Policy Statement

Chromosomal microarray analysis (CMA) may be considered medically necessary as first-line testing in the initial evaluation (see Policy Guidelines) of individuals with any of the following:

- Apparent nonsyndromic developmental delay/intellectual disability
- Autism spectrum disorder
- Multiple congenital anomalies not specific to a well-delineated genetic syndrome

Chromosomal microarray (CMA) is considered investigational for the evaluation of all other conditions of delayed development, including but not limited to idiopathic growth or language delay.

Panel testing using next-generation sequencing is considered investigational in all cases of suspected genetic abnormality in children with developmental delay/intellectual disability, autism spectrum disorder, or congenital anomalies.

Policy Guidelines

Use of chromosomal microarray (CMA) testing as outlined in this policy is not intended for use in the prenatal period.

A guideline update from American College of Medical Genetics (Schaefer et al, 2013) stated 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 (CMA) testing.

Recommendations from the American College of Medical Genetics (Manning and Hudgins [2010]) on array-based technologies and their clinical utilization for detecting chromosomal abnormalities include the following: “Appropriate follow-up is recommended in cases of chromosome imbalance identified by CMA, to include cytogenetic/FISH [fluorescent in situ hybridization] 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.

Genetics Nomenclature Update

The Human Genome Variation Society nomenclature is used to report information on variants found in DNA and serves as an international standard for DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). The Society’s nomenclature is recommended by the Human Variome Project, the HuMan Genome Organization, and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology—"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"—to describe variants identified that cause Mendelian disorders.
Table PG1. Nomenclature to Report on Variants Found in DNA

<table>
<thead>
<tr>
<th>Previous</th>
<th>Updated</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>Disease-associated variant</td>
<td>Disease-associated change in the DNA sequence</td>
</tr>
<tr>
<td>Variant</td>
<td>Change in the DNA sequence</td>
<td></td>
</tr>
<tr>
<td>Familial variant</td>
<td>Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives</td>
<td></td>
</tr>
</tbody>
</table>

Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification

<table>
<thead>
<tr>
<th>Variant Classification</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>Disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>Likely disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Variant of uncertain significance</td>
<td>Change in DNA sequence with uncertain effects on disease</td>
</tr>
<tr>
<td>Likely benign</td>
<td>Likely benign change in the DNA sequence</td>
</tr>
<tr>
<td>Benign</td>
<td>Benign change in the DNA sequence</td>
</tr>
</tbody>
</table>

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

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.

Coding

There is specific CPT coding for CMA testing:

- **81228**: Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number variants (e.g., bacterial artificial chromosome [BAC] or oligo-based comparative genomic hybridization [CGH] microarray analysis)
- **81229**: Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants for chromosomal abnormalities

Codes 81228 and 81229 cannot be reported together.

There are specific CPT codes for genomic sequencing panels for X-linked intellectual disability that meet the criteria in the code descriptor:

- **81470**: X-linked intellectual disability (XLID) (e.g., syndromic and non-syndromic XLID); genomic sequence analysis panel, must include sequencing of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCR1L, RPS6KA3, and SLC16A2
- **81471**: X-linked intellectual disability (XLID) (e.g., syndromic and non-syndromic XLID); duplication/deletion gene analysis, must include analysis of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCR1L, RPS6KA3, and SLC16A2

If testing is performed that does not meet the above code descriptors, it would be reported with the unlisted molecular pathology code 81479.

Description

Chromosomal microarray (CMA) testing has been proposed for detection of genetic imbalances in infants or children with characteristics of developmental delay/intellectual disability, autism spectrum disorder, 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.

**Related Policies**

- Chromosomal Microarray Testing for the Evaluation of Pregnancy Loss
- Invasive Prenatal (Fetal) Diagnostic Testing
- Whole Exome and Whole Genome Sequencing for Diagnosis of Genetic Disorders

**Benefit Application**

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal mandates (e.g., Federal Employee Program [FEP]) prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

**Regulatory Status**

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Lab tests for CMA testing and NGSs are available under the auspices of Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration (FDA) has chosen not to require any regulatory review of this test.

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

**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 the FDA through the de novo 510(k) process. The 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. The 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 offers an NGS ASD panel of genes targeting genetic syndromes that include autism or autistic features.

Greenwood Genetics Center offers an NGS panel for syndromic autism that includes 83 genes.

### Rationale

**Background**

**Developmental Delay/Intellectual Disability**

Developmental delay (DD) is diagnosed in children 5 years or younger who show a significant delay in 2 or more developmental domains: gross or fine motor, speech/language, cognitive, social/personal, and activities of daily living. DD can precede the development of intellectual disability (ID) as the child ages.

ID is manifest by significant limitations in intellectual functioning and adaptive behavior. It is diagnosed at or after age 5 (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.

Prevalence estimates of DD and ID range from 1% to 3%. 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. Inheritance of ID can be autosomal-dominant, recessive, or X-linked; and most nonsyndromic 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. 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. The recommended approach to evaluation in DD/ID begins with a 3-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)¹ as the presence of:\n\begin{itemize}
\item Persistent deficits in social communication and social interaction across multiple contexts,
\item Restricted, repetitive patterns of behavior, interests, or activities,
\item Symptoms must be present in the early developmental period (typically recognized in the first 2 years of life), and
\item Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning.
\end{itemize}

In 2010, the estimated prevalence of ASD among 8-year-olds was 14.7 per 1000 or 1 in 68.⁸ ASD is 4 to 5 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 2. 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 evidence review—is used.

<table>
<thead>
<tr>
<th>Gene (Syndrome)</th>
<th>Patient Selection</th>
<th>Yield, %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMR1 (fragile X)</td>
<td>Unselected autism</td>
<td>3-10</td>
<td>Schaefer and Mendelsohn (2008)¹²</td>
</tr>
<tr>
<td>MECP2 (Rett)</td>
<td>Females with nonsyndromic autism, intellectual disability, and cerebral palsy</td>
<td>3-13</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>Autism with macrocephaly</td>
<td>≤17</td>
<td>Butler et al (2005)¹³</td>
</tr>
</tbody>
</table>

**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 4 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 3 to 5 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%. 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, 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 2 alleles for genes of interest are tagged with different fluorescent dyes. Comparative fluorescence 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 2 copies of a chromosome from 1 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>
<thead>
<tr>
<th>Test</th>
<th>Resolution in Kilobases(^a)</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karyotyping</td>
<td>3000-5000 kb</td>
<td>Genome-wide</td>
</tr>
<tr>
<td>CMA</td>
<td>(\approx 50) kb</td>
<td>Genome-wide</td>
</tr>
<tr>
<td>FISH</td>
<td>(\approx 500) to 1000 kb (depending on probe)</td>
<td>Targeted</td>
</tr>
</tbody>
</table>

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

\(^a\) One kb = 1000 bases, 1000 kb = 1 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. 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. 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. 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.” The proposal defined levels of evidence describe how well or how poorly detected variants or CNVs correlate with phenotype.

**Literature Review**

This review has been informed by a Blue Cross Blue Shield Association Technology Evaluation Center (TEC) Special Report (2009) on array comparative genomic hybridization (aCGH) and a TEC Special Report (2015) on chromosomal microarray (CMA) testing for the genetic evaluation of patients with global developmental delay (DD), intellectual disability (ID), and autism spectrum disorder (ASD).

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.
Chromosomal Microarray Testing

Clinical Context and Test Purpose
The purpose of CMA testing is to identify a genetic cause for patients with DD/ID, ASD, and congenital anomalies. A genetic diagnosis may end a diagnostic odyssey, improve treatment, facilitate the management of associated medical conditions, and permit carrier testing to assess risks to future offspring.

The question addressed in this evidence review is: Does CMA testing lead to a diagnosis in patients with DD/ID, ASD, or congenital anomalies that results in changes in management and improves health outcomes?

The following PICOTS were used to select literature to inform this review.

Patients
The relevant population of interest is children with DD/ID, ASD, and congenital anomalies for whom the cause of the disorder has not been identified despite other established methods such as karyotyping.

Interventions
The relevant intervention of interest is CMA testing.

Comparators
The following practice is currently being used to diagnose DD/ID, ASD, and congenital anomalies: karyotyping.

Outcomes
The potential beneficial outcomes of interest are diagnostic yield with avoidance of future testing, changes in management that lead to an improvement in health outcomes, and identification of unaffected carriers.

Potential harmful outcomes are those resulting from a false-positive or false-negative test result. False-positive test results can lead to an incorrect diagnosis and inappropriate treatment. False-negative test results can lead to the absence of appropriate treatment and continuation of the diagnostic odyssey.

Timing
Follow-up to monitor for outcomes varies from immediately after testing diagnosis to long-term health outcomes subsequent to management changes.

Setting
CMA testing is typically administered in a tertiary care setting. Referral for genetic counseling is important for the explanation of genetic disease, heritability, genetic risk, test performance, and possible outcomes.

Study Selection Criteria
For the evaluation of clinical validity of the CMA test, studies that met the following eligibility criteria were considered: case series or cohort studies that enrolled 20 or more patients with clinical diagnoses DD/ID or ASD with known or suspicion of genetic abnormalities, with or without negative results by conventional cytogenetic evaluation, and performed CMA testing on enrolled patients. Studies were also included if they examined management decisions and/or patient outcomes based on genetic evaluation results.

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist.
This evidence review focuses on the clinical validity and clinical utility.

**Clinically Valid**
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

**Case Series or Cohort Studies**
Several studies have conducted CMA testing on samples with known chromosomal abnormalities using standard karyotyping and are summarized in Table 3. The median diagnostic yield in DD/ID patients from 21 studies published after 2012 was 19%. The median diagnostic yield was 12.3% in patients with ASD from 4 studies published after 2012; for this compilation, studies differed considerably in array resolution and in patient selection criteria. Most studies included patients with prior normal studies (e.g., karyotype and FMR1 testing). However, it is difficult to assess phenotype severity across studies owing to reporting and how samples were assembled. For a recent comparison, investigators reported diagnostic yield from 1133 children enrolled in the U.K. Deciphering Developmental Disorders study for whom a diagnosis was not established prior to CMA testing. Using both CMA and exome sequencing, a diagnostic yield of 27% was achieved.

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Diagnoses</th>
<th>Patient Description</th>
<th>Previous Normal Studies</th>
<th>Yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aradhya et al</td>
<td>20</td>
<td>DD/ID</td>
<td>DD/ID and either dysmorphic features, CA, or growth retardation</td>
<td>G-banded karyotype, FISH</td>
<td>30.0</td>
</tr>
<tr>
<td>Aradhya et al</td>
<td>20</td>
<td>DD/ID</td>
<td>As above</td>
<td>As above</td>
<td>50.0</td>
</tr>
<tr>
<td>Baldwin et al</td>
<td>211</td>
<td>DD/ID/ASD</td>
<td>Various, including idiopathic DD/ID, dysmorphic features, CA, ASD, or syndromal phenotype</td>
<td>G-banded karyotype (“many”)</td>
<td>15.6</td>
</tr>
<tr>
<td>Ballif et al</td>
<td>6946</td>
<td>DD/ID</td>
<td>Various clinical presentations, most commonly DD, dysmorphic features, and/or MCA</td>
<td>Karyotype, subtelomere FISH</td>
<td>2.4</td>
</tr>
<tr>
<td>Ballif et al</td>
<td>3600</td>
<td>DD/ID</td>
<td>Consecutive cases with diverse range of DD or MR features</td>
<td>Not specified</td>
<td>5.1</td>
</tr>
<tr>
<td>Bartnik et al</td>
<td>256</td>
<td>DD/ID</td>
<td>DD/ID with or without dysmorphic features, additional neurodevelopmental abnormalities, and/or CA</td>
<td>G-banded karyotype, fragile X testing</td>
<td>27.0</td>
</tr>
<tr>
<td>Bartnik et al</td>
<td>112</td>
<td>DD/ID</td>
<td>ID accompanied by dysmorphic features and/or CA</td>
<td>G-banded karyotype, fragile X testing, MPLA</td>
<td>21.4</td>
</tr>
<tr>
<td>Battaglia et al</td>
<td>349</td>
<td>DD/ID/ASD</td>
<td>Idiopathic DD/ID/ASD or dysmorphia</td>
<td>FISH or karyotype</td>
<td>22.1</td>
</tr>
<tr>
<td>Bremer et al</td>
<td>223</td>
<td>ASD</td>
<td>151 diagnosed ASD with normal karyotype, 1 nonpathogenic inherited balanced translocation, 72 patients who had not received karyotyping</td>
<td>Karyotype</td>
<td>8.1</td>
</tr>
<tr>
<td>Bruno et al</td>
<td>117</td>
<td>DD/ID</td>
<td>Idiopathic MR and/or CA</td>
<td>Karyotype (400 to 650–band level)</td>
<td>15.4</td>
</tr>
<tr>
<td>Chong et al</td>
<td>115</td>
<td>DD/ID/ASD/CA</td>
<td>105 patients with DD/ID/ASD/CA recruited by clinical genetics services</td>
<td>Karyotype</td>
<td>19.0</td>
</tr>
<tr>
<td>Christian et al</td>
<td>397</td>
<td>ASD</td>
<td>Nonsyndromic autism, subset of AGRE subjects (Roswell Park Cancer Institute)</td>
<td>Karyotype</td>
<td>11.6</td>
</tr>
<tr>
<td>Coulter et al</td>
<td>1792</td>
<td>DD/ID/ASD</td>
<td>DD/ID/ASD, CA, dysmorphic features, seizures, hypotonia</td>
<td>“Majority” karyotype</td>
<td>7.3</td>
</tr>
<tr>
<td>Study</td>
<td>N</td>
<td>Diagnoses</td>
<td>Patient Description</td>
<td>Previous Normal Studies</td>
<td>Yield, %</td>
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</tr>
<tr>
<td>D'Amours et al (2014)</td>
<td>21</td>
<td>CA</td>
<td>DD/ID with or without CA</td>
<td>Karyotype</td>
<td>14.3</td>
</tr>
<tr>
<td>de Vries et al (2005)</td>
<td>100</td>
<td>DD/ID</td>
<td>Idiopathic MR</td>
<td>G-banded karyotype, subtelomere MPLA</td>
<td>10.0</td>
</tr>
<tr>
<td>Eriksson et al (2015)</td>
<td>162</td>
<td>ASD</td>
<td>Suspected ASD</td>
<td>Karyotype (unclear precise proportion but &lt; half)</td>
<td>8.6</td>
</tr>
<tr>
<td>Fliges et al (2012)</td>
<td>131</td>
<td>DD/ID/ASD</td>
<td>Consecutive patients with normal karyotype but presenting with chromosomal phenotypes: malformation syndromes, syndromic and nonsyndromic ID, and ASD</td>
<td>Karyotype</td>
<td>25.2</td>
</tr>
<tr>
<td>Friedman et al (2006)</td>
<td>100</td>
<td>DD/ID</td>
<td>Idiopathic ID</td>
<td>Karyotype</td>
<td>11.0</td>
</tr>
<tr>
<td>Friedman et al (2009)</td>
<td>100</td>
<td>ID</td>
<td>Moderate-to-severe idiopathic DD/MR with CA</td>
<td>Uncertain</td>
<td>16.0</td>
</tr>
<tr>
<td>Harada et al (2004)</td>
<td>69</td>
<td>DD/ID</td>
<td>Idiopathic MR, with or without MCA</td>
<td>Karyotype (400-band level)</td>
<td>5.8</td>
</tr>
<tr>
<td>Iourov et al (2012)</td>
<td>54</td>
<td>ID/ASD/CA</td>
<td>Highly selected patients from group of 2426 patients based on clinical and cytogenic data</td>
<td>G-banded karyotype</td>
<td>28.0</td>
</tr>
<tr>
<td>Krepisch-Santos et al (2006)</td>
<td>95</td>
<td>DD/ID</td>
<td>Syndromic MR or other</td>
<td>G-banded karyotype, FMR1 (in some)</td>
<td>15.8</td>
</tr>
<tr>
<td>Lay-Son et al (2015)</td>
<td>40</td>
<td>DD/ID/othe r</td>
<td>Patients had at least 2 of the following: MCAs, facial dysmorphism, DD/ID</td>
<td>Karyotype, 4 (10%) patients had abnormality on karyotype but it did not convey a definite cause of patients' disorder</td>
<td>25.0</td>
</tr>
<tr>
<td>Lee et al (2013)</td>
<td>190</td>
<td>DD/ID</td>
<td>Retrospective chart review of patients at single center with idiopathic DD/ID</td>
<td>G-banded karyotype</td>
<td>13.7</td>
</tr>
<tr>
<td>Lu et al (2007)</td>
<td>1726</td>
<td>DD/ID</td>
<td>DD/ID, dysmorphic, or MCA features</td>
<td>G-banded karyotype and/or FISH</td>
<td>5.2</td>
</tr>
<tr>
<td>Madrigal et al (2007)</td>
<td>54</td>
<td>DD/ID</td>
<td>Idiopathic MR; 52 from families with X-linked inherited MR; 2 with suspicion of X chromosome deletion</td>
<td>Karyotype, FMR1</td>
<td>11.6</td>
</tr>
<tr>
<td>Study</td>
<td>N</td>
<td>Diagnoses</td>
<td>Patient Description</td>
<td>Previous Normal Studies</td>
<td>Yield, %</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------</td>
<td>-----------</td>
<td>--------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>McGrew et al (2012)</td>
<td>97</td>
<td>ASD</td>
<td>Retrospective review of EMR for patients with ASD or pervasive DD NOS</td>
<td>Uncertain (karyotype?)</td>
<td>6.2</td>
</tr>
<tr>
<td>Menten et al (2006)</td>
<td>140</td>
<td>DD/ID</td>
<td>Idiopathic MR and MCA</td>
<td>Karyotype, subtelomere MPLA (n=31)</td>
<td>13.6</td>
</tr>
<tr>
<td>Nava et al (2014)</td>
<td>194</td>
<td>ASD</td>
<td>ASD</td>
<td>Karyotype, fragile X testing, FISH</td>
<td>1.5</td>
</tr>
<tr>
<td>Nicholl et al (2014)</td>
<td>1700</td>
<td>DD/ID/ASD</td>
<td>1453 unrelated patients prospectively referred for investigation of DD/ID/ASD and 247 epilepsy cases</td>
<td>Uncertain</td>
<td>11.5</td>
</tr>
<tr>
<td>Pickering et al (2008)</td>
<td>1176</td>
<td>DD/ID/CA</td>
<td>Consecutive cases referred for idiopathic DD/MR/MCA or other dysmorphism</td>
<td>Karyotype (30 with visible chromosomal abnormality), FISH in some patients</td>
<td>9.8</td>
</tr>
<tr>
<td>Preiksaitiene et al (2014)</td>
<td>211</td>
<td>DD/ID</td>
<td>Syndromic and nonsyndromic cases of unknown etiology of DD/ID</td>
<td>FISH, MLPA, or karyotype</td>
<td>13.7</td>
</tr>
<tr>
<td>Saam et al (2008)</td>
<td>490</td>
<td>DD/ID</td>
<td>Retrospective review of patients who had received aCGH for autism</td>
<td>Uncertain</td>
<td>17.6</td>
</tr>
<tr>
<td>Schaefer et al (2010)</td>
<td>68</td>
<td>ASD</td>
<td>Mild-to-severe idiopathic MR and dysmorphism and/or family history; patients scored &gt;3 on de Vries Chec klist (2001)</td>
<td>Spectral karyotype (n=11), subtelomere FISH (n=30)</td>
<td>9.8</td>
</tr>
<tr>
<td>Schoumans et al (2005)</td>
<td>41</td>
<td>DD/ID</td>
<td>Nonsyndromic autism; majority from AGRE or NIMH Center for Collaborative Genetic Studies on Mental Disorders</td>
<td>Karyotype</td>
<td>7.2</td>
</tr>
<tr>
<td>Sebat et al (2007)</td>
<td>195</td>
<td>ASD</td>
<td>Consecutive patients with DD or MR</td>
<td>Karyotype (94%), FISH (20%) where prior testing available</td>
<td>5.6</td>
</tr>
<tr>
<td>Sharp et al (2006)</td>
<td>1500</td>
<td>DD/ID</td>
<td>Idiopathic MR with or without dysmorphism or MCA</td>
<td>Karyotype, subtelomere FISH (n=255)</td>
<td>5.5</td>
</tr>
<tr>
<td>Shaw-Smith et al (2004)</td>
<td>50</td>
<td>DD/ID</td>
<td>Idiopathic MR and dysmorphism or other features</td>
<td>Karyotype, subtelomere (n=41)</td>
<td>14.0</td>
</tr>
<tr>
<td>Shen et al (2007)</td>
<td>211</td>
<td>DD/ID</td>
<td>ASD</td>
<td>Not selected by prior results</td>
<td>8.1</td>
</tr>
<tr>
<td>Shen et al (2010)</td>
<td>848</td>
<td>ASD</td>
<td>Idiopathic MR and/or dysmorphism or MCAs</td>
<td>G-banded karyotype, fragile X</td>
<td>7.0</td>
</tr>
<tr>
<td>Shevell et al (2008)</td>
<td>94</td>
<td>DD/ID</td>
<td>DD</td>
<td>G-banded karyotype, fragile X, FMR1, neuroimaging</td>
<td>6.4</td>
</tr>
</tbody>
</table>
Three additional studies published in 2016 are summarized in Table 4.94-97 In the study by Xu et al (2018), the overall yield was 13.6% in 434 patients in China.94 In the study by Sansovic et al (2017), the overall yield was 22% in 334 patients in Croatia.95 In the first study by Ho et al (2016), the overall detection rate of CNVs was 29.4 (9.2% pathogenic, 20.2% variant of uncertain significance [VUS]) in 5487 patients.96 In the second study by Ho et al (2016), the overall detection rate of CNVs was 28.1% (8.6% pathogenic, 19.4% VUS) in 10351 consecutive patients, with an average of 1.2 reportable CNVs per individual.97 Overlap of patients in the 2 reports is unclear.

### Table 4. Diagnostic Yield of Studies of Patients With DD, ID, and ASD Published After 2015

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Diagnoses</th>
<th>Patient Description</th>
<th>Previous Normal Studies</th>
<th>Yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xu et al (2018)94</td>
<td>434</td>
<td>DD/ID/ASD</td>
<td>Children in China with DD/ID/ ASD (371 boys, 63 girls; mean age, 6 y; range, 4 mo to 17 y)</td>
<td>Uncertain</td>
<td>13.6 (all)</td>
</tr>
<tr>
<td>Sansovic et al (2017)95</td>
<td>337</td>
<td>DD/ID/ASD or CAs</td>
<td>Children in Croatia with DD/ID/ASD or CA (median age, 7 y; range, 1 mo to 25 y)</td>
<td>Some patients had previous classical cyogenetic and molecular</td>
<td>21.6 (all)</td>
</tr>
</tbody>
</table>
### Study Diagnoses Patient Description Previous Normal Studies Yield, %
---
Ho et al (2016)\(^{36}\) 5487 DD/ID/ASD DD/ID/ASD with or without multiple CAs, speech/language delay Uncertain • 29.4 (all) • 33 (excluding ASD) • 25 (only ASD)  
Ho et al (2016)\(^{37}\) 10,351 DD/ID/ASD or multiple CAs DD/ID/ASD or multiple CAs Uncertain • 28.1 (all) • 33 (excluding ASD) • 24.4 (only ASD)

ASD: autism spectrum disorder; CA: congenital anomaly; DD: developmental delay; ID: intellectual disability.

Table 5. Diagnostic Yield Studies in Patients With Congenital Anomalies

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Diagnoses</th>
<th>Patient Description</th>
<th>Previous Normal Studies</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hu et al (2016)(^{38})</td>
<td>119</td>
<td>Idiopathic short stature</td>
<td>Height of the individual is below 2 SDS of the corresponding mean height for a given age, sex, and population group, and no known causes can be found</td>
<td>Uncertain</td>
<td>3/119 (2.5%) identified with a pathogenic CNV</td>
</tr>
<tr>
<td>Lu et al (2008)(^{39})</td>
<td>638</td>
<td>Birth defects</td>
<td>Neonates with possible chromosomal abnormality, ambiguous genitalia, dysmorphic features, multiple congenital anomalies, congenital heart disease</td>
<td>Uncertain</td>
<td>109 (17.1%) patients were identified with clinically significant CNVs most of which would not have been defined by karyotyping</td>
</tr>
</tbody>
</table>

CNV: copy number variant; SDS: standard deviation score.

**Clinically Useful**
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

**Direct Evidence**
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

**Chain of Evidence**
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

As noted, CMA testing has a higher diagnostic yield than standard karyotyping, which is an accepted test in the evaluation of DD/ID, ASD, and congenital anomalies. In some cases, disorders are defined by the presence of a genetic variant or genetic testing can contribute to the diagnosis.

In some cases, a specific diagnosis leads to management changes that are either standard of care or are likely to lead to improvements in outcomes.
Changes in Management

A reasonable body of literature has evaluated whether the establishment of a definitive diagnosis in patients with DD/ID, ASD, and/or congenital anomalies leads to changes in management that are likely to improve outcomes. Of particular interest in the use of CMA testing to make a specific genetic diagnosis in a patient with DD/ID, ASD, and/or congenital anomalies is the effect of that diagnosis on the patient’s family. Because many affected patients will be evaluated for testing in childhood, the implications of testing on family members and the reciprocal effect on the patient are considerations.

Results of 6 retrospective studies that examined a potential impact of CMA results on clinical decisions are summarized in Table 6. These studies collectively indicate that identified pathogenic variants can prompt clinical actions potentially impacting morbidity. Less clear is how often outcomes will be improved and in which cases interventions might have occurred in the absence of testing. The proportion that may benefit will depend on the variants identified as well as diagnostic yield, which in turn depends on phenotype severity. Studies did not report on any follow-up or management changes in patients without identified pathogenic variants. In addition to reducing morbidity, bringing closure to a diagnostic odyssey is a reason for genetic testing cited by parents and noted as an outcome in case series and reports. For example, Turner et al (2008) found a median of 16.5 years from the initial medical contact to identify a causal variant in 38 extended families with fragile X syndrome. Saam et al (2008) noted that CMA testing may influence that odyssey.

Parents cite obtaining services and support as a reason for testing, but how the frequency can impact outcomes is difficult to quantify. The studies reviewed convey a set of intermediate outcomes likely to favorably affect the health of some children. Lacking are end-to-end studies following children at presentation to final outcomes. In addition to these studies, Lingen et al (2016) has reported a benefit for maternal quality of life if aCGH tests succeed to clarify the etiologic diagnosis in an affected child.

Table 6. Studies Reporting Management Changes After CMA Testing

<table>
<thead>
<tr>
<th>Study</th>
<th>Dates Testing</th>
<th>Patients (Tests)</th>
<th>Diagnostic Yield, n (%) or %</th>
<th>Pathogenic, n</th>
<th>Actionable, n (%)</th>
<th>Clinical Management Changes, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hayeems et al (2015)</td>
<td>2009-2011</td>
<td>752 (DD and/or CA)</td>
<td>114 (15.2)&lt;sup&gt;f&lt;/sup&gt; 72 (9.6)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>114</td>
<td>79.6% (364/457) with reportable results 62.4% (184/295) with benign results received medical recommendations</td>
<td>Specialist consults: 221 (37.7) Imaging: 125 (21.3) Lab tests: 70 (12.0) Surveillance: 88 (15.0) Family: 82 (14.0)</td>
</tr>
<tr>
<td>Henderson et al (2014)&lt;sup&gt;68&lt;/sup&gt;</td>
<td>2009-2012</td>
<td>1780 (DD/ID/ASD)</td>
<td>12.7</td>
<td>187</td>
<td>102 (54.5)</td>
<td>Referral: 84 (44.9) Screening: 11 (5.9) Imaging: 38 (20.3) Lab tests: 29 (15.5)</td>
</tr>
<tr>
<td>Tao et al (2014)&lt;sup&gt;85&lt;/sup&gt;</td>
<td>2011-2013</td>
<td>327 DD/ID/ASD</td>
<td>11.3</td>
<td>9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Ellison et al (2012)&lt;sup&gt;105&lt;/sup&gt;</td>
<td>2004-2011</td>
<td>46,298 DD/ID, dysmorphic, neurobehavioral, others</td>
<td>5.4</td>
<td>1259</td>
<td>441 (35)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Clinically actionable responses included additional specific tests for monitoring specific disorders</td>
</tr>
<tr>
<td>Coulter et al (2011)&lt;sup&gt;39&lt;/sup&gt;</td>
<td>2009-2010</td>
<td>1792 DD/ID/ASD or CA</td>
<td>7.3&lt;sup&gt;a&lt;/sup&gt; 5.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>121&lt;sup&gt;b,c&lt;/sup&gt; 73&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>65 (53.7)&lt;sup&gt;a&lt;/sup&gt; 25 (34.2)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Referral: 67 (60)&lt;sup&gt;a&lt;/sup&gt; and 11 (29)&lt;sup&gt;b&lt;/sup&gt; Imaging: 25 (22)&lt;sup&gt;a&lt;/sup&gt; and 9 (24)&lt;sup&gt;b&lt;/sup&gt; Lab tests: 20 (18)&lt;sup&gt;a&lt;/sup&gt; and 18 (47)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Study | Dates Testing | Patients (Tests) | Diagnosti c Yield, n (%) or % | Pathogen ic, n | Actionable, n (%) | Clinical Management Changes, n (%)
--- | --- | --- | --- | --- | --- | ---
Saam et al (2008) | 2005-2007 | 490 DD/ID | 17.6 | 48 | 34 (70.8) | • Referral: 7 (14.6)
• Screening: 8 (16.7)
• Avoid further genetic testing: 12 (25)
• Improved access to services: 12 (25)
• Reproductive recurrence risk: 17 (35.4)

ASD: autism spectrum disorder; CA: congenital anomaly; CMA: chromosomal microarray; DD: developmental delay; ID: intellectual disability.

a Abnormal.
b Possibly significant.
c Percentages as reported in the publication—denominators varied from 121 and 73.
d Assumed to be from pathogenic results oligonucleotide arrays.
e Of the 215 patients with DD/ID or ASD.
f Clinically significant.
g Uncertain, likely clinically significant.

Reproductive Decision Making
Risk estimates for recurrence of disease in future births can be altered considerably by information from the genetic diagnosis (see Table 7). Having a child with ASD appears to impact reproductive decision making or so-called reproductive stoppage. For example, Hoffmann et al (2014) examined reproductive stoppage in families with ASD using the California Department of Developmental Services database linked to birth certificates. Between 1990 and 2003, 19,710 families with 39,361 siblings and half-siblings were identified. Birth histories in these families were then compared with a control group (matched 2:1 by sex, birth year, maternal age, ethnicity/race, and county). Investigators found fertility rates in case and control families similar in the 2 years following the birth of a child with ASD, but, in the subsequent years, the rate was 33% (95% confidence interval, 30% to 37%) lower in families having a child affected by ASD.

Table 7. Sibling Recurrence Risk After Identification of Different Types of Genomic Abnormalities Associated With ASD

<table>
<thead>
<tr>
<th>Type of Genetic Abnormality</th>
<th>Clinical Example</th>
<th>Sibling Recurrence Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant single-gene disorder with full penetrance</td>
<td>Tuberous sclerosis: involves abnormalities of the skin, brain, and heart; associated with ID and AS</td>
<td>50% if parent carries the disease-causing variant (i.e., not a de novo variant)</td>
</tr>
<tr>
<td>Recessive single-gene disorder</td>
<td>Smith-Lemli-Opitz syndrome: congenital multiple anomaly syndrome; associated with ASD</td>
<td>25%</td>
</tr>
<tr>
<td>X-linked single-gene disorder</td>
<td>Fragile X syndrome: most common cause of mental retardation; associated with ASD</td>
<td>Brother: 50%</td>
</tr>
<tr>
<td>Copy number variant</td>
<td>Prader-Willi syndrome/ Angelman syndrome (15q11-q13 duplication)</td>
<td>Same as population prevalence if de novo (i.e., not found in parents)</td>
</tr>
</tbody>
</table>

ASD: autism spectrum disorder; ID: intellectual disability.

Section Summary: Chromosomal Microarray Testing
The evidence use of CMA testing for a definitive diagnosis in individuals with DD/ID, ASD, and/or congenital anomalies consists of studies reporting on the yield of a positive test in affected individuals, combined with a chain of evidence to support the clinical utility of testing. The testing yield varies by the underlying population tested, but is generally higher than 10% with higher rates in patients with congenital anomalies. While direct evidence of improved outcomes with CMA compared with karyotype 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. For children with idiopathic growth or language delay, clinical validity has not been established and there is no direct or indirect evidence to support clinical utility.

**Next-Generation Sequencing Panel Testing**

**Clinical Context and Test Purpose**

The purpose of gene panel testing with next-generation sequencing (NGS) is to identify a genetic cause for patients with DD/ID, ASD, and congenital anomalies. A genetic diagnosis may end a diagnostic odyssey, improve treatment, facilitate the management of associated medical conditions, and permit carrier testing to assess risks to future offspring.

The question addressed in this evidence review is: Does gene panel testing with NGS lead to a diagnosis in patients with DD/ID, ASD, or congenital anomalies that results in changes in management and improves health outcomes?

The following PICOTS were used to select literature to inform this review.

**Patients**

The relevant population of interest is children with DD/ID, ASD, and congenital anomalies for whom the cause of the disorder has not been identified after CMA testing.

**Interventions**

The relevant intervention of interest is gene panel testing with NGS.

**Comparators**

The following test is currently being used to diagnose DD/ID, ASD, and congenital anomalies: CMA testing.

**Outcomes**

The potential beneficial outcomes of interest are the identification of a genetic bases of the disorder, avoidance of future testing, changes in management that lead to an improvement in health outcomes, and identification of unaffected carriers.

Potential harmful outcomes are those resulting from a false-positive or false-negative test result. False-positive test results can lead to an incorrect diagnosis and inappropriate treatment. False-negative test results can lead to the absence of appropriate treatment and continuation of the diagnostic odyssey.

**Timing**

Follow-up to monitor for outcomes varies from immediately following testing to identify diagnostic accuracy to long-term health outcomes subsequent to management changes.

**Setting**

NGS testing is typically administered in a tertiary care setting. Referral for genetic counseling is important for the explanation of genetic disease, heritability, genetic risk, test performance, and possible outcomes.

**Study Selection Criteria**

For the evaluation of clinical validity of the gene panel testing with NGS, studies that meet the following eligibility criteria were considered: case series or cohort studies that enrolled 20 or more patients with clinical diagnoses DD/ID or ASD with known or suspicion of genetic abnormalities, with or without negative results by CMA testing on enrolled patients. Studies were also included if they examined management decisions and/or patient outcomes based on genetic evaluation results.
**Technically Reliable**
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

**Clinically Valid**
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

**Case Series or Cohort Studies**
Several studies have assessed NGS panel testing on samples from patients with ID with negative aCGH testing. Table 8 summarizes the diagnostic yield. For example, Grozeva et al (2016) reported that NGS targeted testing resulted in an 11% additional diagnostic yield beyond the 10% to 15% yield from aCGH alone. However, Kalsner et al (2018) reported no increase in yield using an NGS panel.

### Table 8. Diagnostic Yield Studies Published

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Diagnoses</th>
<th>Patient Description</th>
<th>Previous Normal Studies</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalsner et al (2018)</td>
<td>100</td>
<td>ASD</td>
<td>Consecutive children referred to a single U.S. neurogenetics clinic with confirmed diagnosis of ASD without a known genetic diagnosis suspected to be causative of ASD</td>
<td>Performed concurrently with CMA</td>
<td>• CMA yield: 12% (included pathogenic CNVs and VUS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• NGS panel yield: 11% (included pathogenic or likely pathogenic variants [VUS likely pathogenic])</td>
</tr>
<tr>
<td>Grozeva et al (2015)</td>
<td>986</td>
<td>M-to-S ID</td>
<td>996 patients with M-to-S ID from the U.K. (70%), Australia, Spain, U.S., and Italy. Studied phenotypes included: 905 cases with congenital heart disease; 911 cases with ciliopathy, coloboma, neuromuscular disease, severe insulin resistance, congenital thyroid disease.</td>
<td>Negative CMA testing at 500 kb resolution, and testing for fragile X and Prader-Willi or Angelman syndrome</td>
<td>• 11% (likely pathogenic rare variant)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• 8% (likely pathogenic)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• loss-of-function variant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• 3% (known pathogenic missense variant)</td>
</tr>
<tr>
<td>Redin et al (2014)</td>
<td>166</td>
<td>ID</td>
<td>ID patients with or without associated autistic-like features, fragile X, and other specific genetic conditions</td>
<td>Negative for aCGH</td>
<td>Overall diagnostic yield: 25% with 26 causative variants (16 X-linked, 10 de novo in autosomal-dominant genes).</td>
</tr>
</tbody>
</table>

aCGH: array comparative genomic hybridization; ASD: autism spectrum disorder; CHD: congenital heart disease; CMA: chromosomal microarray; CNV: copy number variant; ID: intellectual disability; M-to-S: moderate-to-severe; NGS: next-generation sequencing; VUS: variant of uncertain significance.

**Clinically Useful**
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

**Direct Evidence**
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.
No peer-reviewed, full-length randomized trials on the clinical utility of the commercially available NGS panels for ID/DD or ASD were identified.

**Chain of Evidence**
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Because the clinical validity of NGS testing in these populations has not been established, a chain of evidence supporting the clinical utility of NGS cannot be constructed.

**Section Summary: Next-Generation Sequencing Panel Testing**
It is arguable that a chain of evidence for the use of CMA testing in evaluating DD/ID, ASD, and/or congenital anomalies would apply to NGS panels. However, the clinical validity of NGS panels is less well-established than for CMA. The testing yield and likelihood of an uncertain result are variable, based on the gene panel, gene tested, and patient population. There are real risks of uninterpretable and incidental results. Therefore, current evidence does not permit conclusions whether NGS panel testing improves outcomes.

**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 also 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.

**Supplemental Information**

**Clinical Input from Physician Specialty Societies and Academic Medical Centers**
While the various physician specialty societies and academic medical centers may collaborate with and make recommendations during this process, through the provision of appropriate reviewers, input received does not represent an endorsement or position statement by the physician specialty societies or academic medical centers, unless otherwise noted.

**2011 Input**
In response to requests from Blue Cross Blue Shield Association, clinical input was received from 2 physician specialty societies and 2 academic medical centers in 2011. Input focused on the
clinical utility of chromosomal microarray (CMA) testing. As in 2010, reviewers supported the use of CMA testing for the diagnosis in patients with developmental delay and autism spectrum disorder. Reviewers acknowledged the lack of evidence in the literature on clinical utility, such as the lack of literature demonstrating improved outcomes as a result of testing. Reviewers cited multiple anecdotal and theoretical clinical situations in which management changes were made based on the results of CMA testing. Reviewers also agreed that this test was widely used in standard care with the support of the genetics community.

2010 Input
In response to requests from Blue Cross Blue Shield Association, clinical input was received through 3 physician specialty societies and 2 academic medical centers in 2010. Those providing input supported use of targeted CMA testing in children with developmental delay, intellectual disability, and autism spectrum disorder in several situations. There was less support for whole-genome array testing. However, targeted array testing is now primarily available for prenatal analysis, whereas whole-genome arrays are recommended as standard.

Practice Guidelines and Position Statements
American Academy of Pediatrics
The American Academy of Pediatrics (2014) issued a clinical report on the optimal medical genetics evaluation of a child with developmental delays (DD) or intellectual disability (ID).109 Regarding chromosomal microarray (CMA) testing, this report stated:

“CMA now should be considered a first tier diagnostic test in all children with [global] GDD/ID for whom the causal diagnosis is not known.... CMA is now the standard for diagnosis of patients with GDD/ID, as well as other conditions, such as autism spectrum disorders or multiple congenital anomalies.”

American Academy of Child and Adolescent Psychiatry
The American Academy of Child and Adolescent Psychiatry (2014) updated its guidelines on the assessment and treatment of children and adolescents with autism spectrum disorder (ASD).110 The Academy recommended that “all children with ASD should have a medical assessment, which typically includes physical examination, a hearing screen, a Wood’s lamp examination for signs of tuberous sclerosis, and genetic testing, which may include G-banded karyotype, fragile X testing, or chromosomal microarray.”

American Academy of Neurology and Child Neurology Society
The American Academy of Neurology and the Child Neurology Society (2011) updated their guidelines on the evaluation of unexplained DD and ID with information on genetic and metabolic (biochemical) testing to accommodate advances in the field.4 The guidelines concluded that CMA testing has the highest diagnostic yield in children with DD/ID, that the “often complex results require confirmation and careful interpretation, often with the assistance of a medical geneticist,” and that CMA should be considered the “first-line” test. The guidelines acknowledged that “Research is sorely lacking on the medical, social, and financial benefits of having an accurate etiologic diagnosis.”

American College of Medical Genetics
The American College of Medical Genetics (ACMG; 2010) published guidelines on array-based technologies and their clinical utilization for detecting chromosomal abnormalities.111 CMA testing for copy number variants was recommended as a first-line test in the initial postnatal evaluation of individuals with the following:

A. Multiple anomalies not specific to a well-delineated genetic syndrome
B. Apparently nonsyndromic DD/ID
C. ASD.

Other ACMG guidelines have addressed the design and performance expectations for clinical microarrays and associated software20 and for the interpretation and reporting of copy number
variants, both intended for the postnatal setting. A 2013 update included recommendations on the validation of microarray methodologies for both prenatal and postnatal specimens. The guideline revisions from ACMG (2013) stated that a stepwise or tiered approach to the clinical genetic diagnostic evaluation of ASD is recommended, with the first tier including fragile X syndrome and CMA, and the second tier MECP2 and PTEN testing. The guidelines stated that:

“this approach will evolve with continued advancements in diagnostic testing and improved understanding of the ASD phenotype. Multiple additional conditions have been reported in association with an ASD phenotype, but none of these has been evaluated in a large prospective cohort. Therefore, a future third tier of evaluation is a distinct possibility. Further studies would be needed to elevate the evidence to the point of recommended testing. Alternatively, advances in technology may permit bundling of individual tests into an extended, more readily accessible, and less expensive platform. The accumulating evidence using next-generation sequencing (third tier testing) will increase the diagnostic yield even more over the next few years.”

U.S. Preventive Services Task Force Recommendations
Not applicable.

Medicare National Coverage
There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

Ongoing and Unpublished Clinical Trials
A search of ClinicalTrials.gov in September 2018 did not identify any ongoing or unpublished trials that would likely influence this review.

References

17. Moeschler JB. Medical genetics diagnostic evaluation of the child with global developmental delay or intellectual disability. Curr Opin Neurol. Apr 2008;21(2):117-122. PMID 18317267


**Documentation for Clinical Review**

**Please provide the following documentation (if/when requested):**

- History and physical and/or consultation notes including:
  - Birth records (if applicable)
  - Diagnosis
  - Genetic counseling notes
  - Treatment plan
- Specific test(s) requested

**Post Service**

- Laboratory report(s)
### Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy. Inclusion or exclusion of codes does not constitute or imply member coverage or provider reimbursement.

**MN/IE**

The following services may be considered medically necessary in certain instances and investigational in others. Services may be considered medically necessary when policy criteria are met. Services may be considered investigational when the policy criteria are not met or when the code describes application of a product in the position statement that is investigational.

<table>
<thead>
<tr>
<th>Type</th>
<th>Code</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>CPT®</td>
<td>0012U</td>
<td>Germline disorders, gene rearrangement detection by whole genome next-generation sequencing, DNA, whole blood, report of specific gene rearrangement(s)</td>
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<tr>
<td>CPT®</td>
<td>81228</td>
<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number variants (e.g., bacterial artificial chromosome [BAC] or oligo-based comparative genomic hybridization [CGH] microarray analysis)</td>
</tr>
<tr>
<td>CPT®</td>
<td>81229</td>
<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants for chromosomal abnormalities</td>
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<tr>
<td>CPT®</td>
<td>81470</td>
<td>X-linked intellectual disability (XLID) (e.g., syndromic and non-syndromic XLID); genomic sequence analysis panel, must include sequencing of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCR1L, RPS6KA3, and SLC16A2</td>
</tr>
<tr>
<td>CPT®</td>
<td>81471</td>
<td>X-linked intellectual disability (XLID) (e.g., syndromic and non-syndromic XLID); duplication/deletion gene analysis, must include analysis of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCR1L, RPS6KA3, and SLC16A2</td>
</tr>
<tr>
<td>HCPCS</td>
<td>S3870</td>
<td>Comparative genomic hybridization (CGH) microarray testing for developmental delay, autism spectrum disorder and/or intellectual disability</td>
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</table>

| ICD-10 Procedure | None |

### Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

<table>
<thead>
<tr>
<th>Effective Date</th>
<th>Action</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>07/06/2012</td>
<td>BCBSA Medical Policy adoption</td>
<td>Medical Policy Committee</td>
</tr>
<tr>
<td>07/31/2015</td>
<td>Coding update</td>
<td>Administrative Review</td>
</tr>
<tr>
<td>10/30/2015</td>
<td>Policy title change from Chromosomal Microarray Analysis (CMA) for the Genetic Evaluation of Patients with Developmental Delay/Intellectual Disability or Autism Spectrum Disorder</td>
<td>Medical Policy Committee</td>
</tr>
</tbody>
</table>
Definitions of Decision Determinations

**Medically Necessary:** A treatment, procedure, or drug is medically necessary only when it has been established as safe and effective for the particular symptoms or diagnosis, is not investigational or experimental, is not being provided primarily for the convenience of the patient or the provider, and is provided at the most appropriate level to treat the condition.

**Investigational/Experimental:** A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

**Split Evaluation:** Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member’s health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member’s eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department. Please call (800) 541-6652 or visit the provider portal at www.blueshieldca.com/provider.

Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.