

# Protocol

## Genetic Testing for Developmental Delay and Autism Spectrum Disorder

(20459, 20483, 20481)

*(Content previously found in: Genetic Testing for FMR1 Mutations [Including Fragile X Syndrome], Genetic Testing for Rett Syndrome and Genetic Testing, Including Chromosomal Microarray Analysis and Next-Generation Sequencing Panels, for the Evaluation of Developmental Delay/Intellectual Disability, Autism Spectrum Disorder, and/or Congenital Anomalies Protocols)*

<b>Medical Benefit</b>		<b>Effective Date:</b> 07/01/17	<b>Next Review Date:</b> 09/17
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### **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: <ul style="list-style-type: none"> <li>• With developmental delay/intellectual disability</li> <li>• With autism spectrum disorder</li> <li>• With multiple congenital anomalies not specific to a well-delineated genetic syndrome</li> </ul>	Interventions of interest are: <ul style="list-style-type: none"> <li>• Chromosomal microarray analysis testing</li> </ul>	Comparators of interest are: <ul style="list-style-type: none"> <li>• Karyotyping</li> </ul>	Relevant outcomes include: <ul style="list-style-type: none"> <li>• Test accuracy</li> <li>• Test validity</li> <li>• Changes in reproductive decision making</li> <li>• Morbid events</li> <li>• Resource utilization</li> </ul>
Individuals: <ul style="list-style-type: none"> <li>• With developmental delay/intellectual disability</li> <li>• With autism spectrum disorder</li> <li>• With multiple congenital anomalies not specific to a well-delineated genetic syndrome</li> </ul>	Interventions of interest are: <ul style="list-style-type: none"> <li>• Next-generation sequencing panel testing</li> </ul>	Comparators of interest are: <ul style="list-style-type: none"> <li>• Chromosomal microarray testing</li> </ul>	Relevant outcomes include: <ul style="list-style-type: none"> <li>• Test accuracy</li> <li>• Test validity</li> <li>• Changes in reproductive decision making</li> <li>• Morbid events</li> <li>• Resource utilization</li> </ul>
Individuals: <ul style="list-style-type: none"> <li>• With intellectual disability, developmental delay, or autism spectrum disorder</li> </ul>	Interventions of interest are: <ul style="list-style-type: none"> <li>• <i>FMR1</i> variant testing</li> </ul>	Comparators of interest are: <ul style="list-style-type: none"> <li>• Standard clinical evaluation without genetic testing</li> </ul>	Relevant outcomes include: <ul style="list-style-type: none"> <li>• Test accuracy</li> <li>• Test validity</li> <li>• Resource utilization</li> </ul>
Individuals: <ul style="list-style-type: none"> <li>• Who are asymptomatic with a family history of fragile X syndrome or intellectual disability</li> </ul>	Interventions of interest are: <ul style="list-style-type: none"> <li>• <i>FMR1</i> variant testing</li> </ul>	Comparators of interest are: <ul style="list-style-type: none"> <li>• Standard clinical evaluation without genetic testing</li> </ul>	Relevant outcomes include: <ul style="list-style-type: none"> <li>• Test accuracy</li> <li>• Test validity</li> <li>• Changes in reproductive decision making</li> </ul>

Populations	Interventions	Comparators	Outcomes
seeking reproductive counseling			
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 fragile X test result seeking further counseling on carrier status risk	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 ovarian failure before age 40, with clinical suspicion of fragile X-associated ovarian failure	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 neurologic symptoms consistent with fragile X-associated tremor or ataxia syndrome	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 signs and/or symptoms of Rett syndrome	Interventions of interest are: • Genetic testing for Rett syndrome mutations	Comparators of interest are: • Standard diagnostic workup without genetic testing	Relevant outcomes include: • Test accuracy • Test validity • Other test performance measures • Symptoms • Health status measures • Quality of life
Individuals: • Who are asymptomatic and have a family member with Rett syndrome	Interventions of interest are: • Genetic testing for Rett syndrome mutations	Comparators of interest are: • No genetic testing	Relevant outcomes include: • Test accuracy • Test validity • Other test performance measures • Changes in reproductive decision making • Symptoms
Individuals: • With a child with Rett syndrome and they are considering further offspring	Interventions of interest are: • Genetic testing for Rett syndrome mutations	Comparators of interest are: • No genetic testing	Relevant outcomes include: • 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 mutations in the *MECP2* (methyl-CpG-binding protein 2) gene. Genetic testing is available to determine whether a pathogenic mutation exists 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. While direct evidence of improved outcomes with CMA compared with karyotyping is lacking, 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: end a long diagnostic odyssey, result in a reduction in morbidity for certain conditions with initiation of surveillance or management of associated comorbidities, and may impact future reproductive decision making for parents and potentially the affected child. The evidence is sufficient to determine qualitatively 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 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. 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 fragile X syndrome (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.

The evidence for NGS panel testing in individuals who have DD/ID, ASD, or multiple congenital anomalies not specific to a well-delineated genetic syndrome is lacking. Relevant outcomes are test accuracy and validity, changes in reproductive decision making, morbid events, and resource utilization. The evidence is insufficient to determine the effects of the technology on health outcomes.

The evidence for genetic testing to confirm a diagnosis in patients who have signs and/or symptoms of Rett syndrome (RTT) includes case series. Relevant outcomes are test accuracy and validity, other test performance measures, symptoms, health status measures, and quality of life. *MECP2* mutations are found in most patients with RTT, particularly those who present with classical clinical features of RTT. The diagnostic accuracy of mutation testing for RTT cannot be determined with absolute certainty given the lack of a true criterion standard for RTT diagnosis, but testing appears to have high sensitivity and specificity. Diagnostic testing has clinical utility when signs and symptoms of Rett syndrome are present, but a definitive diagnosis cannot be made without genetic testing. Confirming a diagnosis may alter some aspects of management and may eliminate the need for further diagnostic workup. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

The evidence for genetic testing of asymptomatic family members who have a family member with RTT to determine future risk of disease includes case series. Relevant outcomes are test accuracy and validity, other test performance measures, changes in reproductive decision making, and symptoms. Testing of asymptomatic family members is not likely to improve outcomes. It is unlikely that family members who do not exhibit developmental delay or other signs/symptoms of RTT will have a pathogenic mutation. The evidence is insufficient to determine the effects of the technology on health outcomes.

The evidence for genetic testing of individuals who have a child with RTT to determine carrier status in the preconception or prenatal period includes case series. Relevant outcomes are test accuracy and validity, other test performance measures, and changes in reproductive decision making. Carrier testing (preconception or prenatal) in a couple who have had a child with RTT or intellectual disability due to a *MECP2* mutation is not likely to improve outcomes. The risk of a family having a second child with the disorder is less than 1%, except in the rare situation where the mother carries the mutation, and the impact on decision making is uncertain. The evidence is insufficient to determine the effects of the technology on health outcomes.

## 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.
- Rett syndrome testing to confirm a diagnosis in a female child with developmental delay and signs/symptoms of Rett syndrome, but when there is uncertainty in the clinical diagnosis.

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 their 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.

All other indications for mutation testing for Rett syndrome, including prenatal screening and testing of family members, are considered **investigational**.

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

Whole genome, whole exome or multigene panel analysis by next-generation sequencing is considered **investigational** in all cases of suspected genetic abnormality in children with developmental delay/intellectual disability/autism spectrum disorder.

## Policy Guidelines

### *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*

The 2013 guidelines update from American College of Medical Genetics (ACMG) states that a stepwise 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 analysis (CMA).

In some cases of CMA, 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 fragile X syndrome 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).

#### **Background**

CMA can identify genomic abnormalities that are associated with a wide range of developmental disabilities, including cognitive impairment, behavioral abnormalities, and congenital abnormalities. CMA can detect copy number variants (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.<sup>1,2</sup>

#### *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.<sup>3</sup> 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 such as 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 makes it unlikely that individual SNPs will have high predictive value.<sup>4</sup>

Guidelines for patients with ID/DD, ASD, and/or congenital anomalies, such as those published by the American Academy of Pediatrics<sup>5</sup> (AAP) and the American Academy of Neurology<sup>6</sup> (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. 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 follows<sup>6</sup>:

- "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, 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.<sup>7</sup> 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.

#### 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.

SNPs are the most common genetic variation among people and occur normally throughout the DNA. Each SNP represents a difference in a single nucleotide. On average, SNPs occur every 300 nucleotides. SNPs can act as "biological markers," in that they may identify genes associated with disease. Most SNPs 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.<sup>8</sup>

#### 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, but also recommends 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 accurately delineate breakpoints.

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.<sup>9-11</sup>
- A pathogenic etiology is additionally supported when a CNV includes a gene known to cause the phenotype when inactivated (microdeletion) or overexpressed (microduplication).<sup>10</sup>
- 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 kb to one Mb.<sup>11-14</sup>
- 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.<sup>9,15</sup>

ACMG has also published guidelines for the interpretation and reporting of CNVs in the postnatal setting, to promote consistency among laboratories and CMA results.<sup>16</sup> 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 2016, there were over 53,000 subjects with individual-level data in the database.<sup>17</sup> 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 ISCA 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.<sup>18</sup> In this study, the frequency of pathogenic CNVs was 17.1%.

#### Next-Generation Sequencing

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 a variety of sizes—from the entire genome (whole-genome sequencing) to small subsets of genes (targeted sequencing). NGS allows the detection of SNPs, 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>Dx</sup> 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<sup>®</sup> 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<sup>®</sup> 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.<sup>1</sup> A considerable number of children evaluated for autism have been found to have *FMR1* premutations (55-200 CGG repeats).<sup>2</sup>

### 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 repeats<sup>3</sup>:

- 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.<sup>3-5</sup> 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*

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.<sup>1</sup> 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 the ability to engage in social interaction. Purposeful use of the hands is replaced by repetitive stereotyped hand movements, sometimes described as hand-wringing.<sup>1</sup> Other clinical manifestations include seizures, disturbed breathing patterns with hyperventilation and periodic apnea, scoliosis, growth retardation and gait apraxia.<sup>2</sup>

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.

The diagnosis of RTT remains a clinical one, using diagnostic clinical criteria that have been established for the diagnosis of classic and variant RTT.<sup>1-3</sup>

#### 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.<sup>1</sup> 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 ≈ 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, antiarrhythmics, anesthetic agents and certain antibiotics.

In a mouse model of RTT, genetic manipulation of mutated *MECP2* has demonstrated reversibility of the genetic defect.<sup>4,5</sup>

### 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.<sup>6</sup> 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%.<sup>7</sup> *MECP2* mutation type is associated with disease severity.<sup>8</sup> 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.<sup>9,10</sup>

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.<sup>1,6,11</sup>

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 this test.

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.<sup>6,7</sup>

### Related Protocols

Chromosomal Microarray Testing for the Evaluation of Early Pregnancy Loss and Intrauterine Fetal Demise  
Invasive Prenatal (Fetal) Diagnostic Testing

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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.**

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