The following Protocol contains medical necessity criteria that apply for this service. It is applicable to Medicare Advantage products unless separate Medicare Advantage criteria are indicated. If the criteria are not met, reimbursement will be denied and the patient cannot be billed. Preauthorization is required. Please note that payment for covered services is subject to eligibility and the limitations noted in the patient’s contract at the time the services are rendered.

Description

Chromosomal microarray (CMA) testing has been proposed for detection of genetic imbalances in infants or children with characteristics of developmental delay/intellectual disability (DD/ID) or autism spectrum disorder (ASD). G-banded karyotyping has for many years been the standard first-line test for this purpose. G-banded karyotyping allows visualization and analysis of chromosomes for chromosomal rearrangements including genomic gains and losses. Chromosomal microarray (CMA) analysis performs a similar, although non-visual, analysis at a much higher resolution. As a result, CMA has the potential to increase the diagnostic yield in this population and change clinical interpretation in some cases.

Next-generation sequencing (NGS) panel testing allows for simultaneous analysis of a large number of genes and has been proposed as a way to identify single gene causes of syndromes that have autism as a significant clinical feature, in patients with normal CMA testing.

Background

Children with signs of neurodevelopmental delays or disorders in the first few years of life may eventually be diagnosed with intellectual disability or autism syndromes, serious and lifelong conditions that present significant challenges to families and to public health. Cases of developmental delay/intellectual disability (DD/ID) and of autism spectrum disorder (ASD) may be associated with genetic abnormalities. For children with clear, clinical symptoms and/or physiologic evidence of syndromic neurodevelopmental disorders, diagnoses are based primarily on clinical history and physical examination, and then may be confirmed with targeted genetic testing of specific genes associated with the diagnosed syndrome. However, for children who do not present with an obvious syndrome, who are too young for full expression of a suspected syndrome, or who may have an atypical presentation, genetic testing may be used as a basis for establishing a diagnosis.

Current guidelines for these patients, such as those published by the American Academy of Pediatrics (AAP) and the American Academy of Neurology (AAN), recommend cytogenetic evaluation to look for certain kinds of chromosomal abnormalities that may be causally related to their condition. The AAN guidelines note that only in occasional cases will an etiologic diagnosis lead to specific therapy that improves outcomes but suggest the
more immediate and general clinical benefits of achieving a specific genetic diagnosis from the clinical viewpoint, as follows (1):

- limit additional diagnostic testing;
- anticipate and manage associated medical and behavioral comorbidities;
- improve understanding of treatment and prognosis; and
- allow counseling regarding risk of recurrence in future offspring and help with reproductive planning.

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

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

Conventional methods of cytogenetic analysis, including karyotyping (e.g., G-banded) and fluorescence in situ hybridization (FISH), have relatively low resolution and a low diagnostic yield (i.e., proportion of tested patients found to have clinically relevant genomic abnormalities), leaving the majority of cases without identification of a chromosomal abnormality associated with the child’s condition. CMA analysis is a newer cytogenetic analysis method that increases the chromosomal resolution for detection of CNVs, and, as a result, increases the genomic detail beyond that of conventional methods. CMA results are clinically informative in the same way as results derived from conventional methods, and thus CMA represents an extension of standard methods with increased resolution. NGS has been proposed to detect single gene causes of autism and possibly identify a syndrome that involves autism in patients with normal array-based testing.

CMA analysis to determine genetic etiology

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 (non-single nucleotide polymorphisms [SNP], see following) cannot detect balanced CNVs (equal exchange of material between chromosomes) or sequence inversions (same sequence is present in reverse base pair order) because the fluorescence intensity would not change.

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

There are some differences in CMA technology, most notably in the various types of microarrays. They can differ first by construction; earliest versions were used of DNA fragments cloned from bacterial artificial chromosomes

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(BAC). These have been largely replaced by oligonucleotide (oligos; short, synthesized DNA) arrays, which offer better reproducibility. Finally, arrays that detect hundreds of thousands of single nucleotide polymorphisms (SNP) across the genome have some advantages as well. Oligo/SNP hybrid arrays have been constructed to merge the advantages of each. Regardless of the array components used, all microarrays allow the deposition of many thousands of short, DNA probe sequences on a small, solid surface in an orderly fashion. The location of each known probe sequence allows the identification of the test sequence bound to it, and when compared to a control sequence, the identification of missing sequences or sequences with extra copies (i.e., copy number variants).

Microarrays may be prepared by the laboratory utilizing 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. (2)

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

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

To determine clinical relevance (consistent association with a disease) of CNV findings, the following actions are taken:

- CNVs are confirmed by another method (e.g., FISH, 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. (3-5)
- A pathogenic etiology is additionally supported when a CNV includes a gene known to cause the phenotype when inactivated (microdeletion) or overexpressed (microduplication). (4)
- 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. (5-8)
- 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. (3, 9)
ACMG has also published guidelines for the interpretation and reporting of CNVs in the postnatal setting, in order to promote consistency among laboratories and CMA results. (10) Three categories of clinical significance are recommended for reporting: pathogenic, benign, and uncertain clinical significance.

In 2008, the International Standards for Cytogenomic Arrays (ISCA) Consortium was organized (Available online at: [https://www.iscaconsortium.org/index.php](https://www.iscaconsortium.org/index.php)); it has 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 individuals with phenotypes including intellectual disability, autism, and developmental delay. As of November 2011, there were over 28,500 total cases in the database. Additional members are planning to contribute data; participating members use an opt-out, rather than an opt-in approach that was approved by the National Institutes of Health (NIH) and participating center institutional review boards. The database is held at NCBI/NIH (National Center for Biotechnology Information/National Institutes of Health) and curated by a committee of clinical genetics laboratory experts. A 2012 update from the ISCA summarizes their experience as a model for ongoing efforts to incorporate phenotypic data with genotypic data to improve the quality of research and clinical care in genetics. (11)

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

The Consortium 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 the ISCA and other public databases) that describe how well or how poorly detected mutations or CNVs are correlated with phenotype. The consortium will apparently coordinate a volunteer effort to describe the evidence for targeted regions across the genome.

The consortium 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.

**Commercially available tests**

CMA: CMA testing is commercially available through many laboratories. The following list is not comprehensive.

Signature genomics offers a postnatal microarray (SignatureChip®OS) and a prenatal microarray (Signature PrenatalChip®TE). Both microarrays target over 245 clinically recognized genetic syndromes; these syndromes are listed on their website. SNP microarray analysis can be ordered to run concurrently with either the prenatal or postnatal microarray.

GeneDx’s GenomeDx is a whole genome array intended for postnatal cases. It also contains SNP probes and also targets at the exon level 65 genes associated with neurodevelopmental disorders.

GeneDx has a Prenatal Targeted Array, enriched in 100 regions associated with common or novel microdeletion and microduplication syndromes, and also contains SNP probes.

NGS: Emory Genetics Laboratory offers a NGS ASD panel of 61 genes that target genetic syndromes that include autism or autistic features. These genes have been associated with nonsyndromic autism and genes associated with conditions involved in the differential diagnosis of Rett syndrome and/or Angelman syndrome. The panel is
offered as tier 2 testing after tier 1 cytogenetics, molecular and biochemical testing which includes array testing, fragile X CGG repeat analysis and biochemical testing for some metabolic conditions.

Greenwood Genetics Center offers a NGS panel that includes 62 genes and flanking introns. The panel includes autosomal and X-linked genes that represent the most common single gene etiologies associated with a syndrome that includes autism as a significant clinical feature. The test is offered as an option for patients with syndromal autism and normal cytogenetic/array-based testing, or as a second tier test for patients with a phenotype that resembles Rett or Angelman syndrome.

Both the Emory and Greenwood Genetics panels use RainDance technology, and the Greenwood Lab panel was developed jointly with Emory.

The Department of Genetics and Genomic Sciences at the Mount Sinai School of Medicine offers a 30-gene sequencing panel.

**Regulatory Status**

CMA analysis is commercially available from several laboratories as a laboratory-developed test. Laboratory-developed tests performed by laboratories licensed for high complexity testing under the Clinical Laboratory Improvement Amendments (CLIA) do not require U.S. Food and Drug Administration (FDA) clearance for marketing.

At a meeting hosted by the FDA in July 2010, the FDA indicated that the Agency will in the future require microarray manufacturers to seek clearance in order to sell their products for use in clinical cytogenetics.

On January 17, 2014, FDA cleared for marketing the Affymetrix CytoScan® Dx Assay. FDA reviewed the Affymetrix CytoScan Dx Assay through its de novo classification process. For the de novo petition, FDA’s review of the CytoScan Dx Assay included an analytic evaluation of the test’s ability to accurately detect numerous chromosomal variations of different types, sizes, and genome locations when compared with several analytically validated test methods. FDA found that the CytoScan Dx Assay could analyze a patient’s entire genome and adequately detect chromosome variations in regions of the genome associated with intellectual and developmental disabilities.

**Policy (Formerly Corporate Medical Guideline)**

**Testing in children**

Chromosomal microarray analysis may be considered **medically necessary** for diagnosing a genetic abnormality in children with apparent nonsyndromic cognitive developmental delay/intellectual disability (DD/ID) or autism spectrum disorder (ASD) according to accepted Diagnostic and Statistical Manual of Mental Disorders-IV criteria when all of the following conditions are met (see Policy Guidelines for definitions):

- Any indicated biochemical tests for metabolic disease have been performed, and results are non-diagnostic, and
- *FMR1* gene analysis (for Fragile X), when clinically indicated, is negative, and
- In addition to a diagnosis of nonsyndromic DD/ID or ASD, the child has one or more of the following:
  - two or more major malformations, or
  - a single major malformation or multiple minor malformations, in an infant or child who is also small-for-dates, or
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- a single major malformation and multiple minor malformations, and
- The results for the genetic testing have the potential to impact the clinical management of the patient, and
- Testing is requested after the parent(s) have been engaged in face-to-face genetic counseling with a healthcare professional who has appropriate genetics training and experience.

Chromosomal microarray analysis is considered investigational in all other cases of suspected genetic abnormality in children with developmental delay/intellectual disability or autism spectrum disorder.

Chromosomal microarray analysis to confirm the diagnosis of a disorder or syndrome that is routinely diagnosed based on clinical evaluation alone (see Policy Guidelines) is not medically necessary.

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

Prenatal Testing

Chromosomal microarray analysis is considered investigational for prenatal genetic testing.

Policy Guideline

Definitions, from the American College of Medical Genetics Guideline, Evaluation of the Newborn with Single or Multiple Congenital Anomalies (12):

- A malformation refers to abnormal structural development.
- A major malformation is a structural defect that has a significant effect on function or social acceptability. Examples: ventricular septal defect or a cleft lip.
- A minor malformation is a structural abnormality that has minimal effect on function or societal acceptance. Examples: preauricular ear pit or partial syndactyly (fusion) of the second and third toes.
- A syndrome is a recognizable pattern of multiple malformations. Syndrome diagnoses are often relatively straightforward and common enough to be clinically recognized without specialized testing. Examples include Down syndrome, neural tube defects and achondroplasia. However, in the very young, or in the case of syndromes with variable presentation, confident identification may be difficult without additional testing.

In some cases of CMA analysis, the laboratory performing the test confirms all reported CNVs with an alternative technology such as fluorescence in situ hybridization (FISH) analysis.

Services that are the subject of a clinical trial do not meet our Technology Assessment Protocol criteria and are considered investigational. For explanation of experimental and investigational, please refer to the Technology Assessment Protocol.

It is expected that only appropriate and medically necessary services will be rendered. We reserve the right to conduct prepayment and postpayment reviews to assess the medical appropriateness of the above-referenced procedures. Some of this Protocol may not pertain to the patients you provide care to, as it may relate to products that are not available in your geographic area.
References

We are not responsible for the continuing viability of web site addresses that may be listed in any references below.


40. Available online at:


