Protocol

Genetic Testing for Developmental Delay and Autism Spectrum Disorder

(20459, 20483, 20481)

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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: • 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 validity • Changes in reproductive decision making • Morbid events • Resource utilization
Individuals: • With developmental delay/ intellectual disability, autism, or multiple congenital anomalies non-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 validity • Changes in reproductive decision making • Morbid events • Resource utilization
Individuals: • With characteristics of fragile X syndrome or a fragile X–associated disorder	Interventions of interest are: • *FMR1* variant testing	Comparators of interest are: • Standard clinical evaluation without genetic testing	Relevant outcomes include: • Test accuracy • Test validity • Resource utilization
Individuals: • Who have a personal or family history of fragile X syndrome who are seeking reproductive counseling	Interventions of interest are: • FMR1 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-associated genes	Comparators of interest are: • Standard clinical management 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	Interventions of interest are:	Comparators of interest are:	Relevant outcomes include: • Test accuracy

Populations	Interventions	Comparators	Outcomes
sisters of an individual with Rett syndrome	• Targeted genetic testing for a known familial Rett Syndrome-associated variant	• Standard clinical management without genetic testing	• Test validity • Other test performance measures • Changes in reproductive decision making • Symptoms
Individuals: • Who are females with a child with Rett syndrome who are considering further childbearing	Interventions of interest are: • Targeted genetic testing for a known familial Rett Syndrome-associated variant	Comparators of interest are: • Reproductive planning without genetic testing	Relevant outcomes include: • Test accuracy • Test validity • Other test performance measures • Changes in reproductive decision making

DESCRIPTION

Chromosomal microarray (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 testing increases the diagnostic yield over karyotyping in children with the aforementioned characteristics, and CMA testing may impact clinical management decisions. Next-generation sequencing panel testing allows for simultaneous analysis of a large number of genes and, in patients with normal CMA testing, the next-generation testing has been proposed as a way to identify single-gene causes of syndromes that have autism as a significant clinical feature.

Fragile X syndrome (FXS) is the most common inherited form of mental disability and known genetic cause of autism. The diagnosis is made with 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 variant exists in RTT-associated genes (e.g., MECP2, FOXG1, or CDLK5) in a patient with clinical features of RTT or 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 validity, changes in reproductive decision making, morbid events, and resource utilization. The available evidence supports test 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 testing 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: it

could end a long diagnostic odyssey; or reduce morbidity for certain conditions by initiating surveillance/ management of associated comorbidities; or it could 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 panel testing, the evidence includes primarily case series. Relevant outcomes are test validity, changes in reproductive decision making, morbid events, and resource utilization. The diagnostic yield associated with next-generation sequencing panel testing in this patient population is not well characterized. The testing yield and likelihood of an uncertain result are variable, based on the gene panel, gene tested, and patient population; additionally, there are risks of uninterpretable and incidental results. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals who have characteristics of FXS or an FXS-associated disorder, the evidence includes studies evaluating the clinical validity of FMR1 variant testing. Relevant outcomes are test accuracy, test validity, and resource utilization. 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, treatment of comorbid conditions may be improved. At a minimum, providing a diagnosis eliminates the need for further diagnostic workup. 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 a personal or family history of FXS who are seeking reproductive counseling, the evidence includes studies evaluating the clinical validity of FMR1 variant testing and the effect on reproductive decisions. Relevant outcomes are test accuracy, test validity, and changes in reproductive decision making. Testing the repeat region of the FMR1 gene in the context of reproductive decision making may include individuals with either a family history of FXS or a family history of undiagnosed intellectual disability, fetuses of known carrier mothers, or affected individuals or their relatives who have had a positive cytogenetic fragile X test result who are seeking further counseling related to the risk of carrier status among themselves or their relatives. DNA testing would accurately identify premutation carriers and distinguish premutation from full mutation carrier women. 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. 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 vs. 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 the 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, determina-

tion 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% 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

DEVELOPMENTAL DELAY/INTELLECTUAL DISORDER AND AUTISM SPECTRUM DISORDER

Chromosomal microarray 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:

If clinically indicated, the individual has had FMR1 gene analysis (for Fragile X), and that testing is negative

AND one of the following is true:

The individual has a diagnosis of autism spectrum disorder; OR

The individual has a diagnosis of apparent non-syndromic developmental delay/intellectual disability; OR

The individual has 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

Genetic testing for Rett syndrome-associated genes (e.g., MECP2, FOXG1, 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, FOXG1, or CDKL5), including carrier testing (preconception or prenatal), in persons with a negative family history and testing of asymptomatic family members to determine future risk of disease, are considered **investigational**.

FRAGILE X SYNDROME

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

- Individuals with characteristics of fragile X syndrome or a fragile X-associated disorder, including:
  - Individuals with intellectual disability, developmental delay, or autism spectrum disorder;
  - Women with primary ovarian insufficiency under the age of 40 in whom fragile X-associated primary ovarian insufficiency is suspected;
  - Individuals with neurologic symptoms consistent with fragile X-associated tremor/ataxia syndrome.
- Individuals who have a personal or family history of fragile X syndrome who are seeking reproductive counseling, including:
  - Individuals who have a family history of fragile X syndrome or a family history of undiagnosed intellectual disability;
  - Affected individuals or relatives of affected individuals who have had a positive cytogenetic fragile X test result who are seeking information on carrier status
  - Prenatal testing of fetuses of known carrier mothers.

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

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

Broad gene panel testing using next-generation sequencing is considered **investigational** in all cases of suspected genetic abnormality in children with DD/ID/ASD.

POLICY GUIDELINES

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

GENETIC COUNSELING

Experts recommend formal genetic counseling for patients who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some patients; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

DEVELOPMENTAL DELAY/INTELLECTUAL DISABILITY

A 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 and if they are normal, then second tier to include MECP2 (Rett Syndrome) and PTEN (autism and other developmental abnormalities, including Cowden syndrome) testing.

Recommendations from the American College of Medical Genetics guidelines (Manning and Hudgins [2010]) on array-based technologies and their clinical utilization for detecting chromosomal abnormalities include the fol-

lowing: "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.

TESTING STRATEGY

Detection of CGG triplet repeats in the FMR1 gene can occur sequentially or in parallel with determination of methylation status:

1. In sequential testing, detection of CGG triplet repeats in FMR1 is performed first. If a large number of repeats (e.g., > 55) is detected, reflex methylation testing can be performed to determine methylation status
2. In parallel testing, detection methods such as methylation-specific polymerase chain reaction allow for detection of both the size of CGG triplet repeats in FMR1 and methylation status.

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 for FMR1 and Rett Syndrome not meeting the criteria above will be considered **not medically necessary**.

BACKGROUND

DEVELOPMENTAL DELAY/INTELLECTUAL DISABILITY

Developmental delay (DD) is diagnosed in children five years or younger who show a significant delay in two or more developmental domains: gross or fine motor, speech/language, cognitive, social/personal, and activities of daily living.[1] DD can precede the development of intellectual disability (ID) as the child ages.[2]

ID is manifest by significant limitations in intellectual functioning and adaptive behavior. It is diagnosed at or after age five (when intelligence testing is considered valid and reliable) but prior to age 18 and is lifelong. The Diagnostic and Statistical Manual of Mental Disorders: Fifth Edition (DSM-5) defines ID as occurring during the developmental period and involving impairments of general mental abilities (e.g., IQ < 70 or 75) that impact adaptive functioning in the conceptual, social, and practical domains.[3]

Prevalence estimates of DD and ID range from 1% to 3%.[4] Both are influenced by genetic, environmental, infectious, and perinatal factors. Approximately 450 genes have been causally related to ID; most genes (≈ 90%) are associated with syndromes.[5] Inheritance of ID can be autosomal-dominant, recessive, or Xlinked; and most non-syndromic genes are located on the X chromosome. Prior to the advent of whole exome and genome sequencing, Willemsen and Kleefstra (2014) concluded that 20% to 40% of ID cases could be attributed to a genetic variant.[6] With the use of whole-genome sequencing, they estimated almost 60% of cases have an identifiable genetic etiology.

Congenital anomalies are frequently present in children with DD and ID. In addition, a suspected etiology can often be established from history and physical examination (in skilled specialists as much as 20% to 40% of cases) without genetic testing.[7] The recommended approach to evaluation in DD/ID begins with a three-generation family history and physical (including neurologic) exam. Subsequent testing is used to confirm a suspected diagnosis (e.g., targeted fluorescent in situ hybridization [FISH] testing for DiGeorge or cri-du-chat syndromes). If

no diagnosis is suspected, fragile X syndrome testing, metabolic testing for inborn errors of metabolism, and chromosomal microarray (CMA) testing (without karyotyping) are recommended—regardless of the presence or absence of dysmorphologic features or congenital anomalies.[1]

AUTISM SPECTRUM DISORDER

DSM-5 defines autism spectrum disorder (ASD)[a] as the presence of[3]:

- Persistent deficits in social communication and social interaction across multiple contexts,
- Restricted, repetitive patterns of behavior, interests, or activities,
- Symptoms must be present in the early developmental period (typically recognized in the first two years of life), and
- Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning.

In 2010, the estimated prevalence of ASD among eight-year-olds was 14.7 per 1000 or one in 68.[8] ASD is four to five times more common in boys than girls, and white children are more often identified with ASD than black or Hispanic children. An accurate diagnosis can generally be made by age two. The evaluation includes developmental screening and diagnostic evaluation (i.e., hearing, vision, neurologic, laboratory testing for metabolic disorders, and genetic testing).

A large body of evidence supports a genetic etiology in ASD. Twin studies estimate heritability between 60% and 90%.[9] A family with an affected child has a 13% to 19% risk for recurrence in subsequent children.[10] Based on Swedish genetic studies, Gaugler et al (2014) concluded that "the bulk of autism arises from genetic variation" (as opposed to environmental causes).[11] Still, although genetic determinants can be heritable, most appear to arise de novo.[9]

For these reasons, a child with ASD is often evaluated with genetic testing. Testing may be targeted when a child has a recognizable syndrome such as those shown in Table 1. Alternatively, high-resolution cytogenetic analysis evaluating multiple genes—the focus of this protocol—is used.

Table 1. Examples of Specific Genes Associated With Disorders That Include Autistic Behaviors

Gene(Syndrome)	Patient Selection	Yield, %	Reference
FMR1 (fragile X)	Unselected autism	3-10	
MECP2 (Rett)	Females with nonsyndromic autism, intellectual disability, and cerebral palsy	3-13	Schaefer and Mendelsohn (2008)[12]
PTEN	Autism with macrocephaly	≤ 17	Butler et al (2005)[13]

DIAGNOSTIC TESTING

Karyotyping and FISH

The goal of a cytogenetic evaluation is to identify chromosomal imbalances that cause a disorder. The most common imbalances are copy number variants (CNVs) or deletions and duplications of large segments of genomic material. CNVs are common in DD/ID and ASD but more often reflect normal genetic variation.[14] However, de novo CNVs are observed about four times more frequently in children with ASD than in normal individuals.[9] Less frequently, other abnormalities such as balanced translocations (i.e., exchanges of equally sized DNA loci between chromosomes) may be pathogenic. For many well described syndromes, the type and location of the associated chromosomal abnormality have been established by studying large patient samples. For others, few patients with similar abnormalities may have been evaluated to establish genotype-phenotype correlation.

Finally, in some patients, cytogenetic analysis will discover chromosomal abnormalities that require study to determine their significance.

Prior to the advent of CMAs, the initial step in cytogenetic analysis was G-banded karyotyping, which evaluates all chromosomes. High-resolution G-banding can detect changes as small as three to five megabases in size, although standard G-banding evaluates more than 10 megabases changes. In children with DD/ID, a review by Stankiewicz and Beaudet (2007) found G-banded karyotyping diagnostic in approximately 3% to 5%.[15] In ASD, high-resolution karyotyping appears to identify abnormalities in up to 5% of cases.[16]

In contrast, molecular cytogenetic techniques can detect small submicroscopic chromosomal alterations. FISH, a targeted approach, is used to identify specific chromosomal abnormalities associated with suspected diagnoses such as DiGeorge syndrome. Prior to CMAs, FISH was also used to screen the rearrangement-prone subtelomeric regions. Subtelomeric FISH was found to identify abnormalities in children with DD and ID,[17] diagnostic in approximately 5% to 6% of those with negative karyotypes, but uncommonly in ASD.[18]

Chromosomal Microarrays

Two types of CMAs are considered here: array comparative genomic hybridization (aCGH) and single nucleotide variants (SNV) arrays. The aCGH approach uses DNA samples from a patient and a normal control. Each is labeled with distinct fluorescent dyes (red or green). The labeled samples are then 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 simultaneously. 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 (a deletion) or has the normal sequence plus additional genomic material within that genomic location (e.g., a duplication), the sequence imbalance is detected as a difference in fluorescence intensity (Korf and Rehm [2013][19] offer an illustrative graphic). For this reason, aCGH cannot detect balanced chromosomal translations (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. A portion of the increased diagnostic yield from CMA over karyotyping comes from the discovery that 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.

Like aCGH, SNV arrays detect CNVs. In an SNV array, the two alleles for genes of interest are tagged with different florescent dyes. Comparative florescence intensity will be increased when there are duplications and diminished with deletions. The resolution provided by aCGH is higher than with SNV arrays. In addition, aCGH has better signal-to-background characteristics than SNV arrays. In contrast to aCGH, SNV arrays will also identify long stretches of DNA homozygosity, which may suggest uniparental disomy or consanguinity. Uniparental disomy occurs when a child inherits two copies of a chromosome from one parent and no copies from the other parent. Uniparental disomy can lead to syndromes such as Angelman and Prader-Willi.

Table 2 summarizes the cytogenetic tests used to evaluate children with DD/ID and autism. The table emphasizes the large difference in resolution between karyotyping and CMA.

Table 2. Resolution and Analysis Comparison of FISH, Karyotyping, and CMA Analysis

Test	Resolution in Kilobases[a]	Analysis
Karyotyping	3000-5000 kb	Genome-wide
CMA	≈ 50 kb	Genome-wide
FISH	≈ 500 to 1000 kb (depending on probe)	Targeted

CMA: chromosomal microarray; FISH: fluorescent in situ hybridization; kb: kilobases.

[a] One kb = 1000 bases, 1000 kb = one Mb.

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 laboratory-developed and commercially available platforms prompted the American College of Medical Genetics to publish guidelines for the design and performance expectations for clinical microarrays and associated software in the postnatal setting.[20]

Next-Generation Sequencing

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, insertions, and deletions. With higher resolution comes higher likelihood of detection of variants of uncertain significance.

GENETIC ASSOCIATIONS WITH DD/ID AND ASD

For common phenotypes and syndromes, the pathogenicity of CNVs may be supported by considerable evidence; for uncommon phenotypes and uncommon CNVs determining pathogenicity requires a systematic evaluation that includes parental studies, examining databases for reported associations, and considering the molecular consequences of the identified variant. Parental studies (e.g., "trio" testing of affected child, father, and mother) can identify an inherited CNV from an unaffected parent and therefore considered benign.[21] A variety of databases index the clinical implications of CNVs and their associations with a particular phenotype. CNVs are continuously cataloged and, with growth in CMA testing and improved resolution, databases have become increasingly extensive (e.g., DECIPHER [https://decipher.sanger.ac.uk], ClinVar [http://www.ncbi.nlm.nih.gov/clinvar/]). For uncommon CNVs, in addition to reports of CNV-phenotype associations, the location and size of the CNV can offer clues to pathogenicity; larger CNVs are more often pathogenic and the role of affected genes in brain circuitry and effect of CNV on gene expression can implicate pathogenicity. Although uncommon, an observed phenotype can result from unmasking a mutated recessive allele on the unaffected (non-CNV) chromosome.[22] Other considerations when determining pathogenicity include CNV dosage, X linkage, number of reports in the literature of an association between CNV and phenotype, and findings in "normal" individuals.

The American College of Medical Genetics has published guidelines for evaluating, interpreting, and reporting pathogenicity reflecting these principles.[23] The recommended categories of clinical significance for reporting are pathogenic, uncertain clinical significance (likely pathogenic, likely benign, or no subclassification), or benign. The International Standards for Cytogenomic Arrays Consortium more recently proposed "an evidence-based approach to guide the development of content on chromosomal microarrays and to support interpretation of clinically significant copy number variation."[24] The proposal defined levels of evidence describe how well or how poorly detected variants or CNVs correlate with phenotype.

FRAGILE X SYNDROME

Fragile X Syndrome

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 hyperexcit-

ability, manifested by hyperactivity, increased sensitivity to sensory stimuli, and a high incidence of epileptic seizures.

Fragile X syndrome (FXS) is associated with the expansion of the CGG trinucleotide repeat in the fragile X mental retardation 1 (FMR1) gene on the X chromosome. FXS is associated with the 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, which is believed to play a key role in early brain development and brain function.

Fragile X-Associated Disorders

Patients with a premutation (55-200 CGG repeats) may develop an FMR1-related disorder, such as fragile X–associated tremor or ataxia syndrome or, in women, fragile X–associated premature ovarian insufficiency (FXPOI). Fragile X–associated tremor or ataxia syndrome 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. FXPOI is characterized by ovarian failure before the 40 years of age. Full mutation: greater than 200-230 CGG repeats (methylated).

Diagnosis

DNA studies are used to test for FXS. Cytogenetic testing was used before identification of the FMR1 gene and is significantly less accurate than the current DNA test. Genotypes of individuals with symptoms of FXS and individuals at risk for carrying the variant can be determined by examining the size of the trinucleotide repeat segment and methylation status of the FMR1 gene. Two main approaches are used: polymerase chain reaction (PCR) and Southern blot analysis.

PCR analysis uses flanking primers to amplify a fragment of DNA spanning the repeat region. Thus, the sizes of PCR products are indicative of the approximate number of repeats present in each allele of the individual being tested. The efficiency of PCR is inversely related to the number of CGG repeats, so large mutations are more difficult to amplify and may fail to yield a detectable product in the PCR assay. This, and the fact that no information is obtained about FMR1 methylation status, are limitations of the PCR approach. On the other hand, PCR analysis permits accurate sizing of alleles in the normal zone, the "gray zone," and premutation range on small amounts of DNA in a relatively short turnaround time. Also, the assay is not affected by skewed X-chromosome inactivation.[112, 113]

The difficulty in fragile X testing is that the high fraction of GC bases in the repeat region makes it extremely difficult for standard PCR techniques to amplify beyond 100 to 150 CGG repeats. Consequently, Southern blot analysis is commonly used to determine the number of triplet repeats in FXS and methylation status. Alternatives to Southern blotting for determining FMR1 methylation status have been developed. They include methylation-sensitive PCR and methylation-specific melting curve analysis.[3-6] One test currently available in Europe (FastFraX; TNR Diagnostics, Singapore) combines a direct triplet repeat-primed PCR with melting curve analysis for detecting CGG expansions.[118]

In 2011, a panel of genotyping reference materials for FXS was developed and is expected to be stable over many years and available to all diagnostic laboratories. A panel of five genomic DNA samples (normal female, female premutation, male premutation, male full mutation, and female full mutation) was endorsed by the European Society of Human Genetics and approved as an International Standard by the Expert Committee on Biological Standardization at the World Health Organization.

Treatment

Current approaches to therapy are supportive and symptom-based. Psychopharmacologic intervention to mod-

ify 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 the home and school settings.

RETT SYNDROME

Rett syndrome (RTT) is a severe neurodevelopmental disorder primarily affecting girls, with an incidence of one in 10,000 female births, making it among the most common genetic causes of intellectual disability in girls.[128] In its typical form, RTT is characterized by apparently normal development for the first six to 18 months of life, followed by regression of intellectual functioning, acquired fine and gross motor skills, and social skills. Purposeful use of the hands is replaced by repetitive stereotyped hand movements, such as hand-wringing.[128] Other clinical manifestations include seizures, disturbed breathing patterns with hyperventilation and periodic apnea, scoliosis, growth retardation, and gait apraxia.[129]

There is wide variability in the rate of progression and severity of the disease. In addition to the typical (or classic) form of RTT, there are recognized atypical variants. Three distinct atypical variants have been described: preserved speech, early seizure, and congenital variants. RTT occurring in males is also considered a variant type and is associated with somatic mosaicism or Klinefelter (XXY) syndrome. A small number of RTT cases in males arising from the MECP2 exon 1 variant have been reported. Diagnostic criteria for typical (or classic) RTT and atypical (or variant) RTT have been established.[128-130] 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

Currently, there are no specific treatments that halt or reverse disease progression, 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.[128] A multidisciplinary approach is usually applied, with specialist input from dietitians, physical therapists, occupational therapists, speech therapists, and music therapists. Regular monitoring for scoliosis (seen in ≈ 87% of patients by age 25 years) and possible heart abnormalities, particularly cardiac conduction 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 to control 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 the MECP2 gene has demonstrated reversibility of the genetic defect.[131, 132]

Genetics

RTT is an X-linked dominant genetic disorder. Pathogenic variants in the MECP2 gene, which is thought to con-

trol 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 classic RTT have pathogenic variants in the MECP2 gene. More than 200 pathogenic variants in MECP2 have been associated with RTT.[133] However, eight of the most commonly occurring missense and nonsense variants account for almost 70% of all cases; small C-terminal deletions account for approximately 10%; and large deletions, 8% to 10%.[134] MECP2 variant type is associated with disease severity.[135] Whole duplications of the MECP2 gene have been associated with a 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.[136, 137]

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 variant, which arises 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 variant 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 variant in MECP2 does not necessarily equate to a diagnosis of RTT. Rare cases of MECP2 variants 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.[131, 136, 142] Recent studies have revealed that different classes of genetic variants in MECP2 result in variable clinical phenotypes and overlap with other neurodevelopmental disorders.[140-142]

A proportion of patients with a clinical diagnosis of RTT do not appear to have pathogenic variants in the MECP2 gene. Two other genes (CDKL5, 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.

Asuragen offers the Xpansion Interpreter® test, which analyzes AGG sequences that interrupt CGG repeats and may stabilize alleles, protecting against expansion in subsequent generations.[119, 120]

CMA TESTING

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

In January 2014, the Affymetrix CytoScan® Dx Assay (Thermo Fisher Scientific) was cleared by 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 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 kilobases (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) 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 offers multiple tests (CMA and NGS) designed for diagnosing 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 SNV probes. The expanded NGS panel for neurodevelopmental disorders includes assesses 196 genes.

LabCorp 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

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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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