Regence

Medical Policy Manual

**Topic:** Genetic Testing, including Chromosomal Microarray Analysis (CMA) and Next Generation Sequencing Panels, for the Genetic Evaluation of Patients with Developmental Delay/Intellectual Disability or Autism Spectrum Disorders

**Date of Origin:** August 2010

**Section:** Genetic Testing

**Last Reviewed Date:** April 2014

**Policy No:** 58

**Effective Date:** July 1, 2014

**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**

Chromosomal microarray (CMA) testing has been proposed for detection of genetic imbalances in infants or children with characteristics of developmental delay/intellectual disability or autism spectrum disorders. 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. 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 mental retardation 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 disorders (ASDs) 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. 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, which are 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 and fluorescence in situ hybridization (FISH), have relatively low resolution and a low diagnostic yield, leaving the majority of cases without identification of a chromosomal abnormality associated with the child’s condition. NGS detects single gene causes of autism, and may identify a syndrome that involves autism in patients with normal array-based testing.

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-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. 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 post-natal setting. [3] 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 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 the clinical relevance (consistent association with a disease) of CNV findings, the following actions are taken:

3 – GT58
• CNVs are confirmed by another method (e.g., FISH, multiplex ligation-dependent probe amplification (MLPA), polymerase chain reaction (PCR)).

• CNVs detected are checked against public databases and, if available, against private databases maintained by the laboratory. Known pathogenic CNVs associated with the same or similar phenotype as the patient are assumed to explain the etiology of the case; known benign CNVs are assumed to be nonpathogenic.\[4-6\]

• A pathogenic etiology is additionally supported when a CNV includes a gene known to cause the phenotype when inactivated (microdeletion) or overexpressed (microduplication).\[6\]

• 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.\[4,7-9\]

• 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.\[5,10\]

ACMG has also published guidelines for the interpretation and reporting of CNVs in the post-natal setting, in order to promote consistency among laboratories and CMA results.\[2\] Three categories of clinical significance are recommended for reporting: pathogenic, benign, and uncertain clinical significance.

**Regulatory Status**

CMA and NGS analysis are 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.

**Commercially Available Tests**

**CMA**

• 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.

• The FDA cleared for marketing the Affymetrix CytoScan® Dx Assay. The FDA reviewed the Affymetrix CytoScan Dx Assay through its de novo classification process. For the de novo petition, the FDA’s review of the CytoScan Dx Assay included an analytical evaluation of the test’s ability to accurately detect numerous chromosomal variations of different types, sizes, and genome locations when compared to several analytically validated test methods. The 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.

**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 non-syndromic 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 2nd 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.

### MEDICAL POLICY CRITERIA

I. 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 V criteria when all of the following conditions are met:

A. When clinically indicated, FMR1 gene analysis (for Fragile X) is negative; and

B. In addition to a diagnosis of nonsyndromic DD/ID or ASD, the child has one or more of the following malformations (see Policy Guidelines for definitions):

   1. Two or more major malformations; or

   2. A single major malformation or multiple minor malformations in an infant who is also small-for-dates; or

   3. A single major malformation and multiple minor malformations; and

C. The results for the genetic testing have the potential to impact the clinical management of the patient; and

D. 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
expertise.

II. 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.

III. Chromosomal microarray analysis to confirm the diagnosis of a disorder or syndrome that is routinely diagnosed based on clinical evaluation alone is **not medically necessary**.

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

V. Chromosomal microarray analysis is considered **investigational** for **prenatal** genetic testing.

**POLICY GUIDELINES**

Definitions, from the American College of Medical Genetics Guideline, Evaluation of the Newborn with Single or Multiple Congenital Anomalies:[11]

- A malformation refers to abnormal structural development.
- A major malformation is a structural defect that has a significant effect on function or social acceptability. Example: 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.

**SCIENTIFIC EVIDENCE**

This policy is based on a BlueCross BlueShield Association Technology Evaluation Center (TEC) Special Report on array comparative genomic hybridization.[12] Since that Report was written, the technology has rapidly increased in resolution, and chromosomal microarray has become the term of general use to accommodate all variations in the technology. Increased resolution arrays have been quickly translated to clinical services with a resulting increase in diagnostic yield, but also an increase in the potential for results of undetermined significance. Surveys conducted 2 to 3 years ago indicated that there is a lack of consensus between laboratories in the interpretation and reporting of CNVs, particularly those that are challenging.[13] The International Standards for Cytogenomic Arrays (ISCA) Consortium database now offers increased standardization and classification of CNVs that have been previously reported, and should improve consensus in reporting.

**Diagnosis of Developmental Delay/Intellectual Disability or Autism Spectrum Disorders**
The diagnosis of developmental delay (DD) is reserved for children younger than age 5 years who have significant delay in two or more of the following developmental domains: gross or fine motor, speech/language, cognitive, social/personal, and activities of daily living. The diagnosis implies DD that may be significant and may predict life-long disability, although not all children diagnosed with DD will later be diagnosed with intellectual disability (ID).

Intellectual disability is a life-long disability diagnosed at or after age 5 when intelligence quotient (IQ) testing is considered valid and reliable. The Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association (DSM-V), defines patients with ID as having an IQ less than 70, onset during childhood, and dysfunction or impairment in more than two of areas of adaptive behavior or systems of support.

Autism spectrum disorder (ASD) is defined by a persistent impairment in reciprocal social communication and social interaction, and restricted, repetitive patterns of behavior, interests, or activities. The symptoms of ASD are present from early childhood and limit or impair everyday functioning. Autism spectrum disorder includes disorders previously referred to as early infantile autism, childhood autism, Kanner’s autism, high-functioning autism, atypical autism, pervasive developmental disorder not otherwise specified, childhood disintegrative disorder, and Asperger’s disorder. Many individuals with ASD also have intellectual impairment and/or language impairment, and some have motor deficits, as outlined by the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders (DSM-V), 5th Edition.

Some children present with features of both DDID and of ASDs. For example, Yeargin-Allsopp et al reported that nearly 70% of children with a validated diagnosis of ASDs, sampled from 5 metropolitan Atlanta counties, had cognitive impairment. The evaluation pathway depends on the pediatrician, consulting specialists, and their consensus on the primary neurodevelopmental diagnosis.

**Post-natal CMA Analysis**

Several studies (see Appendix B in reference 8) have conducted CMA analysis on samples with known chromosomal abnormalities by standard karyotyping. In general, currently available CMA clinical services achieve near 100% sensitivity for known chromosomal abnormalities. False-positive rates (i.e., CNVs of undetermined clinical significance) on known normal samples were inconsistently reported and could not be summarized. One study evaluated the analytic validity of an oligo array and reported 99% sensitivity and 99% specificity with a resolution of 300–500 Kb for 10 selected cases with different known chromosomal abnormalities.

Several studies reported the diagnostic yield of CMA analysis in DD/ID or ASDs patients with a normal standard karyotype and in several cases normal FMR1 gene analysis and/or subtelomere FISH screening (see Appendix C in reference 8). Overall, diagnostic yield ranged from 5% to 16.7% in DD/ID patients and from 3.4% to 11.6% in patients with ASDs; for this comparison, studies differed considerably in array resolution and in patient selection criteria. This compares well with a synthesis of studies recently published by the ISCA consortium, reporting an average diagnostic yield of 12.2% across 33 studies. Hochstenback et al. reported a CMA diagnostic yield of 19% for 36,325 DD/ID cytogenetic referrals in the Netherlands; and Shen et al. reported a 7% diagnostic yield among 933 ASD referrals. Cooper et al. studied CMA analyses from over 15,000 individuals with DD/ID, ASDs, and/or various congenital abnormalities and compared them to CMA analyses from over 8,000 unaffected controls, finding a significant excess of large CNVs among cases compared to controls. Using a common cutoff for CNV size, about 26% of cases had a CNV larger than 400 kilobases (kb) compared to about 12% of...
controls, suggesting that CNVs of this size account for approximately 14% of cases. CNVs larger than 400 kb were also significantly more common among cases with multiple congenital abnormalities.

Since the introduction of CMA analysis in about 2005, 18 new genomic disorders have been described, more than doubling the number of disorders described in the previous 20 years.[21] Using CMA in place of conventional cytogenetic testing would have missed 0.6-0.8% of all cases, i.e., those with balanced translocations.[18,22]

A portion of the increased diagnostic yield from CMA analysis comes from the discovery that some chromosomal rearrangements that appear balanced (and therefore not pathogenic) by G-banded karyotype analysis are found to have small imbalances with greater resolution. It has been estimated that 40% of apparently balanced de novo or inherited translocations with abnormal phenotype are associated with cryptic deletion if analyzed by CMA.[23] This contradicts earlier assumptions about inherited, apparently balanced rearrangements and shows that microarray analysis can allow for a less subjective and more accurate interpretation of an abnormal banding pattern.[24]

Neither standard cytogenetic analysis nor CMA analysis have been systematically studied for impact on clinical outcomes other than diagnosis[25,26]; Schaefer and Mendelsohn[27] acknowledge, for example, that a genetic diagnosis “typically will not change interventions for the [autism] patient.” Rather, clinical utility of genetic testing is primarily inferred based on the value of diagnosis to the family, estimation of recurrence risk, and on the importance of early detection and early intervention.[26] Two studies indirectly addressed clinical outcomes other than diagnosis as a result of aCGH testing:

- Saam et al.[28] interviewed 14 physicians (2 neurologists, 12 medical geneticists) regarding management changes as a result of positive CMA test results from the University of Utah Cytogenetics Laboratory for 48 patients with DD or ID and normal karyotypes. Only 29% of patients had no management changes reported. For significant proportions of patients, the diagnostic odyssey was ended. However, this study was only a survey and did not attempt to quantitate the diagnostic tests avoided. The authors also reported that 14.6% of patients with genetic diagnoses were referred to medical specialists, and 25% had improved access to insurance and educational services, but the study did not assess the benefits of specialist referrals or screening for comorbidities on patient outcomes, or describe and quantitate the improvement in access to community services.

- Coulter et al. identified and reviewed, over the course of one year, the medical records of all patients at a tertiary children’s hospital who had CMA results showing an abnormal variant or a variant of possible significance.[29] A Board-certified medical geneticist reviewed the clinical notes from the ordering provider and abstracted recommendations for clinical actions (a specialist referral, imaging study, diagnostic test, or medication prescription) made specifically as a result of the CMA result. Of 1792 patients for whom CMA was ordered during the year reviewed, 131 had an abnormal variant and 104 had a variant of possible significance. Of these, 121 and 73 patients were included in the analysis. Overall, patients with an abnormal variant had a significantly higher rate of recommended clinical action (54%) than patients with a variant of possible significance (34%; p=0.01). Among patients with an abnormal variant and a diagnosis of DD/ID or congenital anomalies, about two-thirds of patients were referred for additional clinical action based on the CMA results, whereas referrals were made for 27% of patients with ASDs and an abnormal variant. Referral rates were similar for patients with a CMA result of a variant of possible significance, with the exception of patients with congenital anomalies, who were referred for additional clinical action only 17% of the time. Patients younger than 2 years were significantly more likely to have clinical anomalies and were significantly more likely to
have abnormal variants. Cases were described in which ancillary CMA results suggested clinical interventions for the present or future regarding possible co-morbid conditions. In no patients, however, were referrals linked to actual patient outcomes; the authors report that this study is ongoing.

Risk estimates for recurrence of disease in future births can be altered considerably by information from the genetic diagnosis. For example, the average sibling recurrence risk in ASDs is 5%.[30] However, if the cause is a dominant single gene disorder with full penetrance and a parent is a carrier, the sibling risk is 50%. If the disorder is recessive but characteristics are otherwise the same, the sibling risk is 25%. If the cause is Fragile X, the recurrence risk in a brother is 50%, while a sister may be only mildly affected but will have a carrier risk of up to 50%. However, in the case of a de novo CNV (i.e., not carried by either parent), the sibling risk remains low, at the population average.

Knowledge of recurrence risk is expected to lead to improved future reproductive decision-making in families with children affected with DD/ID or ASD associated with specific mutations. Turner et al.[31] studied the reproductive decisions of women from 38 families characterized by male members with mental retardation and a pattern consistent with chromosome X-linked transmission. Most of the women in these families spent many years knowing that they were at some risk of being carriers and of having a boy with ID. Prior to the availability of pathogenic mutation analysis, the birth rate for these families was below average for the district (United Kingdom-New South Wales), 1 in 27 versus 1 in 11 per year, respectively. After pathogenic mutation status was determined, both carriers and non-carriers (previously thought to be at risk) of the mutation had children at same rate with 74% of carriers choosing prenatal genetic evaluation. While the results of this study are suggestive, they do not show that knowledge of recurrence risk directly affected reproductive decisions. Saam et al.[28], in the survey described previously, reported that recurrence risk evaluation was possible in about one-third of families after positive aCGH results, but did not study the impact of recurrence risk evaluation on reproductive planning.

As noted in the Description, guidelines emphasize the importance of cytogenetic evaluation to look for certain kinds of mutations that may be linked to specific conditions for early diagnosis and intervention. However, the benefits of early intervention for these disorders are uncertain. Few randomized trials have been conducted and the interventions differ considerably, indicating that the field is still early in researching the critical elements of effective early intervention. For well-characterized genetic syndromes, it is important to incorporate monitoring for co-morbidities known to be associated with the condition. For example, 22q11 microdeletion syndrome (includes diGeorge and velo-cardio-facial syndromes) is associated with development of hearing impairment in a significant proportion of patients, with subsequent delayed speech.[32] Velo-cardio-facial syndrome is also associated with heart defects. (30) Klinefelter syndrome may first be detected as developmental delay in early childhood; androgen treatment is an important component of therapy.[30]

Ellison and colleagues reported on the clinical utility of CMA in a total of 46,298 postnatal patients.[33] Testing was for a variety of indications, including intellectual disability/developmental delay, congenital anomalies, dysmorphic features and neurobehavioral problems. The authors tallied the detection of abnormalities associated with actionable clinical features (i.e., diagnoses which would likely lead to changes in clinical management). A total of 2,088 diagnoses were made of 118 clinically actionable disorders; of these, it was estimated that 94% would likely have been missed by routine karyotyping. Examples of clinically actionable responses to the diagnoses included an electrocardiogram and cardiology referral for those at risk for long QT syndrome, glucose monitoring and endocrine referral for those at increased risk of diabetes, renal ultrasound for those at risk for renal pathology and platelet
count monitoring for those at risk for thrombocytopenia. A subset of cases was monitored for physician response to the microarray finding, and appropriate clinical action was taken more than 90% of the time.

Prenatal CMA Analysis

Prenatal fetal karyotyping is a routine test when the fetus is believed to be at high risk for a chromosomal abnormality as a result of a structural abnormality identified during an ultrasound exam, because of family history, or for other reasons agreed on by the patient and physician. However, karyotyping provides useful information in only a small percentage of these cases. Consistent with the increased diagnostic yield of CMA analysis, many laboratories are now providing this service in the prenatal setting. Currently, the microarrays used in this setting are most often targeted arrays, to reduce the number of results of uncertain significance and thus reduce parent anxiety and difficulties in decision-making. However, whole-genome analysis is also available.

Hillman et al. conducted a prospective cohort study and systematic review and meta-analysis.[34] The cohort study involved 243 women undergoing CMA 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 variant of unknown significance rate of 2.1% (95% CI, 1.3-3.3%). The meta-analysis included studies through December 2012, that reported on prenatal microarray testing that were performed for any indication and was not limited to cases referred for abnormal fetal ultrasound findings. Twenty-five studies were included, 17 of which were not included in their 2011 systematic review.[35] The detection rate in the meta-analysis was 10% (95% CI, 8-13) with a variant of unknown significance rate of 1.4% (95% CI, 0.5-3.7%).

Hillman et al. conducted a systematic review and meta-analysis of studies reporting CMA analysis results in the prenatal setting or in the immediate post-natal setting following pregnancy termination for structural abnormalities detected by ultrasound.[35] A total of 751 participants in 8 studies were included for the overall meta-analysis; 409 of these had fetal anomalies using ultrasound. Overall, CMA analysis detected 3.6% more chromosomal imbalances than karyotyping when CMA results of unknown significance were included (1.1%). The CMA excess detection rate was higher in those with fetal anomalies by ultrasound, at 5.2% including results of unknown significance (1.9%). CMA analysis failed to detect one case of triploidy, and, as would be expected of the standard CMA technology, also failed to detect 14 cases of balanced translocations. The authors noted the benefit of the additional detection by CMA but also the increase in results of unknown significance, and discuss the difficulties of interpretation in conjunction with prenatal decision-making. In recognition of the limitations and disadvantages of CMA in the prenatal setting, the American Congress of Obstetricians and Gynecologists published a Committee Opinion in November, 2009, recommending against CMA as a replacement for classic cytogenetics.[36]

Wapner et al. conducted a prospective study to evaluate the accuracy, efficacy and incremental yield of CMA as compared with karyotyping for routine prenatal diagnosis.[37] 4,406 women who were undergoing routine prenatal diagnosis in one of 29 diagnostic centers by either CVS or amniocentesis had a sample split in two for standard karyotyping and CMA. Indications for prenatal diagnosis included AMA (46.6%), a positive aneuploidy screening result (18.8%), structural anomalies detected by U/S (25.2%) and other indications (9.4%). CMA analysis was successful in 98.8% of the fetal samples. Authors suggest that CMA identified clinically significant cytogenetic information as compared with karyotyping and was equally efficacious in identifying aneuploidies and unbalanced rearrangements but did not identify balanced translocations and triploidies.
Breman et al. evaluated the prenatal CMA results on greater than 1,000 fetal samples sent for testing at Baylor College of Medicine Medical Genetics Laboratories received between 2005 and 2011.[38] Results were obtained in 1,115 samples. Parental samples were obtained concurrently to exclude maternal cell contamination and assist interpretation of copy number variations. In 881 (79%) of the samples, no deletions or duplications were observed using prenatal CMA analysis. Copy number changes were detected in 234 (21%) cases. Eighty-five cases (7.6%) were found to have clinically significant genomic imbalances. Authors suggest from this study that the detection rate of CMA for prenatal chromosomal abnormalities exceed that of conventional karyotype analysis and continues to improve with higher resolution arrays, while maintaining a low frequency of results of unclear clinical significance.

In summary, in prenatal specimens, targeted or lower resolution arrays have the ability to detect the majority of clinically relevant alterations while maintaining a low rate of results with unclear significance. However, this is associated with an increased risk of missing a pathogenic abnormality in a region not covered sufficiently by a targeted array. In addition, many of the known genomic disorders that can be detected on targeted arrays are not associated with readily detectable fetal abnormalities on prenatal ultrasound examinations. Higher resolution arrays have a greater chance of detecting a larger number of alterations but also will have more results of unknown clinical significance.

Next Generation Sequencing (NGS)

No peer-reviewed, full-length publications of the commercially available NGS ASD panels were identified. Without data from published studies, it is not possible to determine the following:

- Analytic validity (technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent),
- Clinical validity (the diagnostic performance of the test [sensitivity, specificity, positive and negative predictive values] in detecting clinical disease), or
- Clinical utility (how test results are used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes).

Further, no published data on the rate of variants of unknown significance using NGS panels for autism have been identified.

Clinical Practice Guidelines

American Academy of Neurology and the Practice Committee of the Child Neurology Society

The American Academy of Neurology and the Practice Committee of the Child Neurology Society Evidence Report concludes that “microarray is the genetic test with the highest diagnostic yield in children with unexplained DD/ID” (based on Class III studies). In addition, the report notes that microarray testing can identify only CNVs and is insufficiently sensitive for detecting disorders caused by other mechanisms (e.g., inversions, balanced insertions, polyploidy etc.). Finally, per the report the often complex results of this testing require confirmation and careful interpretation, often with the assistance of a medical geneticist.[1] The guidelines acknowledge that “Research is sorely lacking on the medical, social, and financial benefits of having an accurate etiologic diagnosis.”

American College of Medical Genetics (ACMG)
ACMG published guidelines on array-based technologies and their clinical utilization for detecting chromosomal abnormalities.\(^{[39]}\) The recommendations are based on a limited review of evidence; the findings from a handful of publications are reported, however, study selection criteria are not included and included publications are not critically appraised. Per the guidelines, CMA testing for copy number variation is recommended as a first-line test in the initial postnatal evaluation of individuals with the following:

- Multiple anomalies not specific to a well-delineated genetic syndrome
- Apparently non-syndromic developmental delay/ intellectual disability
- Autism spectrum disorders

ACMG recommends against use of CMA in cases of multiple miscarriages.

The guideline warns that the clinicians need to be aware of the “different clinical platforms, the variation in resolution among arrays and information each provides.” Also, the guideline notes the limitations of CMA testing (e.g., cannot identify balanced chromosomal rearrangements such as translocations or inversions). Additional ACMG guidelines have been published for the design and performance expectations for clinical microarrays and associated software\(^{[33]}\) and for the interpretation and reporting of CNVs,\(^{[2]}\) both intended for the post-natal setting.

A 2013 guidelines update from the ACMG states that a stepwise or tiered approach to the clinical genetic diagnostic evaluation of autism spectrum disorder is recommended, with the recommendation being for first-tier to include FXS and CMA, and second tier to include MECP2 and PTEN testing\(^{[40]}\). The guideline states that “this approach will evolve with continued advancements in diagnostic testing and improved understanding of the ASD phenotype. Multiple additional conditions have been reported in association with an ASD phenotype, but none of these has been evaluated in a large prospective cohort. Therefore, a future third tier of evaluation is a distinct possibility. Further studies would be needed to elevate the evidence to the point of recommended testing. Alternatively, advances in technology may permit bundling of individual tests into an extended, more readily accessible, and less expensive platform”. The accumulating evidence using next-generation sequencing (third tier testing) “will increase the diagnostic yield even more over the next few years.”

American Congress of Obstetricians and Gynecologists (ACOG)

In 2013, ACOG and the Society for Maternal-Fetal Medicine offered the following recommendations for the use of chromosomal microarray analysis in prenatal diagnosis:\(^{[41]}\)

- In patients with a fetus with one or more major structural abnormalities identified on ultrasonographic examination and who are undergoing invasive prenatal diagnosis, chromosomal microarray analysis is recommended. This test replaces the need for fetal karyotype.
- In patients with a structurally normal fetus undergoing invasive prenatal diagnostic testing, either fetal karyotyping or a chromosomal microarray analysis can be performed.
- Most genetic mutations identified by chromosomal microarray analysis are not associated with increasing maternal age; therefore, the use of this test for prenatal diagnosis should not be restricted to women aged 35 years and older.
- In cases of intrauterine fetal demise or stillbirth when further cytogenetic analysis is desired, chromosomal microarray analysis on fetal tissue (i.e., amniotic fluid, placenta, or
products of conception) is recommended because of its increased likelihood of obtaining results and improved detection of causative abnormalities.

- Limited data are available on the clinical utility of chromosomal microarray analysis to evaluate first-trimester and second-trimester pregnancy losses; therefore, this is not recommended at this time.

**International Standard Cytogenomic Array (ISCA) Consortium**

The ISCA Consortium published a Consensus Statement in which they recommend offering CMA as the first-tier genetic test, in place of G-banded karyotype, for patients with unexplained DD/ID, ASDs, or multiple congenital anomalies (MCA).[9] However, the guideline also acknowledges that CMA is still not widely accepted. “Except in special cases, such as those involving family history of multiple miscarriages, a karyotype is not cost effective in a child with DD/ID, ASDs, or MCA and a negative array study. CMA testing is not inexpensive, but the cost is less than the cost of a G-banded karyotype plus a customized FISH test such as subtelomeric FISH, and the yield is greater.”

**Summary**

**Chromosomal Microarray Analysis for the Evaluation of Children**

Evidence on the clinical benefit of CMA testing is largely anecdotal. Cases have been documented in which the information derived from testing ends a long diagnostic odyssey, aids in planning for surveillance or management of associated comorbidities, and assists in future reproductive decision-making. While systematic studies of the impact of CMA analysis on patient outcomes are lacking, the improvement in diagnostic yield has been demonstrated, and feedback from physician specialty societies, academic medical centers, and in respected guidelines is consistent in supporting the clinical benefit of CMA testing for defined populations. As a result, CMA may be considered medically necessary in individuals with developmental delay, intellectual disabilities or autism spectrum disorders who meet the policy criteria.

**Prenatal Chromosomal Microarray Analysis**

When used in prenatal cases where there is an abnormality detected on ultrasound and a normal karyotype, CMA testing will detect clinically relevant abnormalities in a small percentage of cases. However, the incremental benefit in health outcomes that results from detecting such abnormalities in the prenatal period is not clear. For routine screening of pregnant women, the yield of abnormal findings is less and how patient management is benefited as a result of CMA testing in detecting chromosomal abnormalities in prenatal specimens is unknown. The potential risk for findings of uncertain clinical significance may result in parental anxiety and challenges in genetic counseling. Therefore, the use of CMA analysis in the prenatal setting is considered investigational.

**Next Generation Sequencing Panels**

Published data on analytic and clinical validity, clinical utility and variants of unknown significance using next generation sequencing (NGS) panels in this setting are lacking. Therefore, 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.

**REFERENCES**


42. BlueCross BlueShield Association Medical Policy Reference Manual "Chromosomal Microarray Analysis (CMA) for the Genetic Evaluation of Patients with Developmental Delay/Mental Retardation or Autism Spectrum Disorder." Policy No. 2.04.59

CROSS REFERENCES

*Genetic Testing for FMR1 mutations (including Fragile X Syndrome)*, Genetic Testing, Policy No. 43