



Invasive Prenatal Fetal Diagnostic Testing for Chromosomal Abnormalities

Effective: July 1, 2024

Next Review: April 2025

Last Review: June 2024

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Testing for chromosomal abnormalities, typically using chromosomal microarray (CMA), may be performed in the context of invasive prenatal fetal diagnostic testing or fetal tissue testing to confirm the presence of a pathogenic abnormality after it has been determined by prenatal screening that the fetus is at increased risk for a genetic condition.

MEDICAL POLICY CRITERIA

Notes:

- This policy does not address karyotyping, which may be considered medically necessary.
- Please refer to the Cross References section below for genetic testing not addressed in this policy, including but not limited to whole exome or genome sequencing and reproductive carrier testing.

Testing for chromosomal abnormalities (e.g., chromosomal microarray analysis) for fetal diagnosis may be considered **medically necessary** in the setting of invasive diagnostic prenatal fetal testing (i.e., not cell-free DNA testing), or for fetal tissue testing when an anomaly has been detected by ultrasound.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

LIST OF INFORMATION NEEDED FOR REVIEW

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variant(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
 - History and physical exam including any relevant diagnoses related to the genetic testing
 - Conventional testing and outcomes
 - Conservative treatments, if any
 - Date of sample collection

CROSS REFERENCES

1. [Preimplantation Genetic Testing of Embryos](#), Genetic Testing, Policy No. 18
2. [Genetic and Molecular Diagnostic Testing](#), Genetic Testing, Policy No. 20
3. [Noninvasive Prenatal Testing to Determine Fetal Aneuploidies and Microdeletions using Cell-Free DNA](#), Genetic Testing, Policy No 44
4. [Chromosomal Microarray Analysis \(CMA\) or Copy Number Analysis for the Genetic Evaluation of Patients with Developmental Delay, Intellectual Disability, Autism Spectrum Disorder, or Congenital Anomalies](#), Genetic Testing, Pol. No. 58
5. [Evaluating the Utility of Genetic Panels](#), Genetic Testing, Policy No. 64
6. [Whole Exome and Whole Genome Sequencing](#), Genetic Testing, Policy No. 76
7. [Genetic Testing for the Evaluation of Products of Conception and Pregnancy Loss](#), Genetic Testing, Policy No. 79
8. [Reproductive Carrier Screening for Genetic Diseases](#), Genetic Testing, Policy No. 81
9. [Maternal Serum Analysis for Risk of Preterm Birth](#), Laboratory, Policy No. 75

BACKGROUND

The focus of this evidence review is on the use of CMA as an invasive diagnostic testing methodology in the prenatal (fetal) setting.

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

Genetic disorders are generally categorized into three 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 ANALYSIS

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 can identify abnormalities at the level of the chromosome and measures gains and losses of DNA segments (known as copy number variants [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 co-hybridized 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 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 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 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 single nucleotide variants (SNVs, also known as single nucleotide polymorphisms, or SNPs) 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 indicate 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 VUS

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, 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 (<https://clinicalgenome.org/>).

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

The consortium recently proposed “an evidence-based approach to guide the development of content on chromosomal microarrays and to support interpretation of clinically significant copy number variation.” The proposal 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.

COMMERCIALY AVAILABLE TESTS

Many academic and commercial laboratories offer CMA testing and sequencing-based tests in the prenatal setting. 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 following is not inclusive; it is only an example of some laboratories that offer CMA and sequencing-based testing. The test should be cleared or approved by the Food and Drug Administration or performed in a Clinical Laboratory Improvement Amendment–certified laboratory.

GeneDx offers prenatal CMA for copy number abnormalities in fetuses with ultrasound abnormalities. The targeted CMA includes oligonucleotide probes placed throughout the genome and within 100 common or novel microdeletion and microduplication syndromes, as well as those involving subtelomeric regions and any other intrachromosomal region greater

than 1.5 Mb. This array also contains SNV probes covering chromosomes known to contain uniparental disomy. Exon-level probe coverage is added to some genes associated with some monogenic disorders.

GeneDx also offers a whole genome array that contains oligonucleotide probes for areas throughout the genome and within more than 220 targeted regions. This array detects CNVs greater than 200 kb across the entire genome and between 500 bp and 15 kb in targeted regions. Approximately 65 genes associated with neurodevelopmental disorders are targeted at the exon level. This array also contains SNV probes throughout the genome to detect some types of uniparental disomy (UPD).

ARUP laboratory provides former Signature Genomics clients with prenatal tests, including targeted CMA with SNV coverage.

Many laboratories 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).

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, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature^[1] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

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 bioethical communities that prenatal testing should not include testing for late-onset/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.

CMA is now considered standard of care for women undergoing invasive prenatal testing. Therefore, no further evidence will be added to this policy. Please see below for a summary of the current evidence.

SUMMARY OF EVIDENCE

The evidence for CMA testing in patients who are undergoing invasive diagnostic prenatal (fetal) testing includes systematic reviews, meta-analyses and prospective cohort and retrospective analyses of the diagnostic yield compared with karyotyping. Relevant outcomes reported are test accuracy and validity, and changes in reproductive decision making. CMA testing has been shown to have a higher rate of detection of pathogenic chromosomal

abnormalities than karyotyping. CMA testing is associated with a certain percentage of results that have unknown clinical significance; however, this can be minimized by the use of targeted arrays 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. 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 (n=1,800) being 5.0%. For pregnancies in which CMA was performed for other 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%.

Changes in reproductive decision making could include decisions regarding continuation of the pregnancy, enabling for 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 recommends CMA testing in women who are undergoing an invasive diagnostic procedure. Therefore, the evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

PRACTICE GUIDELINE SUMMARY

THE AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS COMMITTEE ON GENETICS AND THE SOCIETY FOR MATERNAL FETAL MEDICINE

In December 2016 (reaffirmed in 2023), the American Congress of Obstetricians and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine published a Committee Opinion (No. 682),^[2] offering the following recommendations for the use of chromosomal microarray analysis in prenatal diagnosis:

- Chromosomal microarray analysis ... 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 ... 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.

The American College of Obstetricians and Gynecologists (ACOG) published Practice Bulletin No. 162 in May 2016,^[3] stating:

- In all patients at risk of aneuploidy or at risk of having a pregnancy affected by a genetic disorder, “karyotype or microarray analysis should be offered in every case, although performing karyotype or microarray may not be necessary in a low risk patient.”
- “In patients with a major structural abnormality found on ultrasound examination, CVS or amniocentesis with chromosomal microarray should be offered.” Chromosomal microarray is now recommended as the primary test for these patients, replacing karyotyping.
- “Chromosomal microarray analysis should be available to women undergoing invasive diagnostic testing for any indication.”
- “If a structural abnormality is strongly suggestive of a particular aneuploidy in the fetus, karyotype analysis with or without FISH may be offered before chromosomal microarray analysis.”
- Chromosomal microarray analysis can be used to confirm an abnormal FISH test.

International Society for Prenatal Diagnosis:^[4]

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. This initial position statement was replaced in 2022. 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."

SUMMARY

There is enough research to show that testing for chromosomal abnormalities in the setting of invasive diagnostic prenatal fetal testing or ultrasound-detected fetal anomalies informs reproductive decision-making including decisions regarding continuation of the pregnancy, birthing decisions, and enabling for timely treatment of a condition that could be treated medically or surgically either in utero or immediately after birth. In addition, clinical practice guidelines recommend this testing in women who are undergoing invasive diagnostic prenatal fetal testing. Therefore, fetal testing for chromosomal abnormalities may be considered medically necessary when undergoing invasive diagnostic prenatal fetal testing or when a fetal anomaly has been detected by ultrasound.

REFERENCES

1. den Dunnen JT, Dalgleish R, Maglott DR, et al. HGVS Recommendations for the Description of Sequence Variants: 2016 Update. *Human mutation*. 2016;37(6):564-9. PMID: 26931183
2. American College of Obstetricians, Gynecologists Committee on Genetics. Committee Opinion No. 682: Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology. Dec [cited 5/24/2024]. 'Available from:' <https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2016/12/microarrays-and-next-generation-sequencing-technology-the-use-of-advanced-genetic-diagnostic-tools-in-obstetrics-and-gynecology>.
3. Practice Bulletin No. 162: Prenatal Diagnostic Testing for Genetic Disorders. *Obstetrics and gynecology*. 2016;127(5):e108-22. PMID: 26938573
4. Van den Veyver IB, Chandler N, Wilkins-Haug LE, et al. International Society for Prenatal Diagnosis Updated Position Statement on the use of genome-wide sequencing for prenatal diagnosis. *Prenatal diagnosis*. 2022;42(6):796-803. PMID: 35583085

CODES

NOTE: The appropriate codes for reporting CMA are 81228 for CMA alone, and 81229 for CMA testing that includes single nucleotide polymorphism (SNP) analysis. It is not appropriate to report code 81422 for CMA.

Codes	Number	Description
CPT	0469U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis for chromosomal abnormalities, copy number variants, duplications/deletions, inversions, unbalanced translocations, regions of homozygosity (ROH), inheritance pattern that indicate uniparental disomy (UPD), and aneuploidy, fetal sample (amniotic fluid, chorionic villus sample, or products of conception), identification and categorization of genetic variants, diagnostic report of fetal results based on phenotype with maternal sample and paternal sample, if performed, as comparators and/or maternal cell contamination
	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
	81349	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities
	81405	Molecular Pathology Procedure Level 6
HCPCS	None	

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