

Protocol

Genetic Testing for Developmental Delay and Autism Spectrum Disorder

(20459, 20483, 20481)

Medical Benefit		Effective Date: 01/01/18	Next Review Date: 09/18
Preauthorization	Yes	Review Dates: 09/10, 09/11, 03/12, 03/13, 03/14, 11/14, 11/15, 09/16, 05/17, 09/17	

Preauthorization is required.

The following protocol contains medical necessity criteria that apply for this service. The criteria are also applicable to services provided in the local Medicare Advantage operating area for those members, unless separate Medicare Advantage criteria are indicated. If the criteria are not met, reimbursement will be denied and the patient cannot be billed. Please note that payment for covered services is subject to eligibility and the limitations noted in the patient's contract at the time the services are rendered.

Populations	Interventions	Comparators	Outcomes
Individuals: • With developmental delay/intellectual disability, autism, or congenital anomalies not specific to a genetic syndrome	Interventions of interest are: • Chromosomal microarray testing	Comparators of interest are: • Karyotyping	Relevant outcomes include: • Test accuracy • Test validity • Changes in reproductive decision making • Morbid events • Resource utilization
Individuals: • With developmental delay/intellectual disability, autism, or multiple congenital anomalies not specific to a genetic syndrome	Interventions of interest are: • Next-generation sequencing panel testing	Comparators of interest are: • Chromosomal microarray testing	Relevant outcomes include: • Test accuracy • Test validity • Changes in reproductive decision making • Morbid events • Resource utilization
Individuals: • With intellectual disability, developmental delay, or autism spectrum disorder	Interventions of interest are: • <i>FMR1</i> variant testing	Comparators of interest are: • Standard clinical evaluation without genetic testing	Relevant outcomes include: • Test accuracy • Test validity • Resource utilization
Individuals: • Who are asymptomatic with a family history of fragile X syndrome or intellectual disability seeking reproductive counseling	Interventions of interest are: • <i>FMR1</i> variant testing	Comparators of interest are: • Standard clinical evaluation without genetic testing	Relevant outcomes include: • Test accuracy • Test validity • Changes in reproductive decision making
Individuals: • With known <i>FMR1</i> mutation carrier status and current pregnancy seeking prenatal testing	Interventions of interest are: • <i>FMR1</i> variant testing	Comparators of interest are: • Standard clinical evaluation without genetic testing	Relevant outcomes include: • Test accuracy • Test validity • Changes in reproductive decision making
Individuals: • Who are asymptomatic with a positive cytogenetic	Interventions of interest are: • <i>FMR1</i> variant testing	Comparators of interest are: • Standard clinical evaluation without genetic testing	Relevant outcomes include: • Test accuracy • Test validity

Populations	Interventions	Comparators	Outcomes
fragile X test result seeking further counseling on carrier status risk			<ul style="list-style-type: none"> Changes in reproductive decision making
Individuals: <ul style="list-style-type: none"> With ovarian failure before age 40, with clinical suspicion of fragile X-associated ovarian failure 	Interventions of interest are: <ul style="list-style-type: none"> <i>FMR1</i> variant testing 	Comparators of interest are: <ul style="list-style-type: none"> Standard clinical evaluation without genetic testing 	Relevant outcomes include: <ul style="list-style-type: none"> Test accuracy Test validity Changes in reproductive decision making
Individuals: <ul style="list-style-type: none"> With neurologic symptoms consistent with fragile X-associated tremor or ataxia syndrome 	Interventions of interest are: <ul style="list-style-type: none"> <i>FMR1</i> variant testing 	Comparators of interest are: <ul style="list-style-type: none"> Standard clinical evaluation without genetic testing 	Relevant outcomes include: <ul style="list-style-type: none"> Test accuracy Test validity Changes in reproductive decision making
Individuals: <ul style="list-style-type: none"> With signs and/or symptoms of Rett syndrome 	Interventions of interest are: <ul style="list-style-type: none"> Genetic testing for Rett syndrome-associated genes 	Comparators of interest are: <ul style="list-style-type: none"> Standard clinical management without genetic testing 	Relevant outcomes include: <ul style="list-style-type: none"> Test accuracy Test validity Other test performance measures Symptoms Health status measures Quality of life
Individuals: <ul style="list-style-type: none"> Who are asymptomatic sisters of an individual with Rett syndrome 	Interventions of interest are: <ul style="list-style-type: none"> Targeted genetic testing for a known familial Rett Syndrome-associated variant 	Comparators of interest are: <ul style="list-style-type: none"> Standard clinical management without genetic testing 	Relevant outcomes include: <ul style="list-style-type: none"> Test accuracy Test validity Other test performance measures Changes in reproductive decision making Symptoms
Individuals: <ul style="list-style-type: none"> Who are females with a child with Rett syndrome who are considering further childbearing 	Interventions of interest are: <ul style="list-style-type: none"> Targeted genetic testing for a known familial Rett Syndrome-associated variant 	Comparators of interest are: <ul style="list-style-type: none"> Reproductive planning without genetic testing 	Relevant outcomes include: <ul style="list-style-type: none"> Test accuracy Test validity Other test performance measures Changes in reproductive decision making

Description

Chromosomal microarray analysis (CMA) testing has been proposed for detection of genetic imbalances in infants or children with characteristics of developmental delay/intellectual disability (DD/ID), autism spectrum disorder (ASD), and/or congenital anomalies. CMA increases the diagnostic yield over karyotyping in this population and may impact clinical management decisions. Next-generation sequencing (NGS) panel testing allows for simultaneous analysis of a large number of genes and has been proposed as a way to identify single-gene causes of syndromes that have autism as a significant clinical feature, in patients with normal CMA testing.

Fragile X syndrome (FXS) is the most common inherited form of mental disability and known genetic cause of autism. The diagnosis includes use of a genetic test that determines the number of CGG repeats in the fragile X gene, *FMR1*. *FMR1* variant testing has been investigated in a variety of clinical settings, including in the evaluation of individuals with a personal or family history of intellectual disability, developmental delay, or autism

spectrum disorder and in reproductive decision-making in individuals with known FMR1 variants or positive cytogenetic fragile X testing. FMR1 variants also cause premature ovarian failure and a neurologic disease called fragile X-associated ataxia or tremor syndrome.

Rett syndrome (RTT), a neurodevelopmental disorder, is usually caused by pathogenic variants in the methyl-CpG-binding protein 2 (*MECP2*) gene. Genetic testing is available to determine whether a pathogenic mutation variant exists in RTT-associated genes (e.g., *MECP2*, *FOXG1*, or *CDLK5*) in a patient with clinical features of RTT or in a patient's family member.

Summary of Evidence

For individuals who have DD/ID, ASD, or multiple congenital anomalies not specific to a well-delineated genetic syndrome who receive CMA testing, the evidence includes primarily case series. Relevant outcomes are test accuracy and validity, changes in reproductive decision making, morbid events, and resource utilization. The available evidence supports test accuracy and validity. Although systematic studies of the impact of CMA on patient outcomes are lacking, the improvement in diagnostic yield over karyotyping has been well-demonstrated. Direct evidence of improved outcomes with CMA compared with karyotyping is lacking. However, for at least a subset of the disorders potentially diagnosed with CMA in this patient population, there are well-defined and accepted management steps associated with positive test results. Further, there is evidence of changes in reproductive decision making as a result of positive test results. The information derived from CMA testing can accomplish the following: end a long diagnostic odyssey; or reduce morbidity for certain conditions by initiating surveillance/management of associated comorbidities; or it could potentially impact future reproductive decision making for parents. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who have DD/ID, ASD, or multiple congenital anomalies not specific to a well-delineated genetic syndrome who receive next-generation sequencing (NGS) panel testing, the evidence includes primarily case series. Relevant outcomes are test accuracy and validity, changes in reproductive decision making, morbid events, and resource utilization. The rates of variants of uncertain significance associated with NGS panel testing in this patient population are not well-characterized. The yield of testing and likelihood of an uncertain result is variable, based on gene panel, gene tested, and patient population; additionally there are real risks of uninterpretable and incidental results. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals who have intellectual disability, developmental delay, or autism spectrum disorder, who are asymptomatic with a family history of FXS or intellectual disability seeking reproductive counseling, with known FMR1 variant carrier status and current pregnancy seeking prenatal testing, who are asymptomatic with a positive cytogenetic fragile X test result seeking further counseling on carrier status risk, with ovarian failure before age 40 with clinical suspicion of fragile X-associated ovarian failure, or with neurologic symptoms consistent with fragile X-associated tremor or ataxia syndrome who receive FMR1 variant testing, the evidence includes studies evaluating the analytic and clinical validity of FMR1 variant testing. Relevant outcomes are test accuracy and validity, and/or resource utilization, and/or changes in reproductive decision making. The analytic sensitivity and specificity for diagnosing these disorders have been demonstrated to be sufficiently high. The evidence demonstrates that FMR1 variant testing can establish a definitive diagnosis of FXS and fragile X-related syndromes when the test is positive for a pathogenic variant. Following a definitive diagnosis, management may change in various ways. At minimum, providing a diagnosis eliminates the need for further diagnostic workup. Results may aid in management of psychopharmacologic interventions, assist in informed reproductive decision making, or both. A chain of evidence supports improved outcomes following FMR1 variant testing. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who have signs and/or symptoms of RTT who receive genetic testing for RTT-associated genes, the evidence includes case series and prospective cohort studies. Relevant outcomes are test accuracy and validity, other test performance measures, symptoms, health status measures, and quality of life. Methyl-CpG-binding protein 2 (MECP2) variants are found in most patients with RTT, particularly in those who present with classic clinical features of RTT. The diagnostic accuracy of genetic testing for RTT cannot be determined with absolute certainty given variable clinical presentations of typical versus atypical RTT, but testing appears to have high sensitivity and specificity. Genetic testing has clinical utility when signs and symptoms of RTT are present to establish a specific genetic diagnosis. Identification of a specific class or type of pathogenic variant may alter some aspects of management and may eliminate or necessitate surveillance for different clinical manifestations of disease. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are asymptomatic sisters of an individual with RTT who receive targeted genetic testing for a known familial RTT-associated variant, the evidence includes case series and prospective cohort studies. Relevant outcomes are test accuracy and validity, other test performance measures, changes in reproductive decision making, symptoms, and symptoms. Targeted familial variant testing of asymptomatic sisters can eliminate or necessitate surveillance given the variability of clinical presentation in girls due to X-chromosome inactivation and clinical severity based on the type of pathogenic variant present. In sisters of reproductive age, determination of carrier status can eliminate or necessitate prenatal testing and inform reproductive decision making. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are females with a child with RTT who are considering future childbearing who receive targeted genetic testing for a known familial RTT-associated variant, the evidence includes cases series and prospective cohort studies. Relevant outcomes are test accuracy and validity, other test performance measures, and changes in reproductive decision making. Targeted familial variant testing of a woman with a child with RTT to determine carrier status may inform prenatal testing and reproductive decision making. In the rare situation where the mother carries a pathogenic variant, all future offspring have a 50% chance of being affected, with males typically presenting with more severe disease. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

Policy

CMA testing may be considered **medically necessary** as first-line evaluation when genetic evaluation is desired as opposed to first obtaining a karyotype.

Genetic Testing for Evaluation of Developmental Delay/Intellectual Disorder/Autism Spectrum Disorder (DD/ID/ASD) by chromosomal microarray analysis may be considered **medically necessary** in the postnatal period following complete clinical and biochemical evaluation (when these evaluations are non-diagnostic) under the following conditions:

- When FMR1 gene analysis (for Fragile X), when clinically indicated, is negative; AND ANY of the following:
 - Autism spectrum disorder; **OR**
 - Apparently non-syndromic developmental delay/intellectual disability; **OR**
 - Multiple congenital anomalies not specific to a well-delineated genetic syndrome, including:
 - Two or more major malformations; **OR**

- A single major malformation or multiple minor malformations, in an infant or child who is also small-for-dates; OR
- A single major malformation and multiple minor malformations; AND
- The results for the genetic testing have the potential to impact the clinical management of the member.

Genetic testing for Rett syndrome-associated genes (e.g., MECP2, FOXP1, or CDKL5) may be considered **medically necessary** to establish a genetic diagnosis of Rett syndrome in a child with developmental delay and signs/ symptoms of Rett syndrome, when a definitive diagnosis cannot be made without genetic testing.

Targeted genetic testing for a known familial Rett syndrome-associated variant may be considered **medically necessary** to determine carrier status of a mother or a sister of an individual with Rett syndrome.

All other indications for genetic testing for Rett syndrome - associated genes (e.g., MECP2, FOXP1, or CDKL5), including carrier testing (preconception or prenatal), and testing of asymptomatic family members to determine future risk of disease, are considered **investigational**.

Genetic testing for FMR1 mutations may be considered **medically necessary** for the following patient populations:

- Individuals of either sex with intellectual disability, developmental delay, or autism spectrum disorder (see Policy Guidelines*).
- Individuals seeking reproductive counseling who have a family history of fragile X syndrome or a family history of undiagnosed intellectual disability (see Policy Guidelines*).
- Prenatal testing of fetuses of known carrier mothers (see Policy Guidelines*).
- Affected individuals or relatives of affected individuals who have had a positive cytogenetic fragile X test result who are seeking further counseling related to the risk of carrier status (see Policy Guidelines**).
- Women with primary ovarian failure under the age of 40 in whom fragile X-associated ovarian failure is suspected.
- Individuals with neurologic symptoms consistent with fragile X-associated tremor/ataxia syndrome.

Genetic testing for FMR1 mutations is considered **investigational** for all other uses.

Whole exome sequencing (WES) may be considered **medically necessary** for the evaluation of unexplained congenital or neurodevelopmental disorder in children when ALL the following criteria are met:

1. The patient has been evaluated by a clinician with expertise in clinical genetics and counseled about the potential risks of genetic testing.
2. There is potential for a change in management and clinical outcome for the individual being tested.
3. A genetic etiology is considered the most likely explanation for the phenotype despite previous genetic testing, such as chromosomal microarray analysis and/or targeted single gene testing, OR when previous genetic testing has failed to yield a diagnosis and the affected individual is faced with invasive procedures/testing as the next diagnostic step, such as muscle biopsy.

Whole exome sequencing is considered **investigational** for the diagnosis of genetic disorders in all other situations.

Whole genome sequencing is considered **investigational** for the diagnosis of genetic disorders.

Chromosomal microarray analysis is considered **investigational** to confirm the diagnosis of a disorder or syndrome that is routinely diagnosed based on clinical evaluation alone.

Policy Guidelines

The genetic testing discussed in this protocol would be necessary only once in a lifetime.

Genetic Counseling

Genetic counseling is primarily aimed at patients who are at risk for inherited disorders, and experts recommend formal genetic counseling in most cases when genetic testing for an inherited condition is considered. The interpretation of the results of genetic tests and the understanding of risk factors can be very difficult and complex. Therefore, genetic counseling will assist individuals in understanding the possible benefits and harms of genetic testing, including the possible impact of the information on the individual's family. Genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing. Genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

Developmental Delay/Intellectual Disability

A 2013 guidelines update from American College of Medical Genetics (Schaefer et al, 2013) stated that a step-wise (or tiered) approach to the clinical genetic diagnostic evaluation of autism spectrum disorder is recommended, with the recommendation being for first tier to include fragile X syndrome and chromosomal microarray (CMA) testing.

Recommendations from the 2010 American College of Medical Genetics guidelines (Manning et al 2010) on array-based technologies and their clinical utilization for detecting chromosomal abnormalities include the following: "Appropriate follow-up is recommended in cases of chromosome imbalance identified by CMA, to include cytogenetic/FISH studies of the patient, parental evaluation, and clinical genetic evaluation and counseling."

In some cases of CMA analysis, the laboratory performing the test confirms all reported copy number variants with an alternative technology, such as fluorescent in situ hybridization analysis.

FMR1

American College of Medical Genetics Recommendations*

According to the American College of Medical Genetics (ACMG) Recommendations, the following is the preferred approach to testing (Sherman et al, 2005):

- "DNA analysis is the method of choice if one is testing specifically for fragile X syndrome (FXS) and associated trinucleotide repeat expansion in the FMR1 gene."
- "For isolated cognitive impairment, DNA analysis for FXS should be performed as part of a comprehensive genetic evaluation that includes routine cytogenetic evaluation. Cytogenetic studies are critical, since constitutional chromosome abnormalities have been identified as frequently or more frequently than fragile X mutations in mentally retarded individuals referred for fragile X testing."
- Fragile X testing is not routinely warranted for children with isolated attention-deficit/hyperactivity disorder (see Subcommittee on Attention-Deficit/Hyperactivity Disorder, Steering Committee on Quality Improvement and Management, 2011).

- “For individuals who are at risk due to an established family history of fragile X syndrome, DNA testing alone is sufficient. If the diagnosis of the affected relative was based on previous cytogenetic testing for fragile X syndrome, at least one affected relative should have DNA testing.”
- “Prenatal testing of a fetus should be offered when the mother is a known carrier to determine whether the fetus inherited the normal or mutant FMR1 gene. Ideally DNA testing should be performed on cultured amniocytes obtained by amniocentesis after 15 weeks’ gestation. DNA testing can be performed on chorionic villi obtained by CVS at 10 to 12 weeks’ gestation, but the results must be interpreted with caution because the methylation status of the FMR1 gene is often not yet established in chorionic villi at the time of sampling. A follow-up amniocentesis may be necessary to resolve an ambiguous result.”
- “If a woman has ovarian failure before the age of 40, DNA testing for premutation size alleles should be considered as part of an infertility evaluation and prior to in vitro fertilization.”
- “If a patient has cerebellar ataxia and intentional tremor, DNA testing for premutation size alleles, especially among men, should be considered as part of the diagnostic evaluation.”

ACMG made recommendations on diagnostic and carrier testing for FXS to provide general guidelines to aid clinicians in making referrals for testing the repeat region of the FMR1 gene. These recommendations include testing of individuals of either sex who have intellectual disability, developmental delay, or autism spectrum disorder, especially if they have any physical or behavioral characteristics of FXS (see Sherman et al, 2005).

Physical and behavioral characteristics of FXS include: typical facial features, such as an elongated face with prominent forehead, protruding jaw, and large ears. Connective tissue anomalies include hyperextensible finger and thumb joints, hand calluses, velvet-like skin, flat feet, and mitral valve prolapse. The characteristic appearance of adult males includes macroorchidism. Patients may show behavioral problems including autism spectrum disorder, sleeping problems, social anxiety, poor eye contact, mood disorders, and hand-flapping or biting. Another prominent feature of the disorder is neuronal hyperexcitability, manifested by hyperactivity, increased sensitivity to sensory stimuli, and a high incidence of epileptic seizures.

****Cytogenetic Testing**

Cytogenetic testing was used before the identification of the FMR1 gene and is significantly less accurate than the current DNA test. The method is no longer considered an acceptable diagnostic method according to ACMG standards (see Monaghan et al, 2013).

Medicare Advantage

For Medicare Advantage genetic testing (excluding whole exome sequencing) for FMR1 and Rett Syndrome not meeting the criteria above will be considered **not medically necessary**.

Background

Developmental Delay/Intellectual Disability and Autism Spectrum Disorder

Children with signs of neurodevelopmental delays or disorders in the first few years of life may eventually be diagnosed with intellectual disability or autism syndromes, serious and lifelong conditions that present significant challenges to families and to public health.

The diagnosis of DD is reserved for children younger than five years of age who have significant delay in two or more of the following developmental domains: gross or fine motor, speech/language, cognitive, social/personal, and activities of daily living. Intellectual disability (ID) is a life-long disability diagnosed at or after five

years of age when IQ testing is considered valid and reliable. The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), of the American Psychiatric Association defined patients with ID as having an IQ less than 70, onset during childhood, and dysfunction or impairment in more than two areas of adaptive behavior or systems of support.

Congenital Anomalies

In the United States, congenital anomalies, which occur in approximately 3% of all newborns, are the leading cause of neonatal morbidity and mortality.³ Genetic factors have been identified as an important cause for congenital anomalies. Common chromosomal aneuploidies (e.g., monosomy X, trisomies 21, 18, and 13) have traditionally been diagnosed in the neonatal period using conventional karyotyping. Improved methods, such as fluorescence in situ hybridization (FISH) using chromosome or locus-specific probes, enable the diagnosis of some of the common microdeletion syndromes (e.g., DiGeorge/velocardiofacial syndrome, cri-du-chat syndrome, and Prader-Willi and Angelman syndromes). However, FISH is applicable only in patients with a strong clinical suspicion of a specific genetic defect, which may be difficult to detect in a neonate with congenital anomalies, because a patient's clinical presentation may be atypical, or have nonspecific phenotypic features that may be shared by several different disorders, or they may lack specific syndromic features that appear at a later age. An improved rate of detection of CNVs has been shown with the use of array comparative genomic hybridization (aCGH).

Genetic Associations with DD/ID, ASD, and Congenital Anomalies

DD/ID and ASD may be associated with genetic abnormalities. For children with clear, clinical symptoms and/or physiologic evidence of syndromic neurodevelopmental disorders, diagnoses are based primarily on clinical history and physical examination, and then may be confirmed with targeted genetic testing of specific genes associated with the diagnosed syndrome. However, for children who do not present with an obvious syndrome, who are too young for full expression of a suspected syndrome, or who may have an atypical presentation, genetic testing may be used as a basis for establishing a diagnosis.

Complex autism, which comprises approximately 20% to 30% of cases of autism, is defined by the presence of dysmorphic features and/or microcephaly. Essential autism, approximately 70% to 80% of cases of autism, is defined as autism in the absence of dysmorphology. Genetic causes of autism include cytogenetically visible chromosomal abnormalities (5%), single-gene disorders (5%), and CNVs (10%-20%). Single-nucleotide polymorphism (SNP) microarrays to perform high-resolution linkage analysis have revealed suggestive regions on certain chromosomes that had not been previously associated with autism. The SNP findings in autism, to date, seem consistent with other complex diseases, in which common variation has modest effect size (odds ratio, less than two), requiring large samples for robust detection. This diagnostic challenge makes it unlikely that individual single nucleotide variants (SNVs) will have high predictive value.⁷

Guidelines for patients with ID/DD, ASD, and/or congenital anomalies, such as those published by the American Academy of Pediatrics (AAP) and the American Academy of Neurology (AAN) with the Child Neurology Society (CNS), recommend cytogenetic evaluation to look for certain kinds of chromosomal abnormalities that may be causally related to their condition.⁷ The joint AAN and CNS guidelines have noted that only occasionally will an etiologic diagnosis lead to specific therapy that improves outcomes, but suggest the more immediate and general clinical benefits of achieving a specific genetic diagnosis from the clinical viewpoint, as follows¹:

- “limit further diagnostic testing”
- “improve understanding of treatment and prognosis”
- “anticipate and manage associated medical and behavioral comorbidities”

- “allow for counseling regarding risk of recurrence, and prevent recurrence through screening for carriers and prenatal testing.”

The AAP and the AAN and CNS joint guidelines have also emphasized the importance of early diagnosis and intervention in an attempt to ameliorate or improve behavioral and cognitive outcomes over time.

At present, a relatively small body of literature has addressed the use of CMA or other genetic testing for predicting disease phenotype or severity.⁷ This is not yet a major clinical use of testing and is not a focus in this review.

Testing to Determine Genetic Etiology

Most commonly, genetic abnormalities associated with neurodevelopmental disorders are deletions and duplications of large segments of genomic material, called CNVs. For many well-described syndromes, the type and location of the chromosomal abnormality have been established with the study of a large number of cases and constitute a genetic diagnosis; for others, only a small number of patients with similar abnormalities may exist to support a genotype-phenotype correlation. Finally, for some patients, cytogenetic analysis will discover entirely new chromosomal abnormalities that will require additional study to determine their clinical significance.

Conventional methods of cytogenetic analysis, including karyotyping (e.g., G-banded) and FISH, have relatively low resolution and a low diagnostic yield (i.e., proportion of tested patients found to have clinically relevant genomic abnormalities), leaving most cases without identification of a chromosomal abnormality associated with the child’s condition. CMA is a newer cytogenetic analysis method that increases the chromosomal resolution for detection of CNVs, and, as a result, increases the genomic detail beyond that of conventional methods. CMA results are clinically informative in the same way as results derived from conventional methods, and thus CMA represents an extension of standard methods with increased resolution.

Next-generation sequencing (NGS) has been proposed to detect single-gene causes of autism and possibly identify a syndrome that involves autism in patients with normal array-based testing.

CMA Testing

The term CMA collectively describes two different array platforms: aCGH and SNP arrays. Both types of arrays can identify loss or gain of DNA (microdeletions or microduplications, respectively), known as CNVs. CMA testing can identify genomic abnormalities associated with a wide range of developmental disabilities, including cognitive impairment, behavioral abnormalities, and congenital abnormalities. CMA testing can detect CNVs, and the frequency of disease-causing CNVs is highest (20%-25%) in children with moderate-to-severe intellectual disability accompanied by malformations or dysmorphic features. Disease-causing CNVs have been identified in 5% to 10% of cases of autism, being more frequent in severe phenotypes.^{1,2}

Array Comparative Genomic Hybridization and Single-Nucleotide Polymorphism

The aCGH uses a DNA sample from the patient and a DNA sample from a normal control. Each is labeled with one color of fluorescent dye (red or green) and the labeled samples are mixed and hybridized to thousands of cloned or synthesized reference (normal) DNA fragments of known genomic locus immobilized on a glass slide (microarray) to conduct thousands of comparative reactions at the same time. CNVs are determined by computer analysis of the array patterns and intensities of the hybridization signals. If the patient sequence is missing part of the normal sequence (deletion) or has the normal sequence plus additional genomic material within that genomic location (e.g., a duplication of the same sequence), the sequence imbalance is detected as a difference in fluorescence intensity. For this reason, aCGH cannot detect balanced CNVs (equal exchange of material between chromosomes) or sequence inversions (same sequence is present in reverse base pair order) because the fluorescence intensity would not change.

SNVs are the most common genetic variation among people and occur normally throughout the DNA. Each SNV represents a difference in a single nucleotide. On average, SNVs occur every 300 nucleotides. SNVs can act as “biological markers,” in that they may identify genes associated with disease. Most SNVs have no deleterious effect, but may predict an individual’s response to certain drugs, susceptibility to environmental factors, and the risk of developing certain diseases. SNPs may also indicate inheritance of disease genes within families.

Like aCGH, SNP arrays also detect CNVs, although the resolution provided by aCGH is better than that with SNP arrays, and, therefore, SNPs are limited in the detection of single exon CNVs. In addition, aCGH has better signal-to-background characteristics than SNP arrays. In contrast to aCGH, SNP arrays will also identify long stretches of DNA homozygosity, which may suggest uniparental disomy (UPD) or consanguinity. UPD occurs when someone inherits two copies of a chromosome from one parent and no copies from the other parent. UPD can lead to syndromes such as Angelman and Prader-Willi. SNP arrays can also detect triploidy, which cannot be detected by aCGH arrays.

A portion of the increased diagnostic yield from CMA over karyotyping comes from the discovery that some chromosomal rearrangements that appear balanced (and therefore not pathogenic) by G-banded karyotype analysis are found to have small imbalances with greater resolution. It has been estimated that 40% of apparently balanced de novo or inherited translocations with abnormal phenotype are associated with cryptic deletion if analyzed by CMA testing.

The various types of microarrays can differ by construction; earliest versions used DNA fragments cloned from bacterial artificial chromosomes. They have been largely replaced by oligonucleotide (oligo; short, synthesized DNA) arrays, which offer better reproducibility. Oligo/SNP hybrid arrays have been constructed to merge the advantages of each.

Microarrays may be prepared by the laboratory using the technology or, more commonly, by commercial manufacturers, and sold to laboratories that must qualify and validate the product for use in their assay, in conjunction with computerized software for interpretation. The proliferation of in-house developed and commercially available platforms prompted the ACMG to publish guidelines for the design and performance expectations for clinical microarrays and associated software in the postnatal setting.⁸

Copy Number Variants

Targeted CMA provides high-resolution coverage of the genome primarily in areas containing known, clinically significant CNVs. The ACMG guideline for designing microarrays recommends probe enrichment in clinically significant areas of the genome to maximize detection of known abnormalities; however, they also recommend against the use of targeted arrays in the postnatal setting. Rather, a broad genomic screen is recommended to identify atypical, complex, or completely new rearrangements, and to delineate breakpoints accurately.

Whole-genome CMA has allowed the characterization of several new genetic syndromes, with other potential candidates currently under study. However, the whole-genome arrays also have the disadvantage of potentially high numbers of apparent false-positive results, because benign CNVs are also found in phenotypically normal populations; both benign and pathogenic CNVs are continuously cataloged and, to some extent, made available in public reference databases to aid in clinical interpretation. Additionally, some new CNVs are neither known to be benign nor causal; these CNVs may require detailed family history and family genetic testing to determine clinical significance and/or may require confirmation by subsequent accumulation of similar cases and so, for a time, may be considered a CNV of undetermined significance (some may eventually be confirmed true positives or causal, others false positives or benign).

To determine clinical relevance (consistent association with a disease) of CNV findings, the following actions are taken:

- CNVs are confirmed by another method (e.g., FISH, multiplex ligation-dependent probe amplification, polymerase chain reaction).
- CNVs detected are checked against public databases and, if available, against private databases maintained by the laboratory. Known pathogenic CNVs associated with the same or similar phenotype as the patient are assumed to explain the etiology of the case; known benign CNVs are assumed to be nonpathogenic.⁹⁻¹¹
- A pathogenic etiology is additionally supported when a CNV includes a gene known to cause the phenotype when inactivated (microdeletion) or overexpressed (microduplication).¹⁰
- The laboratory may establish a size cutoff; potentially pathogenic CNVs are likely to be larger than benign polymorphic CNVs; cutoffs for CNVs not previously reported typically range from 300 kilobase (kb) pairs to one megabase pairs.¹¹⁻¹⁴
- Parental studies are indicated when CNVs of appropriate size are detected and not found in available databases; CNVs inherited from a clinically normal parent are assumed to be benign polymorphisms whereas those appearing de novo are likely pathogenic; etiology may become more certain as other similar cases accrue.^{9, 15}

ACMG has also published guidelines for the interpretation and reporting of CNVs in the postnatal setting, to promote consistency among laboratories and CMA results.¹⁶ Three categories of clinical significance are recommended for reporting: pathogenic, benign, and uncertain clinical significance.

In 2008, the International Standards for Cytogenomic Arrays (ISCA) Consortium was organized; it has established a public database containing deidentified whole-genome microarray data from a subset of the ISCA Consortium member clinical diagnostic laboratories. Array analysis was carried out on subjects with phenotypes including DD/ID and ASD. As of August 2017, there were over 54,000 subjects with individual-level data in the database.¹⁷ Additional members are planning to contribute data; participating members use an opt-out, rather than an opt-in approach that was approved by the National Institutes of Health (NIH) and participating center institutional review boards. The database is held at National Center for Biotechnology Information/NIH and curated by a committee of clinical genetics laboratory experts. In 2011, Kaminsky et al used data from the Consortium, including 15,749 cases and 10,118 published controls available at the time of analysis, to identify the functional significance of 14 rare CNVs in intellectual and developmental disabilities, and to describe a methodology for assessing for pathologic CNVs.¹⁸ In the Kaminsky study, the frequency of pathogenic CNVs was 17.1%.

Next-Generation Sequencing

NGS has been proposed to detect single-gene causes of autism and possibly identify a syndrome that involves autism in patients with normal array-based testing. NGS involves the sequencing of millions of fragments of genetic material in a massively parallel fashion. NGS can be performed on segments of genetic material of various sizes from the entire genome (whole-genome sequencing) to small subsets of genes (targeted sequencing). NGS allows the detection of SNVs, CNVs, and insertions and deletions. With higher resolution comes higher likelihood of detection of variants of uncertain clinical significance.

Commercially Available Tests

Chromosomal Microarray Analysis

CMA testing is commercially available through many laboratories and includes targeted and whole genome arrays, with or without SNP microarray analysis.

Affymetrix CytoScan® Dx has been cleared by the U.S. Food and Drug Administration (FDA) through the de novo 510(k) process. FDA's review of the CytoScan Dx® Assay included an analytic evaluation of the test's ability to accurately detect numerous chromosomal variations of different types, sizes, and genome locations compared

with several analytically validated test methods. FDA found that the CytoScan Dx[®] Assay could analyze a patient's entire genome and adequately detect chromosome variations in regions of the genome associated with ID/DD. Reproducibility decreased with the CNV gain or loss size, particularly when less than approximately 400 kilobases (kb; generally recommended as the lower reporting limit).

FirstStep^{Dx} PLUS (Lineagen, Salt Lake City, UT) uses Lineagen's custom-designed microarray platform manufactured by Affymetrix. This microarray consists of 1,953,246 unique nonpolymorphic probes and 743,304 SNP probes that come standard on the Affymetrix CytoScan HD[®] microarray, with an additional 83,443 custom probes designed by Lineagen.

Ambry Genetics (Aliso Viejo, CA) offers multiple tests (CMA and NGS) that are designed for ASD and neurodevelopmental disorders.

LabCorp offers the Reveal SNP Microarray-Pediatric for individuals with nonsyndromic congenital anomalies, dysmorphic features, DD/ID, and/or ASD.

Next-Generation Sequencing

A variety of commercial and academic laboratories offer NGS panels designed for the evaluation of ASD, DD/ID, and congenital anomalies, which vary in terms of the numbers of and specific genes tested.

Courtagen (Woburn, MA) offers three NGS panels intended for the assessment of developmental and behavioral phenotypes:

- devSEEK[®] Triome[™]: includes 1119 genes associated with DD/ID and ASD.
- devSEEK[®]: includes 237 genes associated with DD/ID and ASD, with additional testing available for large deletions and duplications.
- devACT[®] Clinical Management Panel: includes 250 genes associated with DD/ID and ASD, focusing on genes associated with actionable clinical management changes, with additional testing available for large deletions and duplications.

Emory Genetics Laboratory offers an NGS ASD panel of genes targeting genetic syndromes that include autism or autistic features.

Greenwood Genetics Center (Greenwood, SC) offers an NGS panel for syndromic autism that includes 83 genes.

Fragile X Syndrome

FXS is the most common cause of heritable intellectual disability, characterized by moderate intellectual disability in males and mild intellectual disability in females. FXS affects approximately one in 4000 males and one in 8000 females. In addition to intellectual impairment, patients present with typical facial features, such as an elongated face with prominent forehead, protruding jaw, and large ears. Connective tissue anomalies include hyperextensible finger and thumb joints, hand calluses, velvet-like skin, flat feet, and mitral valve prolapse. The characteristic appearance of adult males includes macroorchidism. Patients may show behavioral problems including autism spectrum disorders, sleeping problems, social anxiety, poor eye contact, mood disorders, and hand-flapping or biting. Another prominent feature of the disorder is neuronal hyperexcitability, manifested by hyperactivity, increased sensitivity to sensory stimuli, and a high incidence of epileptic seizures.

Approximately 1% to 3% of children initially diagnosed with autism are shown to have FXS, with expansion of the CGG trinucleotide repeat in the *FMR1* gene to full variant size of 200 or more repeats.⁴² A considerable number of children evaluated for autism have been found to have *FMR1* premutations (55-200 CGG repeats).⁴³

Treatment of FXS

Current approaches to therapy are supportive and symptom-based. Psychopharmacologic intervention to modify behavioral problems in a child with FXS may represent an important adjunctive therapy when combined with other supportive strategies including speech therapy, occupational therapy, and special education services. Medication management may be indicated to modify attention deficits, impaired impulse control, and hyperactivity. Anxiety-related symptoms, including obsessive-compulsive tendencies with perseverative behaviors, also may be present and require medical intervention. Emotional lability and episodes of aggression and self-injury may be a danger to the child and others around him or her; therefore, the use of medication(s) to modify these symptoms also may significantly improve an affected child's ability to participate more successfully in activities in home and school settings.

Genetics of FXS

FXS is associated with the expansion of the CGG trinucleotide repeat in the fragile X mental retardation 1 (*FMR1*) gene on the X chromosome. Diagnosis of FXS may include using a genetic test that determines the number of CGG repeats in the fragile X gene. The patient is classified as normal, intermediate (or "gray zone"), premutation, or full mutation based on the number of CGG repeats⁴⁴:

- Full mutation: greater than 200-230 CGG repeats (methylated)
- Premutation: 55-200 CGG repeats (unmethylated)
- Intermediate: 45-54 CGG repeats (unmethylated)
- Normal: 5-44 CGG repeats (unmethylated)

Full mutations are associated with FXS, which is caused by expansion of the *FMR1* gene CGG triplet repeat above 200 units in the 5' untranslated region of *FMR1*, leading to hypermethylation of the promoter region followed by transcriptional inactivation of the gene. FXS is caused by a loss of the fragile X mental retardation protein.

Fragile X-Associated Disorders

Patients with a premutation are carriers and may develop an *FMR1*-related disorder, such as fragile X-associated tremor/ataxia syndrome (FXTAS) or, in women, fragile X-associated premature ovarian insufficiency (FXPOI). FXTAS is a late-onset syndrome, comprising progressive development of intention tremor and ataxia, often accompanied by progressive cognitive and behavioral difficulties, including memory loss, anxiety, reclusive behavior, deficits of executive function, and dementia.

Premutation alleles in females are unstable and may expand to full mutations in offspring. Premutations of fewer than 59 repeats have not been reported to expand to a full mutation in a single generation. Premutation alleles in males may expand or contract by several repeats with transmission; however, expansion to full mutations has not been reported.

Premutation allele prevalence in whites is approximately one in 1000 males and one in 350 females.⁴⁴⁻⁴⁶ Full mutations are typically maternally transmitted. The mother of a child with an *FMR1* mutation is almost always a carrier of a premutation or full mutation. Women with a premutation are at risk of FXPOI and at small risk of FXTAS; they carry a 50% risk of transmitting an abnormal gene, which contains either a premutation copy number (55-200) or a full mutation (> 200) in each pregnancy.

Men who are premutation carriers are referred to as transmitting males. All of their daughters will inherit a premutation, but their sons will not inherit the premutation. Males with a full mutation usually have intellectual disability and decreased fertility.

Rett Syndrome

RTT is a severe neurodevelopmental disorder primarily affecting girls with an incidence of 1:10,000 female births, making it one of the most common genetic causes of intellectual disability in girls.⁶¹ RTT is characterized by apparent normal development for the first six to 18 months of life, followed by the loss of intellectual functioning, loss of acquired fine and gross motor skills and social skills. Purposeful use of the hands is replaced by repetitive stereotyped hand movements, sometimes described as hand-wringing.⁶¹ Other clinical manifestations include seizures, disturbed breathing patterns with hyperventilation and periodic apnea, scoliosis, growth retardation and gait apraxia.⁶²

There is wide variability in the rate of progression and severity of the disease. In addition to the classical form of RTT, there are a number of recognized atypical variants. Variants of RTT may appear with a severe or a milder form. The severe variant has no normal developmental period; individuals with a milder phenotype experience less dramatic regression and milder expression of the characteristics of classical RTT. Diagnostic criteria for typical (or classic) RTT and atypical (or variant) RTT have been established.⁶¹⁻⁶³ For typical RTT, a period of regression followed by recovery or stabilization and fulfillment of all the main criteria are required to meet the diagnostic criteria for classic RTT. For atypical RTT, a period of regression followed by recovery or stabilization, at least two of the four main criteria, plus five of 11 supportive are required to meet the diagnostic criteria of variant RTT.

Treatment of RTT

Currently, there are no specific treatments that halt or reverse the progression of the disease, and there are no known medical interventions that will change the outcome of patients with RTT. Management is mainly symptomatic and individualized, focusing on optimizing each patient's abilities.⁶¹ A multidisciplinary approach is usually applied, with specialist input from dietitians, physiotherapists, occupational therapists, speech therapists and music therapists. Regular monitoring for scoliosis (seen in \approx 87% of patients by age 25 years) and possible heart abnormalities may be recommended. Spasticity can have a major impact on mobility; physical therapy and hydrotherapy may prolong mobility. Occupational therapy can help children develop communication strategies and skills needed for performing self-directed activities (e.g., dressing, feeding, practicing arts and crafts).

Pharmacologic approaches to managing problems associated with RTT include melatonin for sleep disturbances and several agents for the control of breathing disturbances, seizures, and stereotypic movements. RTT patients have an increased risk of life-threatening arrhythmias associated with a prolonged QT interval, and avoidance of a number of drugs is recommended, including prokinetic agents, antipsychotics, tricyclic antidepressants, anti-arrhythmics, anesthetic agents and certain antibiotics.

In a mouse model of RTT, genetic manipulation of mutated *MECP2* has demonstrated reversibility of the genetic defect.^{64, 65}

Genetics of RTT

RTT is an X-linked dominant genetic disorder. Mutations in *MECP2*, which is thought to control expression of several genes including some involved in brain development, were first reported in 1999. Subsequent screening has shown that over 80% of patients with classical RTT have pathogenic mutations in the *MECP2* gene. More than 200 mutations in *MECP2* have been associated with RTT.⁶⁶ However, eight of the most commonly occurring missense and nonsense mutations account for almost 70% of all cases; small C-terminal deletions account for approximately 10%; and large deletions, 8% to 10%.⁶⁷ *MECP2* mutation type is associated with disease severity.⁶⁸ Whole duplications of the *MECP2* gene have been associated with severe X-linked intellectual disability with progressive spasticity, no or poor speech acquisition, and acquired microcephaly. Additionally, the pattern of X-chromosome inactivation influences the severity of the clinical disease in females.^{69, 70}

Because the spectrum of clinical phenotypes is broad, to facilitate genotype-phenotype correlation analyses, the International Rett Syndrome Association has established a locus-specific *MECP2* variation database (RettBASE) and a phenotype database (InterRett).

Approximately 99.5% of cases of RTT are sporadic, resulting from a de novo mutation, which arise almost exclusively on the paternally derived X chromosome. The remaining 0.5% of cases are familial and usually explained by germline mosaicism or favorably skewed X-chromosome inactivation in the carrier mother that results in her being unaffected or only slightly affected (mild intellectual disability). In the case of a carrier mother, the recurrence risk of RTT is 50%. If a mutation is not identified in leukocytes of the mother, the risk to a sibling of the proband is below 0.5% (because germline mosaicism in either parent cannot be excluded).

Identification of a mutation in *MECP2* does not necessarily equate to a diagnosis of RTT. Rare cases of *MECP2* mutations also have been reported in other clinical phenotypes, including individuals with an Angelman-like picture, nonsyndromic X-linked intellectual disability, PPM-X syndrome (an X-linked genetic disorder characterized by psychotic disorders [most commonly bipolar disorder], parkinsonism, and intellectual disability), autism, and neonatal encephalopathy.^{61, 66, 71} Recent studies have revealed that different classes of genetic variants in *MECP2* result in variable clinical phenotypes and overlap with other neurodevelopmental disorders.⁷²⁻⁷⁴

A proportion of patients with a clinical diagnosis of RTT do not appear to have mutations in the *MECP2* gene. Two other genes, *CDKL5* and *FOXG1*, have been shown to be associated with atypical variants.

Regulatory Status

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). Lab tests for CMA, NGS, genetic testing for Rett syndrome and the Xpansion Interpreter[®] test are available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the FDA has chosen not to require any regulatory review of these tests.

In July 2010, FDA indicated that it will in the future require microarray manufacturers to seek clearance to sell their products for use in clinical cytogenetics.

On January 17, 2014, the Affymetrix CytoScan[®] Dx Assay was cleared for marketing by FDA through the de novo classification process. For the de novo petition, FDA's review of the CytoScan[®] Dx Assay included an analytic evaluation of the test's ability to accurately detect numerous chromosomal variations of different types, sizes, and genome locations compared with several analytically validated test methods. FDA found that the CytoScan[®] Dx Assay could analyze a patient's entire genome and adequately detect chromosome variations in regions of the genome associated with intellectual and developmental disabilities. FDA product code: PFX.

Asuragen offers the Xpansion Interpreter[®] test, which analyzes AGG sequences that interrupt CGG repeats and may stabilize alleles, protecting against expansion in subsequent generations.^{6, 7}

CMA Testing

CMA testing is commercially available through many laboratories and includes targeted and whole-genome arrays, with or without SNP microarray analysis.

On January 17, 2014, the Affymetrix CytoScan[®] Dx Assay (Thermo Fisher Scientific, Waltham, MA) has been cleared by the FDA through the de novo 510(k) process. FDA's review of the CytoScan[®] Dx Assay included an analytic evaluation of the test's ability to detect accurately numerous chromosomal variations of different types, sizes, and genome locations compared with several analytically validated test methods. FDA found that the CytoScan[®] Dx Assay could detect copy number variations (CNVs) across the genome and adequately detect CNVs

in regions of the genome associated with ID/DD. Reproducibility decreased with the CNV gain or loss size, particularly when less than approximately 400 kb (generally recommended as the lower reporting limit). As of July 2017, Affymetrix™ has reported 2.69 million markers for copy number, 750,000 biallelic probes, and 1.9 million polymorphic probes (Affymetrix™ was acquired by Thermo Fisher Scientific in 2016). FDA product code: PFX.

FirstStepDx PLUS® (Lineagen, Salt Lake City, UT) uses Lineagen's custom-designed microarray platform manufactured by Affymetrix. As of July 2017, this microarray consists of a 2.8 million probe microarray for the detection of CNVs associated with neurodevelopmental disorders. The array includes probes that come standard on the Affymetrix CytoScan HD® microarray, with an additional 88,435 custom probes designed by Lineagen.

Ambry Genetics (Aliso Viejo, CA) offers multiple tests (CMA and NGS) that are designed for ASD and neurodevelopmental disorders. As of July 2017, the CMA offered by Ambry Genetics includes over 2.6 million probes for copy number and 750,000 SNP probes. The expanded NGS panel for neurodevelopmental disorders includes assesses 196 genes.

LabCorp (Burlington, NC) offers the Reveal® SNP Microarray-Pediatric for individuals with nonsyndromic congenital anomalies, dysmorphic features, DD/ID, and/or ASD. The Reveal® microarray has 2695 million probes as of July 2017.

Next-Generation Sequencing

A variety of commercial and academic laboratories offer NGS panels designed for the evaluation of ASD, DD/ID, and congenital anomalies, which vary in terms of the numbers of and specific genes tested.

Emory Genetics Laboratory (North Decatur, GA) offers an NGS ASD panel of genes targeting genetic syndromes that include autism or autistic features.

Greenwood Genetics Center (Greenwood, SC) offers an NGS panel for syndromic autism that includes 83 genes.

Related Protocols

Chromosomal Microarray Testing for the Evaluation of Early Pregnancy Loss and Intrauterine Fetal Demise

Invasive Prenatal (Fetal) Diagnostic Testing

Whole Exome and Whole Genome Sequencing for Diagnosis of Genetic Disorders

Services that are the subject of a clinical trial do not meet our Technology Assessment Protocol criteria and are considered investigational. *For explanation of experimental and investigational, please refer to the Technology Assessment Protocol.*

It is expected that only appropriate and medically necessary services will be rendered. We reserve the right to conduct prepayment and postpayment reviews to assess the medical appropriateness of the above-referenced procedures. **Some of this protocol may not pertain to the patients you provide care to, as it may relate to products that are not available in your geographic area.**

References

We are not responsible for the continuing viability of web site addresses that may be listed in any references below.

1. Beaudet AL. The utility of chromosomal microarray analysis in developmental and behavioral pediatrics. *Child Dev.* Jan-Feb 2013; 84(1):121-132. PMID 23311723
2. Bi W, Borgan C, Pursley AN, et al. Comparison of chromosome analysis and chromosomal microarray analysis: what is the value of chromosome analysis in today's genomic array era? *Genet Med.* Jun 2013; 15(6):450-457. PMID 23238528
3. Lu XY, Phung MT, Shaw CA, et al. Genomic imbalances in neonates with birth defects: high detection rates by using chromosomal microarray analysis. *Pediatrics.* Dec 2008; 122(6):1310-1318. PMID 19047251
4. Weiss LA. Autism genetics: emerging data from genome-wide copy-number and single nucleotide polymorphism scans. *Expert Rev Mol Diagn.* Nov 2009; 9(8):795-803. PMID 19895225
5. Moeschler JB, Shevell M, Committee on G. Comprehensive evaluation of the child with intellectual disability or global developmental delays. *Pediatrics.* Sep 2014; 134(3):e903-918. PMID 25157020
6. Michelson DJ, Shevell MI, Sherr EH, et al. Evidence Report: Genetic and metabolic testing on children with global developmental delay: Report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology.* Oct 25 2011; 77(17):1629-1635. PMID 21956720
7. Merikangas AK, Segurado R, Heron EA, et al. The phenotypic manifestations of rare genic CNVs in autism spectrum disorder. *Mol Psychiatry.* Nov 2015; 20(11):1366-1372. PMID 25421404
8. Kearney HM, South ST, Wolff DJ, et al. American College of Medical Genetics recommendations for the design and performance expectations for clinical genomic copy number microarrays intended for use in the postnatal setting for detection of constitutional abnormalities. *Genet Med.* Jul 2011; 13(7):676-679. PMID 21681105
9. Rodriguez-Revenga L, Mila M, Rosenberg C, et al. Structural variation in the human genome: the impact of copy number variants on clinical diagnosis. *Genet Med.* Sep 2007; 9(9):600-606. PMID 17873648
10. Vermeesch JR, Fiegler H, de Leeuw N, et al. Guidelines for molecular karyotyping in constitutional genetic diagnosis. *Eur J Hum Genet.* Nov 2007; 15(11):1105-1114. PMID 17637806
11. Stankiewicz P, Beaudet AL. Use of array CGH in the evaluation of dysmorphism, malformations, developmental delay, and idiopathic mental retardation. *Curr Opin Genet Dev.* Jun 2007; 17(3):182-192. PMID 17467974
12. Miller DT, Adam MP, Aradhya S, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet.* May 14, 2010; 86(5):749-764. PMID 20466091
13. Fan YS, Jayakar P, Zhu H, et al. Detection of pathogenic gene copy number variations in patients with mental retardation by genomewide oligonucleotide array comparative genomic hybridization. *Hum Mutat.* Nov 2007; 28(11):1124-1132. PMID 17621639
14. Baldwin EL, Lee JY, Blake DM, et al. Enhanced detection of clinically relevant genomic imbalances using a targeted plus whole genome oligonucleotide microarray. *Genet Med.* Jun 2008; 10(6):415-429. PMID 18496225
15. Zahir F, Friedman JM. The impact of array genomic hybridization on mental retardation research: a review of current technologies and their clinical utility. *Clin Genet.* Oct 2007; 72(4):271-287. PMID 17850622
16. Kearney HM, Thorland EC, Brown KK, et al. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. *Genet Med.* Jul 2011; 13(7):680-685. PMID 21681106
17. Information NCfB. dbGAP: International Standards for Cytogenomic Arrays http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000205.v6.p2. Accessed August, 2016.
18. Kaminsky EB, Kaul V, Paschall J, et al. An evidence-based approach to establish the functional and clinical significance of copy number variants in intellectual and developmental disabilities. *Genet Med.* Sep 2011; 13(9):777-784. PMID 21844811

19. Blue Cross Blue Shield Association Technology Evaluation Center (TEC). TEC Special Report: Array Comparative Genomic Hybridization (aCGH) for the Genetic Evaluation of Patients with Developmental Delay/Mental Retardation and Autism Spectrum Disorder. TEC Assessments. 2009; Volume 24; Tab 10.
20. Blue Cross and Blue Shield Association. Special Report: Chromosomal Microarray for the Genetic Evaluation of Patients With Global Developmental Delay, Intellectual Disability, and Autism Spectrum Disorder TEC Assessments. 2015; Volume 30; Tab 2.
21. Hochstenbach R, van Binsbergen E, Engelen J, et al. Array analysis and karyotyping: workflow consequences based on a retrospective study of 36,325 patients with idiopathic developmental delay in the Netherlands. *Eur J Med Genet.* Jul-Aug 2009; 52(4):161-169. PMID 19362174
22. Shen Y, Dies KA, Holm IA, et al. Clinical genetic testing for patients with autism spectrum disorders. *Pediatrics.* Apr 2010; 125(4):e727-735. PMID 20231187
23. Cooper GM, Coe BP, Girirajan S, et al. A copy number variation morbidity map of developmental delay. *Nat Genet.* Sep 2011; 43(9):838-846. PMID 21841781
24. Tammimies K, Marshall CR, Walker S, et al. Molecular diagnostic yield of chromosomal microarray analysis and whole-exome sequencing in children with autism spectrum disorder. *JAMA.* Sep 1 2015; 314(9):895-903. PMID 26325558
25. Hillman SC, Barton PM, Roberts TE, et al. BAC chromosomal microarray for prenatal detection of chromosome anomalies in fetal ultrasound anomalies: an economic evaluation. *Fetal Diagn Ther.* 2014; 36(1):49-58. PMID 24943865
26. Siu WK, Lam CW, Mak CM, et al. Diagnostic yield of array CGH in patients with autism spectrum disorder in Hong Kong. *Clin Transl Med.* Dec 2016; 5(1):18. PMID 27271878
27. D'Arrigo S, Gavazzi F, Alfei E, et al. The diagnostic yield of array comparative genomic hybridization is high regardless of severity of intellectual disability/developmental delay in children. *J Child Neurol.* May 2016; 31(6):691-699. PMID 26511719
28. Coulter ME, Miller DT, Harris DJ, et al. Chromosomal microarray testing influences medical management. *Genet Med.* Sep 2011; 13(9):770-776. PMID 21716121
29. Ellison JW, Ravnán JB, Rosenfeld JA, et al. Clinical utility of chromosomal microarray analysis. *Pediatrics.* Nov 2012; 130(5):e1085-1095. PMID 23071206
30. Hayeems RZ, Hoang N, Chenier S, et al. Capturing the clinical utility of genomic testing: medical recommendations following pediatric microarray. *Eur J Hum Genet.* Sep 2015; 23(9):1135-1141. PMID 25491637
31. Henderson LB, Applegate CD, Wohler E, et al. The impact of chromosomal microarray on clinical management: a retrospective analysis. *Genet Med.* Sep 2014; 16(9):657-664. PMID 24625444
32. Lingen M, Albers L, Borchers M, et al. Obtaining a genetic diagnosis in a child with disability: impact on parental quality of life. *Clin Genet.* Feb 2016; 89(2):258-266. PMID 26084449
33. Freitag CM, Staal W, Klauck SM, et al. Genetics of autistic disorders: review and clinical implications. *Eur Child Adolesc Psychiatry.* Mar 2010; 19(3):169-178. PMID 19941018
34. Turner G, Boyle J, Partington MW, et al. Restoring reproductive confidence in families with X-linked mental retardation by finding the causal mutation. *Clin Genet.* Feb 2008; 73(2):188-190. PMID 18070138
35. Saam J, Gudgeon J, Aston E, et al. How physicians use array comparative genomic hybridization results to guide patient management in children with developmental delay. *Genet Med.* Mar 2008; 10(3):181-186. PMID 18344707
36. Wood CL, Warnell F, Johnson M, et al. Evidence for ASD recurrence rates and reproductive stoppage from large UK ASD research family databases. *Autism Res.* Feb 2015; 8(1):73-81. PMID 25273900
37. Mount Sinai School of Medicine. Mount Sinai Genetic Testing Laboratory-Molecular Genetics Laboratory. Autism Spectrum Disorder Sequencing Panel - Information Sheet. [http://icahn.mssm.edu/static_files/MSSM/Files/Research/Labs/Genetic%20Testing%20Laboratory/ASD_Info sheet.pdf](http://icahn.mssm.edu/static_files/MSSM/Files/Research/Labs/Genetic%20Testing%20Laboratory/ASD_Info%20sheet.pdf). Accessed August 16, 2016.

38. Grozeva D, Carss K, Spasic-Boskovic O, et al. Targeted next-generation sequencing analysis of 1,000 individuals with intellectual disability. *Hum Mutat.* Dec 2015; 36(12):1197-1204. PMID 26350204
39. Redin C, Gerard B, Lauer J, et al. Efficient strategy for the molecular diagnosis of intellectual disability using targeted high-throughput sequencing. *J Med Genet.* Nov 2014; 51(11):724-736. PMID 25167861
40. Manning M, Hudgins L. Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. *Genet Med.* Nov 2010; 12(11):742-745. PMID 20962661
41. South ST, Lee C, Lamb AN, et al. ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. *Genet Med.* Nov 2013; 15(11):901-909. PMID 24071793
42. Schaefer GB, Mendelsohn NJ, Professional P, et al. Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions. *Genet Med.* May 2013; 15(5):399-407. PMID 23519317
43. Miles JH. Autism spectrum disorders--a genetics review. *Genet Med.* Apr 2011; 13(4):278-294. PMID 21358411
44. Monaghan KG, Lyon E, Spector EB. 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. *Genet Med.* Jul 2013; 15(7):575-586. PMID 23765048
45. Hunter J, Rivero-Arias O, Angelov A, et al. Epidemiology of fragile X syndrome: A systematic review and meta-analysis. *Am J Med Genet A.* Apr 3 2014. PMID 24700618
46. Hersh JH, Saul RA. Health supervision for children with fragile X syndrome. *Pediatrics.* May 2011; 127(5):994-1006. PMID 21518720
47. Nolin SL, Sah S, Glicksman A, et al. Fragile X AGG analysis provides new risk predictions for 45-69 repeat alleles. *Am J Med Genet A.* Apr 2013; 161A(4):771-778. PMID 23444167
48. Yrigollen CM, Mendoza-Morales G, Hagerman R, et al. Transmission of an FMR1 premutation allele in a large family identified through newborn screening: the role of AGG interruptions. *J Hum Genet.* Aug 2013; 58(8):553-559. PMID 23739124
49. ARUP Laboratories. Fragile X (FMR1) with reflex to methylation analysis. <http://ltd.aruplab.com/Tests/Pub/2009033>. Accessed June 17, 2015.
50. ARUP Laboratories. Fragile X (FMR1) with reflex to methylation analysis, fetal. <http://ltd.aruplab.com/Tests/Pub/2009034>. Accessed June 17, 2015.
51. Sherman S, Pletcher BA, Driscoll DA. Fragile X syndrome: diagnostic and carrier testing. *Genet Med.* Oct 2005; 7(8):584-587. PMID 16247297
52. Grasso M, Boon EM, Filipovic-Sadic S, et al. A novel methylation PCR that offers standardized determination of FMR1 methylation and CGG repeat length without southern blot analysis. *J Mol Diagn.* Jan 2014; 16(1):23-31. PMID 24177047
53. Gatta V, Gennaro E, Franchi S, et al. MS-MLPA analysis for FMR1 gene: evaluation in a routine diagnostic setting. *BMC Med Genet.* 2013; 14:79. PMID 23914933
54. Chaudhary AG, Hussein IR, Abuzenadah A, et al. Molecular diagnosis of fragile X syndrome using methylation sensitive techniques in a cohort of patients with intellectual disability. *Pediatr Neurol.* Apr 2014; 50(4):368-376. PMID 24630283
55. Inaba Y, Schwartz CE, Bui QM, et al. Early Detection of Fragile X Syndrome: Applications of a Novel Approach for Improved Quantitative Methylation Analysis in Venous Blood and Newborn Blood Spots. *Clin Chem.* Apr 28 2014. PMID 24778142
56. Lim GX, Loo YL, Mundho RF, et al. Validation of a Commercially Available Screening Tool for the Rapid Identification of CGG Trinucleotide Repeat Expansions in FMR1. *J Mol Diagn.* May 2015; 17(3):302-314. PMID 25776194
57. Rajan-Babu IS, Chong SS. Molecular correlates and recent advancements in the diagnosis and screening of FMR1-related disorders. *Genes (Basel).* Oct 14 2016; 7(10). PMID 27754417

58. Hawkins M, Boyle J, Wright KE, et al. Preparation and validation of the first WHO international genetic reference panel for Fragile X syndrome. *Eur J Hum Genet.* Jan 2011; 19(1):10-17. PMID 20736975
59. American College of Obstetricians Gynecologists Committee on Genetics. ACOG Committee Opinion No. 469: Carrier screening for fragile X syndrome. *Obstet Gynecol.* Oct 2010; 116(4):1008-1010. PMID 20859177
60. Biancalana V, Glaeser D, McQuaid S, et al. EMQN best practice guidelines for the molecular genetic testing and reporting of fragile X syndrome and other fragile X-associated disorders. *Eur J Hum Genet.* Apr 2015; 23(4):417-425. PMID 25227148
61. Williamson SL, Christodoulou J. Rett syndrome: new clinical and molecular insights. *Eur J Hum Genet.* Aug 2006; 14(8):896-903. PMID 16865103
62. Lotan M, Ben-Zeev B. Rett syndrome. A review with emphasis on clinical characteristics and intervention. *Scientific World Journal.* 2006; 6:1517-1541. PMID 17160339
63. Neul JL, Kaufmann WE, Glaze DG, et al. Rett syndrome: revised diagnostic criteria and nomenclature. *Ann Neurol.* Dec 2010; 68(6):944-950. PMID 21154482
64. Guy J, Gan J, Selfridge J, et al. Reversal of neurological defects in a mouse model of Rett syndrome. *Science.* Feb 23 2007; 315(5815):1143-1147. PMID 17289941
65. Robinson L, Guy J, McKay L, et al. Morphological and functional reversal of phenotypes in a mouse model of Rett syndrome. *Brain.* Sep 2012; 135(Pt 9):2699-2710. PMID 22525157
66. Suter B, Treadwell-Deering D, Zoghbi HY, et al. Brief report: MECP2 mutations in people without Rett syndrome. *J Autism Dev Disord.* Mar 2014; 44(3):703-711. PMID 23921973
67. Lane JB, Lee HS, Smith LW, et al. Clinical severity and quality of life in children and adolescents with Rett syndrome. *Neurology.* Nov 15 2011; 77(20):1812-1818. PMID 22013176
68. Cuddapah VA, Pillai RB, Shekar KV, et al. Methyl-CpG-binding protein 2 (MECP2) mutation type is associated with disease severity in Rett syndrome. *J Med Genet.* Mar 2014; 51(3):152-158. PMID 24399845
69. Archer H, Evans J, Leonard H, et al. Correlation between clinical severity in patients with Rett syndrome with a p.R168X or p.T158M MECP2 mutation, and the direction and degree of skewing of X-chromosome inactivation. *J Med Genet.* Feb 2007; 44(2):148-152. PMID 16905679
70. Weaving LS, Williamson SL, Bennetts B, et al. Effects of MECP2 mutation type, location and X-inactivation in modulating Rett syndrome phenotype. *Am J Med Genet A.* Apr 15 2003; 118A(2):103-114. PMID 12655490
71. Liyanage VR, Rastegar M. Rett syndrome and MeCP2. *Neuromolecular Med.* Jun 2014; 16(2):231-264. PMID 24615633
72. Zahorakova D, Lelkova P, Gregor V, et al. MECP2 mutations in Czech patients with Rett syndrome and Rett-like phenotypes: novel mutations, genotype-phenotype correlations and validation of high-resolution melting analysis for mutation scanning. *J Hum Genet.* Jul 2016; 61(7):617-625. PMID 26984561
73. Sheikh TI, Ausio J, Faghfoury H, et al. From function to phenotype: impaired DNA Binding and clustering correlates with clinical severity in males with missense mutations in MECP2. *Sci Rep.* Dec 08 2016; 6:38590. PMID 27929079
74. Schonewolf-Greulich B, Tejada MI, Stephens K, et al. The MECP2 variant c.925C>T (p.Arg309Trp) causes intellectual disability in both males and females without classic features of Rett syndrome. *Clin Genet.* Jun 2016; 89(6):733-738. PMID 26936630
75. ARUP Laboratories. Rett Syndrome (MECP2): sequencing and deletion/duplication. <http://ltd.aruplab.com/Tests/Pub/0051614>. Accessed August 13, 2015.
76. Huppke P, Laccone F, Kramer N, et al. Rett syndrome: analysis of MECP2 and clinical characterization of 31 patients. *Hum Mol Genet.* May 22, 2000; 9(9):1369-1375. PMID 10814718
77. Cheadle JP, Gill H, Fleming N, et al. Long-read sequence analysis of the MECP2 gene in Rett syndrome patients: correlation of disease severity with mutation type and location. *Hum Mol Genet.* Apr 12 2000; 9(7):1119-1129. PMID 10767337
78. Bao X, Downs J, Wong K, et al. Using a large international sample to investigate epilepsy in Rett syndrome. *Dev Med Child Neurol.* Jun 2013; 55(6):553-558. PMID 23421866

79. Bebbington A, Downs J, Percy A, et al. The phenotype associated with a large deletion on MECP2. *Eur J Hum Genet.* Sep 2012; 20(9):921-927. PMID 22473088
80. Halbach N, Smeets EE, Julu P, et al. Neurophysiology versus clinical genetics in Rett syndrome: A multicenter study. *Am J Med Genet A.* Sep 2016; 170(9):2301-2309. PMID 27354166
81. Pidcock FS, Salorio C, Bibat G, et al. Functional outcomes in Rett syndrome. *Brain Dev.* Jan 2016; 38(1):76-81. PMID 26175308
82. Sajan SA, Jhangiani SN, Muzny DM, et al. Enrichment of mutations in chromatin regulators in people with Rett syndrome lacking mutations in MECP2. *Genet Med.* Jan 2017; 19(1):13-19. PMID 27171548
83. Fabio RA, Colombo B, Russo S, et al. Recent insights into genotype-phenotype relationships in patients with Rett syndrome using a fine grain scale. *Res Dev Disabil.* Aug 11 2014; 35(11):2976-2986. PMID 25124696
84. Amir RE, Sutton VR, Van den Veyver IB. Newborn screening and prenatal diagnosis for Rett syndrome: implications for therapy. *J Child Neurol.* Sep 2005; 20(9):779-783. PMID 16225835
85. AAP publications retired and reaffirmed. *Pediatrics.* Dec 2007, reaffirmed in 2010 and 2014; 126:e1622.
86. Johnson CP, Myers SM. Identification and evaluation of children with autism spectrum disorders. *Pediatrics.* Nov 2007; 120(5):1183-1215. PMID 17967920