
Policy Number: 2.04.59  Last Review: 5/2018

Policy
Blue Cross and Blue Shield of Kansas City (Blue KC) will provide coverage for Genetic Testing, Including Chromosomal Microarray Analysis and Next-Generation Sequencing Panels, for the Evaluation of Developmental Delay/Intellectual Disability, Autism Spectrum Disorder, and/or Congenital Anomalies when it is determined to be medically necessary because the criteria shown below are met.

Note: This is a type of genetic testing that may be excluded in some contracts. Verify benefits prior to review for Medical Necessity.

When Policy Topic is covered
Chromosomal microarray analysis may be considered medically necessary as first-line testing in the initial evaluation of individuals with any of the following:
- Apparently nonsyndromic developmental delay/intellectual disability
- Autism spectrum disorder
- Multiple congenital anomalies not specific to a well-delineated genetic syndrome

When Policy Topic is not covered
Chromosomal microarray 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.

Considerations
Use of CMA testing as outlined in this policy is not intended for use in the prenatal period.
A 2013 guidelines 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 2010 American College of Medical Genetics guidelines (Manning et al 2010) on array-based technologies and their clinical utilization for detecting chromosomal abnormalities include the following: “Appropriate follow-up is recommended in cases of chromosome imbalance identified by CMA, to include cytogenetic/FISH studies of the patient, parental evaluation, and clinical genetic evaluation and counseling.”

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

**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 in DNA diagnostics. The nomenclature 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>Variants</td>
<td></td>
<td>Change in the DNA sequence</td>
</tr>
<tr>
<td>Familial variant</td>
<td>Disease-associated variant identified in a proband</td>
<td>for use in subsequent targeted genetic testing in first-degree relatives</td>
</tr>
</tbody>
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**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>----------------------------------</td>
<td>--------------------------------------------------------</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>

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

**Genetic Counseling**

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

**Description of Procedure or Service**

<table>
<thead>
<tr>
<th>Populations</th>
<th>Interventions</th>
<th>Comparators</th>
<th>Outcomes</th>
</tr>
</thead>
</table>
| **Individuals:**  
  - With developmental delay/intellectual disability, autism, or congenital anomalies not specific to a genetic syndrome | Interventions of interest are:  
  - Chromosomal microarray testing | Comparators of interest are:  
  - Karyotyping | Relevant outcomes include:  
  - Test accuracy  
  - Test validity  
  - Changes in reproductive decision making  
  - Morbid events  
  - Resource utilization |
| **Individuals:**  
  - With developmental delay/intellectual disability, autism, or multiple congenital anomalies nonspecific to genetic syndrome | Interventions of interest are:  
  - Next-generation sequencing panel testing | Comparators of interest are:  
  - Chromosomal microarray testing | Relevant outcomes include:  
  - Test accuracy  
  - Test validity  
  - Changes in reproductive decision making  
  - Morbid events  
  - Resource utilization |

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.

For individuals who have DD/ID, ASD, or multiple congenital anomalies not specific to a well-delineated genetic syndrome who receive CMA testing, the evidence includes primarily case series. Relevant outcomes are test accuracy and validity, changes in reproductive decision making, morbid events, and resource utilization. The available evidence supports test accuracy and validity. Although systematic studies of the impact of CMA on patient outcomes are lacking, the improvement in
diagnostic yield over karyotyping has been well-demonstrated. Direct evidence of improved outcomes with CMA compared with karyotyping is lacking. However, for at least a subset of the disorders potentially diagnosed with CMA 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 potentially impact future reproductive decision making for parents. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

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

**Background**

**Developmental Delay/Intellectual Disability and Autism Spectrum Disorder**

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

The diagnosis of developmental delay (DD) is reserved for children younger than 5 years of age who have a significant delay in 2 or more of the following developmental domains: gross or fine motor, speech/language, cognitive, social/personal, and activities of daily living. Intellectual disability (ID) is a lifelong disability diagnosed at or after 5 years of age when IQ testing is considered valid and reliable. The *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)*, of the American Psychiatric Association defined patients with ID as having an IQ less than 70, onset during childhood, and dysfunction or impairment in more than 2 areas of adaptive behavior or systems of support.

According to *DSM-IV*, pervasive developmental disorders (PDD) encompass 5 conditions: autistic disorder, Asperger disorder, pervasive developmental disorder—not otherwise specified (PDD-NOS), childhood disintegrative disorder, and Rett syndrome. Although not mentioned in the *DSM-IV*, autism spectrum disorder (ASD) includes the first three on the list.
One of the major changes between DSM-IV and DSM-5 is the new diagnostic criteria for ASD, which include removing the term pervasive developmental disorders. Researchers found that the separate diagnoses included in PDD were not consistently applied across different clinics and treatment centers. Under DSM-5, ASD now encompasses the previous DSM-IV autistic disorder (autism), Asperger disorder, childhood disintegrative disorder, and PDD-NOS. Anyone diagnosed with one of the PDDs from DSM-IV should still meet the criteria for ASD in DSM-5.

**Congenital Anomalies**

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

**Genetic Associations With DD/ID, ASD, and Congenital Anomalies**

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

Complex autism, which comprises approximately 20% to 30% of cases of autism, is defined by the presence of dysmorphic features and/or microcephaly. Essential autism, approximately 70% to 80% of autism cases, is defined as autism in the absence of dysmorphology. Genetic causes of autism include cytogenetically visible chromosomal abnormalities (5%), single-gene disorders (5%), and CNVs (10%-20%). Single-nucleotide polymorphism (SNP) microarrays to perform high-resolution linkage analysis have revealed suggestive regions on certain chromosomes not previously associated with autism. To date, the SNP findings in autism seem consistent with other complex diseases, in which common variation
has modest effect size (odds ratio, <2), requiring large samples for robust
detection. This diagnostic challenge makes it unlikely that individual single
nucleotide variants (SNVs) will have high predictive value.²

Guidelines for patients with ID/DD, ASD, and/or congenital anomalies, such as
those published by the American Academy of Pediatrics³ (AAP) and the American
Academy of Neurology (AAN) with the Child Neurology Society (CNS), have
recommended cytogenetic evaluation to look for certain kinds of chromosomal
abnormalities that may be causally related to their condition.⁴ The joint AAN and
CNS guidelines have noted that only occasionally will an etiologic diagnosis lead to
specific therapy that improves outcomes, but suggest the more immediate and
general clinical benefits of achieving a specific genetic diagnosis from the clinical
viewpoint, as follows⁴:

- “limit[] further diagnostic testing”
- “improve[e] understanding of treatment and prognosis”
- “anticipat[e] and manag[e] associated medical and behavioral comorbidities”
- “allow[] for counseling regarding risk of recurrence, and prevent[] recurrence
  through screening for carriers and prenatal testing.”

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

At present, a relatively small body of literature has addressed the use of
chromosomal microarray (CMA) or other genetic testing for predicting disease
phenotype or severity.⁵ This is not yet a major clinical use of testing and is not a
focus in this review.

**Testing to Determine Genetic Etiology**

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

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

**CMA Testing**
The term CMA collectively describes 2 different array platforms: aCGH and SNP arrays. Both types of arrays can identify loss or gain of DNA (microdeletions or microduplications, respectively), known as CNVs. CMA testing can identify genomic abnormalities associated with a wide range of developmental disabilities, including cognitive impairment, behavioral abnormalities, and congenital abnormalities. CMA testing can detect CNVs, and the frequency of disease-causing CNVs is highest (20%-25%) in children with moderate-to-severe intellectual disability accompanied by malformations or dysmorphic features. Disease-causing CNVs have been identified in 5% to 10% of cases of autism, being more frequent in severe phenotypes.\(^6\)\(^7\)

**Array Comparative Genomic Hybridization and Single-Nucleotide Polymorphism**
The aCGH technique uses a DNA sample from the patient and a DNA sample from a normal control. Each is labeled with 1 color of fluorescent dye (red or green), and the labeled samples are mixed and hybridized to thousands of cloned or synthesized reference (normal) DNA fragments of known genomic locus immobilized on a glass slide (microarray) to conduct thousands of comparative reactions at the same time. CNVs are determined by computer analysis of the array patterns and intensities of the hybridization signals. If the patient sequence is missing part of the normal sequence (deletion) or has the normal sequence plus additional genomic material within that genomic location (eg, a duplication of the same sequence), the sequence imbalance is detected as a difference in fluorescence intensity. For this reason, aCGH cannot detect balanced CNVs (equal exchange of material between chromosomes) or sequence inversions (the same sequence is present in reverse base pair order) because the fluorescence intensity would not change.

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

Like aCGH, SNP arrays also detect CNVs, although the resolution provided by aCGH is better than that with SNP arrays, and, therefore, SNP arrays are limited in the detection of single exon CNVs. In addition, aCGH has better signal-to-background characteristics than SNP arrays. In contrast to aCGH, SNP arrays will also identify long stretches of DNA homozygosity, which may suggest uniparental disomy (UPD) or consanguinity. UPD occurs when someone inherits 2 copies of a chromosome from 1 parent and no copies from the other parent. UPD can lead to
syndromes such as Angelman and Prader-Willi. SNP arrays can also detect triploidy, which cannot be detected by aCGH arrays.

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

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

Microarrays may be prepared by the laboratory using the technology or, more commonly, by commercial manufacturers, and sold to laboratories that must qualify and validate the product for use in their assay, in conjunction with computerized software for interpretation. The proliferation of 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.8

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

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

- CNVs are confirmed by another method (e.g., FISH, multiplex ligation-dependent probe amplification, polymerase chain reaction).
- CNVs detected are checked against public databases and, if available, against private databases maintained by the laboratory. Known pathogenic CNVs associated with the same or similar phenotype as the patient are assumed to explain the etiology of the case; known benign CNVs are assumed to be nonpathogenic. \(^9\)-\(^11\)
- A pathogenic etiology is additionally supported when a CNV includes a gene known to cause the phenotype when inactivated (microdeletion) or overexpressed (microduplication). \(^10\)
- The laboratory may establish a size cutoff; potentially pathogenic CNVs are likely to be larger than benign polymorphic CNVs; cutoffs for CNVs not previously reported typically range from 300 kilobase (kb) pairs to 1 megabase pairs. \(^11\)-\(^14\)
- Parental studies are indicated when CNVs of appropriate size are detected and not found in available databases; CNVs inherited from a clinically normal parent are assumed to be benign variants whereas those appearing de novo are likely pathogenic; etiology may become more certain as other similar cases accrue. \(^9\),\(^15\)

The American College of Medical Genetics has also published guidelines on the interpretation and reporting of CNVs in the postnatal setting, to promote consistency among laboratories and CMA results. \(^16\) Three categories of clinical significance have been recommended for reporting: pathogenic, benign, and uncertain clinical significance.

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

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

**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 NGS 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, FDA has chosen not to require any regulatory review of this test.

In July 2010, 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 SNP microarray analysis. On January 17, 2014, the Affymetrix CytoScan® Dx Assay (Thermo Fisher Scientific, Waltham, MA) has been cleared by the U.S. Food and Drug Administration (FDA) through the de novo 510(k) process. FDA’s review of the CytoScan® Dx Assay included an analytic evaluation of the test’s ability to detect accurately numerous chromosomal variations of different types, sizes, and genome locations compared with several analytically validated test methods. FDA found that the CytoScan® Dx Assay could detect copy number variations (CNVs) across the genome and adequately detect CNVs in regions of the genome associated with ID/DD. Reproducibility decreased with the CNV gain or loss size, particularly when less than approximately 400 kb (generally recommended as the lower reporting limit). As of July 2017, Affymetrix™ has reported 2.69 million markers for copy number, 750,000 biallelic probes, and 1.9 million polymorphic probes (Affymetrix™ was acquired by Thermo Fisher Scientific in 2016). FDA product code: PFX.

FirstStep Dx PLUS® (Lineagen, Salt Lake City, UT) uses Lineagen’s custom-designed microarray platform manufactured by Affymetrix. As of July 2017, this microarray consists of a 2.8 million probe microarray for the detection of CNVs associated with neurodevelopmental disorders. The array includes probes that come standard on the Affymetrix CytoScan HD® microarray, with an additional 88,435 custom probes designed by Lineagen.
Ambry Genetics (Aliso Viejo, CA) offers multiple tests (CMA and NGS) that are designed for ASD and neurodevelopmental disorders. As of July 2017, the CMA offered by Ambry Genetics includes over 2.6 million probes for copy number and 750,000 SNP probes. The expanded NGS panel for neurodevelopmental disorders includes assesses 196 genes.

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

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

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

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

**Rationale**
This evidence review was created in January 2010 and has been updated regularly with a search of the MEDLINE database. The most recent literature update was performed through June 22, 2017 (see Appendix Table 1 for genetic testing categories). This review has been informed by a 2009 TEC Special Report on array comparative genomic hybridization (aCGH) and a 2015 TEC Special Report on chromosomal microarray (CMA) testing for the genetic evaluation of patients with global developmental delay (DD), intellectual disability (ID), and autism spectrum disorder (ASD).

Validation of the clinical use of any genetic test focuses on 3 main principles: (1) analytic validity, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent; (2) clinical validity, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and (3) clinical utility (ie, how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes). The following is a summary of the key literature.

**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 includes patients 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 relevant comparator of interest is usual care without genetic testing.

**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**
The timeframe for outcome measures varies from immediately following testing diagnosis to long-term health outcomes subsequent to management changes.

**Setting**
Patients suspected of a genetic cause for their disability are typically seen in a tertiary care setting. Referral for genetic counseling is important for explanation of genetic disease, heritability, genetic risk, test performance, and possible outcomes.

**Analytic Validity**
The 2015 TEC Special Report on CMA testing for global DD/ID and ASD reviewed the evidence for the analytic validity of CMA testing, and made the following conclusions: “In summary, acceptable analytic validity is generally assumed for CMA testing based on laboratories meeting quality standards under CLIA [Clinical Laboratory Improvement Amendments], including participation in proficiency testing. Reports of analytic validity specific to CMA are not readily identified. Data supporting the analytic validity of the CytoScan Dx® Assay for use in children with..."
ID and GDD [global developmental delay] were included as part of the FDA [Food and Drug Administration] clearance."²⁰

Clinical Validity
Several studies (see Appendix B of the 2009 TEC Special Report on aCGH¹⁹) have conducted CMA testing on samples with known chromosomal abnormalities by standard karyotyping. In general, currently available CMA clinical services achieve near 100% sensitivity for known chromosomal abnormalities. False-positive rates (ie, copy number variants [CNVs] of uncertain clinical significance) on known normal samples were inconsistently reported and could not be summarized. One study evaluated the clinical validity of an oligonucleotide (oligo) array and reported 99% sensitivity and 99% specificity, with a resolution of 300 to 500 kilobase (kb) pairs for 10 selected cases with different known chromosomal abnormalities.

The literature on the diagnostic yield of DD/ID, ASD, and congenital anomalies consists of a large number of prospective and retrospective case series.

DD/ID or ASD
Several studies have reported on the diagnostic yield of CMA testing in DD/ID or ASD patients with normal standard karyotype and, in several cases, normal FMR1 gene analysis and/or subtelomere fluorescence in situ hybridization (FISH) screening (see Appendix Table 2 of the TEC Special Report on CMA²⁰). As summarized in the 2015 TEC Special Report, 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. This diagnostic yield compares well with a synthesis of studies published in 2010 by the International Standards for Cytogenomic Arrays Consortium, reporting an average diagnostic yield of 12.2% across 33 studies.¹² Hochstenback et al (2009) reported a CMA diagnostic yield of 19% for 36,325 DD/ID cytogenetic referrals in the Netherlands;²¹ and Shen et al (2010) reported a 7% diagnostic yield among 933 ASD referrals.²² Cooper et al (2011) studied CMA testing from over 15,000 individuals with DD/ID, ASD, and/or various congenital abnormalities and compared them with CMA results from over 8000 unaffected controls, finding a significant excess of large CNVs among cases compared with controls.²³ Using a common cutoff for CNV size, about 26% of cases had a CNV larger than 400 kb pairs compared with about 12% of controls, suggesting that CNVs of this size account for approximately 14% of cases. CNVs larger than 400 kb pairs were also significantly more common among cases with multiple congenital abnormalities.

Tammimies et al (2015) reported on the yield of CMA plus whole-exome sequencing (WES) in 258 children with ASD from 2 developmental clinics in Canada, who were stratified on the basis of the presence of associated major and minor congenital and physical anomalies.²⁴ Subjects were evaluated clinically with brain magnetic resonance imaging, intelligence tests, and characterization of birth defects and minor physical anomalies according to standardized scales. Genetic and molecular diagnostic testing occurred in a stepwise manner: all patients were
tested for fragile X syndrome; all girls had MECP2 sequencing; any child with a head circumference 3 standard deviations or more above the mean had PTEN sequencing; and if any syndrome was suspected clinically, relevant targeted sequencing was obtained. Patients underwent CMA testing with a greater than 1 million probes research microarray, with CNVs classified according to American College of Medical Genetics guidelines. For 100 probands, WES was also obtained. All 258 children had some form of microarray, 150 of whom received a clinical microarray testing (125 with oligo arrays, 25 with bacterial artificial chromosome microarray-based CGH), and the remainder of whom received high-resolution research microarray testing. Molecular diagnoses were obtained by CMA in 24 probands (9.8%; 95% confidence interval, 6.4% to 14.3%). Patients with ASD in conjunction with congenital anomalies (“complex ASD”) had significantly more pathogenic CNVs (24.5%) than those with essential ASD (4.2%; p<0.001).

Roberts et al (2014) reported on their experience with the use of the 105,000 and 180,000 oligo microarrays in 215 consecutive patients with either ASD or DD/learning disability who were referred to a single medical for genetic services between 2009 and 2012. Of the 215 patients (140 males, 75 females), 65 had ASD and 150 had learning disability. Abnormal microarray results were found in 45 (21%) patients with a total of 49 CNVs. Thirty-two represented a known diagnostic CNV contributing to the clinical presentation and 17 represented variants of uncertain significance (VUS). Thirteen (20%) of 65 patients with ASD had a CNV compared with 32 (21%) of 150 patients with a learning disability. The 13 patients with ASD had a total of 14 CNVs, 6 recognized as diagnostic and 8 as nondiagnostic. For those patients with a learning disability, 32 had a total of 35 CNVs, 26 of which were classified as a known diagnostic CNV (usually a deletion; n=20) and 9 of which were classified as an unknown nondiagnostic CNV (usually a duplication; n=8).

In 2016, investigators from Lineagen published 2 articles on CMA with FirstStepDx PLUS for ASD and neurodevelopmental disorders. To be considered pathogenic, there had to be at least 2 publications that indicated that haplo-insufficiency or triplo-sensitivity of the region or gene(s) were causative of clinical features. The overall detection rate of CNVs was 28.1% (8.6% pathogenic and 19.4% VUS) in 10,351 consecutive patients, with an average of 1.2 reportable CNVs per individual. In the 5694 patients with ASD, the detection rate was 5.4% pathogenic and 19.0% VUS. The most common referrals were made by neurologists (36%), developmental pediatricians (31%), pediatricians (16%), and medical geneticists (14%). The second report included 5487 patients with neurodevelopmental disorders who had been assessed over a period of 3.5 years. Overlap of patients in the 2 reports is unclear. The detection rates were 9.2% pathogenic and 20.2% VUS, compared with 9.0% pathogenic and 14.2% VUS in the CytoScan HD microarray. Thus, the addition of the custom probes to the standard CytoScan HD microarray significantly increased the VUS rate with little change in the detection of established pathogenic variants.
**Congenital Anomalies**

Lu et al (2008) reported on the frequency of genomic imbalances in neonates with birth defects by using 3 different targeted aCGH platforms using bacterial artificial chromosomes. The study included 638 neonates with various birth defects who had been referred between 2006 and 2007. Overall, 109 (17.1%) patients were identified with clinically significant CNVs, most of which would not have been defined by karyotyping. The clinically significant detection rates for various clinical indications were 66.7% for "possible chromosomal abnormality" with or without "others" (other clinical indications), 33.3% for ambiguous genitalia with or without others, 27.1% for dysmorphic features with multiple congenital anomalies with or without others, 24.6% for dysmorphic features with or without others, 21.8% for congenital heart disease with or without others, 17.9% for multiple congenital anomalies with or without others, and 9.5% for patients referred for other indications not in the defined groups. In all, of the 109 patients in whom clinically significant genomic imbalances or pathogenic CNVs were detected by CMA testing, 14.7% had numeric anomalies including trisomies 21, 18, 13, and 22, and monosomy X. The remaining 85.3% had genomic imbalances that might not have been detected by standard cytogenetic studies, including 33.9% with common microdeletion or microduplication syndromes, 40.4% with genomic imbalances at relatively rare disease loci, and 11.0% with chromosomal mosaicism.

**Idiopathic Growth Retardation**

Short stature has been associated with hundreds of single-nucleotide variants, and with an increased number of CNVs and greater CNV length. One study by Hu et al (2016) was identified that reported the diagnostic yield of CMA in Chinese children with idiopathic short stature. Pathogenic CNVs were identified in 3 of 119 patients, for a diagnostic yield of 2.5%. Possibly pathogenic CNVs were identified in an additional 2 patients.

**Idiopathic Language Delays**

Several case reports were identified on the association of specific genes with nonsyndromic language delay. No studies were identified that evaluated diagnostic yield of CMA for idiopathic language delay.

**Clinical Utility**

Clinical utility is defined as how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes. The clinical utility of genetic testing is considered primarily for the case of diagnostic testing in symptomatic individuals with DD/ID, ASD, and/or congenital anomalies. Direct evidence for the clinical utility of genetic testing to confirm a specific diagnosis in individuals with DD/ID, ASD, and/or congenital anomalies consists of studies reporting on the prevalence of potentially clinically actionable diagnoses, studies of practice behavior after CMA testing, and reports of reproductive behavior of parents of children with DD/ID, ASD, and/or congenital anomalies, which are suggestive but not definitive. A chain of evidence can provide support for clinical utility if all the links in the chain of evidence are intact. The elements...
contributing to the indirect chain are derived from evidence review 2.04.91 (general approach to genetic testing). The following series of questions represents the chain of evidence for diagnostic testing to make a specific genetic diagnosis in patients with DD/ID, ASD, and/or congenital anomalies:

- Can genetic testing confirm the suspected diagnosis?

A variety of genetically based syndromes associated with DD/ID, ASD, and/or congenital anomalies can be diagnosed with CMA testing.

- Can the diagnosis be confirmed by alternative methods without genetic testing?

Not always. 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.

- Does confirmation of diagnosis by genetic testing lead to initiation of other management changes with uncertain impact on outcomes (eg, referrals to specialists and/or ancillary care, initiate screening)? Does confirmation of diagnosis by genetic testing lead to initiation of other management changes that are considered “standard of care” treatment for disorder?

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.

The 2015 TEC Special Report on the use of CMA testing for the genetic evaluation of patients with DD/ID and ASD found the following for the clinical utility of CMA testing:

Studies on the potential impact of CMA testing on clinical decisions “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 ... and noted as an outcome in case series and reports.... 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 cohort studies following children at presentation to final outcomes.”

“There are considerable challenges conducting studies of sufficient size given the underlying genetic heterogeneity, and including follow-up adequate to
observe final health outcomes…. [However] studies examining clinical utility have reported intermediate outcomes and indirect evidence.”

**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.

Coulter et al (2011) identified and reviewed, over the course of a year, the medical records of all patients at a tertiary children’s hospital who had CMA results showing an abnormal variant or a VUS. A board-certified medical geneticist reviewed the clinical notes from the ordering provider and abstracted recommendations for clinical actions (a specialist referral, imaging study, diagnostic test, or medication prescription) made specifically as a result of the CMA result. Of 1792 patients for whom CMA was ordered during the year reviewed, 131 had an abnormal variant and 104 had a VUS. Of these, 121 and 73 patients were included in the analysis. Overall, patients with an abnormal variant had a significantly higher rate of recommended clinical action (54%) than patients with a VUS (34%; p=0.01). Among patients with an abnormal variant and a diagnosis of DD/ID or congenital anomalies, about two-thirds were referred for additional clinical action based on the CMA results, whereas referrals were made for 27% of patients with ASD and an abnormal variant. Referral rates were similar for patients with a CMA result of a VUS, with the exception of patients with congenital anomalies, who were referred for additional clinical action only 17% of the time. Patients younger than 2 years old were significantly more likely to have clinical anomalies and were significantly more likely to have abnormal variants. Cases were described in which ancillary CMA results suggested clinical interventions for the present or future regarding possible comorbid conditions.

Ellison et al (2012) reported on the clinical utility of CMA testing in 46,298 postnatal patients. Testing was for a variety of indications, including DD/ID, congenital anomalies, dysmorphic features, and neurobehavioral problems. The authors tallied the detection of abnormalities associated with actionable clinical features (ie, diagnoses that would likely lead to changes in clinical management). A total of 2088 diagnoses were made for 118 clinically actionable disorders; of these, it was estimated that 94% would likely have been missed by routine karyotyping. Examples of clinically actionable responses to the diagnoses included an electrocardiogram and cardiology referral for those at risk for long QT syndrome, glucose monitoring and endocrine referral for those at increased risk of diabetes, renal ultrasound for those at risk for renal pathology, and platelet count monitoring for those at risk for thrombocytopenia. A subset of cases was monitored for physician response to the microarray finding, and appropriate clinical action was taken more than 90% of the time.

Hayeems et al (2015) retrospectively reviewed subsequent diagnosis and management recommendations for 752 children with congenital anomalies and/or DD who underwent CMA testing at a single academic genetics practice. A sample of 752 patients was constructed from a database of patients who had CMA testing
done and had available clinical records, including all 457 variant cases and a sample of 295 benign cases in a 2.5:1 ratio. Medical recommendations were made following CMA results in 79.8% of patients with reportable results and in 62.0% of those with benign results, most frequently for specialist consultation (40.8%).

Smaller studies have also reported on clinical follow-up for patients who have undergone CMA testing. For example, Henderson et al (2014) reported that, among 38 patients with neurodevelopmental indications for CMA testing and abnormal findings, from a database of 1780 cases referred for CMA testing at a single laboratory over 3 years, 7.9% had cancer-related screening or surveillance recommended, and 36.8% had some additional clinical evaluation, most commonly specialist referral.

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.

In 2016, Lingen et al reported on the effect on parental quality of life of a diagnostic aCGH result in a child with unexplained DD/ID, with or without multiple congenital anomalies. The study included parents and children evaluated at an interdisciplinary pediatric clinic. A validated metric constructed for the assessment of quality of life in parents of chronically ill children was obtained for parents of 65 children with no chromosomal imbalance detected on aCGH and for 34 children with a clear genetic diagnosis on aCGH. The interval between aCGH result and questionnaire ranged from 1 to 4 years. Quality of life scores were 20.17 percentile rank scales higher in mothers of children with diagnostic vs inconclusive aCGH results (effect size, 0.71). Interpretation of these results is limited by the retrospective nature of the study and the potential for response bias.

**Reproductive Decision Making**

Risk estimates for recurrence of disease in future births can be altered considerably by information from the genetic diagnosis. For example, the average sibling recurrence risk in ASD is 5%. However, if the cause is a dominant single-gene disorder with full penetrance and a parent is a carrier, the sibling risk is 50%. If the disorder is recessive but characteristics are otherwise the same, the sibling risk is 25%. If the cause is fragile X, the recurrence risk in a brother is 50%, while a sister may be only mildly affected but will have a carrier risk of up to 50%. However, in the case of a de novo CNV (ie, not carried by either parent), the sibling risk remains low, at the population average.

Knowledge of recurrence risk is expected to lead to improved future reproductive decision making in families with children affected with DD/ID or ASD associated with specific variants. Turner et al (2008) studied the reproductive decisions of women from 38 families characterized by male members with and a pattern consistent with chromosome X-linked transmission. Most women in these families spent many years knowing that they were at some risk of being carriers.
and of having a boy with mental retardation. Before the availability of pathogenic variant analysis, the birth rate for these families was below average for their geographic area, 1 in 27 vs 1 in 11 per year, respectively. After pathogenic variant status was determined, both carriers and noncarriers (previously thought to be at risk) of the variant had children at the same rate, with 74% of carriers choosing prenatal genetic evaluation. While the results of this study are suggestive, they do not show that knowledge of recurrence risk directly affected reproductive decisions. Saam et al (2008), in a survey, reported that recurrence risk evaluation was possible in about one-third of families after positive aCGH results but did not study the impact of recurrence risk evaluation on reproductive planning.37

Wood et al (2015) analyzed reproductive stoppage and ASD recurrence rates within 2 U.K. family databases; the databases comprised 299 families including 660 children (327 diagnosed with ASD).38 In 10% of families, there was more than 1 ASD-affected child and an estimated 24.7% recurrence risk. Reproductive stoppage was examined by comparing statistically whether children with ASD were born later in families than their unaffected siblings. In 132 of the 180 complete families analyzed, the last-born child was more often affected (p<0.05); 40 families had a single child (affected) and 62 families 2 children with only the second affected. Any potential confounding by maternal or paternal age was not reported.

The 2015 TEC Special Report on the use of CMA for the genetic evaluation of patients with DD/ID and ASD made the following comments on the clinical utility of CMA testing in terms of impact on reproductive planning40:

“...[A] child with ASD appears to impact reproductive decision making, or so-called reproductive stoppage.” “Whether it can be attributed to concerns over having another affected child or the caregiving burden of the first affected child is unclear. Regardless, quantifying recurrence risk may assist reproductive decision making, particularly given that recurrence risk may be high—eg, in ASD, as high as 18%. However, establishing a genetic cause may revise the estimated risk considerably....”

**Section Summary: Chromosomal Microarray Testing**
The evidence for 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 yield of testing varies depending on 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 includes patients 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 and CMA testing.

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

Comparators
The relevant comparator of interest is usual care without genetic 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
The time frame for outcome measures varies from immediately following testing to identify diagnostic accuracy to long-term health outcomes subsequent to management changes.

Setting
Patients suspected of a genetic cause for their disability are typically seen in a tertiary care setting. Referral for genetic counseling is important for explanation of genetic disease, heritability, genetic risk, test performance, and possible outcomes.
**Analytic Validity**
Analytic validity is the technical accuracy of the test in detecting a variant that is present or excluding a variant that is absent. No peer-reviewed, full-length publications on the analytic validity of the commercially available NGS panels these disorders were identified.

**Clinical Validity**
Clinical validity is the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease. According to a laboratory’s website, this type of sequencing will pick up more than 97% of DNA variants at the level of a few base pairs; however, for most genes on the panel, the clinical sensitivity of the assay cannot be estimated individually because each gene is a rare cause of ASD.³⁹

In 2015, Grozeva et al reported on the prevalence of variants in 565 genes known or suspected to be involved in ID in 986 individuals with moderate-to-severe ID, using targeted NGS.⁴⁰ The patient cohort was a subset of a larger study of rare diseases, and comprised predominantly (93.8%) of male patients because the sample was originally created to evaluate the contribution of X-linked mutations to ID. Patients in the sample had previously had negative results by routine diagnostic approaches, including CMA testing at 500 kb resolution, and testing for fragile X and Prader-Willi or Angelman syndrome. The panel used consisted of 253 known and 312 candidate ID-associated genes. After manual curation, 107 (11%) individuals were able to receive a definitive diagnosis, including 77 (8%) with a loss of function variant and 30 (3%) with a causal missense variant.

In 2014, Redin et al reported on the yield of targeted high-throughput sequencing for 217 candidate genes in 106 patients with ID of unknown cause after evaluation with CMA and other genetic testing.⁴¹ Overall diagnostic yield was 25%, with 26 causative variants (16 X-linked, 10 de novo in autosomal-dominant genes). No false positives were detected in the 80 candidate variants located in the regions tested for confirmation by Sanger sequencing.

No studies identified reported on rates of VUS in NGS panels for patients with DD/ID, ASD, and/or congenital anomalies.

**Clinical Utility**
Clinical utility is how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes. No peer-reviewed, full-length publications on the clinical utility of the commercially available NGS panels for ASD or ID/DD were identified.

**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, particularly
regarding VUS rates. The yield of testing and likelihood of an uncertain result are variable, based on gene panel, gene tested, and patient population. There are real risks of uninterpretable and incidental results. Therefore, the 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 accuracy and validity, changes in reproductive decision making, morbid events, and resource utilization. The available evidence supports test accuracy and validity. Although systematic studies of the impact of CMA on patient outcomes are lacking, the improvement in diagnostic yield over karyotyping has been well-demonstrated. Direct evidence of improved outcomes with CMA compared with karyotyping is lacking. However, for at least a subset of the disorders potentially diagnosed with CMA 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 potentially impact future reproductive decision making for parents. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who have DD/ID, ASD, or multiple congenital anomalies not specific to a well-delineated genetic syndrome who receive next-generation sequencing panel testing, the evidence includes primarily case series. Relevant outcomes are test accuracy and validity, changes in reproductive decision making, morbid events, and resource utilization. The rates of variants of uncertain significance associated with next-generation sequencing panel testing in this previously described patient population are not well-characterized. The yield of testing and likelihood of an uncertain result is variable, based on gene panel, gene tested, and patient population; additionally, there are 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, clinical input was received from 2 physician specialty societies and 2 academic medical centers while this policy was under review in 2011. Clinical 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 resulted from 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, clinical input was received through 3 physician specialty societies and 2 academic medical centers while this policy was under review in early 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
In 2014, the American Academy of Pediatrics issued a clinical report on the optimal medical genetics evaluation of a child with or global developmental delays (GDD) or intellectual disability (ID). Regarding chromosomal microarray (CMA) testing, this report stated

“CMA now should be considered a first tier diagnostic test in all children with 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
In 2014, the American Academy of Child and Adolescent Psychiatry updated its guidelines on the assessment and treatment of children and adolescents with autism spectrum disorder (ASD). 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
In 2011, the American Academy of Neurology and the Child Neurology Society updated their guidelines on the evaluation of unexplained global developmental delay (DD) and ID with information on genetic and metabolic (biochemical) testing
to accommodate advances in the field.\textsuperscript{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) published guidelines on array-based technologies and their clinical utilization for detecting chromosomal abnormalities in 2010.\textsuperscript{43} CMA testing for copy number variants (CNVs) 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 software\textsuperscript{8} and for the interpretation and reporting of CNVs,\textsuperscript{16} both intended for the postnatal setting. A 2013 update included recommendations on the validation of microarray methodologies for both prenatal and postnatal specimens.\textsuperscript{44}

A 2013 guideline revisions from ACMG stated that a stepwise or tiered approach to the clinical genetic diagnostic evaluation of ASD is recommended, with the recommendation being for first tier to include fragile X syndrome and CMA, and second tier to include \textit{MECP2} and \textit{PTEN} testing.\textsuperscript{45} The guideline 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.”

**International Standard Cytogenomic Array Consortium**
The International Standard Cytogenomic Array Consortium published a consensus statement in which it recommended offering CMA testing as the first-tier genetic test, in place of G-banded karyotype, for patients with unexplained DD/ID, ASD, or multiple congenital anomalies (MCA). “Except in special cases, such as those involving family history of multiple miscarriages, a karyotype is not cost effective in a child with DD/ID, ASD, or MCA and a negative array study. CMA testing is not inexpensive, but the cost is less than the cost of a G-banded karyotype plus a
customized fluorescent in situ hybridization (FISH) test such as subtelomeric FISH, and the yield is greater.”

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 July 2017 did not identify any ongoing or unpublished trials that would likely influence this review.

References

**Billing Coding/Physician Documentation Information**

81228  Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number variants (eg, 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

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

Comparative genomic hybridization (CGH) microarray testing for developmental delay, autism spectrum disorder and/or intellectual disability.

ICD10 Codes

- **F84.0** Autistic disorder
- **F80.0-** Specific developmental disorders of speech and language; code range
- **F80.9**
- **F81.0-** Specific developmental disorders of scholastic skills; code range
- **F81.9**
- **F82** Specific developmental disorder of motor function
- **F88** Other disorders of psychological development
- **F89** Unspecified developmental disorder, unspecified
- **H93.25** Central auditory processing disorder
- **R48.0** Dyslexia and alexia
- **F70-** Intellectual disabilities; code range
- **F79**

Policy Implementation/Update Information

- **5/1/15** New Policy. Considered Investigational.
- **10/1/15** Policy statements changed that CMA may be considered medically necessary for apparent nonsyndromic developmental delay/intellectual disability, autism spectrum disorder, and multiple anomalies not specific to a well-delineated genetic syndrome. This includes removing Investigational and Not Medically Necessary Statements on this topic.
- **5/1/16** No policy statement changes.
- **10/1/17** The first medically necessary statement was revised to remove the “postnatal” term; A second statement was added that chromosomal microarray analysis is investigational for the evaluation of all other conditions of delayed development, including but not limited to idiopathic growth or language delay.
- **5/1/18** No policy statement changes.

Appendix Table 1. Categories of Genetic Testing Addressed in 2.04.59

<table>
<thead>
<tr>
<th>Category</th>
<th>Addressed</th>
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<tbody>
<tr>
<td>1. Testing of an affected individual’s germline to benefit the individual</td>
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<tr>
<td>1a. Diagnostic</td>
<td>X</td>
</tr>
<tr>
<td>1b. Prognostic</td>
<td></td>
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<tr>
<td>1c. Therapeutic</td>
<td></td>
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</tbody>
</table>
2. Testing cancer cells from an affected individual to benefit the individual
   2a. Diagnostic
   2b. Prognostic
   2c. Therapeutic

3. Testing an asymptomatic individual to determine future risk of disease

4. Testing of an affected individual’s germline to benefit family members

5. Reproductive testing
   5a. Carrier testing: preconception
   5b. Carrier testing: prenatal
   5c. In utero testing: aneuploidy
   5d. In utero testing: mutations
   5e. In utero testing: other
   5f. Preimplantation testing with in vitro fertilization

State and Federal mandates and health plan contract language, including specific provisions/exclusions, take precedence over Medical Policy and must be considered first in determining eligibility for coverage. The medical policies contained herein are for informational purposes. The medical policies do not constitute medical advice or medical care. Treating health care providers are independent contractors and are neither employees nor agents Blue KC and are solely responsible for diagnosis, treatment and medical advice. No part of this publication may be reproduced, stored in a retrieval system or transmitted, in any form or by any means, electronic, photocopying, or otherwise, without permission from Blue KC.