Invasive Prenatal (Fetal) Diagnostic Testing

Policy Number: 2.04.116  
Origination: 1/2015  
Last Review: 1/2018  
Next Review: 1/2019

Policy

Blue Cross and Blue Shield of Kansas City (Blue KC) will provide coverage for Invasive Prenatal (Fetal) Diagnostic Testing when it is determined to be medically necessary because the criteria shown below are met.

Note: Verify the member has maternity benefits prior to review for Medical Necessity. If no maternity benefit the testing is considered a contract exclusion.

When Policy Topic is covered

Chromosomal Microarray

In patients who are undergoing invasive diagnostic prenatal (fetal) testing, chromosome microarray (CMA) testing may be considered medically necessary, as an alternative to karyotyping (see Considerations).

Single-Gene Disorders

Invasive diagnostic prenatal (fetal) testing for molecular analysis for single-gene disorders may be considered medically necessary when a pregnancy has been identified as being at high risk:

1. For autosomal dominant conditions, at least 1 of the parents has a known pathogenic variant.
2. For autosomal recessive conditions:
   a. Both parents are suspected to be carriers or are known to be carriers, OR
   b. One parent is clinically affected and the other parent is suspected to be or is a known carrier.
3. For X-linked conditions: A parent is suspected to be or is a known carrier.

AND, ALL of the following are met:

a. The natural history of the disease is well understood, and there is a reasonable likelihood that the disease is one with high morbidity in the homozygous or compound heterozygous state, AND
b. The variants has high penetrance, AND
c. The genetic test has adequate sensitivity and specificity to guide clinical decision making and residual risk is understood, AND
An association of the marker with the disorder has been established.

**When Policy Topic is not covered**

**Single-Gene Disorders**
If the above criteria for molecular analysis for single-gene disorders are not met, invasive diagnostic prenatal (fetal) testing is considered **investigational**.

**Next-Generation Sequencing**
The use of next-generation sequencing in the setting of invasive prenatal testing is considered **investigational**.

**Considerations**

**Chromosomal Microarray**
The American College of Obstetricians and Gynecologists have recommended chromosome microarray (CMA) testing be performed in patients who are undergoing invasive prenatal diagnostic testing and that if:

- Prenatal CMA analysis is recommended for a patient with a fetus with 1 or more major structural abnormalities identified on ultrasonographic examination and who is undergoing invasive prenatal diagnosis. This test typically can replace the need for fetal karyotype.
- In a patient with a structurally normal fetus who is undergoing invasive prenatal diagnostic testing, either fetal karyotyping or CMA analysis can be performed.

**Fetal Structural Malformations**
Fetal malformations identified by ultrasound, characterized as major or minor malformations, whether isolated or multiple, may be part of a genetic syndrome, despite a normal fetal karyotype.

Major malformations are structural defects that have a significant effect on function or social acceptability. They may be lethal or associated with possible survival with severe or moderate immediate or long-term morbidity. Examples by organ system include: genitourinary: renal agenesis (unilateral or bilateral), hypoplastic/cystic kidney; cardiovascular: complex heart malformations; musculoskeletal: osteochondrodysplasia/osteogenesis imperfecta, clubfoot, craniosynostosis; CNS: anencephaly, hydrocephalus, myelomeningocele; facial clefts; body wall: omphalocele/gastroschisis; respiratory: cystic adenomatoid lung malformation.

**Single-Gene Disorders**
An individual may be suspected of being a carrier if there is a family history of or ethnic predilection for a disease. Carrier screening is not recommended if the carrier rate is less than 1% in the general population.

In most cases, before a prenatal diagnosis using molecular genetic testing can be offered, the family-specific mutation must be identified, either in an affected
relative or carrier parent(s). Therefore, panel testing in this setting would not be considered appropriate.

In some cases, the father may not be available for testing, and the risk assessment to the fetus will need to be estimated without knowing the father’s genetic status.

**CPT Coding**
The following CPT codes might be used for chromosomal microarray testing:
- 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: interrogation of genomic regions for copy number and single nucleotide polymorphism (SNV) variants for chromosomal abnormalities

**Genetics Nomenclature Update**
Human Genome Variation Society (HGVS) nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). HGVS nomenclature is recommended by HGVS, the Human Variome Project, and the HUman Genome Organization (HUGO).

The American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) standards and guidelines for interpretation of sequence variants represent expert opinion from ACMG, AMP, and 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>
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</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>Disease-associated variant</td>
<td>Disease-associated change in the DNA sequence</td>
</tr>
<tr>
<td></td>
<td>Disease-associated variant</td>
<td>Change in the DNA sequence</td>
</tr>
<tr>
<td></td>
<td>Familial variant</td>
<td>Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives</td>
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</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>
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</table>
ACMG: 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.

The following CPT codes might be used for chromosomal microarray testing:

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: interrogation of genomic regions for copy number and single nucleotide polymorphism (SNV) variants for chromosomal abnormalities.

CPT code 81405 includes:

Cytogenomic constitutional targeted microarray analysis of chromosome 22q13 by interrogation of genomic regions for copy number and single nucleotide polymorphism (SNV) variants for chromosomal abnormalities.

There is also a CPT code for a genomic sequencing procedure panel (ie, next-generation sequencing) for X-linked intellectual disability:

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.

**Description of Procedure or Service**

<table>
<thead>
<tr>
<th>Population</th>
<th>Intervention</th>
<th>Comparator</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals: Who are undergoing invasive prenatal (fetal) testing</td>
<td>Interventions of interest are: Chromosomal microarray analysis</td>
<td>Comparators of interest are: Karyotyping</td>
<td>Relevant outcomes include: Test accuracy, Test validity, Changes in reproductive decision making</td>
</tr>
<tr>
<td>Individuals: Who are undergoing invasive prenatal (fetal) testing</td>
<td>Interventions of interest are: Molecular testing for single-gene disorders</td>
<td>Comparators of interest are: No molecular testing</td>
<td>Relevant outcomes include: Test accuracy, Test validity, Changes in reproductive decision making</td>
</tr>
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</table>
Invasive prenatal (fetal) diagnostic testing may be used to identify pathogenic genetic alterations in fetuses at increased risk based on prenatal screening or in women who choose to undergo diagnostic testing due to other risk factors. This evidence review only addresses the use of chromosomal microarray testing, molecular diagnosis of single-gene disorders, and next-generation sequencing.

For individuals who are undergoing invasive diagnostic prenatal (fetal) testing who receive chromosomal microarray (CMA) analysis, the evidence includes a systematic review and meta-analysis and prospective cohort and retrospective analyses comparing the diagnostic yield of CMA testing with that of karyotyping. Relevant outcomes are test accuracy, test validity, and changes in reproductive decision making. CMA testing has a higher detection rate of pathogenic chromosomal alterations than karyotyping. CMA testing can yield results that have uncertain clinical significance; however, such results can be minimized by the use of targeted arrays, testing phenotypically normal parents for the copy number variant (CNV), and the continued accumulation of pathogenic variants in international databases. The highest yield of pathogenic CNVs by CMA testing has been found in fetuses with malformations identified by ultrasound. Changes in reproductive decision making could include decisions on continuation of a pregnancy, enabling timely treatment of a condition that could be treated medically or surgically either in utero or immediately after birth, and birthing decisions. The American College of Obstetricians and Gynecologists has recommended CMA testing in women who are undergoing an invasive diagnostic procedure. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are undergoing invasive diagnostic prenatal (fetal) testing who receive molecular testing for single-gene disorders, the evidence includes case series that may report disorders detected and test validity. Relevant outcomes are accuracy, test validity, and changes in reproductive decision making. The analytic validity in the diagnosis of single-gene disorders depends on the individual variant tested. In general, it is necessary to identify the particular variant(s) in the affected parent(s) so that the particular variant(s) can be sought for prenatal diagnosis. When a familial variant is known, the analytic validity of testing for this variant is expected to be high, approaching 100% accuracy. For clinical validity, when there is a known pathogenic familial variant, the sensitivity and specificity of testing for the variant in other family members is expected to be very high. Changes in reproductive decision making could include decisions on continuation of the pregnancy, facilitating timely treatment of a condition medically or surgically either in utero or immediately after birth, decisions concerning place of delivery (ie, tertiary care center), and route of delivery. The evidence is sufficient to
determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are undergoing invasive diagnostic prenatal (fetal) testing who receive next-generation sequencing (NGS), the evidence is lacking. Relevant outcomes are test accuracy, test validity, and changes in reproductive decision making. There are concerns about interpretation of data generated by NGS and the data’s clinical relevance. The analytic and clinical validity of NGS in the prenatal setting are unknown. The evidence is insufficient to determine the effects of the technology on health outcomes.

**Background**

**Prenatal Genetic Testing Methodologies**

The focus of this evidence review is the use of certain invasive prenatal genetic testing methodologies in the prenatal (fetal) setting to provide a framework for evaluating the clinical utility of diagnosing monogenic disorders in this setting. The purpose of prenatal genetic testing is to identify conditions that might affect the fetus, newborn, or mother to inform pregnancy management—eg, prenatal treatment, decisions about delivery location and personnel, or pregnancy termination.

Invasive fetal diagnostic testing can include obtaining fetal tissue for karyotyping, fluorescence in situ hybridization (FISH), CMA testing, quantitative polymerase chain reaction (qPCR), next-generation sequencing (NGS), and multiplex ligation-dependent probe amplification (MLPA).

This policy will only address the following:

- the diagnosis of copy number variants using CMA technology
- the diagnosis of single-gene disorders, most of which are due to point mutations or very small deletions and use molecular methods to diagnose (mainly PCR, but also MLPA)
- NGS

This policy applies only if there is not a separate Medical Policy Reference Manual (MPRM) policy that outlines specific criteria for diagnostic testing. If a separate MPRM policy does exist, then the criteria for medical necessity in that policy supersede the guidelines in this policy. This policy does NOT cover the use of

- prenatal carrier testing
- preimplantation genetic diagnosis or screening
- noninvasive prenatal testing
- testing in the setting of fetal demise

Genetic disorders are generally categorized into 3 main groups: chromosomal, single gene, and multifactorial. Single-gene disorders (also known as monogenic) result from errors in a specific gene, whereas those that are chromosomal include larger aberrations that are numerical or structural.
Invasive prenatal testing refers to the direct testing of fetal tissue, typically by chorionic villus sampling (CVS) or amniocentesis. Invasive prenatal procedures are typically performed in pregnancies of women who have been identified as having a fetus at increased risk for a chromosomal abnormality, or if there is a family history of a single-gene disorder.

**Chromosomal Microarray**

Chromosomal microarray (CMA) technology has several advantages over karyotyping, including improved resolution (detection of smaller chromosomal variants that are undetectable using standard karyotyping) and therefore can result in potentially higher rates of detection of pathogenic chromosomal abnormalities. However, there are disadvantages to CMA, including the detection of variants of unknown clinical significance and the fact that it cannot detect certain types of chromosomal abnormalities, including balanced rearrangements.

CMA analyzes abnormalities at the chromosomal level and measures gains and losses of DNA (known as CNVs) throughout the genome. CMA analysis detects CNVs by comparing a reference genomic sequence (“normal”) with the corresponding patient sequence. Each sample has a different fluorescent label so that they can be distinguished, and both are cohybridized to a sample of a specific reference (also normal) DNA fragment of known genomic locus. If the patient sequence is missing part of the normal sequence (deletion) or has the normal sequence plus additional genomic material within that genomic location (e.g., a duplication of the same sequence), the sequence imbalance is detected as a difference in fluorescence intensity. For this reason, standard CMA (non-SNVs, see the following) cannot detect balanced CNVs (equal exchange of material between chromosomes) or sequence inversions (same sequence is present in reverse base pair order) because the fluorescence intensity would not change.

CMA analysis uses thousands of cloned or synthesized DNA fragments of known genomic locus immobilized on a glass slide (microarray) to conduct thousands of comparative reactions at the same time. The prepared sample and control DNA are hybridized to the fragments on the slide, and CNVs are determined by computer analysis of the array patterns and intensities of the hybridization signals. Array resolution is limited only by the average size of the fragment used and by the chromosomal distance between loci represented by the reference DNA fragments on the slide. High resolution oligonucleotide arrays are capable of detecting changes at a resolution of up to 50 to 100 Kb.

**Types of CMA Technologies**

There are some differences in CMA technology, most notably in the various types of microarrays. They can differ first by construction; earliest versions were used of DNA fragments cloned from BAC. These have been largely replaced by oligonucleotide (oligos; short, synthesized DNA) arrays, which offer better reproducibility. Finally, arrays that detect hundreds of thousands of SNVs across the genome have some advantages as well. A SNV is a DNA variation in which a single nucleotide in the genomic sequence is altered. This variation can occur between two different individuals or between paired chromosomes from the same
individual and may or may not cause disease. Oligo/SNV hybrid arrays have been constructed to merge the advantages of each.

The two types of microarrays both detect CNVs, but they identify different types of genetic variation. The oligo arrays detect CNVs for relatively large deletions or duplications, including whole chromosome duplications (trisomies), but cannot detect triploidy. SNV arrays provide a genome-wide copy number analysis, and can detect consanguinity, as well as triploidy and uniparental disomy.

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

At this time, no guidelines exist as to whether targeted or genome-wide arrays should be used, or what regions of the genome should be covered. Both targeted and genome-wide arrays search the entire genome for CNVs, however, targeted arrays are designed to cover only clinically significant areas of the genome. The ACMG guideline for designing microarrays recommends probe enrichment in clinically significant areas of the genome to maximize detection of known abnormalities. Depending on the laboratory that develops a targeted array, it can include as many or as few microdeletions and microduplication syndromes as thought to be needed. The advantage, and purpose, of targeted arrays is to minimize the number of variants of unknown significance (VUS).

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 relevance.

Clinical Relevance of CMA Findings and Variants of Unknown Significance

CNVs are generally classified as pathogenic (known to be disease-causing), benign or a VUS.

A VUS is defined as a CNV that:

- has not been previously identified in a laboratory’s patient population, or
- has not been reported in the medical literature, or
- is not found in publicly available databases, or
- does not involve any known disease-causing genes.
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, MLPA, polymerase chain reaction [PCR]).
- 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.
- A pathogenic etiology is additionally supported when a CNV includes a gene known to cause the phenotype when inactivated (microdeletion) or overexpressed (microduplication).
- The laboratory may establish a size cutoff; potentially pathogenic CNVs are likely to be larger than benign polymorphic CNVs; cutoffs for CNVs not previously reported typically range from 300 kb to 1 Mb.
- Parental studies are indicated when CNVs of appropriate size are detected and not found in available databases; CNVs inherited from a clinically normal parent are assumed to be benign polymorphisms whereas those appearing de novo are likely pathogenic; etiology may become more certain as other similar cases accrue.

In 2008, the International Standards for Cytogenomic Arrays (ISCA) Consortium was organized; it established a public database containing deidentified whole genome microarray data from a subset of the ISCA Consortium member clinical diagnostic laboratories. Array analysis was carried out on subjects with phenotypes including intellectual disability, autism, and developmental delay. As of June 2016, there were over 53,900 total cases in the database. Data are currently hosted on ClinGen.¹

Use of the database includes an intralaboratory curation process, whereby laboratories are alerted to any inconsistencies among their own reported CNVs or other mutations, as well as any not consistent with the ISCA “known” pathogenic and “known” benign lists. The intralaboratory conflict rate was initially about 3% overall; following release of the first ISCA curated track, the intralaboratory conflict rate decreased to about 1.5%. A planned interlaboratory curation process, whereby a group of experts curates reported CNVs/mutations across laboratories, is currently in progress.

The consortium 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 defines levels of evidence (from the literature and/or ISCA and other public databases) that describe how well or how poorly detected variants or CNVs correlate with phenotype.

ISCA is also developing vendor-neutral recommendations for standards for the design, resolution, and content of cytogenomic arrays using an evidence-based approach.
process and an international panel of experts in clinical genetics, clinical laboratory genetics, genomics, and bioinformatics.

**Single-Gene (Mendelian) Disorders**
Single-gene (Mendelian) disorders include those with an inheritance mode of autosomal dominant or recessive, X-linked dominant or recessive. Women may be identified as being at increased risk for having a fetus with an inherited genetic condition because of previously affected pregnancies, a family history in a suggestive pattern of inheritance, or being a member of a subpopulation with elevated frequencies of certain autosomal recessive conditions.

Most Mendelian disorders are caused by SNVs or very small deletions or duplications. Monogenic variants are diagnosed by molecular methods, mainly PCR for SNVs, but also other methods like MLPA for very small deletions and duplications. There are approximately 5000 known disorders that are inherited in this fashion. Diagnostic tests are currently available for most of the common monogenic disorders, as well as for a number of the more rare disorders. For most single-gene disorders, testing in the prenatal setting requires knowledge of the familial variants.

**Next-Generation Sequencing**
NGS has been used to identify pathogenic variants in disease-associated genes in many Mendelian disorders. Approximately 85% of known disease-causing variants occur within the 1% of the genome that encodes for proteins (exome). Therefore, whole exome sequencing can cost-effectively capture the majority of protein-coding regions. However, there remain concerns about technical complexity, coverage, bioinformatics, interpretation, VUSs, as well as ethical issues.²

**Commercially Available Tests**
Many academic and commercial laboratories offer CMA testing and testing for single-gene disorders. The following is not inclusive and is only an example of some laboratories that offer CMA testing. The test should be cleared or approved by FDA, or performed in a Clinical Laboratory Improvement Amendment (CLIA)–certified laboratory.

**Definitions**

**Amniocentesis**
A test that removes a small amount of fluid that surrounds the fetus and can be used for genetic testing of the fetus or the measurement of certain biochemical markers. Traditional amniocentesis is usually performed between weeks 15 and 20 of gestation.

**Aneuploidy**
A chromosomal abnormality in which the number of chromosomes is abnormal, either having more or less than the normal 46 chromosomes (44 autosomal, 2 sex chromosomes).
**Autosomal**
Any chromosome other than the sex-chromosomes (X and Y).

**Chorionic Villus Sampling**
CVS is generally performed after 9 weeks of gestation. It involves obtaining chorionic villi through transcervical or transabdominal access to the placenta. (Chorionic villi are of fetal origin, and are vascular processes that emerge from the outer sac that surrounds the developing fetus and provide for exchange between the fetal and maternal circulation).

**Chromosomal Inversion**
A chromosome inversion occurs when 2 breaks occur in the same chromosome and the intervening genetic material is inverted before the breaks are repaired. Even though no genetic material is lost or duplicated, and the person may not show abnormalities at the phenotypic level, gene function may be altered by the rearrangement, and carriers of inversions may have children with abnormalities.

**Chromosomal Translocation/Rearrangement**
A chromosomal translocation refers to an abnormal rearrangement of chromosomes. There are 2 main types: a reciprocal translocation, which occurs when 2 fragments break off from 2 different chromosomes, and they change places; and a Robertsonian translocation, in which 1 chromosome becomes attached to another. Approximately 1 in 500 people have a translocation. In reciprocal and Robertsonian translocations, no chromosome material is gained or lost (which is called a balanced translocation). Most people who carry a balanced translocation are phenotypically normal, but they are at risk of having a child with an unbalanced translocation. With an unbalanced translocation, there is either an extra piece of 1 chromosome and/or a missing piece of another chromosome, which can lead to a child with learning disabilities, developmental delay, and health problems.

**Cytogenetics**
The study of chromosomes.

**Imprinted Genes**
Usually, both copies of each gene (1 copy of each gene inherited from each parent) are active. Sometimes, only 1 copy is active, which depends on parent of origin; this is what is referred to as genomic imprinting. In genes that undergo genomic imprinting, certain segments of DNA undergo methylation. Imprinted genes tend to cluster in the same regions of chromosomes. Two major clusters of imprinted genes have been identified on chromosomes 11 and 15. Prader-Willi and Angelman syndrome are caused by UPD or other errors in imprinting involving genes on chromosome 15. Beckwith-Wiedemann syndrome is associated with abnormalities of imprinted genes on chromosome 11.

**Karyotyping**
A test that examines chromosomes in a sample of cells (ie, from amniotic fluid and CVS), and can count the number of chromosomes and look for large structural
changes in chromosomes. A regular human cell has 46 chromosomes, 44 autosomes, and 2 sex chromosomes which specify gender (XX=female, XY=male).

**Structural Chromosome Abnormality**
There is a normal number of chromosomes (46), however, a segment(s) of chromosome(s) are missing (deleted), extra (inserted), or rearranged (translocated or inverted).

**Subtelomeric Rearrangements**
Subtelomeric regions (present on most chromosomes) are prone to rearrangements that have been suggested to represent a high proportion of abnormalities in individuals with idiopathic intellectual disability.

**Triploidy**
A chromosome number of 69 (3 copies of each chromosome).

**Trisomy**
The presence of an extra chromosome (eg, trisomies 13, 18, 21 [Down syndrome]).

**Uniparental Disomy**
Normally, for each of the 23 pairs of chromosomes, 1 is inherited from the mother and the other from the father. UPD is an abnormal situation in which both chromosomes in a pair are inherited from 1 parent, and the other parent’s chromosome from that pair is missing. UPD for most chromosomes is without consequence, but for some chromosomes, it can result in a genetic disorder. The most well-known conditions that result from UPD include Prader-Willi syndrome and Angelman syndrome.

**REGULATORY STATUS**
Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, FDA has chosen not to require any regulatory review of this test.

**Rationale**
This evidence review was originally created in October 2014 and has been updated regularly with searches of the MEDLINE database. The most recent literature review was performed through February 23, 2017. This review is based on the published literature, the general principles of prenatal (fetal) testing, and accepted practice guidelines (see Appendix Table 1 for genetic testing categories).

There are many ethical considerations in testing a fetus for a condition that is of adult-onset. In general, there is consensus in the medical and bioethics communities that prenatal testing should not include testing for late- or adult-onset conditions, or for diseases for which there is a known intervention that
would lead to improved health outcomes, but would only need to be started after the onset of adulthood.

Ideally, peer-reviewed literature on the performance and indications for the test should be available. The evaluation of a genetic test focuses on 3 main principles: (1) analytic validity (technical accuracy of a test in detecting a variant that is present or in excluding a variant that is absent); (2) clinical validity (diagnostic performance of a test [sensitivity, specificity, positive and negative predictive values] in detecting clinical disease); and (3) clinical utility (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).

**Chromosomal microarray Testing**

**Clinical Context and Test Purpose**
The purpose of chromosomal microarray (CMA) testing (copy number variants [CNVs]) in patients who are undergoing invasive prenatal testing is to inform reproductive decisions.

The question addressed in this evidence review is: What is the analytic validity, clinical validity, and clinical utility of CMA testing of invasively obtained fetal samples?

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

**Patients**
The relevant population of interest is women undergoing invasive prenatal testing.

**Interventions**
The relevant intervention of interest is testing by CMA.

**Comparators**
The relevant comparator of interest is karyotyping.

**Outcomes**
The primary outcomes are test accuracy and test validity (ie, diagnostic yield); an accurate result will inform reproductive decision making. The premise of obtaining a test is that a woman or couple desires a result for the purposes of pregnancy decisions. Clinical management decisions may include continuation of the pregnancy, enabling timely treatment of a condition that could be treated medically or surgically either in utero or immediately after birth, and birthing decisions.

**Timing**
Prenatal (fetal) testing may be performed for the purpose of anticipatory guidance and management, either during pregnancy or at the time of delivery.
Setting
The relevant setting of interest is an obstetrics practice.

Analytic Validity
Analytic validity is the ability of a test to accurately and reliably measure the marker of interest. Measures of analytic validity include sensitivity (detection rate), specificity (1 – false-positive rate), reliability (repeatability of test results), and assay robustness (resistance to small changes in preanalytic or analytic variables).

In general, acceptable analytic validity is generally assumed for CMA testing in laboratories meeting quality standards under the Clinical Laboratory Improvement Amendments (CLIA), including participation in proficiency testing.

Clinical Validity
Most of the literature on CMA testing in the prenatal (fetal) setting consists of prospective and retrospective analyses of CMA findings compared to conventional karyotyping, either in patients with known karyotype results or in patients with concurrently performed karyotyping and CMA. CMA analysis has been proposed as being used as either a first tier test (in place of or in conjunction with karyotype) or as a second tier test (after a negative karyotype).

Jansen et al (2015) conducted a systematic review and meta-analysis of the additional diagnostic gain of array comparative genomic hybridization (aCGH) compared with standard karyotyping and 22q11 microdeletion ascertainment by fluorescence in situ hybridization (FISH) in prenatally diagnosed cardiac malformations. Thirteen studies with 1131 cases of congenital heart disease (CHD) were included from a literature search through September 2014. A meta-analysis identified an incremental yield of 7.0% (95% confidence interval [CI], 5.3% to 8.6%) for the detection of CNVs using aCGH, excluding aneuploidy and 22q11 microdeletion cases. A subgroup analysis showed a 3.4% (95% CI, 0.3% to 6.6%) incremental yield in isolated CHD cases, and 9.3% (95% CI, 6.6% to 12%) when extracardiac malformations were present. Overall, an incremental yield of 12% (95% CI, 7.6% to 16%) was found when 22q11 deletion cases were included. The rate of variants of uncertain significance (VUS) was 3.4% (95% CI, 2.1% to 4.6%).

Armengol et al (2012) conducted a comparative study of available technologies, including karyotyping and CMA, for detection of chromosomal abnormalities after invasive prenatal sampling. Multiple testing techniques were performed on the same sample. The study included 900 women with the main indications for testing being abnormal ultrasound findings, altered biochemical screening, family history of a chromosomal disorder or other genetic condition, and advanced maternal age (AMA). A total of 57 (6.3%) clinically relevant chromosomal aberrations were found, with CMA testing having the highest detection rate, 32% above other methods. Most VUSs could be classified as likely benign after proving they were inherited. Cross-validation was provided by the simultaneous use of multiple
techniques, and additional molecular techniques were performed in the follow-up of some of the alterations identified by CMA.

The reported diagnostic accuracy results for karyotyping and CMA were as follows:

- **Karyotyping:** sensitivity of 76.4% (95% CI, 63.0% to 87.0%); specificity of 99.9% (99.2% to 99.9%); positive predictive value (PPV) of 97.7% (87.7% to 99.9%); negative predictive value (NPV) of 98.3% (97.1% to 99.1%); and diagnostic accuracy of 98.2% (97.1% to 99.0%).

- **CMA analysis:** sensitivity of 98.2% (90.4% to 99.9%); specificity of 99.7% (99.1% to 99.9%); PPV of 96.5% (87.9% to 99.5%); NPV of 99.9% (99.3% to 100%); and diagnostic accuracy of 99.7% (99.0% to 99.9%).

Shaffer et al (2012) reported on the results of microarray testing for prenatal diagnosis in over 5000 prospectively collected prenatal samples received from 2004 to 2011 for a variety of indications. They used aCGH microarrays targeted to known chromosomal syndromes, with later versions providing backbone coverage of the entire genome. Cases were stratified by test result (normal, VUS, abnormal) and indication for the study, and compared with karyotyping results. Of 5003 prenatal specimens, 56% were referred with normal karyotypes, 13% had known abnormal karyotypes, 16% had karyotypes performed concurrently with microarray testing, and 15% had unknown karyotype status. Indications for microarray testing included a known abnormal karyotype (n=648), family history of a parent known to carry a chromosomal rearrangement or imbalance (n=62), fetal demise (n=417), abnormal ultrasound (n=2858) (detailed in another study by the same group), abnormal first- or second-trimester screen (n=77), other family history of a genetic condition (n=487), AMA (n=346), parental anxiety (n=95), or other/not specified (n=13). The overall detection rate of clinically significant results with microarray testing was 5.3%. The detection rate of clinically significant CNVs was 5.5% among cases with known normal karyotypes. After excluding the cases of fetal demise, the VUS rate was 4.2%, but if only de novo CNVs were considered (the rate was 0.39%).

In the other study, Shaffer et al (2012) retrospectively analyzed 2858 pregnancies with abnormal ultrasound findings (as stratified by organ system). Most cases had previously normal karyotypes (n=2052 [72%]). The remaining had karyotyping performed concurrently with microarray testing (n=465 [16%]) or had unknown or failed karyotypes (n=341 [12%]). Ultrasound anomalies were categorized in several ways: multiple structural anomalies, structural anomalies involving a single-organ system, isolated abnormalities of growth, isolated abnormal amniotic fluid volume, single or multiple soft marker(s), or multiple nonstructural anomalies (eg, intrauterine growth restriction). Soft markers included choroid plexus cysts, echogenic foci in the heart or bowel, isolated short long bones, absent nasal bones, sandal gap between the first and second toes, fifth finger clinodactyly, single umbilical artery, and persistent right umbilical vein. The average maternal age at the time of testing was 31.8 years. Most tests were whole genome, oligoarrays (n=2161 [76%]), and the remaining were bacterial artificial chromosome–based arrays, either with coverage of the whole genome (n=506
Overall, with microarray testing, 6.5% showed clinically significant results, and 4.8% had VUS. For the cases with a previously normal karyotype, the detection rate for significant CNVs was similar (6.2%). Clinically significant genomic alterations were identified in cases with a single ultrasound anomaly (n=99/1773 [5.6%]), anomalies in 2 or more organ systems (n=77/808 [9.5%]), isolated growth abnormalities (n=2/76 [2.6%]), and soft markers (n=2/77 [2.6%]). Certain anomalies, either in isolation or with additional anomalies, had higher detection rates: holoprosencephaly (n=9/85 [10.6%]), posterior fossa defects (n=21/144 [14.6%]), skeletal anomalies (n=15/140 [10.7%]), ventricular septal defect (n=14/132 [10.6%]), hypoplastic left heart (n=11/68 [16.2%]), and cleft lip/palate (n=14/136 [10.3%]).

Hillman et al (2013) conducted a prospective cohort study and systematic review. The cohort study involved 243 women undergoing CMA testing and karyotyping for a structural abnormality detected on prenatal ultrasound. There was an excess detection rate of abnormalities by CMA of 4.1% over conventional karyotyping, with a VUS rate of 2.1% (95% CI, 1.3% to 3.3%). Meta-analysis included studies through December 2012 that reported on prenatal microarray testing performed for any indication and was not limited to cases referred for abnormal fetal ultrasound findings. Twenty-five studies were included, with a collective number of 18,113 samples analyzed. The detection rate in the meta-analysis was 10% (95% CI, 8% to 13%). The VUS rate was 1.4% (95% CI, 0.5% to 3.7%) when any indication for prenatal CMA testing was meta-analyzed and 2.1% (95% CI, 1.3 to 3.3) when the indication for the CMA testing was an abnormal ultrasound finding.

Wapner et al (2014) conducted a prospective study to evaluate the accuracy, efficacy, and incremental yield of CMA testing compared with karyotyping for routine prenatal diagnosis. A total of 4406 women undergoing routine prenatal diagnosis in 1 of 29 diagnostic centers by either chorionic villus sampling (CVS) or amniocentesis had a sample split in 2 for standard karyotyping and CMA analysis. Indications for prenatal diagnosis included AMA (46.6%), a positive aneuploidy screening result (18.8%), structural anomalies detected by ultrasound (25.2%), and other indications (9.4%). CMA analysis was successful in 98.8% of the fetal samples. The primary analysis classified microarray results as being true positive, true negative, false positive, or false negative relative to the findings by karyotyping. Secondary outcomes included the occurrence and classification of CNVs identified by microarray in the presence of a normal karyotype and the ability of CMA testing to identify uncommon cytogenetic abnormalities found on karyotyping. Two array platforms were used, 1 covering targeted regions of known disease association and 1 genome-wide single-nucleotide variant (SNV) assay. The data for the second platform were masked by the analysis software to emulate the same resolution and coverage as the first platform; therefore, review of the SNVs was not performed. Microarray analysis of DNA from maternal and paternal blood samples was used to determine whether CNVs detected in fetal samples were inherited. All de novo array findings seen in samples with a normal karyotype were confirmed by a second method, preferentially FISH. Deletions and duplications identified exclusively by means of microarray analysis were classified as “pathogenic” when they encompassed a region implicated in a well-described
abnormal phenotype, and all other deletions and duplications were classified as being of “uncertain clinical significance.” A total of 4282 samples were included in the primary analysis. Of these, common autosomal aneuploidies were identified in 317 (7.4%) and sex chromosome aneuploidies were identified in 57 (1.3%) by standard karyotyping. CMA testing identified all of these aneuploidies. None of the balanced rearrangements identified on karyotyping was identified with CMA, nor did CMA identify any of the triploid samples (0.4%).

Of the 3822 cases with a normal karyotype, on microarray, 1399 samples were identified as having CNVs; of these, 88.2% were classified as common benign and 0.9% were on the predetermined list of pathogenic CNVs. The cases of uncertain clinical significance were adjudicated by a clinical advisory committee, which reclassified them as likely to be benign (1.8% of all 1399 samples) or of potential clinical significance (1.6% of all 1399 samples). Overall, 96 (2.5%; 95% CI, 2.1% to 3.1%) of the 3822 fetal samples with normal karyotypes had a microdeletion or duplication of clinical significance.

In subgroup analysis of 755 women with normal karyotypes and fetuses with suspected growth or structural anomalies, 45 (6.0%; 95% CI, 4.5% to 7.9%) had clinically relevant findings on microarray. These included CNVs that were predetermined as known pathogenic, as well as those classified by the clinical advisory committee as clinically relevant. In this population with structural abnormalities identified on ultrasound, CNVs of uncertain clinical significance, but likely benign, were found in 16 patients (2.1%). Of the women tested for AMA, 1.7% (95% CI, 1.2 to 2.4) had a clinically relevant finding on microarray, as did 1.6% (95% CI, 0.9 to 2.9) of women who tested positive on Down syndrome screening. Recurrent CNVs associated with autism and neurocognitive alterations were detected in 1.3% of karyotypically normal pregnancies—3.6% with and 0.8% without structural anomalies.

In summary, the Wapner study included 3822 patients with normal karyotype and the following indications for prenatal diagnosis: AMA (n=1966), positive Down syndrome screen (n=729), anomaly on ultrasound (n=755), and other (n=372). CMA testing provided additional clinically relevant CNVs and VUS rate of:

- AMA: 1.7% (95% CI, 1.2 to 2.4) and 1.9%
- Positive Down screen: 1.6% (95% CI, 0.9 to 2.9) and 1.8%
- Ultrasound anomaly: 6.0% (95% CI, 4.5 to 7.9) and 2.1%

Breman et al (2012) evaluated the prenatal CMA results of more than 1000 fetal samples sent for testing at the medical genetics laboratories of an academic institution between 2005 and 2011. A total of 1124 specimens were received, of which reportable results were obtained in 1115. Maternal blood samples were required with every fetal sample (and paternal if possible) to exclude maternal cell contamination and to assist with interpretation of CNVs. CMA testing was performed on DNA extracted from amniotic fluid, CVS, or cultured cells (amniocytes/CVS) in most cases. The gestational ages for direct amniotic fluid samples ranged from 14 to 36 weeks; samples from pregnancies more than 16
weeks of gestation provided the most optimal DNA yield. Samples were submitted for either standard cytogenetic studies (karyotype with or without aneuploidy FISH) plus CMA, or for CMA only with a karyotype analysis having been performed elsewhere. For those samples, unless only DNA was submitted, a culture was established in this university’s laboratories so that any CMA findings could be confirmed by an independent method (FISH, karyotype, or other).

The most common clinical indications were abnormal ultrasound findings (n=410) and AMA (n=394). Other indications included a previous child with or a family history of a genetic disorder or chromosome abnormality (n=137), further workup of a known chromosomal abnormality detected by karyotype or FISH (n=61), parental concern (n=61), an abnormal maternal serum screen (n=37), and other or unclassified (n=4). Twelve cases had no indication provided. The cases spanned 5 years, over which time different types of targeted clinical arrays were used, with progressively increasing complexity and sensitivity. Targeted bacterial artificial chromosomes were used for 282 samples, and all others with targeted oligonucleotide arrays.

In 881 (79%) of the 1115 samples, no deletions or duplications were observed using prenatal CMA analysis. Copy number changes were detected in 234 (21%) cases. Of these, 131 (11.7%) were classified as likely benign. Eighty-five (7.6%) cases were found to have clinically significant genomic imbalances. Twenty-seven microdeletion or microduplication findings (2.4% of total cases; 32% of abnormal cases) were small gains or losses below the resolution of prenatal karyotype analysis, and would not have been detected by conventional chromosome studies alone. Of these, family history was the indication for testing in 8 cases, an abnormal FISH result was the indication for 1 case, and the remaining 18 abnormal findings were unanticipated. Eighteen (1.6%) of the 1115 specimens had results of uncertain clinical significance. An additional 17 cases were found to have multiple inherited CNVs interpreted as likely benign familial variants. The indications yielding the greatest number of clinically significant findings by microarray analysis were abnormal karyotype/FISH (42.6%), a family history of chromosomal abnormality (9.5%), all abnormal prenatal ultrasound findings (9.3%), abnormal serum screening (5.4%), and AMA (1.3%).

In summary, the overall detection rate in the Breman study for clinically significant CNVs was 7.6%; the detection rate was 4.2% when the abnormal cases that had a previously identified chromosome abnormality or a known familial genomic imbalance were excluded. In 1.7% of the cases, abnormal results were obtained that were neither anticipated before microarray analysis nor detectable by conventional prenatal chromosome analysis. The clinical significance of the microarray results could not be determined in 1.7% of cases.

A 2014 review by Wapner et al summarized the existing literature of the largest studies that reported the estimates of detectable pathogenic CNVs according to indication for CMA testing. 

For studies that included all high-risk pregnancies (which were primarily because of abnormal ultrasound abnormalities), the range of pathogenic CNV detection was 2.6% to 7.8%, with a combination of all studies
Papoulidis et al (2015) compared the diagnostic yield of conventional karyotype with aCGH in 1763 prenatal samples. Samples of trophoblastic tissue (n=458) and amniotic fluid (n=1305) were examined. Pathogenic alterations were identified in 125 (7.1%) and a VUS in 13 (0.7%). The incremental diagnostic yield from aCGH was 0.9%. Incremental improvements were greatest when test indications were second trimester ultrasound markers (incremental improvement, 1.5%) or structural anomalies (1.3%), but lower with increased nuchal translucency (0.5%). The authors concluded: “The present study indicates that routine implementation of aCGH offers an incremental yield over conventional karyotype analysis, which is also present in cases with ‘milder’ indications, further supporting its use as a first-tier test.”

Lovrecic et al (2016) evaluated the clinical usefulness of prenatal CMA testing for small (submicroscopic) imbalances (CNV) in 218 fetuses across a range of indications for testing. In fetuses with ultrasound findings, diagnostic yield of CMA testing was 10% or 7.7% more than was obtained with karyotyping. Similar to other studies, diagnostic yield varied by indication for testing. For example, a pathogenic CNV rate was found in 6.3% of fetuses with intrauterine growth retardation and 16.7% of fetuses with multiple anomalies. The results support an increase in the diagnostic yield with CMA testing over conventional karyotyping.

Robson et al (2017) reported results of the U.K. Evaluation of Array Comparative genomic Hybridisation (EACH) study, a multicenter cohort study including an economic and qualitative substudy. Enrolled women underwent quantitative fluorescent polymerase chain reaction (QF-PCR) and conventional karyotyping after CVS (55.8%), amniocentesis (40.8%), or fetal blood sampling (2.7%). Testing indications included isolated nuchal translucency (≥3.5mm) or any structural anomaly detected on ultrasound at 11 to 14 weeks. Nine laboratories performed testing with an identical oligonucleotide-CGH array. Between March 2012 and May 2014, 1718 women were recruited and results from 1123 analyzed. Irrespective of indication for testing, results were observed as shown in Table 1.

<table>
<thead>
<tr>
<th>Karotype</th>
<th>CMA Testing</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>–</td>
<td>15 (1.3%)</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>58 (5.2%)</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>42 (3.7%)</td>
</tr>
<tr>
<td>–</td>
<td>VUS</td>
<td>38 (3.4%)</td>
</tr>
</tbody>
</table>

CMA: chromosomal microarray; + pathogenic alteration; – benign alteration; VUS: variant of uncertain significance.

Similar to other studies, results varied according to indication for testing. The authors concluded: “The results suggest that CMA is a robust, acceptable and probably cost-effective diagnostic test and should replace karyotyping in care
pathways when the indication for fetal testing is one or more structural anomalies or an isolated NT [nuchal translucency] of ≥ 3.5 mm on an ultrasound scan after a normal QF-PCR result.”

**Clinical Utility**
The premise of undergoing an invasive prenatal procedure and its attendant risks is that a test result will inform pregnancy decisions. Accordingly, evidence in addition to clinical validity is not required to support clinical utility.

**Section Summary: Chromosomal Microarray Testing**
CMA testing has been shown to have a higher rate of detection (diagnostic yield) of pathogenic chromosomal abnormalities than karyotyping. CMA testing is associated with some VUSs. However, VUSs can be minimized by the use of targeted arrays, testing phenotypically normal parents for the CNV, and the continued accumulation of pathogenic variants in relevant databases.

**Single-Gene Disorders**

**Clinical Context and Test Purpose**
The purpose of testing for single-gene disorders in patients who are undergoing invasive prenatal testing is to inform reproductive decisions.

The question addressed in this evidence review is: What is the analytic validity, clinical validity, and clinical utility of testing for single-gene disorders using invasively obtained fetal samples?

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

**Patients**
The relevant population of interest is women undergoing invasive prenatal testing.

**Interventions**
The relevant intervention of interest is molecular testing (eg, genotyping).

**Comparators**
The relevant comparator of interest is no molecular testing.

**Outcomes**
The primary outcomes are test accuracy and test validity (ie, diagnostic yield); an accurate result will inform reproductive decision making. The premise of obtaining a test is that a woman or couple desires a result for the purposes of pregnancy decisions. Clinical management decisions may include continuation of the pregnancy, enabling timely treatment of a condition that could be treated medically or surgically either in utero or immediately after birth, and birthing decisions.
Timing
Prenatal (fetal) testing may be performed for the purpose of anticipatory guidance and management, either during pregnancy or at the time of delivery.

Setting
The relevant setting of interest is an obstetrics practice.

Analytic Validity
In general, acceptable analytic validity is generally assumed for CMA testing in laboratories meeting quality standards under CLIA, including participation in proficiency testing. The analytic validity in the diagnosis of single-gene disorders depends on the individual variant being tested. In general, it is necessary to identify the particular variant(s) in the affected parent(s) so that the particular variant(s) can be sought for prenatal diagnosis. When a familial variant is known, the analytic validity of testing for this variant is expected to be high, approaching 100% accuracy.

Clinical Validity
For clinical validity, when there is a known pathogenic familial variant, the sensitivity and specificity for testing for the variant in other family members is expected to be very high. That a prenatal diagnosis established from fetal tissue is accurate is broadly accepted. For example, in a case series of spinal muscular atrophy, Kocheva et al (2008) tested Macedonian families. Using restriction fragment length polymorphism (RFLP) analysis of 12 prenatal diagnostic CVS samples, 4 fetuses were determined to be homozygous for exons 7 and 8 of the SMN1 gene and 8 fetuses were normal. The 8 fetuses were carried to term and their unaffected state confirmed; 4 pregnancies were terminated and the deletions subsequently confirmed. Also relying on RFLP analysis, Chen et al (2007) reported agreement between invasive prenatal testing results in 4 Chinese aborted fetuses homozygous for SMN1 variants and 7 (3 normal, 4 carrier) live births.

Clinical Utility
As with CMA testing, the premise of undergoing an invasive prenatal procedure and its attendant risks is that a test result will inform pregnancy decisions. Accordingly, evidence in addition to clinical validity is not required to support clinical utility.

Section Summary: Testing for Single-Gene Disorders
The analytic validity in the diagnosis of single-gene disorders depends on the individual variant tested. In general, it is necessary to identify the particular variant(s) in the affected parent(s) so that the particular variant(s) can be sought for prenatal diagnosis. When a familial variant is known, the analytic validity of testing for this variant is expected to be high, approaching 100% accuracy. For clinical validity, when there is a known pathogenic familial variant, the sensitivity and specificity of testing for the variant in other family members is expected to be very high. Changes in reproductive decision making could include decisions on continuation of the pregnancy, facilitating timely treatment of a condition.
medically or surgically either in utero or immediately after birth, decisions concerning place of delivery (ie, tertiary care center), and route of delivery.

Next-Generation Sequencing

Clinical Context and Test Purpose
The purpose of next-generation sequencing (NGS) in patients who are undergoing invasive prenatal testing is to inform reproductive decisions.

The question addressed in this evidence review is: What is the analytic validity, clinical validity, and clinical utility of testing for single-gene disorders using invasively obtained fetal samples?

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

**Patients**
The relevant population of interest is women undergoing invasive prenatal testing.

**Interventions**
The relevant intervention of interest is NGS.

**Comparators**
The relevant comparator of interest is CMA testing (CNV) or genotyping for single-gene disorders.

**Outcomes**
The primary outcomes are test accuracy and test validity (ie, diagnostic yield); an accurate result will inform reproductive decision making. The premise of obtaining a test is that a woman or couple desires a result for the purposes of pregnancy decisions. Clinical management decisions may include continuation of the pregnancy, enabling timely treatment of a condition that could be treated medically or surgically either in utero or immediately after birth, and birthing decisions.

**Timing**
Prenatal (fetal) testing may be performed for the purpose of anticipatory guidance and management, either during pregnancy or at the time of delivery.

**Setting**
The relevant setting of interest is an obstetrics practice.

**Analytic Validity**
The analytic validity of NGS in the prenatal setting is unknown.

**Clinical Validity**
The clinical validity of NGS in the prenatal setting is unknown.

**Clinical Utility**
Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Preferred evidence comes from randomized controlled trials.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. It is not possible to construct an indirect chain of evidence for clinical utility due to the lack of clinical validity.

Section Summary: NGS Testing on Invasively Obtained Fetal Tissue
NGS can include multigene panel testing, as well as whole exome and whole genome sequencing. While the use of NGS has been accepted in certain noninvasive prenatal testing settings, its use in the setting of invasive prenatal testing for detecting CNVs and single-gene variants is still considered investigational, and includes concerns about interpretation of the data generated and the data’s clinical relevance. Evidence on the use of NGS in the invasive prenatal setting is lacking.

Summary of Evidence
For individuals who are undergoing invasive diagnostic prenatal (fetal) testing who receive chromosomal microarray (CMA) analysis, the evidence includes a systematic review and meta-analysis and prospective cohort and retrospective analyses comparing the diagnostic yield of CMA testing with that of karyotyping. Relevant outcomes are test accuracy, test validity, and changes in reproductive decision making. CMA testing has a higher detection rate of pathogenic chromosomal alterations than karyotyping. CMA testing can yield results that have uncertain clinical significance; however, such results can be minimized by the use of targeted arrays, testing phenotypically normal parents for the copy number variant (CNV), and the continued accumulation of pathogenic variants in international databases. The highest yield of pathogenic CNVs by CMA testing has been found in fetuses with malformations identified by ultrasound. Changes in reproductive decision making could include decisions on continuation of a pregnancy, enabling timely treatment of a condition that could be treated medically or surgically either in utero or immediately after birth, and birthing decisions. The American College of Obstetricians and Gynecologists has recommended CMA testing in women who are undergoing an invasive diagnostic procedure. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are undergoing invasive diagnostic prenatal (fetal) testing who receive molecular testing for single-gene disorders, the evidence includes case series that may report disorders detected and test validity. Relevant outcomes are accuracy, test validity, and changes in reproductive decision making. The analytic validity in the diagnosis of single-gene disorders depends on the individual variant tested. In general, it is necessary to identify the particular variant(s) in the affected parent(s) so that the particular variant(s) can be sought for prenatal
diagnosis. When a familial variant is known, the analytic validity of testing for this variant is expected to be high, approaching 100% accuracy. For clinical validity, when there is a known pathogenic familial variant, the sensitivity and specificity of testing for the variant in other family members is expected to be very high. Changes in reproductive decision making could include decisions on continuation of the pregnancy, facilitating timely treatment of a condition medically or surgically either in utero or immediately after birth, decisions concerning place of delivery (i.e., tertiary care center), and route of delivery. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are undergoing invasive diagnostic prenatal (fetal) testing who receive next-generation sequencing (NGS), the evidence is lacking. Relevant outcomes are test accuracy, test validity, and changes in reproductive decision making. There are concerns about interpretation of data generated by NGS and the data's clinical relevance. The analytic and clinical validity of NGS in the prenatal setting are unknown. The evidence is insufficient to determine the effects of the technology on health outcomes.

Supplemental Information

Practice Guidelines and Position Statements
The American College of Obstetricians and Gynecologists and the Society for Maternal Fetal Medicine have offered recommendations in 2016 on the use of chromosomal microarray testing and next-generation sequencing in prenatal diagnosis:

- “Chromosomal microarray analysis is a method of measuring gains and losses of DNA throughout the human genome. It can identify chromosomal aneuploidy and other large changes in the structure of chromosomes that would otherwise be identified by standard karyotype analysis, as well as submicroscopic abnormalities that are too small to be detected by traditional modalities.
- Most genetic changes identified by chromosomal microarray analysis that typically are not identified on standard karyotype are not associated with increasing maternal age; therefore, the use of this test can be considered for all women, regardless of age, who undergo prenatal diagnostic testing.
- Prenatal chromosomal microarray analysis is recommended for a patient with a fetus with one or more major structural abnormalities identified on ultrasonographic examination and who is undergoing invasive prenatal diagnosis. This test typically can replace the need for fetal karyotype.
- In a patient with a structurally normal fetus who is undergoing invasive prenatal diagnostic testing, either fetal karyotyping or a chromosomal microarray analysis can be performed.
- Comprehensive patient pretest and posttest genetic counseling from an obstetrician–gynecologist or other health care provider with genetics expertise regarding the benefits, limitations, and results of chromosomal microarray analysis is essential.
Chromosomal microarray analysis should not be ordered without informed consent, which should include discussion of the potential to identify findings of uncertain significance, nonpaternity, consanguinity, and adult-onset disease. The routine use of whole-genome or whole-exome sequencing for prenatal diagnosis is not recommended outside of the context of clinical trials until sufficient peer-reviewed data and validation studies are published.”

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

**Medicare National Coverage**
There is no national coverage determination (NCD). In the absence of an NCD, coverage decisions are left to the discretion of local Medicare carriers.

**Ongoing and Unpublished Clinical Trials**
A search of ClinicalTrials.gov in April 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 (SNV) variants for chromosomal abnormalities

**81405** Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)

**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

ICD10 Codes

**O28.5** Abnormal chromosomal and genetic finding on antenatal screening of mother

**O35.1xx0-** Maternal care for (suspected) chromosomal abnormality in fetus, code range

**O35.1xx9** Maternal care for (suspected) hereditary disease in fetus, code range

**O35.2xx0-** Maternal care for (suspected) hereditary disease in fetus, code range

**O35.2xx9**

Additional Policy Key Words

N/A

Policy Implementation/Update Information

1/1/2015 New Policy. Invasive prenatal (fetal) diagnostic testing is medically necessary using CMA and for single-gene disorders when criteria for each category are met. NGS is considered investigational.

1/1/2016 No policy statement changes.

1/1/2017 No policy statement changes.

1/1/2018 No policy statement changes.
### Appendix

#### Appendix Table 1. Categories of Genetic Testing

<table>
<thead>
<tr>
<th>Category</th>
<th>Addressed</th>
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</thead>
<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></td>
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<tr>
<td>1b. Prognostic</td>
<td></td>
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<tr>
<td>1c. Therapeutic</td>
<td></td>
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<tr>
<td>2. Testing cancer cells from an affected individual to benefit the individual</td>
<td></td>
</tr>
<tr>
<td>2a. Diagnostic</td>
<td></td>
</tr>
<tr>
<td>2b. Prognostic</td>
<td></td>
</tr>
<tr>
<td>2c. Therapeutic</td>
<td></td>
</tr>
<tr>
<td>3. Testing an asymptomatic individual to determine future risk of disease</td>
<td></td>
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<tr>
<td>4. Testing of an affected individual’s germline to benefit family members</td>
<td></td>
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<tr>
<td>5. Reproductive testing</td>
<td></td>
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<tr>
<td>5a. Carrier testing: preconception</td>
<td></td>
</tr>
<tr>
<td>5b. Carrier testing: prenatal</td>
<td></td>
</tr>
<tr>
<td>5c. In utero testing: aneuploidy</td>
<td>X</td>
</tr>
<tr>
<td>5d. In utero testing: familiar variants</td>
<td>X</td>
</tr>
<tr>
<td>5e. In utero testing: other</td>
<td>X</td>
</tr>
<tr>
<td>5f. Preimplantation testing with in vitro fertilization</td>
<td></td>
</tr>
</tbody>
</table>

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.