragile X mental retardation 1; MECP2, methyl CpG binding protein 2; mTOR, mammalian target of rapamycin; PCR, polymerase chain reaction; PTEN, phosphatase and tensin homolog; QTc, corrected QT interval; TSC1, tuberous sclerosis 1; TSC2, tuberous sclerosis 2. Adapted with permission from Myers SM, Challman TD. Autism Spectrum Disorders. In: Voigt RG, Macias MM, Myers SM, eds. Developmental and Behavioral Pediatrics. Elk Grove Village, IL: American Academy of Pediatrics; 2011:249–291.
## Table 15: Selected ASD Risk Genes Identified or Confirmed in Whole-Exome Studies

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Name</th>
<th>Broad Functional Categorization</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN2A</td>
<td>sodium channel, voltage-gated, type II, α subunit</td>
<td>Synaptic functions (e.g., ion channels, neurotransmitter receptors, cell adhesion molecules, microtubule assembly, scaffolding proteins, actin cytoskeleton)</td>
</tr>
<tr>
<td>GRIN2B</td>
<td>glutamate receptor, ionotropic, N-methyl-D-aspartate 2B</td>
<td>Synaptic functions (e.g., ion channels, neurotransmitter receptors, cell adhesion molecules, microtubule assembly, scaffolding proteins, actin cytoskeleton)</td>
</tr>
<tr>
<td>KATNAL2</td>
<td>katanin p60 subunit A-like 2</td>
<td>Synaptic functions (e.g., ion channels, neurotransmitter receptors, cell adhesion molecules, microtubule assembly, scaffolding proteins, actin cytoskeleton)</td>
</tr>
<tr>
<td>ANK2</td>
<td>ankyrin 2, neuronal</td>
<td>Synaptic functions (e.g., ion channels, neurotransmitter receptors, cell adhesion molecules, microtubule assembly, scaffolding proteins, actin cytoskeleton)</td>
</tr>
<tr>
<td>DSGAM</td>
<td>Down syndrome cell adhesion molecule</td>
<td>Synaptic functions (e.g., ion channels, neurotransmitter receptors, cell adhesion molecules, microtubule assembly, scaffolding proteins, actin cytoskeleton)</td>
</tr>
<tr>
<td>NRXN1</td>
<td>neurexin 1</td>
<td>Synaptic functions (e.g., ion channels, neurotransmitter receptors, cell adhesion molecules, microtubule assembly, scaffolding proteins, actin cytoskeleton)</td>
</tr>
<tr>
<td>SHANK2</td>
<td>SH3 and multiple ankyrin repeat domains 2</td>
<td>Synaptic functions (e.g., ion channels, neurotransmitter receptors, cell adhesion molecules, microtubule assembly, scaffolding proteins, actin cytoskeleton)</td>
</tr>
<tr>
<td>SHANK3</td>
<td>SH3 and multiple ankyrin repeat domains 3</td>
<td>Synaptic functions (e.g., ion channels, neurotransmitter receptors, cell adhesion molecules, microtubule assembly, scaffolding proteins, actin cytoskeleton)</td>
</tr>
<tr>
<td>PTEN</td>
<td>phosphatase and tensin homolog</td>
<td>Intracellular signaling, activity-dependent synaptic protein synthesis and degradation</td>
</tr>
<tr>
<td>SYNGAP1</td>
<td>synaptic Ras GTPase activating protein 1</td>
<td>Intracellular signaling, activity-dependent synaptic protein synthesis and degradation</td>
</tr>
<tr>
<td>DYRK1A</td>
<td>dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A</td>
<td>Intracellular signaling, activity-dependent synaptic protein synthesis and degradation</td>
</tr>
<tr>
<td>POGZ</td>
<td>pogo transposable element with ZNF domain</td>
<td>Intracellular signaling, activity-dependent synaptic protein synthesis and degradation</td>
</tr>
<tr>
<td>CUL3</td>
<td>cullin 3</td>
<td>Intracellular signaling, activity-dependent synaptic protein synthesis and degradation</td>
</tr>
<tr>
<td>GHD2</td>
<td>chromodomain helicase DNA binding protein 2</td>
<td>Transcription regulation, chromatin remodeling</td>
</tr>
<tr>
<td>GHD8</td>
<td>chromodomain helicase DNA binding protein 8</td>
<td>Transcription regulation, chromatin remodeling</td>
</tr>
<tr>
<td>ADNP</td>
<td>activity-dependent neuroprotector homeobox</td>
<td>Transcription regulation, chromatin remodeling</td>
</tr>
<tr>
<td>ARID1B</td>
<td>AT rich interactive domain 1B (SW1-like)</td>
<td>Transcription regulation, chromatin remodeling</td>
</tr>
<tr>
<td>ASH1L</td>
<td>ASH1 (absent, small, or homeotic)-like</td>
<td>Transcription regulation, chromatin remodeling</td>
</tr>
<tr>
<td>KDM5B</td>
<td>lysine-specific demethylase 5B</td>
<td>Transcription regulation, chromatin remodeling</td>
</tr>
<tr>
<td>KMT2C</td>
<td>lysine-specific methyltransferase 2C</td>
<td>Transcription regulation, chromatin remodeling</td>
</tr>
<tr>
<td>SETD5</td>
<td>SET domain containing 5</td>
<td>Transcription regulation, chromatin remodeling</td>
</tr>
</tbody>
</table>


* Also involved in microtubule dynamics at the synapse.
### SUPPLEMENTAL TABLE 16 Selected Metabolic Conditions That May (Rarely) Be Associated With an ASD Phenotype

<table>
<thead>
<tr>
<th>Disorders of amino acid metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylketonuria (untreated)</td>
</tr>
<tr>
<td>Homocystinuria</td>
</tr>
<tr>
<td>Branched-chain ketoacid dehydrogenase kinase deficiency</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disorders of γ-aminobutyric acid metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinic semialdehyde dehydrogenase deficiency</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disorders of cholesterol metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smith-Lemli-Opitz syndrome (7-dehydrocholesterol reductase deficiency)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disorders associated with cerebral folate deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate receptor 1 gene mutations</td>
</tr>
<tr>
<td>Dihydrofolate reductase deficiency</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disorders of creatine transport or metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine-glycine aminotransferase deficiency</td>
</tr>
<tr>
<td>Guanidinoacetate methyltransferase deficiency</td>
</tr>
<tr>
<td>X-linked creatine transporter deficits</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disorders of carnitine biosynthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-N-methyllysine dioxygenase deficiency</td>
</tr>
<tr>
<td>Disorders of purine and pyrimidine metabolism</td>
</tr>
<tr>
<td>Adenylosuccinate lyase deficiency</td>
</tr>
<tr>
<td>Adenosine deaminase deficiency</td>
</tr>
<tr>
<td>Cytosolic 5′-nucleotidase superactivity</td>
</tr>
<tr>
<td>Dihydropyrimidine dehydrogenase deficiency</td>
</tr>
<tr>
<td>Phosphoribosyl pyrophosphate synthetase superactivity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lysosomal storage disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanfilippo syndrome (mucopolysaccharidosis type III)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mitochondrial disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial DNA mutations</td>
</tr>
<tr>
<td>Nuclear DNA mutations</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotinidase deficiency</td>
</tr>
<tr>
<td>Urea cycle defects</td>
</tr>
</tbody>
</table>


### SUPPLEMENTAL REFERENCES

ACMG TECHNICAL STANDARD

Laboratory testing for fragile X, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG)

Elaine Spector1,2, Andrea Behlmann3, Kathryn Kronquist2, Nancy C. Rose4, Elaine Lyon5, Honey V. Reddi6,7 and ACMG Laboratory Quality Assurance Committee8*

Note: This document supersedes the Technical Standards and Guidelines for Fragile X Testing: The First of a Series of Disease-Specific Supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics and Genomics (2001),1 the Technical Standards and Guidelines for Fragile X Testing: A Revision to the Disease-Specific Supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics and Genomics (2005)2 and the ACMG Standards and Guidelines for Fragile X testing: a revision to the disease-specific supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics and Genomics (2013).3 It is designed for genetic testing professionals who are already familiar with the disease and the methods of analysis.

Disclaimer: This technical standard is designed primarily as an educational resource for clinical laboratory geneticists to help them provide quality clinical laboratory genetic services. Adherence to this technical standard is voluntary and does not necessarily assure a successful medical outcome. This technical standard should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinical laboratory geneticist should apply his or her own professional judgment to the specific circumstances presented by the individual patient or specimen. Clinical laboratory geneticists are encouraged to document in the patient’s record the rationale for the use of a particular procedure or test, whether or not it is in conformance with this technical standard. They also are advised to take notice of the date any particular technical standard was adopted, and to consider other relevant medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures.

Molecular genetic testing of the FMR1 gene is commonly performed in clinical laboratories. Pathogenic variants in the FMR1 gene are associated with fragile X syndrome, fragile X–associated tremor ataxia syndrome (FXTAS), and fragile X–associated primary ovarian insufficiency (FXPOI). This document provides updated information regarding FMR1 pathogenic variants, including prevalence, genotype–phenotype correlations, and variant nomenclature. Methodological considerations are provided for Southern blot analysis and polymerase chain reaction (PCR) amplification of FMR1, including triplet repeat–primed and methylation-specific PCR.

The American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee has the mission of maintaining high technical standards for the performance and interpretation of genetic tests. In part, this is accomplished by the publication of the document ACMG Technical Standards for Clinical Genetics Laboratories, which is now maintained online (http://www.acmg.net). This subcommittee also reviews the outcome of national proficiency testing in the genetics area and may choose to focus on specific diseases or methodologies in response to those results. Accordingly, the subcommittee selected fragile X syndrome to be the first topic in a series of supplemental sections, recognizing that it is one of the most frequently ordered genetic tests and that it has many alternative methods with different strengths and weaknesses. This document is the fourth update to the original standards and guidelines for fragile X testing that were published in 2001, with revisions in 2005 and 2013, respectively.

This version
Clarifies the clinical features associated with different FMR1 variants (Section 2.3)
Discusses important reporting considerations (Section 3.3.1.3)
Provides updates on technology (Section 4.1)

FX 1: INTRODUCTION

Disease-specific statements are intended to augment the current general American College of Medical Genetics and Genomics (ACMG) Technical Standards for Clinical Genetics Laboratories. Individual laboratories are responsible for meeting the CLIA/College of American Pathologists (CAP) quality assurance standards with respect to appropriate sample documentation, assay validation, general proficiency, and quality control measures.

FX 2: BACKGROUND ON FRAGILE X SYNDROME

FX 2.1: Gene symbol/chromosome locus

FMR1 is the gene symbol recognized by the Human Genome Organisation (HUGO) gene nomenclature committee. Historically, the locus was referred to as FXA. The chromosome locus is Xq27.3.

FX 2.2: OMIM number

The OMIM numbers are as follows: 309550 for the FMR1 gene, 300624 for fragile X syndrome (FXS), 311360 for fragile X–associated primary ovarian insufficiency (FXPOI), and 300623 for fragile X–associated tremor ataxia syndrome (FXTAS).

FX 2.3: Brief clinical description

Pathogenic variants in the FMR1 gene cause a spectrum of disorders, each with a different pathophysiological mechanism leading to a corresponding phenotype ranging from neurodevelopmental problems in childhood to neurodegenerative issues with aging (Table 1). The features of FXS include varying degrees of cognitive deficits, seizures, and certain characteristic physical features such as macro-orchidism and large ears. While not all individuals with the premutation (alleles ranging from ≥55 to ≤200 CGG repeats) demonstrate FXS-related features, some with larger repeat sizes (>100–200 CGG repeats) have been identified with learning difficulties, prominent ears, neuropsychiatric disorders, or intellectual disabilities. Farzin et al. demonstrated a high rate of attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) in boys with FXS. Females with premutations (usually in the range of >80–200 CGG repeats) have approximately a 20% risk for fragile X–associated primary ovarian insufficiency (FXPOI). There is no evidence to support an association between high normal and intermediate repeat range (45–54 repeats) FMR1 alleles with a risk of FXPOI. Older males and females with premutations are at risk for fragile X–associated tremor/ataxia syndrome (FXTAS). FXTAS is a late-onset, progressive development of intention tremor and ataxia often accompanied by progressive cognitive and behavioral deterioration including memory loss, anxiety, reclusive behavior, deficits of executive function, and dementia. The phenotype of FXTAS gets more defined and prevalent with age and with premutation repeat length. Guidelines are available to identify individuals with FXTAS. Adults with heterozygote premutation alleles (both male and female) can express a spectrum of neuropsychiatric problems referred to as fragile X–associated neuropsychiatric disorders (FXAND). The symptoms include anxiety, depression, adult ADHD, addictive behaviors, chronic pain, and fibromyalgia. The risk for phenotypic findings is higher in males than in females with a premutation allele. For more information on these disorders, see the online GeneReviews profile for FMR1-related disorders (https://www.ncbi.nlm.nih.gov/books/NBK1384/) and the National Fragile X Foundation website (http://www.fragilex.org).

FX 2.4: Mode of inheritance

Inheritance of the FMR1 variant is X-linked, although the pattern of FXS is complicated due to the characteristics of the unstable repeat sequence. In typical fragile X families, the variant is a multistep expansion occurring over one or more generations in a region of CGG repeats in the 5’-untranslated region (UTR) of the gene. Small expansions are not generally associated with cognitive deficits in males and females. Large expansions (i.e., large premutations or full mutations) are fully penetrant in all males and many females (depending on X chromosome inactivation). With extremely rare exceptions, the parent of origin of the expansion to the full mutation is female.

FX 2.5: Gene product and mutational mechanism

The gene product of the FMR1 gene is the fragile X mental retardation protein (FMRP), a widely expressed RNA-binding protein. FXS is caused by the deficiency or absence of FMRP. Theoretically, this can occur through any type of deletion or a single base pair change resulting in an inactive variant, but in more than 99% of cases, there is an expansion of a segment of CGG repeats in the 5’ UTR of exon 1 of FMR1. Large CGG expansions in this region are associated with hypermethylation and inhibition of transcription resulting in the absence of FMRP. In individuals with a heterozygous premutation, FMR1 messenger RNA (mRNA) levels are increased, with higher CGG repeat numbers correlating with higher mRNA levels.

FMRP is a selective RNA-binding protein that can form a messenger ribonucleoprotein complex and associate with poly-somes. At the neuroanatomic level, the fragile X brain differs from an unaffected brain by the presence of unusually long and thin dendritic spines in the cortical regions. Excitatory synaptic transmission occurs at the dendritic spines and FMRP appears to associate with polyribosomes within dendritic spines of “wildtype” neurons. From these data, FMRP, which is shown to behave in vitro as an inhibitor of protein translation, is hypothesized to suppress translation of dendritic proteins in

Table 1. Clinical features of FMR1-related disorders.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>OMIM</th>
<th>Main clinical features</th>
<th>Associated FMR1 variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragile X syndrome (FXS)</td>
<td>300624</td>
<td>Cognitive deficit, seizures, macro-orchidism, large ears, autism spectrum disorder</td>
<td>Full-mutation allele (99%), FMR1 sequence variants, FMR1 gene deletion</td>
</tr>
<tr>
<td>Fragile X primary ovarian insufficiency (FXPOI)</td>
<td>311360</td>
<td>Premature ovarian insufficiency, amenorrhea</td>
<td>Premutation</td>
</tr>
<tr>
<td>Fragile X tremor ataxia syndrome (FXTAS)</td>
<td>300623</td>
<td>Tremor, cerebellar gait ataxia, MRI white matter lesions</td>
<td>Premutation</td>
</tr>
<tr>
<td>Fragile X–associated neuropsychiatric disorders (FXAND)</td>
<td>None</td>
<td>Anxiety, depression, ADHD, addictive behavior</td>
<td>Premutation</td>
</tr>
</tbody>
</table>

The main clinical features for each disorder can be variable and are part of a larger clinical spectrum. ADHD attention-deficit/hyperactivity disorder, MRI magnetic resonance imaging.
response to synaptic stimulation signals. There are no known forms of FMRP deficiency that do not map to the FMR1 gene. In the fragile X brain, therefore, translation of certain messages may be exaggerated because the normal inhibition provided by FMRP is absent.

Studies of FMR1 mRNA expression provide evidence that expansion in the premutation range perturbs gene expression and may have pathophysiological consequences, particularly those related to FXS and FXPOI (see Section 2.3). Reductions in the amount of FMRP have been found in both lymphocytes and transformed lymphocytes of premutation heterozygotes. Using a highly sensitive fluorescent assay, Kenneson et al. demonstrated a decrement of FMRP in individuals with expansions only slightly larger than the upper edge of the normal range. The reduction in FMRP is associated with an increase in FMR1 mRNA in individuals with premutations. The understanding of the mechanisms involved in expansion of the CGG repeats into the premutation range and the effects of FMR1 transcription dysregulation continues to evolve.

FX 2.6: Non-CGG repeat expansion variants in the FMR1 gene
Pathogenic variants other than the typical CGG repeat expansion have been described in the FMR1 gene, including single-nucleotide variants (missense and nonsense), splicing and regulatory substitutions, small deletions resulting in frameshift and premature truncation, several gross deletions, and one complex rearrangement, which could be detected by Sanger, exome, and genome sequencing; a growing list of these variants is available in ClinVar. Evidence suggests that intragenic FMR1 variants, although much less frequent than CGG repeat expansions, are also a mutational mechanism leading to FXS. Guidelines for detecting these relatively rare variants are beyond the scope of this document.

FX 2.7: Prevalence and ethnic association of common variants

FX 2.7.1: Full mutations: The genetics and clinical heterogeneity of FXS have made the diagnosis, and therefore the assessment of prevalence, challenging. All major ethnic groups and races appear to be susceptible to expansion of the FMR1 CGG region. Hunter and colleagues carried out a systematic literature review of 5,562 papers of which 54 were identified for inclusion, and a meta-analysis of the prevalence of expanded FMR1 alleles performed.

Studies assessing three types of populations were considered: (1) total population studies that assessed the whole population without selection bias (these studies were typically screening studies of pregnant women and random newborns); (2) normal population studies that assessed healthy individuals without any intellectual disability (studies in the normal populations were used only to assess the carrier frequency of FMR1 variants in females, because these individuals are usually considered to be healthy); and (3) populations with intellectual disability being defined individually in each study. The 54 epidemiologic studies analyzed used only polymerase chain reaction (PCR) and Southern blot analysis for variant detection. The analysis included over 90,000 females and 50,000 males. The prevalence of the full mutation was 1.4 per 10,000 males and 0.9 per 10,000 females or 1:7,000 males and 1:850 females. The prevalence of premutation alleles among the general population was 1:300 females and 1:850 males. The frequency of individuals with the premutation allele in the healthy female population was the same as that in the total female population (34.4 per 10,000 or 1:291). Prevalence estimates for the full mutation from this meta-analysis are lower than those in previous reviews of FXS epidemiological data. Approximately 2.4% of individuals with intellectual disability identified in this study had the full mutation.

FX 2.7.2: Premutations: Different studies have proposed a range of carrier frequencies across varied populations. While a study of 2,300 US women identified 1 in 382 as heterozygotes, a study of 119,000 individuals tested identified the premutation carrier frequency among US females to be 1 in 59. A larger study evaluating approximately 135,000 individuals across a self-reported ethnically diverse population identified a pan-ethnic premutation carrier frequency of 1 in 201. A different study of 21,411 anonymous Canadian females (mothers of newborns) identified 1 in 549 as heterozygotes. Previous screens for the prevalence of premutations (with 55–101 repeats) in French-Canadian women estimated the carrier frequency to be 1 in 259. A subsequent study demonstrated a 1 in 1,760 prevalence of premutation alleles among Canadian males. A study from Israel of 36,483 women who requested screening identified 1 in 157 as heterozygotes. This result is consistent with an earlier study of 9,459 women in Israel that found 1 in 152 with alleles having >54 repeats. In the individuals with no family history of FXS, 1 in 113–152 women were determined to have premutations with a CGG repeat range of 55–101. This estimate of the premutation carrier frequency is approximately twofold higher than that reported in the studies performed in Canada. Toledano-Alhadeff et al. obtained similar values when studying 14,334 preconceptual or pregnant women in Israel, namely, 1 in 113 women with >54 CGG repeats. This study excluded women with a family history of developmental delays. In addition, they found that the premutation heterozygotes were well distributed among all the Jewish ethnic groups, in contrast to a previous study. A fragile X screen of 10,000 newborn males in Taiwan showed a premutation prevalence of 1 in 1,674. Therefore the carrier frequencies vary widely among populations and may be higher than those determined in the French-Canadian population. Among females with FXPOI and simplex cases of adult males with cerebellar ataxia, the FMR1 premutation is identified in 4–6% and 2%, respectively.

FX 2.8: Special testing considerations

FX 2.8.1: Sensitivity and specificity: CGG repeat expansion to full mutations account for >99% of cases of FXS. Therefore, PCR tests that effectively detect and measure the CGG repeat region (up to 200 repeats) of the FMR1 gene are >99% sensitive. As one report demonstrates there also appears to be an association between schizophrenia and mood disorders with low FMRP. FXS should not be confused with the unrelated syndrome associated with the FRAXE locus (MIM 309548/locus MIM 30086).

FX 2.8.2: Indications for testing: The ACMG and the American College of Obstetricians and Gynecologists (ACOG) as well as the National Society for Genetic Counselors (NSGC) have published practice guidelines with recommendations regarding fragile X testing for diagnostic testing and heterozygote detection. These include screening with the appropriate family history of intellectual disability suggestive of FXS or ovarian insufficiency or failure prior to age 40 years. Studies have demonstrated that the diagnostic yield of FX testing in males with intellectual disability and learning delay is about 2.5% and in individuals with autism is ~1.2% suggesting that FX testing may not be indicated as a first-tier test. The identification of a full mutation in a male is considered diagnostic rather than predictive, inasmuch as penetrance of FXS is virtually 100% in males and the age of onset is not variable. The identification of a full mutation in a female may be diagnostic, although <50% of females with full mutations have intellectual disability. They may have some other manifestations of the disease such as avoidance personality, mood, or stereotypic disorders. Nonrandom X-inactivation may explain the milder phenotype in females, although the extent of symptoms cannot be determined by X-inactivation patterns from diagnostic tests since they evaluate the expansion and
methylations in blood and not the brain. The identification of a premutation in an asymptomatic male or female undergoing carrier testing (e.g., preconception carrier screening or due to a family history of intellectual disability) is predictive because FXPOI and FXTAS are not fully penetrant and are dependent on both age and allele size. Population carrier screening and newborn screening for FXS are somewhat controversial and not recommended at this time. It is important that clinicians and laboratorians alike refer to the ACMG/ACOG/NSGC guidelines referred above60 to make clinically relevant decisions.

**FX 2.8.3: Prenatal testing:** This test can be used for prenatal diagnosis in cells obtained from amniocentesis and chorionic villus sampling (CVS). Because methylation is not fully established at the time of CVS, the appearance of full mutations examined by a methylation-specific method may vary in CVS as compared with blood and amniocytes. Laboratories offering testing of chorionic villi must be aware of this tissue’s unique properties:

- Methylation associated with lyonization is usually not present, and methylation associated with full mutations may or may not be present.69 In the past, the hypomethylated status of this locus in this tissue had been thought of as a limitation or possible source of confusion. However, because it is unwarranted to use methylation status or X-inactivation for phenotypic prediction of a full mutation, the possible hypomethylation of this tissue is no disadvantage, provided that the tissue-specific basis of the hypomethylation is understood.70,71 It is acceptable to omit methylation analysis entirely when testing CVS specimens. In the minor fraction of CVS cases with a result that is ambiguous between a large premutation and a small full mutation by size criteria alone, a follow-up amniocentesis may be required.

- The degree of somatic variation in a full-mutation “smear” has a wider range of possibilities than is typically seen in blood specimens, from very limited to extraordinarily diffuse.

- Mosaicism between trophoblasts and somatic cells is theoretically possible. For this reason, when CVS results indicate a premutation, a follow-up amniocentesis has been suggested to rule out mosaicism for a full mutation. However, there are currently no known occurrences of this type of mosaicism.

Genetic counseling regarding the potential limitations of CVS is recommended. Close contact with the laboratory accepting the specimen should be maintained before testing begins and throughout the testing process.

**FX 2.9: Nomenclature**

The use of standard nomenclature is important for the accurate communication of results to health-care providers and is recommended by the ACMG and CAP in accordance with Human Genome Variation Society (HGVS) recommendations. According to HGVS, regarding the nomenclature for short sequence repeats, the designation for the FMR1 triplet repeats varies based on whether the AGG interruptions are being evaluated or not. If the AGG interruptions are not being evaluated, then the designation for the FMR1 triplet repeats starting at position c.-129 based on the coding DNA reference sequence, NM_002024.5 is c.-129CGG[X] for a male, and c.-129CGG[X] for a female. The start of the variable repeat is specified by -129, and CGG indicates the sequence of the repeat unit. The number of triplet repeats present is specified by “X.” If the exact size of the repeat cannot be determined (e.g., full mutations sized by Southern blot analysis), then the square brackets are replaced by parenthesis, (X), to signify uncertainties.72 Current HGVS recommendations do not address nomenclature for nucleotide repeat variants with size mosaicism. Standard nomenclature is recommended, although laboratorians and clinicians may continue to use common variant nomenclature. To avoid confusion, it is acceptable to describe a variant using standard nomenclature followed by the common name in parentheses throughout the report or to use the standard and common name in the beginning of the report (e.g., results) and either the standard or common name subsequently.

<table>
<thead>
<tr>
<th># CGG repeats</th>
<th>Category</th>
<th>Clinical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤44</td>
<td>Normal</td>
<td>No disease association. Rare cases with FMR1 deletion or base change may cause fragile X. Very low risk of CGG repeat expansion to next generation.</td>
</tr>
<tr>
<td>45–54</td>
<td>Intermediate or gray zone</td>
<td>No disease association. Very low risk of CGG expansion to full mutation within one generation.</td>
</tr>
<tr>
<td>&gt;200</td>
<td>Full mutation</td>
<td>Fragile X syndrome: clinical severity influenced by sex, degree of methylation, and level of mosaicism.</td>
</tr>
</tbody>
</table>

**FX 3: GUIDELINES**

**FX 3.1: Definition of normal and variant categories**

There are four allelic forms of the gene: normal, intermediate, premutation, and full mutation (Table 2). The associated number of CGGs for each can be defined based on our current information to date. It must be recognized that the borders of each definition may change with increased empirical data and research as well as the variation observed in laboratories.73

**FX 3.1.1: Normal alleles:** Normal alleles have a range of ~5 to ≤44 repeats. The most common repeat length is 29 or 30 CGG repeats. Normal alleles rarely have meiotic or mitotic instability.74

**FX 3.1.2: Intermediate (gray zone, inconclusive, borderline):** The range of repeats from ≥45 to ≤54 is considered intermediate (also referred to as gray zone, inconclusive, or borderline). Studies have shown that 7.7% of parents with FMR1 alleles in the 40–49 range and 25% of parents with FMR1 alleles in the 50–60 CGG repeat range are likely to pass a changed FMR1 allele to their children. Both expansion and contraction of the CGG repeat size were observed in the next generation.75 Alleles in this range can be considered normal in the sense that such alleles are not associated with FXS and have not been observed to expand to a full mutation in one generation. Although earlier studies suggested an association between alleles in this size range and FXPOI, larger subsequent studies did not support these initial findings.14,15 A small number of patients meeting the criteria for FXTAS with FMR1 intermediate alleles have been described, although larger studies are needed to determine the significance of this finding.16,76
Minor increases and decreases in repeat number can occur when alleles in the intermediate range are passed on, but there is no measurable risk of a child with FXS in the next generation. Alleles of this size may be associated with FXS in future generations or in distant relatives. Intermediate range alleles can be referred to as premutations if they are confirmed by family studies to be traceable to a known full mutation or unambiguous premutation. A gray zone allele of 52 repeats was reported to expand to a premutation allele of 56 repeats in one generation, which subsequently expanded to a full-mutation allele in the next generation. Testing at-risk relatives of individuals with an intermediate allele may determine the stability of the allele in the family. However, the rate of expansion of intermediate alleles is not well understood.

FX 3.1.3: Premutation: Premutation alleles range from ≥55 to ≤200 repeats. These alleles are long repeat tracks that are unstably transmitted from parent to child. Expansions from the premutation size range to the full mutation typically occur during maternal transmission. Due to the possibility of somatic mosaicism, careful examination for mosaicism into the full-mutation range is recommended when a premutation is detected. FMR1 alleles in the premutation size range are not hypermethylated or associated with FXS. Although males and females with premutations and manifestations of some symptoms of FXS have been reported, further studies are needed. Women with premutation alleles are considered to be at risk for having affected children. The smallest FMR1 premutation allele reported to expand to a full mutation in a single generation is 56 repeats. Females who carry an FMR1 premutation should be offered prenatal diagnosis for all pregnancies. All at-risk family members of known heterozygotes should be offered testing to determine their status (Table 3).

FX 3.1.4: Full mutations: Full mutations have more than 200 repeats, typically several hundred to several thousand. There is usually broad somatic variation within each patient. Partial or complete promoter hypermethylation is typically seen in full mutations in most or all cases, except in the case of DNA extracted from CVS (see Section 2.8.3).

FX 3.1.5: Mosaicism: Size mosaics due to aneuploidy and methylation mosaics have been observed at the FMR1 CGG repeat region. When mosaicism is present, tissue-specific differences can be seen. Individuals with size or methylation mosaicism may be higher functioning than individuals with completely methylated full mutations.

FX 3.1.5.1: Size mosaics: This term refers to an individual with subpopulations of full mutations, which are methylated, and premutations, which are unmethylated. Occasionally, there also may be minor subpopulations with near-normal or normal length. For this reason, care must be taken in examining larger alleles when a normal or gray zone allele is detected using standard PCR methods.

FX 3.1.5.2: Methylated mosaics: This term refers to individuals with an FMR1 allele in the full-mutation size range, with subpopulations of cells containing an unmethylated full mutation and other populations of cells containing a methylated full mutation.

FX 3.1.6: AGG interruptions: In stable, normal alleles, the CGG region is interrupted by an AGG triplet after every 9 or 10 CGG repeats. The AGG triplets are thought to anchor the region during replication and prevent strand slippage. Premutation alleles, in contrast, are less likely to contain AGGs and have long stretches of uninterrupted CGGs at their 3′ end. The number of AGG interruptions helps predict the risk of expansion from premutations of <100 repeats to full mutations. Recent studies have demonstrated that premutation alleles with no AGGs are at risk for expansion to full mutations in the next generation while alleles that include AGG interruptions are associated with greater intergenerational stability of the repeat. More than 5,000 cases of normal, intermediate, and premutation alleles were surveyed to examine the relationship of the sex of the transmitting parent, repeat size, and pattern of AGG interruptions with allele instability. The instability was strongly influenced by the sex of the transmitting parent and by the number of repeats and location of the AGG interruptions in the parental allele.

Given the emerging role of AGG interruptions, determining the size of the repeat and the AGG interruption structure is relevant when performing family/prenatal counseling for women with premutations. Smaller repeat lengths and the presence of at least two AGGs decrease the risk of expansion in the next generation. Although not routinely performed, direct testing for the AGG triplets can be interrogated via triplet repeat–primed PCR (TP-PCR) or long-read single-molecule sequencing and is available clinically in a limited number of laboratories.

FX 3.2: Methodological considerations

All general guidelines for Southern blot analysis and PCR in the ACMG Technical Standards for Clinical Laboratories apply. The following additional details are specific for fragile X testing. For this test, there are many valid methods with different strengths and weaknesses. Laboratories will likely need to use more than one method because no single method can characterize all aspects of the FMR1 full mutation, and precision in determining allele size varies between PCR and Southern blot analysis. For mosaic samples spanning the premutation and full-mutation ranges, traditional PCR may amplify the premutation population but not the subpopulation with the full mutation. The expected phenotype for an individual with a premutation versus mosaicism for a premutation and full mutation is very different. Therefore, not detecting the full mutation would result in a different risk assessment for fragile X, FXTAS, and FXPOI resulting in the previous recommendation to always perform Southern blot analysis along with traditional PCR. Newer repeat-primed PCR methods or methylation PCR reduce the need to perform Southern blot analysis (see Section 3.2.2.7) on every sample. Additionally, because the fragile X assay is technically challenging due to high GC content, varying size repeats, and size limitations of conventional PCR and Southern blot (especially in the case of small premutations, or unmethylated normal alleles), it is important to ensure that appropriate controls are used while processing clinical samples. Characterized reference material possessing specific FMR1 premutation and full-mutation CGG repeat sizes that can be used across different methodologies may be obtained from the CDC Genetic Testing Reference Materials (Get-Rm) Program (https://www.cdc.gov/labquality/get-rm/index.html) through the Coriell Institute for Medical Research (https://www.coriell.org/).
### Table 3. Suggested comments for the reporting of fragile X results.

<p>| Variant                        | Interpretation                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Clinical Significance for the Patient                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Clinical Significance for the Patient's Family                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Recommendations                                                                                                                                                                                                                                                                                                                                                                                                  |
|-------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full mutation, heterozygote female | (1) This patient possesses a full fragile X expansion mutation with greater than 200 CGG repeats on one FMR1 allele as determined by triple repeat-primed PCR. (2) This patient possesses a full fragile X expansion mutation with approximately [<strong>] CGG repeats on one FMR1 allele. Southern blot analysis identified a pattern consistent with hypermethylation in the FMR1 gene. The second FMR1 allele contains [</strong>] CGG repeats, which falls within the normal range (≥5–≤44 CGG repeats). | These results indicate that this patient possesses the common trinucleotide repeat expansion variant observed in the majority of patients affected with fragile X syndrome. Females with full mutations have variable clinical presentations, ranging from no detectable deficits to clinical symptoms as severe as affected males. Mothers of children with full mutations carry a premutation or a full mutation in one of their FMR1 genes and are at risk to have other affected children. With each pregnancy, female carriers of full mutations have a 50% chance of passing the mutation on to their child (daughters and sons). | Genetic counseling and FMR1 DNA testing are recommended for at-risk family members to determine the size of their FMR1 allele(s). Prenatal diagnosis in future pregnancies should be considered. | Genetic counseling and FMR1 DNA testing are recommended for at-risk family members to determine the size of their FMR1 allele(s). Prenatal diagnosis in future pregnancies should be considered. |
| Full mutation, male            | (1) This patient possesses a full fragile X expansion mutation with greater than 200 CGG repeats on one FMR1 allele as determined by triple repeat-primed PCR. (2) This patient possesses a full fragile X mutation with approximately [*<strong>] CGG repeats. Southern blot analysis identified a pattern consistent with hypermethylation in the FMR1 gene. | This result is consistent with a clinical diagnosis of fragile X syndrome. Mothers of children with full mutations carry a premutation or a full mutation in one of their FMR1 genes and are at risk to have other affected children. | | |
| Premutation heterozygote, female | One allele of this patient’s FMR1 gene contains [</strong>] CGG repeats, which falls within the premutation range (≥55–≤200 CGG repeats). The second FMR1 allele contains [**] CGG repeats, which falls within the normal range (≥5–≤44 CGG repeats). | Females carrying a premutation allele do not have fragile X syndrome, but they are at an increased risk for fragile X-associated primary ovarian insufficiency (FXPOI), which is defined as menopause prior to the age of 40. Approximately 20% of female premutation heterozygotes have FXPOI, although the rate varies with expanded repeat length; the greatest prevalence of FXPOI is between 80 and 100 CGG repeats. Women are also at risk for fragile X-associated tremor ataxia syndrome (FXTAS), an adult-onset neurodegenerative disorder that occurs in approximately 16% of women who are premutation heterozygotes. Premutation heterozygotes are also at increased risk for fragile X-associated neuropsychiatric disorders (FXAND). | The premutation allele may have been inherited from either parent. Males can pass a premutation allele to female children, however, in male transmission the size of the premutation allele remains stable. When premutation alleles are transmitted from females to their children, expansion of the premutation allele into the full-mutation range can occur. Recent studies have demonstrated that premutation alleles with no AGGs are at risk for expansion to full mutations in the next generation while alleles that include AGGs interruptions are associated with greater intergenerational stability of the repeat. | Genetic counseling and FMR1 DNA testing are recommended for at-risk family members to determine the size of their FMR1 allele(s). Females with premutations may be referred for determination of AGG interruptions. Prenatal diagnosis in future pregnancies should be considered. |</p>
<table>
<thead>
<tr>
<th>Variant</th>
<th>Interpretation</th>
<th>Clinical Significance for the Patient</th>
<th>Clinical Significance for the Patient's Family</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premutation, male</td>
<td>This patient's <em>FMR1</em> gene contains [*] CGG repeats, which falls within the premutation range (≥55–≤200 CGG repeats).</td>
<td>Males with premutation alleles do not have fragile X syndrome but they are at risk for fragile X-associated tremor ataxia syndrome (FXTAS), an adult-onset neurodegenerative disorder that occurs in approximately 40% of men with a premutation. Premutation heterozygotes are also at increased risk for fragile X–associated neuropsychiatric disorders (FXAND).</td>
<td>In males, fragile X premutations are maternally inherited. When premutation alleles are transmitted from females to their children, expansion of the premutation allele into the full-mutation range can occur. Recent studies have demonstrated that premutation alleles with no AGGs are at risk for expansion to full mutations in the next generation, while alleles that include AGG interruptions are associated with greater intergenerational stability of the repeat. In the next generation, all daughters of males carrying fragile X premutations will inherit the premutation allele and will be at risk for having sons with fragile X syndrome and fragile X heterozygote daughters.</td>
<td>Genetic counseling and <em>FMR1</em> DNA testing are recommended for at-risk family members to determine the size of their <em>FMR1</em> allele(s). Prenatal diagnosis in future pregnancies should be considered.</td>
</tr>
<tr>
<td>Intermediate range, female heterozygote</td>
<td>One allele of this patient's <em>FMR1</em> gene contains [<em>] CGG repeats, which falls within the intermediate range (≥45–≤54 CGG repeats). The second <em>FMR1</em> allele contains [</em>] CGG repeats, which falls within the normal range (≥25–≤44 CGG repeats).</td>
<td>No <em>FMR1</em>-related disorders are associated with patients possessing an intermediate range allele.</td>
<td>Studies have shown that 7.7% of parents with <em>FMR1</em> alleles in the 40–49 range and 25% of parents with <em>FMR1</em> alleles in the 50–60 CGG repeat range are likely to pass a changed <em>FMR1</em> allele to their children. Both expansion and contraction of the CGG repeat size were observed in the next generation.</td>
<td>Genetic counseling and <em>FMR1</em> DNA testing are recommended for at-risk family members to determine the size of their <em>FMR1</em> allele(s). Prenatal diagnosis in future pregnancies should be considered.</td>
</tr>
<tr>
<td>Intermediate range, male</td>
<td>This patient's <em>FMR1</em> gene contains [*] CGG repeats, which falls within the intermediate range (≥45–≤54 CGG repeats).</td>
<td>No <em>FMR1</em>-related disorders are associated with patients possessing an intermediate range allele.</td>
<td>Studies have shown that 7.7% of parents with <em>FMR1</em> allele in the 40–49 range and 25% of parents with <em>FMR1</em> alleles in the 50–60 CGG repeat range are likely to pass a changed <em>FMR1</em> allele to their children. Both expansion and contraction of the CGG repeat size were observed in the next generation.</td>
<td>Genetic counseling and <em>FMR1</em> DNA testing are recommended for at-risk family members to determine the size of their <em>FMR1</em> allele(s). Prenatal diagnosis in future pregnancies should be considered.</td>
</tr>
<tr>
<td>Normal range, female</td>
<td>This patient's <em>FMR1</em> alleles contain [<em>] and [</em>] CGG repeats, both of which fall within the normal range (≥25–≤44 CGG repeats).</td>
<td>No <em>FMR1</em>-related disorders are associated with patients possessing normal range alleles.</td>
<td>Genetic counseling and <em>FMR1</em> DNA testing are recommended for at-risk family members to determine the size of their <em>FMR1</em> allele(s). Prenatal diagnosis in future pregnancies should be considered.</td>
<td>Genetic counseling is recommended.</td>
</tr>
<tr>
<td>Normal range, male</td>
<td>This patient's <em>FMR1</em> allele contains [*] CGG repeats, which falls within the normal range (≥25–≤44 CGG repeats).</td>
<td>No <em>FMR1</em>-related disorders are associated with patients possessing normal range alleles.</td>
<td>Genetic counseling is recommended. If clinically indicated, <em>FMR1</em> sequencing and/or deletion analysis may be considered.</td>
<td>Genetic counseling is recommended.</td>
</tr>
</tbody>
</table>
FX 3.2.1.2: Controls should be included to confirm the proper choice and activity of restriction enzymes and probe. They should ideally represent the more difficult to recognize genotypes. To verify digestion and hybridization parameters, a normal control will suffice. However, in fragile X blot analyses, the abnormal controls are extremely important because they provide quality control on the resolution of small premutations and the detectability of diffuse smears. Refer to Section 3.2 for a discussion of reference materials. Laboratories should use verified reference materials to confirm their results.

FX 3.2.1.3: For female patients, it should be noted that the degree of separation between two differently sized normal alleles could appear identical to that between a normal and a premutation allele (e.g., 20 and 44 repeats vs. 35 and 59 repeats). A Southern blot analysis with superior resolution and appropriate size standards or controls is required to distinguish between these possibilities (Fig. 1). Alternatively, most PCR-based methods can provide the required resolution. Similar considerations apply to detection of premutation alleles in normal transmitting males.

FX 3.2.1.4: Because full mutations can be extremely diffuse and faint, signal to noise ratios must be very good. Laboratories are advised to be aware of the many different appearances of full mutations. Full mutations are not likely to be overlooked in males, inasmuch as the normal signal will be absent (or light, in size mosaics), but full mutations can be easily missed in females if the background is poor. Skewed X-inactivation may also present problems in the use of Southern blot analyses performed with methylation-sensitive restriction enzymes in the detection of females with premutations or full mutations.

FX 3.2.1.5: Migration distances should be interpreted using a standard ladder such as lambda Hind III fragments or a set of carefully chosen, independently tested human references.

FX 3.2.1.6: The following guidelines refer to methylation analysis using two different restriction enzymes, one of which is methylation-sensitive.

FX 3.2.1.6.1: In DNA extracted from tissues other than chorionic villi, methylation analysis reveals the degree of hypermethylation in full mutations and shows the distribution of X-inactivation in any female with two distinguishable alleles. Southern blot analysis with the addition of methylation-sensitive enzyme digestion can

- Help discriminate between premutations and full mutations for the rare alleles that fall near the boundary (i.e., around 200 repeats).
- Detect rare individuals who are methylation mosaics.

FX 3.2.1.6.2: In DNA extracted from tissues other than chorionic villi, the results of routine methylation analysis and PCR are sometimes confounded by an abnormal karyotype such as 45,X or 47,XXX. Individuals with testicular feminization (XY females) will have a male methylation pattern. In these cases, sex chromosome constitution should be confirmed. Interpretation of results should take the individual’s karyotype into account, when it is available to the molecular laboratory.

FX 3.2.1.6.3: In DNA extracted from tissues other than chorionic villi, methylation analysis increases the difficulty of detecting females with small premutations who have highly skewed X-inactivation. Double digestion with a methylation-sensitive restriction enzyme causes the signal from each allele in a female to be split into active and inactive bands, forming four bands in a heterozygous female. When X-inactivation is balanced in a heterozygote, the two active bands are readily seen, although the two inactive bands may co-migrate. However, if X-inactivation is heavily skewed, there may be only two visible bands of the predominant X population. This result is challenging to interpret particularly when the premutation is predominantly inactive because then it appears only in the upper region of the gel, where resolution is considerably poorer. For an example of a heterozygote with extremely skewed X-inactivation, see lane 13 of Fig. 1. Lanes 3 and 4 show two females with oppositely skewed X-inactivation. The above data are true for the use of the StBl21.2 probe. Use of other probes such as pE5.1 will yield an additional small control band.

FX 3.2.1.6.4: FMR1 methylation status should not be used to predict severity in fetal or newborn cases, regardless of whether the DNA was extracted from amniocytes, chorionic villi, or blood.

FX 3.2.1.6.5: In DNA extracted from chorionic villi, the FMR1 region usually does not have methylation associated with X-inactivation, and it may or may not have hypermethylation associated with full mutations if the CVS procedure was performed before 12.5 weeks’ gestation. When testing DNA extracted from chorionic villi, methylation analysis is optional. Incidentally, methylation analysis before 12.5 weeks of gestation can serendipitously alert a laboratory to maternal cell contamination in chorionic villi specimens. Inasmuch as methylation associated with X-inactivation is usually not present at this locus in tissue obtained via CVS, a strong normal inactive band can be a sign of possible maternal cell contamination. Other explanations for such a band include X-inactivation in some fetal cells or incomplete digestion. Further investigation is merited in such cases.

FX 3.2.2: PCR methods

FX 3.2.2.1: Several sets of primers, PCR conditions, and methods of separation and detection have been published. Other primers and methods can be used if equivalence is demonstrated.
keeping in mind regions such as deletion hotspots, particularly in primer design. PCR can be performed by incorporating a fluorescently labeled primer followed by capillary electrophoresis (CE). Regardless of the locus, any PCR can theoretically fail to detect an allele if there is a polymorphism at the primer binding site. There are no known polymorphisms that would affect any of the commonly used primers. Patient amplicon sizes should be determined using a size standard. For CE, a standard fluorescently labeled size marker can be used.

**FX 3.2.2.2:** Controls representing the genotypes to be distinguished should be used for each run. Refer to Section 3.2 for information on reference materials. The upper limit of allele size that can be successfully detected should be known, and a control corresponding to that size should be included in each run.

Laboratories should confirm the size of their control DNA by sequencing (if possible) or by using verified reference materials.

**FX 3.2.2.3:** Amplification of GC-rich regions is difficult, and special conditions are required. The difficulty increases with increasing numbers of CGG repeats; therefore, many PCR strategies do not attempt to detect large alleles. In such a system, it is not possible to tell the difference between a female who is homozygous for a normal allele and one who has a large nonamplifiable second allele. Similarly, patients who are mosaics for premutations and full mutations may appear to have only premutations.

**FX 3.2.2.4:** When a PCR strategy can detect large alleles, amplification nevertheless may favor the smaller allele in any specimen with multiple alleles, i.e., females and mosaics.

![Triplet repeat primed PCR results](image)

**Fig. 2** Triplet repeat primed PCR results. Triplet repeat–primed polymerase chain reaction (TP-PCR) using a two-primer system (a) and a three-primer system (b). (a) Top panel: female with 20 and 31 CGG repeats. Middle panel: male with 103 CGG repeats. Bottom panel: male with size mosaicism from ~140 to 800 CGG repeats (inset: reduced y-axis to better visualize baseline). (b) Left panel: male with 32 CCG repeats. Middle panel: female with 20 and 64 CGG repeats. Right panel: female with 29 and >200 CGG repeats.
Such methods should be validated with heterozygous females and mosaics, in addition to males. Because of disproportionate amplification, PCR is not reliable for determining the ratio of different species in a mosaic individual. In PCR amplification of samples from females and mosaics, heteroduplexes can form due to technical artifacts, particularly if denaturing gels are used and should be duly considered.

FX 3.2.2.5: Basic PCR amplification is not affected by methylation. Although PCR tests specifically modified to detect methylation status have been described,104,105 the original PCR strategies that have been in use for many years are completely independent of methylation.

FX 3.2.2.6: When a PCR strategy is used to detect full mutations, the presence of a deletion hotspot in the CGG repeat region should be noted.106 Primers located within the deletion hotspot may result in failure to detect the expanded allele. Primers located upstream of the deletion hotspot may result in apparent size mosaicism.

FX 3.2.2.7: Triplet repeat–primed PCR:
FX 3.2.2.7.1: TP-PCR allows rapid detection of PCR products formed by a chimeric primer binding inside a triplet repeat region. In TP-PCR for fragile X, one primer is anchored completely outside of the CGG repeat region, whereas the other overlaps the CGG repeat and the adjacent nonrepeated sequence. A third primer can be anchored outside of the CGG region that, when paired with the opposite anchored primer, will amplify “over” the CGG repeat. This will increase the amount of full-length product from the largest CGG repeat allele and in some assays enables accurate sizing of alleles up to 200 CGG repeats. From the chimeric primer annealing at each CGG repeat, multiple amplicons are made, forming products each with a length differing by three bases. The application of TP-PCR for fragile X testing has been described in multiple studies.107–113

FX 3.2.2.7.2: Although products can be separated by ethidium bromide–stained agarose gels to detect “smears,” combining TP-PCR with single base resolution fragment analysis, the “smear” on a lower resolution agarose gel becomes characteristic “stutters” or “ladders” that are easily visualized. The stuttering will end at the allele with the greatest number of CGG repeats. An increase of this product can be seen, particularly if the third primer is used. For alleles with >200 repeats, a “compression” product can be seen, which can be used as a marker for an allele with >200 repeats, although the fragment cannot be sized (Fig. 2a). If a third primer is used, full mutations appear as a compression (or compacted) product. These are seen by CE as a compacted product of ~200 CGG repeats, thereby indicating a full mutation (Fig. 2b).

FX 3.2.2.7.3: PCR followed by CE at a single base resolution has a high analytical sensitivity and specificity for detecting expanded alleles. A threshold can be set to distinguish premutation and full-mutation (and intermediate, if desired) alleles from normal alleles. If no expansion is detected, no further testing is necessary. Alleles with a stuttering pattern past a threshold (>150 repeats) and consistent with an expansion can be tested further to determine methylation status (by Southern blot analysis or methylation-sensitive PCR) or to determine size (traditional PCR and/or Southern blot analysis). Therefore, the simple yes/no answer for the presence of expansions can eliminate the need for Southern blot analysis in samples with normal sized and intermediate sized \( FMR1 \) allele(s).

FX 3.2.2.7.4: TP-PCR resolves the challenges associated with apparent homozygous females, because the normal allele will not outcompete the expanded allele. The increased sensitivity of the TP-PCR assay also resolves the difficulty of detecting mosaics in males because mosaicism can be detected up to ~10%,109,110 Laboratories using TP-PCR are encouraged to define the sensitivity of their assay using DNA with a normal \( FMR1 \) allele titrated with serial dilutions of full-mutation \( FMR1 \) DNA.

FX 3.2.2.7.5: Controls for various stages of processing need to be included as part of the testing workflow, including a no DNA control, a sensitivity control (particularly for the detection of mosaics and full expansions), and a rotating group of patient controls of different genotypes or by using verified reference materials.98 Refer to Section 3.2 for information on reference materials.

FX 3.2.3: Non–Southern blot methods for methylation detection
FX 3.2.3.1: Methylation-specific PCR: Several methods besides Southern blot analysis have been described to determine methylation. Methylation-specific PCR involves the differential treatment of DNA with methylation-specific restriction enzymes followed by allele-specific PCR and resolution of the PCR products with CE.106,113 This method not only determines methylation status but also \( FMR1 \) allele size up to 250 repeats; however, to size alleles with >250 repeats accurately, Southern blot analysis is needed.

FX 3.2.3.2: Multiplexed ligation probe amplification (MLPA): MLPA has been described to identify males with methylated fragile X alleles.114 In this method, sequence-specific probes are hybridized to methylated and unmethylated alleles. Probes are simultaneously ligated and digested with a methylation-sensitive restriction endonuclease. A universal PCR primer set will amplify only probes that are ligated and undigested, indicating methylated alleles.

FX 3.2.3.3: Real-time PCR: Real-time PCR has also been described with TaqMan chemistry and by melt curve analysis, using methylation-specific PCR. TaqMan chemistry amplification separates methylated and unmethylated specific alleles and provides a ratio based on amplification cycle thresholds. Using melt analysis, however, methylated and unmethylated alleles are amplified simultaneously due to differences of GC content, but this can be resolved by differences in melting temperature between methylated and unmethylated alleles.115 These methods have high analytical sensitivity and specificity for detecting methylation in males but are less sensitive and specific in females.

FX 3.3: Interpretations
FX 3.3.1: In addition to the items described in the general ACMG Technical Standards for Clinical Genetics Laboratories (https://www.nature.com/gim/articles?type=acmg-standards-and-guidelines), the following elements should be included in the report.

FX 3.3.1.1: State whether the method used was PCR, Southern blot analysis, or both. If Southern blot analysis is the method of evaluation, state the restriction enzymes and probes that were used. If PCR, describe the PCR method used (e.g., TP-PCR) and method used for separation and detection (e.g., CE).

FX 3.3.1.2: State the ranges (per literature and guidelines) and analytical precision (as determined by the testing laboratory during validation) for the different categories of normal, intermediate (gray zone, borderline, inconclusive), premutation, and full mutation.

FX 3.3.1.2.1: Note that it is not necessarily obvious that the borderline category (intermediate/gray) refers to the border between normal and premutation and not to the border between premutation and full mutation. Similarly note that the term instability, which is often used regarding borderline allele calls to describe minor intergenerational or mitotic changes, may unintentionally suggest a risk of having an affected child or personal late-onset symptoms.

FX 3.3.1.3: Classify the patient’s result using the defined categories and HGVS nomenclature (refer to Section 2.9). Common nomenclature can be included for clarity. The term size mosaic should be used for alleles that have significant subpopulations in both the premutation and full-mutation range. Caution is needed not to suggest that a sample with size mosaicism has multiple
alleles. The term subpopulation of an allele is recommended. See Table 3 for example interpretation paragraphs for use in reporting.

FX 3.3.1.4: All positive results should state that genetic counseling is recommended, and testing is available for at-risk family members.

FX 3.3.2: The following descriptive elements may appear, with caution:

FX 3.3.2.1: The size of the alleles may be reported and could be of clinical use for individuals who are heterozygous for the premutation. The premutation allele size may be used for risk assessment in determining the chance of expansion in the offspring of these individuals and in determining the chance of FXS or FXPOI. If so, the precision used in quoting the size must be supportable by the precision of the size marker used, the sharpness of the bands or peaks, degree of stutter, and so on. It may be appropriate to state a range or use qualifying terms such as “approximately.” Descriptions such as “positive for an allele with 55–200 repeats” are ambiguous and should not be included on the laboratory report.

The CAP/ACMG Biochemical and Molecular Genetics Resource Committee published results of laboratory performance on the CAP proficiency surveys for molecular genetic testing for fragile X conducted between 2001 and 200923 and the acceptable range for sizing CGG repeats for fragile X is based on these results. Acknowledging the technical limitations of size analysis, the ACMG supports the following grading criteria for the CAP/ACMG proficiency testing survey: consensus size ±5 repeats for alleles with <55 repeats, consensus size ±10 repeats for alleles with 56–100 repeats, and consensus size ±2 SDs for alleles with >100 repeats.

FX 3.3.2.2: Description of methylation may be provided. The two kinds of methylation must be clearly distinguished: methylation due to X-inactivation and hypermethylation of full mutations. The term methylation mosaic or incomplete methylation may be used if not all molecules in a full mutation are hypermethylated.

FX 3.3.2.3: Occasionally unexpected patterns are seen that may not fit within the descriptions provided here. In those cases, a detailed description may be helpful. For example, methylation PCR may exhibit a pattern of size and methylation mosaicism with subpopulations of premutations (<200 CGG repeats), which are methylated, and full mutations (>200 CGG repeats), which are unmethylated.

FX 3.3.3: Helpful points on alternative diagnoses may be included

FX 3.3.3.1: There are rare forms of FMRP deficiency not caused by CGG expansion, which may not be detected by this test.

FX 3.3.3.2: Intellectual disability associated with other fragile X sites, FXR1, or other gene variants will not be detected with this test.

FX 3.3.3.3: DNA analysis for FXS should be performed as part of a comprehensive genetic evaluation that includes chromosomal microarray and a five-cell screen for chromosome rearrangement analysis as recommended by ACMG.

FX 3.3.4: Comments on phenotype, if included, should be abstract rather than case specific. The following concepts apply:

FX 3.3.4.1: All males with full mutations have FXS to some degree. The severity cannot be predicted from the size of the full mutation, but if premutations are also present or if the majority of the full-mutation molecules are unmethylated, the phenotype may be less severe.

FX 3.3.4.2: Females with full mutations exhibit a wide spectrum of phenotypes. They may be as severely affected as a male with an expanded fragile X allele (which is itself a range of phenotypes). Females with full mutations may also exhibit very mild learning disabilities or have no detectable deficits. The severity cannot be predicted from the size of the full mutation, nor can it be predicted from the pattern of X-inactivation in blood.

FX 3.3.4.3: Individuals with heterozygous premutations should not be interpreted as unaffected. Females who carry a premutation are at risk for FXPOI and FXTAS. Males with the premutation are at risk for FXTAS. Both sexes are at risk for FXAND. If an individual referred for diagnostic testing due to intellectual disability, autism, or learning disability is found to carry a premutation, the upper end of the premutation is often associated with these problems, because FMRP levels are lower than normal above 120 repeats. FMRP deficiency or mosaicism for a full mutation can be detected.

FX 3.3.4.4: Individuals with intermediate alleles should be interpreted as unaffected. Even more so than a premutation, an intermediate allele is considered a coincidence when found in an individual referred for diagnostic testing due to intellectual disability, learning disability, or autism. FMRP deficiency or mosaicism for a full mutation can be investigated by methylation-sensitive Southern blot analysis but with less likelihood of success because intermediate alleles are common in the general population.

FX 3.3.5: Comments on reproductive risk, if included, should be abstract rather than case specific. The following concepts apply:

FX 3.3.5.1: All affected males and most affected females inherit their variant from their mothers. Mothers carry either a premutation or full-mutation allele. Females with heterozygous premutations may have inherited their FMR1 allele from either their mother or father.

FX 3.3.5.2: Women with full mutations have a theoretical 50% chance of passing on the full mutation with each pregnancy.

FX 3.3.5.3: Women with premutations have a 50% chance of passing on the fragile X variant with each pregnancy. If it is passed on, the chance the allele will increase to a full mutation depends on its size in the mother and the number of AGG interruptions. Probabilities range from 3% for maternal alleles with CGG repeats from 55 to 59 (1/23 transmissions) to ~100% for maternal alleles with 90 CGGs and above. The smallest allele known to expand to full mutation is 56 repeats. Laboratories should be familiar with publications on this topic, including any current publications.

FX 3.3.5.4: Men with premutations will almost always pass premutation alleles to all their daughters. An extremely rare phenomenon involves males with premutations who have had daughters with full mutations, apparently due to gonadal mosaicism for full mutations. The sons of men with the premutation are not at risk for developing the FXS or FXTAS since they inherit their father’s Y chromosome.

FX 3.3.5.5: To date, there have been no reports of males or females with heterozygous intermediate alleles having offspring with an FMR1 allele in the full-mutation range. Instability may be identified if the allele can be traced through the family to a known full mutation or unambiguous premutation. In the absence of such a connection, it may be possible to show meiotic instability or a specific repeat sequence pattern (absence of AGG interruptions) that is at higher risk for instability. Testing for AGG status is available in a limited number of laboratories.

FX 4: ALTERNATIVE TESTING METHODS

FX 4.1: Next-generation sequencing (NGS)

Testing for FMR1 repeats is included in expanded carrier testing using NGS for multiple genes. Inherent limitations of short read NGS technology include difficulties sequencing across GC-rich regions, ineffective mapping of repetitive elements, and in the case of capture-based technology, PCR amplification bias of smaller alleles compared to larger full-mutation FMR1 alleles.
To combat these constraints, multiple algorithms have been designed to identify clinically relevant repeat expansions from short read sequence data. However, these attempts demonstrated poor sensitivity and specificity performance in detection of FMR1 expanded alleles.\textsuperscript{122,123} Currently, short read NGS technology cannot reliably detect expanded FMR1 alleles and should not be used to rule out or confirm any FMR1-related disorders. Advances in genome testing, using PCR-free methods, reduces some of the difficulties in sequencing through repetitive regions. Combined with new analysis software, repeat disorders may be identified from PCR-free genomes.\textsuperscript{124–126}

although genome sequencing for fragile X is cost prohibitive for expanded carrier testing. In those applications, FMR1 testing is often performed separately.

Single-molecule, real-time (SMRT) long-read sequencing is able to sequence through a full-mutation allele of 750 CGG repeats (~2 kb) and may be used to distinguish the number and location of AGG interruptions.\textsuperscript{94,127} Long-read technology is not yet widely available for clinical use, though this may change as error rate and costs decrease, and more bioinformatics tools become available for clinical application. Currently, TP-PCR and Southern blot methods remain the gold standards for identification of expanded FMR1 alleles and CGG repeat quantification.

**FX 4.2:** Cytogenetic evaluation

Testing for the fragile site FRAXA at Xq27 is no longer an acceptable diagnostic method. Clinical and analytical specificity and sensitivity are both insufficient.

**FX 4.3:** Protein analysis

Immunohistochemical staining for FMRP is a valid diagnostic method in lymphocytes.\textsuperscript{71} Willemsen et al. demonstrated that staining for the FMRP protein in chorionic villus samples could be used as an alternative prenatal diagnostic method for detection of full mutations in male fetuses.\textsuperscript{56} The situation is more complicated in female fetuses for which some chorionic villi may be completely positive and others from the same sample may be completely negative for FMRP staining. The authors’ data shed light on the timing of X-inactivation in chorionic villus cells of the female fetus. The diagnostic application of this method is not recommended at this time for the prenatal diagnosis of females carrying FMR1 full mutations.

**FX 5: POLICY STATEMENTS**

**FX 5.1**

The American College of Medical Genetics and Genomics issued a policy statement titled Fragile X Syndrome: Diagnosis and Carrier Testing in 1994,\textsuperscript{128} which was updated in October 2005.\textsuperscript{63} This document is also available online (http://www.acmg.net). These Standards are in general agreement with that statement.

**FX 5.2**

The NSGC also published practice guidelines to assist genetic counselors in providing accurate risk assessment and appropriate educational and supportive counseling for individuals with positive test results and families affected by FMR1-associated disorders.\textsuperscript{64} Additionally, in 2017, ACOG issued a Committee Opinion, No. 691, on carrier screening for genetic conditions, including fragile X syndrome.\textsuperscript{65} The Standards presented here are in general agreement with those opinions.

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Age intolerance from clinical molecular testing for fragile X syndrome. N.C.R. and E.L. declare no competing interests. E.S., A.B., K.K., and H.V.R. direct or work in laboratories that offer clinical molecular testing for fragile X syndrome. 

**ADDITIONAL INFORMATION**

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**COMPETING INTERESTS**

E.S., A.B., K.K., and H.V.R. direct or work in laboratories that offer clinical molecular genetic testing for fragile X syndrome. N.C.R. and E.L. declare no competing interests.