2.04.116	Invasive Prenatal (Fetal) Diagnostic Testing		
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Section:	2.0 Medicine	**Page:**	Page 1 of 25

Policy Statement

Chromosomal Microarray Testing

I. In individuals who are undergoing invasive diagnostic prenatal (fetal) testing, chromosome microarray testing may be considered **medically necessary** as an alternative to karyotyping (see Policy Guidelines).

Single-Gene Disorders

II. Invasive diagnostic prenatal (fetal) testing for molecular analysis for single-gene disorders may be considered **medically necessary** when **all** of the following criteria have been met:
   A. A pregnancy has been identified as being at high risk for **any** of the following:
      1. Autosomal dominant conditions, at least one of the parents has a known pathogenic variant.
      2. Autosomal recessive conditions in **either** of the following:
         a. Both parents are suspected to be carriers or are known to be carriers
         b. One parent is clinically affected and the other parent is suspected to be or is a known carrier
      3. X-linked conditions: A parent is suspected to be or is a known carrier
   B. 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
   C. Any variants have high penetrance
   D. The genetic test has adequate sensitivity and specificity to guide clinical decision making and residual risk is understood
   E. An association of the marker with the disorder has been established

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

Next-Generation Sequencing

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

NOTE: Refer to Appendix A to see the policy statement changes (if any) from the previous version.

Policy Guidelines

Fetal 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; central nervous system: anencephaly, hydrocephalus, myelomeningocele; facial clefts; body wall: omphalocele/gastroschisis; and 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 familial variant 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.

Genetics Nomenclature Update

The 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). The Society's nomenclature is recommended by the Human Variome Project, the Human Genome Organization (HUGO), and the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) 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

Previous	Updated	Definition
Mutation	Disease-associated variant	Disease-associated change in the DNA sequence
	Variant	Change in the DNA sequence
	Familial variant	Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives

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

Variant Classification	Definition
Pathogenic	Disease-causing change in the DNA sequence
Likely pathogenic	Likely disease-causing change in the DNA sequence
Variant of uncertain significance	Change in DNA sequence with uncertain effects on disease
Likely benign	Likely benign change in the DNA sequence
Benign	Benign change in the DNA sequence

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.

Coding

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

- **81228:** Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number variants, comparative genomic hybridization [CGH] microarray analysis
- **81229:** Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants, comparative genomic hybridization (CGH) microarray analysis

CPT code **81405** includes:
Cytogenomic constitutional targeted microarray analysis of chromosome 22q13 by interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants for chromosomal abnormalities.

There are also CPT codes for a genomic sequencing procedure panels (i.e., next-generation sequencing) for X-linked intellectual disability:

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

Effective October 1, 2022, there is a new HCPCS code that represents IriSight™ Prenatal Analysis – Proband by Variantyx Inc. Per the manufacturer, this test would be ordered when amniocentesis has been determined to be medically necessary due to fetal ultrasound abnormalities. DNA is isolated and sequenced. The test assesses for variants in the DNA sequence and correlates these variants with the fetus's phenotype. Variants best matching the phenotype are evaluated for pathogenicity based on ACMG guidelines.

- **0335U**: Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, fetal sample, identification and categorization of genetic variants

Effective October 1, 2022, there is a new HCPCS code that represent IriSight™ Prenatal Analysis – Comparator by Variantyx Inc. Per the manufacturer, this gene sequencing panel is indicated when an amniocentesis has been determined to be medically necessary due to fetal ultrasound abnormalities. An amniotic fluid sample is submitted for IriSight™ Prenatal Analysis to assess the fetus for constitutional/ heritable genomic changes potentially responsible for the abnormalities identified by ultrasound.

- **0336U**: Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, blood or saliva, identification and categorization of genetic variants, each comparator genome (e.g., parent)

Description

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 (CMA) testing, molecular diagnosis of single-gene disorders, and next-generation sequencing.

Related Policies

- Carrier Screening for Genetic Diseases
- Genetic Testing for Developmental Delay/Intellectual Disability, Autism Spectrum Disorder, and Congenital Anomalies
- Noninvasive Prenatal Screening for Fetal Aneuploidies, Microdeletions, and Twin Zygosity Using Cell-Free Fetal DNA
- Preimplantation Genetic Testing

Benefit Application

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

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

Regulatory Status

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

Rationale

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 (e.g., 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, chromosomal microarray (CMA) testing, quantitative polymerase chain reaction (PCR), next-generation sequencing, and multiplex ligation-dependent probe amplification.

This evidence review only addresses the following:

- the diagnosis of copy number variants (CNVs) using CMA technology
- the diagnosis of single-gene disorders, most of which are due to single nucleotide variants (SNVs) or very small deletions, and use molecular methods to diagnose (mainly PCR but also multiplex ligation-dependent probe amplification)
- Next-generation sequencing

This evidence review applies only if there is not a separate evidence review that outlines specific criteria for diagnostic testing. If a separate evidence review exists, then the criteria in it supersede the guidelines herein. This evidence review does NOT cover the use of:

- prenatal carrier testing (Blue Shield of California Medical Policy: Carrier Screening for Genetic Diseases)
- preimplantation genetic diagnosis or screening (Blue Shield of California Medical Policy: Preimplantation Genetic Testing)
- noninvasive prenatal testing (Blue Shield of California Medical Policy: Noninvasive Prenatal Screening for Fetal Aneuploidies, Microdeletions, and Twin Zygosity Using Cell-Free Fetal DNA)

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. Both procedures increase the risk of miscarriage. Chorionic villus sampling utilizes placental cells that are derived from the same fertilized egg as the fetus. The chorionic villi are collected for genetic evaluation under ultrasound guidance without entering the amniotic sac. During amniocentesis, a small sample of the fluid that surrounds the fetus is removed. This fluid contains cells that are shed primarily from the fetal skin, bladder, gastrointestinal tract, and amnion. Typically, CVS is done at earlier gestation than amniocentesis. Most times only one procedure is done; however, sometimes CVS has ambiguous results from maternal cell contamination or placental mosaicism such that amniocentesis might additionally be needed for clarification. Invasive prenatal procedures are usually 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. For confirming positive cell-free DNA results, amniocentesis might be preferred over CVS to avoid potential false-positive results due to confined placental mosaicism[1,2,].

Chromosomal Microarray Testing

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 higher rates of detection of pathogenic chromosomal abnormalities. However, there are disadvantages to CMA testing, including the detection of variants of uncertain significance (VUS) 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 testing 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 the 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 (the 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 loci immobilized on a glass slide (microarray) to conduct thousands of comparative reactions at the same time. The prepared sample and control DNA is 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 Chromosomal Microarray Technologies

There are differences in CMA technology, most notably in the various types of microarrays. They can differ first by construction; the earliest versions used DNA fragments cloned from a bacterial artificial chromosome. They 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. An SNV is a DNA variation in which a single nucleotide in the genomic sequence is altered. This variation can occur between 2 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 2 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 and Genomics to publish guidelines for the design and performance expectations for clinical microarrays and associated software in the postnatal setting.

At this time, no guidelines have shown 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 American College of Medical Genetics guidelines for designing microarrays has recommended probe enrichment in clinically significant areas of the genome to maximize the 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 VUS.

Whole-genome CMA analysis has allowed for the characterization of several new genetic syndromes, with other potential candidates currently under study. However, 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 Chromosomal Microarray Findings and Variants of Uncertain Significance

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

A CNV that is considered a VUS:

- 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., fluorescence in situ hybridization, multiplex ligation-dependent probe amplification, 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 kilobases to 1 megabase.
- 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.

The International Standards for Cytogenomic Arrays (ISCA) Consortium (2008) was organized; it established a public database containing de-identified 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 July 2018, nearly 10500 "expert reviewed" variants are listed in the ClinVar database. Data are currently hosted on ClinGen.[3,]

Use of the database includes an intralaboratory curation process, whereby laboratories are alerted to any inconsistencies among their own reported CNVs or other variants, as well as any inconsistency with the ISCA "known" pathogenic and "known" benign lists. The intralaboratory conflict rate was initially about 3% overall; following the 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/variants 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 the 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 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 multiplex ligation-dependent probe amplification for very small deletions and duplications. Approximately 5000 known disorders 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

Next-generation sequencing has been used to identify pathogenic variants in disease-associated genes in many Mendelian disorders. Approximately 85% of known disease-causing variants occur within 1% of the genome that encodes for proteins (exome). Therefore, whole-exome sequencing can cost-effectively capture the majority of protein-coding regions. However, concerns remain about technical complexity, coverage, bioinformatics, interpretation, VUSs, as well as ethical issues.[4,]

Commercially Available Tests

Many academic and commercial laboratories offer CMA testing and single-gene disorder testing. Many laboratories also offer reflex testing, which may be performed with microarray testing added if karyotyping is normal or unable to be performed (due to no growth of cells). The test should be cleared or approved by the U.S. Food and Drug Administration, or performed in a Clinical Laboratory Improvement Amendment-certified laboratory.

Literature Review

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

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

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.

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: Does CMA testing improve the net health outcome in individuals undergoing invasive prenatal testing?

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest is patients undergoing invasive prenatal testing.

Interventions
The relevant intervention of interest is CMA testing.

Comparators
The following practice is currently being used to make decisions about prenatal testing: karyotyping.

Outcomes
The primary outcomes are test accuracy and test validity (i.e., 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 the 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. Prenatal (fetal) testing may be performed for the purpose of anticipatory guidance and management, either during pregnancy or at the time of delivery.

Study Selection Criteria
For the evaluation of clinical validity of the CMA testing, studies that meet the following eligibility criteria were considered:

- Reported on the detection of pathogenic chromosomal abnormalities of the technology
- Included a suitable reference standard (karyotyping)
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

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

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

Review of Evidence
Systematic Reviews
Sun et al (2021) conducted a meta-analysis on the incidence of chromosomal abnormalities and additional diagnostic gain of CMA compared with standard karyotyping in the detection of cases of fetal ventriculomegaly (VM).[5,] Twenty-three articles involving 1635 patients were included based on a literature search through April 2020. Meta-analysis determined that the incidence of chromosomal abnormalities in VM was 9% (95% confidence interval [CI], 5% to 12%) and incremental yield of CMA in VM was 11% (95% CI, 7% to 16%), demonstrating an improvement in the detection rate of abnormalities. The incidences of chromosomal abnormalities in mild, severe, isolated, and non-isolated VM were 9% (95% CI, 4% to 16%), 5% (95% CI, 1% to 11%), 3% (95% CI, 1% to 6%), and 13% (95% CI, 4% to 25%) respectively.

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 in prenatally diagnosed cardiac malformations.[6,] Thirteen studies with 1131 cases of congenital heart disease were included from a literature search through September 2014. A meta-analysis identified an incremental yield of 7.0% (95% 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 congenital heart disease 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%).

A review by Wapner et al (2014) summarized the existing literature of the largest studies that reported the estimates of detectable pathogenic CNVs according to the indication for CMA testing.[7,] For studies that included only 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 (n=1800) being 5.0%. For pregnancies in which CMA was performed for only low-risk indications (advanced maternal age, abnormal Down syndrome screening test, parental anxiety), the range of pathogenic CNV detection was 0.5% to 1.6%, with a combination of all studies (n=10,099) being 0.9%.

Hillman et al (2013) conducted a prospective cohort study and systematic review.[8,] 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%). The 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.

Prospective and Retrospective Studies

Various prospective and retrospective cohort studies have compared CMA and karyotype testing.[9,10,11,12,13,14,15,16,17,18,19,20,21,22,]Studies have consistently found CMA testing to have a higher rate of detection (diagnostic yield) of pathogenic chromosomal abnormalities than karyotyping, but results have varied by indication. A sampling of the largest studies is discussed below.

Xiang et al (2021) retrospectively evaluated a cohort to compare the diagnostic yield of CMA testing using a single nucleotide polymorphism (SNP) -array compared to G-banded karyotyping.[19,] There were 4022 women who were evaluated that had chosen to receive CMA and karyotyping simultaneously in their pregnancy. Of these, 151 cases of aneuploidy and 2 cases of triploidy (69,XXX) were identified with both CMA testing and karyotyping. Among 3665 cases with normal results on karyotyping, CMA testing yielded an additional 286 abnormal results (286/3665, 7.8%) including 2 cases of mosaic 45,X, 19 cases of loss of heterozygosity, and 265 cases of microduplication/ microdeletion.

Robson et al (2017) reported on the results of the U.K. Evaluation of Array Comparative genomic Hybridisation (EACH) study, a multicenter cohort study including an economic and qualitative substudy.[9,] Enrolled women underwent quantitative fluorescent polymerase chain reaction and conventional karyotyping after chorionic villus sampling (55.8%), amniocentesis (40.8%), or fetal blood sampling (2.7%). Testing indications included an isolated nuchal translucency (≥3.5 mm) or any structural anomaly detected on ultrasound at 11 to 14 weeks. Nine laboratories performed testing with an identical oligonucleotide-comparative genome hybridization (CGH) array. Between March 2012 and May 2014, 1718 women were recruited, and results from 1123 were analyzed. Irrespective of indication for testing, results were observed as shown in Table 1.

Table 1. Comparison of Karyotype and Chromosomal Microarray Testing Results (EACH Study)

Karyotyping	Chromosomal Microarray Testing	n (%)
Pathogenic alteration	Benign alteration	15 (1.3)

Karyotyping	Chromosomal Microarray Testing	n (%)
Pathogenic alteration	Pathogenic alteration	58 (5.2)
Benign alteration	Pathogenic alteration	42 (3.7)
Benign alteration	Variant of uncertain significance	38 (3.4)

Adapted from Robson et al (2017).[9,]

Similar to other studies discussed below, results varied by 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 1 or more structural anomalies or an isolated NT [nuchal translucency] of ≥ 3.5 mm on an ultrasound scan after a normal QF-PCR [quantitative fluorescent polymerase chain reaction] result."

Lovrecic et al (2016) evaluated the clinical usefulness of prenatal CMA testing for small (sub-microscopic) imbalances (CNV) in 218 fetuses across a range of indications for testing.[10,] In fetuses with ultrasound findings, the 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.

Papoulidis et al (2015) compared the diagnostic yield of conventional karyotyping with aCGH in 1763 prenatal samples.[11,] 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."

Armengol et al (2012) conducted a comparative study of available technologies, including karyotyping and CMA, for the detection of chromosomal abnormalities after invasive prenatal sampling.[12,] 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. 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.

Table 2 reports the data on karyotyping and CMA testing. The diagnostic accuracy was 98.2% (97.1% to 99.0%) for karyotyping and 99.7% (99.0% to 99.9%) CMA testing.

Table 2. Clinical Validity of Karyotyping vs Chromosomal Microarray Testing in Armengol et al (2012)

Study	Initial N	Final N	Clinical Validity (95% Confidence Interval), %			
			Sensitivity	Specificity	PPV	NPV
Armengol et al (2012)[12,]	906[a]	57[a]				
Karyotyping			76.4 (63.0 to 87.0)	99.9 (99.2 to 99.9)	97.7 (87.7 to 99.9)	98.3 (97.1 to 99.1)
CMA testing			98.2 (90.4 to 99.9)	99.7 (99.1 to 99.9)	96.5 (87.9 to 99.5)	99.9 (99.3 to 100)

CMA: chromosomal microarray; NPV: negative predictive value; PPV: positive predictive value.
[a] Fifty-seven variants detected from 906 fetal samples from 900 women.

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.[13,] 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[14,]), abnormal first- or second-trimester screen (n=77), other family history of a genetic condition (n=487), advanced maternal age (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%.

The other study by Shaffer et al (2012) retrospectively analyzed 2858 pregnancies with abnormal ultrasound findings (as stratified by organ system).[14,] 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 (e.g., 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 [18%]) or targeted coverage (n=191 [7%]). 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%]).

Wapner et al (2012) conducted a prospective study to compare the accuracy, efficacy, and incremental yield of CMA testing with karyotyping for routine prenatal diagnosis.[15,] A total of 4406 women undergoing routine prenatal diagnosis in 1 of 29 diagnostic centers by either chorionic villus sampling or amniocentesis had a sample split in 2 for standard karyotyping and CMA testing. Indications for prenatal diagnosis included advanced maternal age (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. 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 the 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 a 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 the micro-array. 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 (2.1%) patients. Of the women tested for advanced maternal age, 1.7% (95% CI, 1.2% to 2.4%) had a clinically relevant finding on the 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 et al (2012) study included 3822 patients with normal karyotype and the following indications for prenatal diagnosis: advanced maternal age (n=1966), positive Down syndrome screen (n=729), an anomaly on ultrasound (n=755), and other (n=372).

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.[16,] 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. 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 fluorescence in situ hybridization 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/fluorescence in situ hybridization (42.6%), a family history of a chromosomal abnormality (9.5%), all abnormal prenatal ultrasound findings (9.3%), abnormal serum screening (5.4%), and advanced maternal age (1.3%). In summary, the overall detection rate in the Breman et al (2012) 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.

Clinically Useful

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

Direct Evidence

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

No randomized trials were identified on the use of CMA testing for this indication.

Chain of Evidence

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

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: Does testing for single-gene disorders improve the net health outcome in individuals undergoing invasive prenatal testing?

The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is patients undergoing invasive prenatal testing.

Interventions

The relevant intervention of interest is molecular testing (e.g., genotyping).

Comparators

The following practice is currently being used to make decisions about prenatal testing: no molecular testing.

Outcomes

The primary outcomes are test accuracy and test validity (i.e., 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 the 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. Prenatal (fetal) testing may be performed for the purpose of anticipatory guidance and management, either during pregnancy or at the time of delivery.

Study Selection Criteria

For the evaluation of clinical validity of testing for single-gene disorders, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of detecting a single-gene disorder
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

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

Review of Evidence
When there is a known pathogenic familial variant, the sensitivity and specificity for testing for the variant in other family members are 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.[23,] Using restriction fragment length polymorphism analysis of 12 prenatal diagnostic chorionic villus sampling 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 was confirmed; 4 pregnancies were terminated and the deletions were subsequently confirmed. Also relying on restriction fragment length polymorphism 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.[24,]

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

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

No randomized trials were identified on testing for single-gene disorders for this indication.

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

As 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: Single-Gene Disorders
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 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 the place of delivery (i.e., tertiary care center), and route of delivery.

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

The question addressed in this evidence review is: Does next-generation sequencing improve the net health outcome in individuals undergoing invasive prenatal testing?

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest is patients undergoing invasive prenatal testing.

Interventions
The relevant intervention of interest is next-generation sequencing.

Comparators
The relevant comparators of interest are CMA testing (CNV) and genotyping.

Outcomes
The primary outcomes are test accuracy and test validity (i.e., 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 the 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. Prenatal (fetal) testing may be performed for the purpose of anticipatory guidance and management, either during pregnancy or at the time of delivery.

Study Selection Criteria
For the evaluation of clinical validity of the next-generation sequencing testing, studies that meet the following eligibility criteria were considered:

- Reported on the detection of pathogenic chromosomal abnormalities of the technology
- Included a suitable reference standard (e.g., CMA, genotyping)
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

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

The clinical validity of next-generation sequencing in the prenatal setting is unknown.

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

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

No randomized trials were identified on the use of next-generation sequencing testing for this indication.

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

It is not possible to construct a chain of evidence for clinical utility due to the lack of clinical validity.

Section Summary: Next-Generation Sequencing Testing

Next-generation sequencing can include multigene panel testing, as well as whole-exome and whole-genome sequencing. While the use of next-generation sequencing has been accepted in certain noninvasive prenatal testing settings, its use in the invasive prenatal testing setting for detecting CNVs and single-gene variants is still uncertain and includes concerns about the interpretation of the data generated and the data's clinical relevance. Evidence on the use of next-generation sequencing in the invasive prenatal setting is lacking.

Summary of Evidence

For individuals who are undergoing invasive diagnostic prenatal (fetal) testing who receive CMA testing, 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, and the continued accumulation of pathogenic variants in international databases. The highest yield of pathogenic copy number variants by CMA testing has been found in fetuses with malformations identified by ultrasound. Changes in reproductive decision-making could include decisions on the 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 an 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 test accuracy, test validity, and changes in reproductive decision-making. For clinical validity, when there is a known pathogenic familial variant, the sensitivity and specificity of testing for the variant in other family members are 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 the place of delivery (i.e., tertiary care center), and route of delivery. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

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

Supplemental Information

The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

Practice Guidelines and Position Statements

Guidelines or position statements will be considered for inclusion in ‘Supplemental Information’ if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine

In 2016, the American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine offered recommendations on the use of chromosomal microarray (CMA) testing and next-generation sequencing in prenatal diagnosis (Committee Opinion Number 682)[25,]:

- "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 a 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."

International Society for Prenatal Diagnosis, et al.

In 2018, the International Society for Prenatal Diagnosis, the Society for Maternal-Fetal Medicine, and the Perinatal Quality Foundation released a joint position statement on the use of prenatal exome and genome-wide sequencing for fetal diagnosis.[26,] This initial position statement was replaced in 2022.[27,] The 2022 position statement provides suggestions for clinical use, as described in the clinical indications below:

1. "The current existing data support that prenatal sequencing is beneficial for the following indications:
    a. A current pregnancy with a fetus having a major single anomaly or multiple organ system anomalies:
        i. For which no genetic diagnosis was found after CMA and a clinical genetic expert review considers the phenotype suggestive of a possible genetic etiology.
        ii. For which the multiple anomaly 'pattern' strongly suggests a single gene disorder with no prior genetic testing. As pES [prenatal exome sequencing] is not currently validated to detect all CNVs [copy number variants], CMA should be run before or in parallel with pES in this scenario.
    b. A personal (maternal or paternal) history of a prior undiagnosed fetus (or child) affected with a major single or multiple anomalies:
        i. With a recurrence of similar anomalies in the current pregnancy without a genetic diagnosis after karyotype or CMA for the current or prior undiagnosed pregnancy. Point a.i. above also applies in these circumstances.

ii. When such parents present for preconception counseling and no sample is available from the affected proband, or if a fetal sample cannot be obtained in an ongoing pregnancy, it is considered appropriate to offer sequencing for both biological parents to look for shared carrier status for autosomal recessive mutations that might explain the fetal phenotype. However, where possible, obtaining tissue from a previous abnormal fetus or child for pES is preferable.

2. There is currently no evidence that supports routine testing (including upon parental request) on fetal tissue obtained from an invasive prenatal procedure (amniocentesis, CVS, cordocentesis, other) for indications other than fetal anomalies.
    a. There may be special settings when prenatal sequencing in the absence of a fetal phenotype visible on prenatal imaging can be considered, such as with a strong family history of a recurrent childhood-onset severe genetic condition with no prenatal phenotype in previous children for whom no genetic evaluation was done and is not possible. Such scenarios should be reviewed by an expert multidisciplinary team preferentially in the context of a research protocol. If sequencing is done for this indication, it must be done as trio sequencing, using an appropriate analytical approach."

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 June 2022 did not identify any ongoing or unpublished trials that would likely influence this review.

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Documentation for Clinical Review

Please provide the following documentation:

- History and physical and/or consultation notes including:
  - Reason for test
  - Type/name of test
  - Family history including known variant or carrier status of parents
  - Documentation of high risk pregnancy and why it is high risk
  - Evidence-based support for genetic test or specific gene(s) of interest

Post Service (in addition to the above, please include the following):

- Laboratory report(s)

Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy.

The following codes are included below for informational purposes. Inclusion or exclusion of a code(s) does not constitute or imply member coverage or provider reimbursement policy. Policy Statements are intended to provide member coverage information and may include the use of some codes for clarity. The Policy Guidelines section may also provide additional information for how to interpret the Policy Statements and to provide coding guidance in some cases.

Type	Code	Description
CPT®	0335U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, fetal sample, identification and categorization of genetic variants ***(Code effective 10/1/2022)***
	0336U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, blood or saliva, identification and categorization of genetic variants, each comparator genome (e.g., parent) ***(Code effective 10/1/2022)***
	81228	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number variants, comparative genomic hybridization [CGH] microarray analysis ***(Code revision effective 1/1/2022)***

Type	Code	Description
	81229	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants, comparative genomic hybridization (CGH) microarray analysis *(Code revision effective 1/1/2022)*
	81329	SMN1 (survival of motor neuron 1, telomeric) (e.g., spinal muscular atrophy) gene analysis; dosage/deletion analysis (e.g., carrier testing), includes SMN2 (survival of motor neuron 2, centromeric) analysis, if performed
	81336	SMN1 (survival of motor neuron 1, telomeric) (e.g., spinal muscular atrophy) gene analysis; full gene sequence
	81337	SMN1 (survival of motor neuron 1, telomeric) (e.g., spinal muscular atrophy) gene analysis; known familial sequence variant(s)
	81405	Molecular Pathology Procedure Level 6
	81470	X-linked intellectual disability (XLID) (e.g., syndromic and non-syndromic XLID); genomic sequence analysis panel, must include sequencing of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3, and SLC16A2
	81471	X-linked intellectual disability (XLID) (e.g., syndromic and non-syndromic XLID); duplication/deletion gene analysis, must include analysis of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3, and SLC16A2
HCPCS	None	

Policy History

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

Effective Date	Action
06/01/2017	BCBSA Medical Policy adoption
10/01/2018	Policy revision without position change
02/01/2019	Coding update
10/01/2019	Policy revision without position change
10/01/2020	Annual review. No change to policy statement. Literature review updated.
01/01/2021	Coding update
10/01/2021	Annual review. No change to policy statement. Literature review updated.
02/01/2022	Coding update
10/01/2022	Annual review. . Policy statement, guidelines and literature updated.
11/01/2022	Coding update.

Definitions of Decision Determinations

Medically Necessary: Services that are Medically Necessary include only those which have been established as safe and effective, are furnished under generally accepted professional standards to treat illness, injury or medical condition, and which, as determined by Blue Shield, are: (a) consistent with Blue Shield medical policy; (b) consistent with the symptoms or diagnosis; (c) not furnished primarily for the convenience of the patient, the attending Physician or other provider; (d) furnished at the most appropriate level which can be provided safely and effectively to the patient; and (e) not

more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of the Member's illness, injury, or disease.

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

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

Prior Authorization Requirements and Feedback (as applicable to your plan)

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

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department at (800) 541-6652, or the Transplant Case Management Department at (800) 637-2066 ext. 3507708 or visit the provider portal at www.blueshieldca.com/provider.

We are interested in receiving feedback relative to developing, adopting, and reviewing criteria for medical policy. Any licensed practitioner who is contracted with Blue Shield of California or Blue Shield of California Promise Health Plan is welcome to provide comments, suggestions, or concerns. Our internal policy committees will receive and take your comments into consideration.

For utilization and medical policy feedback, please send comments to: MedPolicy@blueshieldca.com

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

Appendix A

POLICY STATEMENT	
BEFORE Red font: Verbiage removed	**AFTER** Blue font: Verbiage Changes/Additions
Invasive Prenatal (Fetal) Diagnostic Testing 2.04.116 **Policy Statement:** **Chromosomal Microarray Testing** In patients who are undergoing invasive diagnostic prenatal (fetal) testing, chromosome microarray testing may be considered **medically necessary** as an alternative to karyotyping (see Policy Guidelines). **Single-Gene Disorders** Invasive diagnostic prenatal (fetal) testing for molecular analysis for single-gene disorders may be considered **medically necessary** when **all** of the following criteria have been met: I. A pregnancy has been identified as being at high risk for **any** of the following: A. Autosomal dominant conditions, at least one of the parents has a known pathogenic variant B. Autosomal recessive conditions in **either** of the following: 1. Both parents are suspected to be carriers or are known to be carriers 2. One parent is clinically affected and the other parent is suspected to be or is a known carrier C. X-linked conditions: A parent is suspected to be or is a known carrier II. 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 III. Any variants have high penetrance IV. The genetic test has adequate sensitivity and specificity to guide clinical decision making and residual risk is understood V. An association of the marker with the disorder has been established	**Invasive Prenatal (Fetal) Diagnostic Testing 2.04.116** **Policy Statement:** **Chromosomal Microarray Testing** I. In individuals who are undergoing invasive diagnostic prenatal (fetal) testing, chromosome microarray testing may be considered **medically necessary** as an alternative to karyotyping (see Policy Guidelines). **Single-Gene Disorders** II. Invasive diagnostic prenatal (fetal) testing for molecular analysis for single-gene disorders may be considered **medically necessary** when **all** of the following criteria have been met: A. A pregnancy has been identified as being at high risk for **any** of the following: 1. Autosomal dominant conditions, at least one of the parents has a known pathogenic variant. 2. Autosomal recessive conditions in **either** of the following: a. Both parents are suspected to be carriers or are known to be carriers b. One parent is clinically affected and the other parent is suspected to be or is a known carrier 3. X-linked conditions: A parent is suspected to be or is a known carrier B. 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 C. Any variants have high penetrance D. The genetic test has adequate sensitivity and specificity to guide clinical decision making and residual risk is understood E. An association of the marker with the disorder has been established

POLICY STATEMENT	
BEFORE Red font: Verbiage removed	**AFTER** Blue font: Verbiage Changes/Additions
If the above criteria for molecular analysis of 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**.	III. If the above criteria for molecular analysis of single-gene disorders are not met, invasive diagnostic prenatal (fetal) testing is considered **investigational**. **Next-Generation Sequencing** IV. The use of next-generation sequencing in the setting of invasive prenatal testing is considered **investigational**.