Medical Policy Manual

**Topic:** Chromosomal Microarray Analysis (CMA) and Next-generation Sequencing Panels, for the Genetic Evaluation of Patients with Developmental Delay/Intellectual Disability, Autism Spectrum, or Congenital Disorders  
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**Section:** Genetic Testing  
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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. CMA increases the diagnostic yield over karyotyping in this population and may impact clinical management decisions.

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. 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. Therefore, 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.

Background
Developmental Delay/Intellectual Disability and Autism Spectrum Disorders

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.

The diagnosis of developmental delay (DD) is reserved for children younger than 5 years of age 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.[1]

Intellectual disability (ID) 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.[2]

Some children present with features of both DD/ID and of ASDs. For example, Yeargin-Allsopp et al[3] 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.

Diagnostic Testing to Determine Genetic Etiology

Most commonly, genetic abnormalities associated with neurodevelopmental disorders are deletions and duplications of large segments of genomic material, called copy number variants (CNVs). For many well-described syndromes, the type and location of the chromosomal abnormality have been established with the study of a large number of cases and constitute 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.

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Conventional methods of cytogenetic analysis, including karyotyping (e.g., G-banded) and FISH, have relatively low resolution and a low diagnostic yield (i.e., proportion of tested patients found to have clinically relevant genomic abnormalities), leaving most cases without identification of a chromosomal abnormality associated with the child’s condition.

**Chromosomal Microarray**

Chromosomal microarray (CMA) 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.

CMA can identify genomic abnormalities that are associated with a wide range of developmental disabilities, including cognitive impairment, behavioral abnormalities and congenital abnormalities. CMA can detect copy number variants (CNVs) and the frequency of disease-causing CNVs is highest (20-25%) in children with moderate to severe intellectual disability accompanied by malformations or dysmorphic features. Disease-causing CNVs have been identified in 5-10% of cases of autism, being more frequent in severe phenotypes.[4]

The term chromosomal microarray (CMA) collectively describes two different array platforms: array comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) arrays. Both types of arrays can identify loss or gain of DNA (microdeletions or microduplications, respectively), known as copy number variants (CNVs).

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.[5] 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.[6]

**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**

CMA testing is commercially available through many laboratories and includes targeted and whole-genome arrays, with or without SNP microarray analysis.

- 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.

- FirstStepDx PLUS uses Lineagen’s custom-designed microarray platform manufactured by Affymetrix. This microarray consists of 1,953,246 unique nonpolymorphic probes and 743,304 SNP probes that come standard on the Affymetrix CytoScan HD® microarray, with an additional 83,443 custom probes designed by Lineagen. FirstStepDx PLUS is an integrated service that combines proprietary genetic testing, reporting, and genetic counseling.

- Ambry Genetics offers a 180 K oligo array and a combined SNP + CGH array and states that the tests should be considered for all individuals with syndromic or non-syndromic conditions that may be caused by genomic imbalance.

- LabCorp offers the Reveal SNP microarray-Pediatric and states that the test is intended for individuals with non-syndromic congenital anomalies, dysmorphic features, DD, mental retardation, ID and/or ASD.

**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**

Note: This policy applies to “reflex” chromosomal microarray analysis (reflex CMA).

I. Chromosomal microarray analysis may be considered **medically necessary** in children and adolescents (17 years or younger) when any of the following conditions are met:

   A. Apparent nonsyndromic cognitive developmental delay/ intellectual disability (DD/ID); or

   B. Autism spectrum disorder (ASD); or

   C. Multiple congenital anomalies not specific to a well-delineated genetic syndrome. Congenital anomaly is defined as an anomaly that is present at birth.

II. Chromosomal microarray analysis may be considered **medically necessary** in patients undergoing invasive diagnostic prenatal (fetal) testing as an alternative to karyotyping.

III. Chromosomal microarray is considered **not medically necessary** when either of the above criteria (I. or II.) are not met.

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.

**POLICY GUIDELINES**

- It is critical that the list of information below is submitted for review to determine if the policy criteria are met. 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 mutations 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
   - Conventional testing and outcomes
Conservative treatment provided, if any

- Definitions, from the American College of Medical Genetics Guideline, Evaluation of the Newborn with Single or Multiple Congenital Anomalies:[7]
  - 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 was initially based on a BlueCross BlueShield Association Technology Evaluation Center (TEC) Special Report on array comparative genomic hybridization.[8] 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.[9] 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**

**Postnatal 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.[10]

- 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.

- Roberts and colleagues reported their experience with the use of the 105 K and 180K oligonucleotide microarrays in 215 consecutive patients that were referred with either autism or autism spectrum disorders (ASD) or developmental delay/learning disability for genetic services to a single medical center between 2009 and 2012. Of the 215 patients (140 males and 75 females), 65 had ASD and 150 had a learning disability. Abnormal microarray results were found in 45 patients (21%) with a total of 49 CNVs. Thirty two represented a known diagnostic CNV contributing to the clinical presentation and 17 represented variants of unknown significance. Thirteen out of 65 patients (20%) with ASD had a CNV compared with 32 out of 150 patients (21%) with a learning disability. The thirteen patients with ASD had a total of 14 CNVs, 6 recognized as diagnostic and 8 as non-diagnostic. For those patients with a learning disability, 32 had a total of 35 CNVs, 26 of which were classified as a known diagnostic CNV [usually a deletion; n=20], and 9 were classified as an unknown non-diagnostic CNV [usually a duplication; n=8]. A higher percentage of individuals with a learning disability had clinical findings of seizures, dysmorphic features and microcephaly, but this was not statistically significant.

- Lu and colleagues reported on the frequency of genomic imbalances in neonates with birth defects by using 3 different targeted aCGH platforms using bacterial artificial chromosomes. The study included 638 neonates with various birth defects who were referred between March 2006 and September 2007. Overall, 109 (17.1%) patients were identified with clinically significant CNVs, the majority of which would not have been defined by karyotyping. The clinically significant detection rates for various clinical indications were 66.7% for "possible chromosomal abnormality"+/-"others" (other clinical indications), 33.3% for ambiguous genitalia+/-others, 27.1% for dysmorphic features with multiple congenital anomalies+/-others, 24.6% for dysmorphic features+/-others, 21.8% for congenital heart disease+/-others, 17.9% for multiple congenital anomalies+/-others, and 9.5% for patients referred for other indications that were not in the defined groups. In all, of the 109 patients in whom clinically significant genomic imbalances or pathogenic CNVs were detected by CMA, 14.7% had numerical anomalies including trisomy 21, 18, 13, 22 and monosomy X. The remaining 85.3% had genomic imbalances that may not have been detected by standard cytogenetic studies, including 33.9% with common microdeletion or microduplication syndromes, 40.4% with genomic imbalances at relatively rare disease loci and 11.0% with chromosomal mosaicism.

Clinical Utility of CMA Testing

Neither standard cytogenetic analysis nor CMA analysis have been systematically studied for impact on clinical outcomes other than diagnosis; Schaefer and Mendelsohn 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. Two studies indirectly addressed clinical outcomes other than diagnosis as a result of aCGH testing:
Saam et al.\cite{20} 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.\cite{21} 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%.\cite{22} 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.\cite{23} 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.\[20\], 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 section, 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 velocardio-facial syndromes) is associated with development of hearing impairment in a significant proportion of patients, with subsequent delayed speech.\[24\] Velo-cardio-facial syndrome is also associated with heart defects.\[22\] Klinefelter syndrome may first be detected as developmental delay in early childhood; androgen treatment is an important component of therapy.\[22\]

- Ellison and colleagues reported on the clinical utility of CMA in a total of 46,298 postnatal patients.\[25\] 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.

A 2015 TEC Special Report on the use of CMA for the genetic evaluation of patients with DD/ID and ASD found the following for the clinical utility of CMA testing:\[26\]:

- Studies on the potential impact of CMA on clinical decisions “collectively indicate that identified pathogenic variants can prompt clinical actions potentially impacting morbidity. Less clear is how often outcomes will be improved and in which cases interventions might have occurred in the absence of testing. The proportion that may benefit will depend on the variants identified as well as diagnostic yield, which in turn depends on phenotype severity. Studies did not report on any follow-up or management changes in patients without identified pathogenic variants. In addition to reducing morbidity, bringing closure to a diagnostic odyssey is a reason for genetic testing … and noted as an outcome in case series and reports. Parents cite obtaining services and support as a reason for testing, but the frequency and can impact on outcomes is difficult to quantify. The studies reviewed convey a set of intermediate outcomes likely to favorably affect the health of some children. Lacking are end-to-end cohort studies following children at presentation to final outcomes.” “There are considerable challenges conducting studies of sufficient size given the underlying genetic heterogeneity, and including follow-up adequate to observe final health outcomes”; however, “studies examining clinical utility have reported intermediate outcomes and indirect evidence.”
“In addition, outside readily recognizable syndromes, pathogenic variants identified represent a collection of rare disorders. Ascertaining improved net health outcome for rare diseases is not easy. Both conditions and outcomes can be heterogeneous. The evidence reviewed here reflects the accompanying uncertainty, but supports a perspective that identifying a pathogenic variant can: (1) impact the search for a diagnosis, (2) inform follow-up that can benefit a child by reducing morbidity and rarely potential mortality, and (3) affect reproductive planning for parents and later potentially the affected child. There are also likely circumstances where other family members may be impacted owing to the nature of the variant and subsequent cascade (family member) testing. The downsides to testing can include detecting nonpaternity, an incorrect diagnosis, and findings of uncertain significance—how often they occur is uncertain. It is difficult to quantify lower or upper bounds for any potential improvement in the net health outcome owing in part to heterogeneity of disorders, rarity, and outcome importance that may differ according to identified variants. The strong expert opinion in recommending initial CMA testing over other approaches … together with the indirect evidence for benefit following testing, supports concluding that the net health outcome can be improved.

“…[A] child with ASD appears to impact reproductive decision making, or so-called reproductive stoppage.” “Whether it can be attributed to concerns over having another affected child or the caregiving burden of the first affected child is unclear. Regardless, quantifying recurrence risk may assist reproductive decision making, particularly given that recurrence risk may be high—e.g., in ASD, as high as 18%. However, establishing a genetic cause may revise the estimated risk considerably….”

Wood et al. analyzed reproductive stoppage and ASD recurrence rates within 2 U.K. family databases—299 families including 660 children (327 diagnosed with ASD).[27] In 10% of families, there was more than 1 ASD-affected child and an estimated 24.7% recurrence risk. Reproductive stoppage was examined by comparing statistically whether children with ASD were born later in families than their unaffected siblings. In 132 of the 180 complete families analyzed, the last-born child was more often affected (p<0.05); 40 families had a single child (affected) and 62 families 2 children with only the second affected. Any potential confounding by maternal or paternal age was not reported.

Prenatal CMA Analysis

The clinical utility of invasive prenatal (fetal) diagnostic testing is determined by how the results of the test would have an impact on management decisions and health outcomes. Prenatal (fetal) testing may be performed for the purpose of anticipatory guidance and management, either during the pregnancy or at the time of delivery.

Clinical management decisions may include the following:

• 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.
• birthing decisions.

Most of the literature on the use of CMA in the prenatal (fetal) setting consists of prospective and retrospective analyses of CMA findings compared with conventional karyotyping, either in patients with known karyotype results or in patients with concurrently performed karyotyping and CMA. CMA has been proposed as being used as either a first tier test (in place of or in conjunction with karyotype) or as a second tier test (after a negative karyotype).
Jansen et al. conducted a systematic review and meta-analysis of the additional diagnostic gain of array comparative genomic hybridization (CGH) compared with standard karyotyping and 22q11 by fluorescence in situ hybridization (FISH) in prenatally diagnosed cardiac malformations. Thirteen studies with 1131 cases of congenital heart disease (CHD) were included, with a literature search through September 2014. A meta-analysis identified an incremental yield of 7.0% (95% confidence interval [CI], 5.3% to 8.6%) for the detection of copy number variants using aCGH, excluding aneuploidy and 22q11 microdeletion cases. A subgroup analysis showed a 3.4% (95% CI, 0.3% to 6.6%) incremental yield in isolated CHD cases, and 9.3% (95% CI, 6.6% to 12%) when extracardiac malformations were present. Overall, an incremental yield of 12% (95% CI, 7.6% to 16%) was found when 22q11 deletion cases were included. The rate of variants of unknown significance (VUS) was 3.4% (95% CI, 2.1% to 4.6%).

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

The reported diagnostic accuracy for karyotyping and CMA was as follows:

- **Karyotyping**: sensitivity of 76.4% (95% CI, 63.0 to 87.0); specificity of 99.9% (99.2 to 99.9); positive predictive value (PPV) of 97.7 (87.7 to 99.9); negative predictive value (NPV) of 98.3 (97.1 to 99.1); and diagnostic accuracy of 98.2 (97.1 to 99.0).
- **CMA**: sensitivity of 98.2 (90.4 to 99.9); specificity of 99.7 (99.1 to 99.9); PPV of 96.5 (87.9 to 99.5); NPV of 99.9 (99.3 to 100); and diagnostic accuracy of 99.7 (99.0 to 99.9).

Shaffer et al. reported the results of microarray testing for prenatal diagnosis in over 5000 prospectively collected prenatal samples received from 2004 to 2011 for a variety of indications. They used CGH microarrays targeted to known chromosomal syndromes with later versions providing backbone coverage of the entire genome. Cases were stratified according to the test result (normal, VUS, abnormal) and indication for the study, and compared with karyotyping results. Of 5003 prenatal specimens, 56% were referred with normal karyotypes, 13% had known abnormal karyotypes, 16% had karyotypes performed concurrently with microarray testing, and 15% had unknown karyotype status. Indications for microarray testing included a known abnormal karyotype (n=648), family history of a parent known to carry a chromosomal rearrangement or imbalance (n=62), fetal demise (n=417), abnormal ultrasound (n=2858) [further detailed in the next study], abnormal first- or second-trimester screen (n=77), other family history of a genetic condition (n=487), AMA (n=346), parental anxiety (n=95), or other/not specified (n=13). The overall detection rate of clinically significant results with microarray testing was 5.3%. The detection rate of clinically significant CNVs was 5.5% among cases with known normal karyotypes. After excluding the cases of fetal demise, the VUS rate was 4.2%, but if only de novo CNVs were considered (the rate was 0.39%).
• Shaffer et al. performed a retrospective analysis of 2858 pregnancies, with abnormal ultrasound findings (as stratified by organ system). Most cases had previously normal karyotypes (n=2052 [72%]). The remaining had karyotyping performed concurrently with microarray testing (n=465 [16%]) or had unknown or failed karyotypes (n=341 [12%]). Ultrasound anomalies were categorized in several ways: multiple structural anomalies, structural anomalies involving a single-organ system, isolated abnormalities of growth, isolated abnormal amniotic fluid volume, single or multiple soft marker(s), or multiple nonstructural anomalies (e.g., IUGR). Soft markers included choroid plexus cysts, echogenic foci in the heart or bowel, isolated short long bones, absent nasal bones, sandal gap between the first and second toes, fifth finger clinodactyly, single umbilical artery, and persistent right umbilical vein. The average maternal age at the time of testing was 31.8 years. Most tests were whole genome, oligoarrays (n=2161 [76%]), and the remaining were bacterial artificial chromosome–based arrays, either with coverage of the whole genome (n=506 [18%]) or targeted coverage (n=191 [7%]). Overall, with microarray testing, 6.5% showed clinically significant results, and 4.8% had VOUS. For the cases with a previously normal karyotype, the detection rate for significant CNVs was similar (6.2%). Clinically significant genomic alterations were identified in cases with a single ultrasound anomaly (n=99/1773 [5.6%]), anomalies in 2 or more organ systems (n=77808 [9.5%]), isolated growth abnormalities (n=276 [2.6%]), and soft markers (n=277 [2.6%]). Certain anomalies, either in isolation or with additional anomalies, had higher detection rates: holoprosencephaly (n=9/85 [10.6%]), posterior fossa defects (n=21/144 [14.6%]), skeletal anomalies (n=15/140 [10.7%]), ventricular septal defect (n=14/132 [10.6%]), hypoplastic left heart (n=11/68 [16.2%]), and cleft lip/palate (n=14/136 [10.3%]).

• Hillman et al. conducted a prospective cohort study and systematic review and meta-analysis. 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. 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. 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.

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

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

In summary, the study included 3822 patients with normal karyotype and the following indications for prenatal diagnosis: AMA (n=1966), positive Down syndrome screen (n=729), anomaly on ultrasound (n=755), and other (n=372). CMA provided additional clinically relevant CNVs (95% CI) and VOUS rate of:

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

Breman et al. 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.[36] 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 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.

**Conclusion**

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 minimalized by the use of targeted arrays, testing phenotypically normal parents for the CNV, and the continued accumulation of pathogenic variants in international databases.
Next-generation Sequencing

No peer-reviewed, publications of the commercially available next-generation sequencing (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. 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 and Genomics

The American College of Medical Genetics and Genomics (ACMG) published guidelines on array-based technologies and their clinical utilization for detecting chromosomal abnormalities. The recommendations are based on a limited review of evidence and the findings are 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[39] and for the interpretation and reporting of CNVs,[40] 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.[41] 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

In 2013, the American Congress of Obstetricians and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine offered the following recommendations for the use of chromosomal microarray analysis in prenatal diagnosis:[42]

- “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 Consortium

The International Standard Cytogenomic Array (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).[11] 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

Evidence on the clinical benefit of chromosomal microarray (CMA) testing primarily consist of large case series. Although systematic studies of the impact of CMA on patient outcomes are lacking, the improvement in diagnostic yield over karyotyping has been well-demonstrated, and studies have documented that the information derived from CMA testing can: end a long diagnostic odyssey, result in a reduction in morbidity for certain conditions with initiation of surveillance or management of associated comorbidities, and may impact future reproductive decision making for parents and potentially the affected child. In addition, the improvement in diagnostic yield has been demonstrated, and clinical practice guidelines from several specialty societies are consistent in supporting the clinical benefit of CMA testing for defined populations. As a result, CMA may be considered medically necessary when specific criteria are met.

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


26. Blue Cross and Blue Shield Association Technology Evaluation Center (TEC). Special Report: Chromosomal Microarray for the Genetic Evaluation of Patients With Global Developmental...
Delay, Intellectual Disability, and Autism Spectrum Disorder. . TEC Assessments. 2015;30:Tab 2. PMID:

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**CROSS REFERENCES**

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

*Genetic and Molecular Diagnostic Testing*, Genetic Testing, Policy No. 20

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<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants for chromosomal abnormalities</td>
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