



# Cigna Medical Coverage Policy

**Subject Comparative Genomic Hybridization Testing (Chromosomal Microarray Analysis) for Autism Spectrum Disorders, Developmental Delay, Intellectual Disability and Multiple or Unspecified Congenital Anomalies**

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## Coverage Policy

**Please refer to the applicable benefit plan document to determine benefit availability and the terms, conditions and limitations of coverage. Under some benefit plans, coverage for genetic screening and/or testing may be excluded or restricted. If coverage for genetic testing is available, the following conditions of coverage apply.**

**Cigna covers comparative genomic hybridization testing (chromosomal microarray analysis) for evaluation of a fetus when conventional cytogenetic testing (e.g., karyotyping) is normal or cannot be performed for EITHER of the following indications:**

- abnormal fetal anatomic findings pathognomonic of a genetic abnormality
- fetal demise with congenital anomalies

**Cigna covers comparative genomic hybridization testing (chromosomal microarray analysis) as medically necessary in a child age 13 years or under for ANY of the following indications:**

- autism spectrum disorder in which the phenotypic characteristics of a specific genetic disorder are absent
- non-syndromic developmental delay or intellectual disability in which the phenotypic characteristics of a specific genetic disorder are absent
- multiple congenital anomalies not specific to a well-delineated genetic syndrome

**All individuals undergoing genetic testing for the above indications should have both pre- and post-test genetic counseling with a board-certified or board-eligible medical geneticist or a licensed or certified genetic counselor.**

**Cigna does not cover comparative genomic hybridization testing (chromosomal microarray analysis) for any other indication, including preimplantation genetic diagnosis (PGD), because it is considered experimental, investigational or unproven.**

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## General Background

Gene technology is advancing rapidly, outpacing clear indications for when new or emerging technologies provide clinical utility. Four conventional platforms utilized for gene profiling include immunohistochemistry (IHC), fluorescent in situ hybridization (FISH), polymerase chain reaction (PCR), and high resolution karyotyping (G-Banding). IHC involves designing monoclonal antibodies that bind to the molecule being assessed. Formalin-fixed paraffin-embedded tissue is stained with the antibodies and the expression of the protein is assessed under a microscope. FISH is an established technique that labels specific regions of deoxyribonucleic acid (DNA), using sequence specific oligonucleotides (i.e., short sequences of DNA) to identify chromosomal deletions, additions or rearrangements. Because FISH uses individual probes, it reveals DNA aberrations of only the probe-targeted segments. Locus-specific FISH detects subtelomeric and interstitial submicroscopic chromosomal arrangements (usually 3–5 megabases [Mb] in size) associated with particular phenotypes. When high resolution G-banding is used, chromosomes are first treated with trypsin, an enzyme that degrades proteins. The chromosomes are then stained with Giemsa which produces a banding pattern of light and dark stripes enabling identification of each chromosome. G-band karyotyping is limited to a resolution of 5–10 Mb. PCR is an established laboratory method used to make numerous copies of a specific DNA sequence, utilizing pairs of oligonucleotide primers to replicate and alternate rounds of DNA. Real-time polymerase chain reaction, also called quantitative real time polymerase chain reaction (Q-PCR/qPCR/qrt-PCR) or kinetic polymerase chain reaction (KPCR), is a PCR technology used to simultaneously amplify and quantify the targeted DNA molecule. In reverse transcriptase PCR (RT-PCR) an RNA strand is reverse transcribed into its DNA complement (cDNA). Methylation-specific PCR (MSP) assesses the methylation status of DNA (American Association of Clinical Chemistry [AACC], 2010; Kibel and Reiter, 2007).

Conventional cytogenetic testing is used to identify balanced rearrangements (e.g., translocations or inversions), alterations in chromosome structure, sequence alterations, copy number changes (deletion, duplication and amplification), single-base pair mutation, 20% or lower level of mosaicism, and some types of polyploidy, including triploidy and tetraploidy. Conventional cytogenetic tests identify known genetic abnormalities associated with specific clinical syndromes. These tests may be used when a specific clinical syndrome is suspected.

Chromosomal microarray analysis (CMA) includes comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) arrays. CGH is a microarray based cytogenetic technology used for the detection of submicroscopic genomic copy number variations (CNVs: e.g., deletion and duplication) of DNA. Comparative genomic hybridization (CGH) is also known as chromosomal microarray analysis (CMA) and molecular karyotyping.

The test has the capacity to examine the genome for CNVs at high resolution (e.g. 40 to >1000 times). A further modification of CGH includes array comparative genomic hybridization (aCGH) which is able to examine larger sized regions, compared to traditional CGH, with high resolution. When a microarray is used to identify CNVs, its sensitivity approaches 100%, while its false positive rate has been reported to be as high as 7%. Due to its ability to examine the entire genome at high resolution and specifically target copy number variations (CNVs), CGH has been proposed to provide 10%-15% more information than conventional testing in some circumstances when CNV is the etiologic mutagenic defect. In contrast to conventional cytogenetic tests, CGH does not identify balanced rearrangements (e.g., translocations or inversions), alteration in chromosome structure that is not represented on the array, sequence alterations, single-base pair mutation, 20% or lower level of mosaicism, and some types of polyploidy including triploidy and tetraploidy.

SNP arrays detect genotypes across the entire genome. The arrays utilize two oligonucleotides with one matching each of the two variant alleles. The genotype is then determined by a single-base extension reaction or differential hybridization to oligonucleotides that distinguish a perfect match from a single-base mismatch. For SNP arrays only the patient's DNA is hybridized to the slide and then compared to control data. SNP arrays also detect copy number changes. The advantages of SNP arrays include the capacity to detect uniparental disomy (inheritance of two copies of a chromosome or chromosome segment from one parent) and detection of low-level mosaicism. SNP can also detect triploidy (Beaudet, 2013; Breman, et al., 2012).

A microarray is a system that allows rapid analysis of thousand of different DNA sequences. The slide is prepared using segments of DNA (i.e., probes) that may be either cloned (e.g., bacterial artificial chromosomes [BACs]) or synthesized (e.g., oligonucleotides [oligos]). The probes selected are those that are known or suspected to be associated with genetic conditions. Different colored fluorescently labeled DNA from a patient and a normal control are placed on the microarray slide. The DNA from the patient and the control compete to hybridize (attach) to their corresponding DNA probes. The slide is analyzed to detect areas of unequal hybridization of the patient compared to the control. Areas of unequal hybridization, mostly large deletions and duplications, signify a DNA alteration (Edelmann and Hirschhorn, 2009; Lee, et al., 2007; Manning and Hudgins, 2007; Burton, 2006).

There are two types of CGH array platforms, the targeted or constitutional array, and the whole genome array. The targeted array contains DNA fragments with clinically significantly known CNVs (e.g., subtelomeric regions) or commonly known chromosomal alterations (e.g., microdeletion/microduplication syndromes). The whole genome, or tiling path, array has a wider coverage over the entire human genome and can discover new CNVs of unknown clinical significance. The whole genome array is proposed to identify an additional 5% of abnormalities compared to the targeted array (BlueCross BlueShield Association [BCBSA], 2009; Edelmann and Hirschhorn, 2009; Burton, 2006).

When CGH identifies a CNV of known clinical significance, conventional testing is typically used to confirm the findings of CGH. If an unknown CNV is detected, a genomic database is accessed to see if the abnormality has been previously reported and whether or not it has been associated with a benign or proposed pathogenic condition. Evaluation of parental samples are sometimes done at that point to determine if the abnormality is inherited or has arisen de novo. CNVs that appear in normal individuals have been reported to be as high as 12%, making diagnostic interpretation and identification of CNVs' clinical significance difficult. Various CMA platforms are currently being used and no one platform has been found to be clearly superior to all of the others for clinical purposes. There is an absence of published clinical standards for coverage and resolution which results in a lack of uniformity in arrays used in various laboratories (Novelli, et al., 2012; Miller, et al., 2010; BCBS, 2009; Pickering et al., 2008; Schaefer, et al., 2008; Burton, 2006).

CGH is an established genetic microarray used for the evaluation of a specific subset in the prenatal and postnatal setting. When conventional cytogenetic testing is normal or cannot be performed, CGH may be used to evaluate a fetus when abnormal anatomic findings are pathognomonic of a genetic abnormality. The results may impact the decision to continue or terminate a pregnancy. CGH may also be used to assess the genetic contributions to fetal demise when congenital anomalies are present and conventional testing is also normal. CGH testing is also being used for genetic evaluations of children, age 13 years or under, diagnosed with autism spectrum disorder, developmental delay and/or intellectual disability in which the phenotypic characteristics of a specific genetic disorder are absent, and/or when multiple or unspecified congenital anomalies are not specific to a well-delineated genetic syndrome. The results of CGH are expected to impact the clinical management of the child by using information gained from CGH that would otherwise not be known. There is insufficient evidence to support CGH any other prenatal indication or for preimplantation genetic diagnosis.

## **U.S. Food and Drug Administration (FDA)**

Approval by the FDA for array comparative genomic hybridization tests is not required. CGH is a laboratory-developed test performed by various Clinical Laboratory Improvement Amendments (CLIA) licensed laboratories. Array platforms, assay protocol, and analysis systems vary from laboratory to laboratory.

## **Evaluation of a Fetus**

Prenatal diagnosis and evaluation of fetal demise include analysis of amniocytes, chorionic villi or fetal cells. Prenatal testing may provide information on which to make decisions about continuation of an affected pregnancy or continuing to birth with better preparation for the postnatal needs of the child. Conventional prenatal testing includes G-banding karyotype, fluorescent in situ hybridization (FISH) on interphase nuclei, quantitative fluorescent PCR (QF-PCR) and multiplex ligation-dependent probe amplification (MLPA). Proposed disadvantages of conventional tests include long turn around times, risk of culture artifacts, use of living cells, and/or low resolution that does not detect some abnormalities. Because of a shorter turn around time, higher resolution and avoidance of culturing amniocytes or chorionic villi, CGH has been proposed for prenatal testing. CGH does not require dividing cells. Disadvantages of prenatal CGH testing is the complexity of the data and the identification of abnormalities of uncertain clinical significance resulting in parental anxiety, if the decision is made to share this information with them, and challenging genetic counseling. Another potential limitation of CGH is the inability to isolate sufficient quantities of fetal DNA, especially from amniotic fluid (Hui and Bianchi, 2013; Hillman, et al., 2013; Armengol, et al., 2012; Breman, et al., 2012; Novelli, et al., 2012, Shaeffer, et al., 2012; Fiorentino, et al., 2011).

CGH has also been proposed for analysis of fetal demise (death of infant in utero). Abnormalities by karyotypes are detected in 6–13% of stillbirths. Chromosome imbalances below the resolution of conventional testing (5–10 Mb) are not typically identified on karyotyping. The primary advantage of microarray analysis for stillbirth or fetal demise is that it can be performed on nonviable or macerated tissue (Reddy, et al., 2012).

Evidence in the published peer-reviewed literature supports CGH prenatal testing in a defined subpopulation when there are abnormal anatomic findings and conventional testing (e.g., karyotyping) is normal. Likewise, CGH is supported for fetal demise with congenital anomalies when conventional karyotype testing is normal or karyotype testing cannot be performed.

**Literature Review:** Hillman et al. (2013) conducted a prospective cohort study (n=243) to compare CMA results to karyotyping results in women who had a structural abnormality on prenatal ultrasound. Patients with multiple soft markers (echogenic bowel, choroid plexus cyst, echogenic cardiac foci and single umbilical artery) and increased nuchal translucency > 3.5mm were included in the study. Patients with single soft markers were excluded. Specimens were obtained by chorionic villus sampling (CVS), fetal blood sampling or amniocentesis. DNAs were hybridized to whole genome BAC microarrays (CytoChip Focus constitutional, BlueGnome, Cambridge, UK<sup>®</sup>). CNVs were classified as benign, variants of unknown significance (VOUS) or pathogenic. A total of 156 samples had no CNVs. A total of 121 CNVs were found in 87 samples. Ninety of the 121 were common benign CNVs, two were uncommon benign CNVs, one VOUS were detected and 17 CNVs were pathogenic. Eight pathogenic CNVs were detected by karyotyping. Nine CNVs considered pathogenic were not found by karyotyping. In five samples, karyotyping revealed an anomaly not reported on CMA.

Hillman et al. (2013) also conducted a systematic review and meta-analysis on the use of prenatal CMA. Twenty-five studies met inclusion criteria. Studies were included if microarray was used on prenatal specimens analyzed during pregnancy or after delivery on postnatal specimens following termination of pregnancy for structural abnormalities detected on an ultrasound scan. Included studies had to allow generation of a comparative table between karyotyping and array. Overall agreement between CMA and conventional karyotyping was 93.4% when CMA and karyotyping were performed for any clinical indication. In 17 studies, conventional karyotyping revealed an additional 0.6% abnormality rate when CMA results were normal. Analysis of a subset of subjects who had structural abnormality on ultrasound revealed that CMA detected 10% more abnormalities than karyotyping (p<0.01) and karyotyping detected 0.8% more abnormalities than CMA. The rate of variants of unknown significance (VOUS) in 17 cohorts was 1.4%. The VOUS rate was higher (2.1% VS. 1.4%) in cases of abnormal scan. In this subgroup analysis, the excess rate of detection by CMA over karyotyping was 10%. The authors noted that the 10% detection rate of abnormalities by CMA may have been due to the small cohort numbers, no parental testing and the high VOUS rates. In more recent studies in which higher CGH resolutions was used, there was an increase in VOUS rate. The authors also stated that additional prospective studies with large sample sizes comparing karyotyping to a

“commercial reproducible array” were needed. The optimum resolution of an array for prenatal testing has not been established. Limitations of this study included the heterogeneity of the studies (e.g., prospective, retrospective, case reports) and the heterogeneity of the arrays used.

Wapner et al. (2012) prospectively compared the outcomes of microarray analysis to standard karyotyping in 4406 prenatal samples. Women presenting with a singleton gestation for chorionic- villus sampling or amniocentesis for advanced maternal age (46.6%), abnormal result on Down’s syndrome screening (18.8%), structural anomalies on ultrasonography (25.2%), and other indications (9.4%) were included in the study. Each sample was divided and standard karyotyping was performed on one portion and the other portion was sent to one of four laboratories for microarray analysis. Two array platforms were consistently used throughout the study. One was designed by the investigators and the other was the Affymetrix Genome-Wide Human SNP Array 6.0, containing 1.8 million oligonucleotide probes. Microarray analysis of nonmosaic samples identified all the aneuploidies and unbalanced rearrangements identified on karyotyping, however, CGH did not identify balanced translocations and fetal triploidy. In 96 samples with a normal karyotype, CGH identified clinically significant deletions or duplications. A total of 6.0% of the samples had a suspected growth or structural anomaly on ultrasound and 1.7% were in women with advanced maternal age or had positive screening results.

Armengol et al. (2012) conducted a prospective, multicenter comparative study (n=900 subjects; 906 samples) to evaluate conventional screening methodologies and chromosomal microarray-based analysis (CMA) for detecting chromosomal abnormalities. All women had been referred for invasive prenatal sampling. The conventional methodologies included: karyotyping, quantitative fluorescent polymerase chain reaction (QF-PCR), and two panels of multiplex ligation-dependent probe amplification (MLPA). CMA was performed with a targeted BAC microarray. Main indications for sampling included: abnormal ultrasound findings, altered biochemical screening, familial history of chromosomopathy or other genetic condition, maternal age greater than age 37, parental anxiety and other exceptional conditions (e.g., high-risk twin pregnancy, suspected viral infection). Pre- and post-test genetic counseling was conducted. Samples were obtained from chorionic villus (CVS), amniotic fluid and fetal blood. Mean turn-around time was seven days for CMA and MLPA, 25 days for karyotyping and two days for QF-PCR. Karyotyping was the most robust technique with eight failures (0.9%), CMA and QF-PCR showed a 1.1% failure rate and MLP had a 10.1% rate. Technical failure occurred when it was not possible to provide a definitive result. A total of 100 chromosomal aberrations were identified from 95 samples with 57 aberrations being clinically significant. The CMA detection rate was 32% above other methods, 13.3% in fetuses with abnormal ultrasounds. Eight conditions had poor prognosis for postnatal development. A total of 21 VOUS were detected. Two VOUS were detected by MLPA, three by kayotyping, none by QF-PCR, and 17 by CMA. Eleven VOUS were considered benign, inherited alterations.

The diagnostic accuracy measures were as follows:

	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic accuracy
QF-PCR	60.71 %	98.80%	97.14%	95.74%	95.83%
CMA	98.21%	99.75%	96.49%	99.88%	99.66%
Karyotype	76.36%	99.86%	97.67%	98.27%	98.23%
Subtelomeric MLPA mix	66.67%	97.28%	73.91%	96.19%	94.11%
RGD MLPA mix	14.58%	97.46%	41.18%	90.33%	88.44%

Following a 20-week normal ultrasound evaluation, decisions were made in six cases of recurrent microduplication to continue pregnancy.

Over a period of seven years, Shaffer et al. (2012a) prospectively evaluated 5003 prenatal samples to assess the usefulness of CGH compared to karyotyping for prenatal diagnosis. Prenatal samples from amniotic fluid, chorionic villi, fetal blood, or products of conception were initially tested using various targeted microarrays. Indications for testing including abnormal ultrasound (n=2534), advanced maternal age, abnormal maternal serum screening, positive family history, parental anxiety and other or nonspecific. As the study progressed, whole-genome arrays were used. Using microarrays, the overall detection rate of clinically significant CNVs was 5.3% (207/3876) among

unbiased, nondemise cases. The detection rate for cases referred with abnormal ultrasounds was 6.5% and 8.5% with fetal demise. These results showed that the rate of CNVs was higher in these two subcategories compared to other reasons for testing (e.g., advanced maternal age, family history, parental anxiety). The overall rate of CNVs with unclear clinical significance was 4.2%. When only de novo CNVs were included, the rate of unclear clinical significance was 0.39%. Of the clinically significant CNVs found by CGH, 71% were below the resolution of conventional karyotyping.

Breman et al. (2012) retrospectively reviewed CGH results of 1115 fetal samples obtained from amniotic fluid or chorionic villus sampling or cultured cells. The primary indications for CGH were abnormal prenatal ultrasound findings (n=410) and advanced maternal age (n=394). Other indications included a previous child with or a family history of a genetic disorder or chromosome abnormality detected by karyotype or FISH, parental concern, abnormal maternal serum screen, other/unclassified indications or unknown. Parental testing was performed when indicated. Whole genome and various oligonucleotide and targeted BAC arrays were used. Detected duplications or losses were evaluated and FISH was used to confirm all de novo CNVs. No deletions or duplications were seen in 881 samples. CNVs were detected in 234 cases. Overall 85 cases (7.6%) of clinically significant CNVs were observed. Eighteen variants (1.6%) were of unclear clinical significance. A total of 45/1075 cases (4.2%) had clinically significant CNVs after 40 abnormal cases with known chromosome abnormalities or familial genomic imbalances were excluded. The most clinically significant findings were in patients with an abnormal karyotype/FISH (26/61, 42.6%), family history of chromosomal abnormality (13/137, 9.5%), abnormal ultrasound (38/410, 9.3%), abnormal serum screening (2/37, 5.4%) and advanced maternal age (5/394, 1.3%). CGH identified 18/1075 (1.7%) cases that had no previously known abnormality or family history had clinically significant genomic changes undetectable by conventional prenatal chromosome analysis.

Shaffer et al. (2012b) retrospectively analyzed 2858 pregnancies with abnormal ultrasounds and normal karyotypes using CGH microarrays. The majority (n=2161) of samples were tested on whole genome, oligonucleotide-based arrays. The remaining samples were tested by targeted or whole genome BAC arrays. A total of 2052 cases had previously tested normal on karyotypes. An additional 465 had karyotyping performed concurrently to microarray or had unknown or failed karyotypes (n=341). An additional 5.6% of clinically significant CNVs were identified in fetuses with anomalies involving a single organ system and in 9.5% of fetuses with anomalies in multiple organ systems. CGH identified clinically significant genomic alterations in 6.5% of cases with one or more abnormal ultrasound findings; the majority was below the resolution of karyotyping.

Lee et al. (2012) conducted a prospective study to evaluate the clinical utility of CGH for prenatal diagnosis in 3171 fetuses. Both CGH and conventional karyotyping were performed on samples from chorionic villi, amniotic fluid, or cord blood. Indications for CGH testing included: maternal age > 34 year, parental anxiety, abnormal sonographic findings, abnormal karyotyping, and abnormal Down syndrome screening risk. Parental blood samples were collected if CNV of unknown clinical significance were detected by CGH. Various array platforms (BAC array or oligonucleotide array) were used across the course of the study. CGH identified 37 (1.2%) numerical chromosome anomalies, 34 (1.2%) microdeletion and duplication and 18 CNVs. A pathological genetic imbalance was found in 33 of 194 (17.0%) fetuses with abnormal prenatal ultrasound, seven deletion or duplication were diagnosed in 50 samples, and six segmental genomic imbalances were found in 39 fetuses with two or more anomalies. Conventional karyotyping identified balanced translocation in 22 fetuses. Five were inherited and not detected by CGH. Karyotyping identified 23 fetuses with abnormal chromosomes, and array CGH helped to determine the origins of the abnormalities in eight of them. CGH identified 85 pathological chromosomal alterations (2.7%). Excluding benign copy number variations, CGH detected 34 fetuses with microdeletion or duplications, and five with VOUS. In total 1.2% (39/3171) more chromosomal imbalances were identified by array CGH than by G-banding karyotyping. Fetuses with one major sonographic anomalies and normal karyotypes had a 10.5% detection rate for a clinically significant copy number alterations and a 15.45% rate in fetuses with anomalies involving two or more organ systems.

Reddy et al. (2012) analyzed 532 samples from stillbirths to determine if microarray analysis would detect abnormalities not found on karyotyping. DNA from frozen specimens was analyzed using Affymetrix GenomeWide Human SNP Array 6.0. Attempted karyotype analyses yielded 375 results with 31 abnormalities identified. Microarray analysis on the same samples yielded 465 results, significantly more than karyotyping (p<0.001). Thirty-two stillbirths (6.9%) were aneuploid, 12 (2.6%) harbored a pathogenic variant, and 25 (5.4%) harbored a variant of unknown significance. Duplications and deletions identified by CGH included abnormalities seen in DiGeorge syndrome and variants of unknown significance. In eight stillborns with variants of unknown significance, there were no anomalies noted on postmortem examination but had substantially abnormal placental findings. Compared to

karyotype analysis, microarray analysis provided improved detection of genomic abnormalities (aneuploidy plus pathogenic variants) ( $p=0.007$ ). When variants of unknown significance were included, the microarray significance ( $p<0.001$ ) was even greater. Of the 157 stillbirths for which karyotype analysis failed to provide a definitive result, 79.6% yielded a definitive microarray result (73.9% were normal or probably benign and 5.7% were abnormal). Of the 31 stillbirths with abnormalities, 25 microarrays were consistent with karyotype results. In a subgroup of stillborns with postmortem examination, microarray detected significantly more abnormalities than karyotype ( $p=0.008$ ). Limitations of the study include concurrent karyotype and microarray testing on the same tissues was not possible, inability to distinguish de novo from inherited variants owing to unavailability of parentally DNA.

Fiorentino et al. (2011a) prospectively compared the results of 1037 samples tested by CGH and G-banding karyotyping to assess the feasibility of using CGH as a first-line test for prenatal diagnosis. Specimen types included amniotic fluid, chorionic villus sampling and cultured amniocytes or DNA extracted from uncultured amniocytes isolated directly from amniotic fluid. CGH was offered as an option to patients considering an invasive prenatal genetic testing procedure and karyotyping. Patients were offered invasive testing due to maternal  $\geq$  age 35 years at the time of conception, abnormal results of maternal serum screening tests, abnormal ultrasound findings (AUS), a known abnormal fetal karyotype (AFK), family history of a genetic condition or chromosome abnormality (FIS), parental anxiety (PA), cell culture failure (CCF) and multiple indications (MI). A total of 96.7% of samples had normal results. In nine out of 34 cases abnormal cases (26.5%), CGH detected pathogenic copy number variations that would not have been detected by standard karyotype. CGH also detected mosaicism at the 10% level. Except for two cases only correctly diagnosed by aCGH, there was complete concordance between karyotyping and CGH. CGH did not detect balanced rearrangements in seven samples that were identified by karyotyping. The turnaround time was  $2.5 \pm 0.6$  days. Parental samples were obtained for possible maternal cell contamination and immediate characterization of potential familial CNVs, where necessary.

**Professional Societies/Organizations:** Based on a review of the recent literature and the knowledge and experience of the working group, the Italian Society of Human Genetics (SIGU) (Novelli, et al., 2012) made the following recommendations for prenatal CGH testing:

- never as a substitute for conventional karyotyping;
- for specific diagnostic purposes in selected pregnancies and not for general screening in all pregnancies;
- only in prenatal cases with specific indications, such as:
  - single (apparently isolated) or multiple sonographic fetal abnormalities;
  - de novo chromosomal rearrangements, even if apparently balanced, detected by standard karyotyping, to investigate the possible presence of cryptic imbalance(s) related to the structural chromosome abnormality;
  - supernumerary marker chromosomes in order to characterize their origin and genetic content.

SIGU recommended using a genome-wide array and parental testing when an uncommon CNV is found.

In a technology update (Duncan, et al., 2011), the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC) and the Prenatal Diagnosis Committee of the of the Canadian College of Medical Geneticists (CCMG) summarized the current literature and made recommendations for aCGH in the prenatal setting. The recommendations included the following:

- Array genomic hybridization is not recommended in pregnancies at low risk for a structural chromosomal abnormality; for example, advanced maternal age, positive maternal serum screen, previous trisomy, or the presence of “soft markers” on fetal ultrasound (III-D: “Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees”. “There is fair evidence to recommend against the clinical preventive action”).
- Array genomic hybridization may be an appropriate diagnostic test in cases with fetal structural abnormalities detected on ultrasound or fetal magnetic resonance imaging; it could be done in lieu of a karyotype if rapid aneuploidy screening is negative and an appropriate turnaround time for results is assured (II-2A: “Evidence from well–designed cohort [prospective or retrospective] or case–control studies, preferably from more than one centre or research group”. “There is good evidence to recommend the clinical preventive action.”).
- Any pregnant woman who qualifies for microarray genomic hybridization testing should be seen in consultation by a medical geneticist before testing so that the benefits, limitations, and possible outcomes of the analysis can be discussed in detail. The difficulties of interpreting some copy number variants should also be discussed. This will allow couples to make an informed decision about whether or not they wish to pursue such prenatal testing (III-A: “Opinions of respected authorities, based on clinical experience,

descriptive studies, or reports of expert committees.” “There is good evidence to recommend the clinical preventive action”).

In a 2009 committee opinion document on CGH in prenatal diagnosis, the American College of Obstetricians and Gynecologists (ACOG) made the following recommendations:

- “Conventional karyotyping remains the principal cytogenetic tool in prenatal diagnosis.
- Targeted array CGH, in concert with genetic counseling, can be offered as an adjunct tool in prenatal cases with abnormal anatomic findings and a normal conventional karyotype, as well as in cases of fetal demise with congenital anomalies and the inability to obtain a conventional karyotype.
- Couples choosing targeted array CGH should receive both pretest and posttest genetic counseling. Follow-up genetic counseling is required for interpretation of array CGH results. Couples should understand that array CGH will not detect all genetic pathologies and that array CGH results may be difficult to interpret.
- Targeted array CGH may be useful as a screening tool; however, further studies are necessary to fully determine its utility and its limitations.”

The College noted that “the information should not be construed as dictating an exclusive course of treatment or procedure to be followed”.

### **Autism Spectrum Disorders (ASD), Developmental Delay, Intellectual Disability, And/Or Multiple Or Unspecified Congenital Anomalies**

In persons with symptoms of autism spectrum disorders (ASD), developmental delay, intellectual disability, and/or multiple or unspecified congenital anomalies, conventional genetic testing has been used as a method to rule out known underlying disease states and or genetic syndromes. CGH has evolved into an established alternative to conventional cytogenetic testing to explore the genome for copy number variants (CNV) in this subpopulation when a specific genetic disorder has not been identified by conventional cytogenetic testing. Studies have reported a 7%–20% higher diagnostic yield with microarray compared to conventional karyotyping. The clinical utility of microarray testing includes: provision of additional information leading to family reassurance, guidance for family planning, early identification of special needs, avoidance of ongoing diagnostic assessment where no clear diagnosis exists, predicted prognosis for the patient, pharmacotherapy and identification of medical risk and the need for ongoing monitoring.

#### **Autism Spectrum Disorders (ASD)**

The autism spectrum disorders (ASDs) are a range of complex behavioral disorders that are also referred to as pervasive developmental disorders (PDD). The disorders range from the condition referred to as autism or autistic disorder to Asperger’s syndrome. Two other disorders in the spectrum are Rett’s disorder, or Rett syndrome, and childhood disintegrative disorder. When a child has symptoms of autistic disorder or Asperger’s syndrome, but does not meet the specific criteria for either, the diagnosis is described as pervasive developmental disorder not otherwise specified (PDD-NOS), also referred to as atypical PDD or atypical autism.

The evaluation for ASDs often requires a multidisciplinary team approach and will depend on the impairments that are present. There is no specific test that can confirm a diagnosis of ASD. Conditions that may warrant genetic testing include situations where the results will directly impact clinical decision-making and/or clinical outcome, and the testing method is considered a proven method for the identification of a genetically-linked inheritable disease. There are selected genetic syndromes that are known etiologies of ASDs such as Angleman syndrome, CHARGE syndrome, de Lange syndrome, Fragile X syndrome, Prader-Willi syndrome, Rett syndrome, MED12 disorders (including Lujan–Fryns syndrome, Smith–Lemli–Opitz syndrome, Smith–Magenis syndrome, Sotos syndrome, Tuberous sclerosis, and PTEN, phosphatase and tensin homolog, Shprintzen syndrome (22q11 deletions), Hypomelanosis of Ito (Schaefer and Mendelsoh, 2013; Shen, et al., 2013; Schaefer and Mendelsoh, 2008; ). When these syndromes are present the American Academy of Medical Genetics (ACMG, 2013) states that no work-up is indicated. CGH is a recognized genetic test for individuals with diagnosed ASDs in which a specific genetic disorder is unknown.

**Literature Review:** Shen et al. (2010) evaluated 933 patients with a predominant diagnosis of autistic disorder (n=477) and pervasive developmental disorder-not otherwise specified (PDD-NOS) (n=454) to compare the outcomes of karyotype testing, aCGH and Fragile X testing. 433 patients out of 461 completed all three tests and an additional 472 patients were added through samples submitted for the study. CGH testing was performed by 244k or 500k whole genome arrays (n=697) or v5.0 single-nucleotide polymorphism arrays (n=108). Karyotyping

identified 19 of 852 patients (2.23%) with abnormal results. Array CGH detected 8 abnormalities in the 19 patients with abnormal karyotype, but 10 of the 19 had balanced rearrangements that appeared normal and were not detected by aCGH. Fragile X testing identified 4 abnormalities. aCGH identified deletions or duplications in 154 of 848 patients (18.2%) with 59 being considered abnormal and possibly significant. A total of 95 abnormalities were considered of unknown significance. Abnormal or possible significant results identified by CGH targeted array were 5.3% and 7.3% by whole genome array. Fifty of the abnormalities noted on aCGH were below the size range detected by karyotype. Twelve of 54 individuals diagnosed with MR had aCGH abnormalities compared to two by karyotype and three by Fragile X testing. Ten of 16 individuals with dysmorphic features had abnormalities detected by aCGH and two by karyotyping. Eight of 36 individuals with seizure disorders had eight aCGH abnormalities and two karyotype abnormalities. aCGH “corrected or clarified ambiguous karyotype results by demonstrating that a 15q duplication was a clinically insignificant repetitive sequence” in one patient and “by precisely defining cytogenetically ambiguous translocation break points” in another patient. One patient with low-level mosaicism was not detected with aCGH, six identified balanced translocation and three de novos were “not necessarily pathogenic.” Although aCGH detected more abnormalities, the authors noted that aCGH could not replace a G-banded karyotype in this population because of the inability of aCGH to detect balanced rearrangements. However, according to the authors, missed diagnosis may occur in 5% of ASD cases without aCGH testing. The impact of aCGH results on clinical management decisions for this patient population was not discussed. Limitations of the study noted by the authors include concerns regarding credibility of diagnosis and bias based on ascertainment of patients through tertiary care centers cannot be excluded.”

To determine the benefit of CGH as a diagnostic tool, Jacquemont et al. (2006) conducted whole-genome CGH using a 1 megabase (Mb) resolution (Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK) on 29 patients with idiopathic syndromic ASD. The patients had normal high-resolution karyotype (approximately 800 bands), biochemical tests and hematological results prior to CGH testing. Thirty-three chromosome gains or losses in 22 patients were identified by CGH. Twenty-three variants were considered normal. The ten remaining abnormalities were considered possible pathogenic and were validated by at least one independent method. Seven rearrangements occurred de novo, and two were inherited from a normal parent. Out of the 29 patients, CGH identified eight clinically relevant abnormalities in eight patients (27.5%). One patient had previously been diagnosed with Williams syndrome (which is due to a copy number variance) and had a normal FISH analyses, but a chromosomal imbalance was found on CGH.

### **Developmental Delay, Intellectual Disability, and Congenital Anomalies**

Developmental delay typically refers to a child younger than age six years who presents with delays in the attainment of developmental milestones at the expected age and demonstrates deficits in learning and adaptation. Global developmental delay involves a significant delay in two or more developmental domains, including gross/fine motor, speech/language, cognition, social/personal, and activities of daily living. The delays may be significant and predictive of the development of cognitive and/or intellectual disability (American Academy of Neurology, 2011; Moeschler, et al., 2006).

According to the American Association on Intellectual and Developmental Disabilities (AAIDD) (2012), intellectual disability, or mental retardation, is a “disability characterized by significant limitations both in intellectual functioning and in adaptive behavior, which covers many everyday social and practical skills. This disability originates before the age of 18”. Generally, the individual has an intelligence quotient (IQ) score of below 70–75 and is compromised in the areas of conceptual skills, social skills, and practical skills.

Intellectual disability can be caused by genetic abnormalities seen in various syndromes such as: Down syndrome, Edwards syndrome, Patau syndrome, Fragile X syndrome, Rett syndrome, Angelman syndrome or Prader-Willi (Prader-Labhart-Willi) syndrome. These individuals may also have various levels of mental retardation (Zelden, et al., 2012).

Multiple or unspecified (i.e., not specific to a well-delineated genetic disorder) congenital anomalies, or birth defects, are morphologic defects present at birth, may present in various patterns, and are usually multifactorial. In 10–15% of cases, anomalies can be attributed to chromosomal aberrations (Maitra and Kumar, 2005). Examples of congenital anomalies include: cleft palate; clubfoot; spina bifida; vision and hearing impairments; and respiratory, renal and cardiac malformations. Congenital anomalies may be coupled with intellectual disability, and developmental delay. Microarray CGH has evolved into an established diagnostic study for this subpopulation.

**Literature Review:** Using data from a laboratory databank, Ellison et al. (2012) retrospectively reviewed 46,298 postnatal patients tested by CGH to assess the degree to which the results provided abnormalities associated with diagnosis that would likely result in changes to the clinical management of the patient. The categories of disorders that were reviewed included: 1) known microdeletion and microduplication syndromes, 2) conditions associated with increased cancer susceptibility and 3) specific actionable phenotypes for which obvious medical intervention was indicated. The type of microarray platforms used to test samples evolved over the course of the study, beginning with bacterial artificial chromosome (BAC) probes with targeted coverage of the genome, to subsequent BAC-based arrays and oligonucleotide-based whole genome arrays and lastly, to a 135K custom designed array. A total of 40 disorders with complex phenotypes including developmental and/or neurologic abnormalities which may be accompanied by congenital malformations and other medical problems (e.g., cardiac, renal, eye abnormalities) were identified in group 1. It was not known if these features were evident at the time of testing and they were rarely listed as an indication for testing. Each of the 40 disorders had at least one clinical feature that would require specific medical follow-up. Group 3 included disorders caused by copy abnormalities that often have multiple clinical features due to the altered doses of multiple genes and would require follow-up. To determine if the medical risks identified by CGH actually resulted in specific clinical action, treating physicians of a subset of 122 cases were queried. Forty-six physicians provided information on 81 patients. Per the survey, it was reported that at least one follow-up action per patient was taken as a result of CGH (e.g., additional diagnostic testing, glucose monitoring, platelet count monitoring, specialty referrals). The authors reported that 35% of all pathogenic copy number changes identified in this study warranted clinical action. Limitations of this study include the retrospective study design, small number of patients for whom follow-up was obtained, follow-up data based on physician query, and heterogeneity of array platforms used.

Hayashi et al. (2011) recruited 536 patients from multiple centers to apply aCGH to the diagnosis of patients with clinically uncharacterized multiple congenital anomalies (MCA) and mental retardation (MR). The patients had normal conventional karyotypes. An in-house targeted array, containing 550 BACs used for the first screening, detected pathogenic CNVs (pCNVs) in 54 cases (10.1%) which were confirmed by FISH. All the CNVs corresponded to known syndromes. In 16 cases with two CNVs (one deletion and one duplication), parental analysis on available samples for three cases confirmed that subtelomeric aberration was inherited in two cases and de novo in one case. The other 14 were known disorders. A second screening of the negative cases was conducted using a genome-wide, high-density array at intervals of approximately 0.7Mb. The higher resolution detected 482 negative cases of which 349 had been analyzed at the time of publication of the study. Sixty-six CNVs in 63 patients (18.1%) were detected and 48 cases were considered pathogenic (13.8%). For the purpose of this study, pathogenic was variously defined as a rising de novo, with or without a clinical correlation, being associated with a variance known to cause a phenotypic disease and those not known to cause disease. In one patient with a variance known to cause a phenotypic disease the diagnosis was discounted due to the clinical identification of another syndrome. It was noted that in one case clinical management was changed.

Sagoo et al. (2009) conducted a systematic review and meta-analysis (n=19 studies; 13,926 patients) investigating patients with learning disabilities (LD) and congenital anomalies who had negative conventional cytogenetic analysis followed by CGH. Studies included case series and cohort studies (n=2–316). Various types of arrays (e.g., oligonucleotide, BAC array, targeted array) were used for sampling of DNA and some studies used more than one type of array. There were variations in the patient selection and testing criteria. The overall diagnostic yield of causal genetic abnormalities was 10% (95% confidence interval: 8–12%). The overall false-positive yield was 7% (95% confidence interval: 5–10%). The authors concluded that the meta-analysis supported CGH as an option for patients with LD and congenital anomalies when other conventional cytogenetic test are negative, but due to the false positive rate stated that “some caution in clinical practice is also required”. They noted that “findings cannot be extrapolated to an unselected group, where LD may be less severe and the likelihood of a genetic cause is less.” They did not support CGH as first-line tests in all patients with LD. Limitations of the study include the heterogeneous patient population, variation in the types of arrays used and the high false positive rates.

Pickering et al. (2008) reported on 1176 patients with unexplained mental retardation/developmental delays and/or MCA, and/or dysmorphic features (DF), mostly with normal (n=1146) or balanced karyotypes and FISH studies who were then analyzed with Spectral™ Chip 2600 (1-Mb) (PerkinElmer LAS Inc., Waltham, MA) and/or Spectral Genomics Constitutional Chip (PerkinElmer LAS Inc., Waltham, MA) CGH. Forty-four of the samples were prenatal analysis from products of conception or amniotic fluid. Abnormal imbalances found by CGH were confirmed with fluorescence in situ hybridization (FISH). CGH detected 163 (13.8%) genomic imbalances. Forty-seven (3.9%) were considered a benign copy number variation or a deletion or duplication of unknown clinical significance. The 116 (9.8%) remaining identified imbalances were considered clinically relevant & related to the subject's abnormal

phenotype. A 7.6% abnormality rate was found using the constitutional chip, a 14.8% abnormality rate was found using the 1-Mb array, and a 15.3% abnormality rate was found when both chips were used. The overall diagnostic yield for abnormalities was 9.8%, an increase of 7.7% using CGH. Four of the 25 abnormal cases had an apparently balanced rearrangement that revealed an unexpected deletion. Five cytogenetically abnormal tests yielded a normal aCGH of which three were balanced translocations, one was a supernumerary marker chromosome and one was a mosaic trisomy. The clinical management of these patients was not discussed nor were specific diagnoses noted. The authors noted that CGH should not be a substitute for traditional karyotyping because it cannot detect balanced chromosomal rearrangements, has limits detecting low-level mosaicism, and cannot identify location or orientation of a duplicated chromosome segment. Because of these limitations, it is recommended that CGH not be used alone and abnormalities should be confirmed by additional testing.

In an effort to detect subtelomeric imbalances, Shao et al. (2008) utilized CGH to evaluate 5380 patients referred for mental retardation, developmental delays, dysmorphic features, MCA, seizure disorders, and/or behavioral abnormalities. CGH V5 (853 BAC clones) was used on the first 4493 patients and CMA V6 (1475 BAC clones) was used for the remaining 887 patients. Of the total population, 2550 had a normal karyotype, 175 had an abnormal karyotype and 2655 had no or unavailable karyotype analysis. All CGH abnormalities, except copy number polymorphisms, were verified by FISH and/or GTG-banding. Parental samples were obtained for patients with CNV of clinical or unknown significance. CNV in subtelomeric regions were identified in 499 (9.3%) patients. In 175 (3.2%) of these patients, the alterations were considered normal. Clinical significance could not be determined in 88 cases due to unavailable parental samples and/or the alterations were small and did not involve a known disease region. The remaining 236 (4.4%) of the alterations were either de novo or inherited. After excluding samples with known karyotypic alterations, the CGH detection rate of abnormalities exceeded that of conventional testing by approximately 3%. Subtelomeric imbalances in 76 patients were found by CGH in patients with a normal karyotype and CGH did not detect apparently balanced translocations or inversions. In this study the most commonly observed duplications were at Xq28 region. The authors concluded that duplication at this region may be a cause of development delay in males. Specific diagnoses made or patient management based on the results of CGH were not discussed.

To determine the possible diagnostic yield of CGH using SignatureChip<sup>®</sup> (Signature Genomic Laboratories, Spokane, WA), Shevell et al. (2008) tested 94 children with global development delay (GDD) who had previously undergone subspecialty evaluation, karyotyping, FMR1 molecular genotyping and neuro-imaging studies with non-diagnostic results". CGH abnormal results were confirmed with FISH. Parents of children with abnormal CGH were tested to distinguish between pathogenic and familial non-pathogenic variants. An abnormality in 12 subjects was revealed on initial CGH. Following familial testing, six were found to be familial, nonpathogenic variants. The results of the remaining six children were felt to be pathogenic and of etiologic significance causally related to the diagnosis of GDD. Only the presence of minor dysmorphic features were significantly predictive of etiologic yield on CGH ( $p=0.05$ ). In this study, CGH had a 6.4% etiologic yield in children with non-syndromal GDD. Specific syndromes associated with abnormalities identified with CGH were not noted by the authors nor was treatment management based on CHG discussed.

To assess the diagnostic utility of CGH, Baris et al. (2007) conducted a retrospective review of 373 patients with normal chromosomal analysis who then underwent CGH testing ( $n=193$ ). The clinical features of the children included global developmental delay/mental retardation (234/352), facial dysmorphism (114/286) and/or multiple congenital anomalies (MCA) (58/372). An abnormal CGH was reported in 36 of the 193 patients (9.7%). Twenty patients had potential pathogenetic imbalances and 16 had CNV. Unbalanced translocations were identified in three patients and mosaic chromosomal trisomies in two patients. FISH analysis confirmed all but six of the CGH abnormalities. Targeted CGH identified 5.4% of all patients with undetectable cytogenetic abnormalities and 11.4% of patient with both facial dysmorphism and MCA. Of the genomic imbalances identified by CGH, two deletions were known to cause Angelman syndrome and one deletion each was known to contribute to velocardiofacial syndrome and X-linked ichthyosis was identified. How these results affected the clinical management of the patients was not discussed. The authors recommended that routine chromosomal analysis should be normal prior to conducting CGH because CGH cannot detect balanced translocation and inversions due to a lack of genomic imbalance.

Engels et al. (2007) used > 6000 or 8000 large insert clone CGHs in an attempt to identify deleted or duplicated genes in 69 patients with unexplained mental retardation, most with congenital anomalies, who had normal karyotypes and FISH analyses. A total of 134 possible microimbalances were detected. Nonpolymorphic array clones with ratios outside the diagnostic thresholds were verified by follow-up FISH. Parental chromosomes were also analyzed. Six most likely causal imbalances were detected, representing a diagnostic yield of 10%. In one

patient, CGH identified a microduplication associated with DiGeorge/VCFS critical region. Other identified abnormalities were not correlated to a specific genetic disorder. The management of these patients was not discussed.

Subramonia-Iyer et al. (2007) conducted a systematic review and meta-analysis of case series that used CGH to investigate patients with mental retardation and congenital anomalies, and/or dysmorphic features who had negative results from conventional cytogenetic analysis. Seven studies including a total of 462 patients (range 20–140) met inclusion criteria. Five studies used a 1-Mb resolution array, one used a 50 kilobase (kb) and another used a specified set of 2173 clones and an average 1.4 Mb resolution. The overall diagnostic yield was 13% (95% confidence interval; 10–17%). A meta-analysis of five studies resulted in a 7% false-positive yield (i.e., identification of abnormalities that are deemed noncausal). The authors stated that the results of this systematic review suggested that CGH is a “promising technology” for investigating patients with mental retardation who have negative results on conventional cytogenetic analysis. They also noted that before widespread use of CGH can be used in clinical practice, agreement should be made on “optimal array resolution, choice of included clones, the most appropriate platforms and the establishment of quality assurance mechanisms.” CGH should also be compared with existing cytogenetic tests and more information is needed regarding the clinical utility of the test. In summary, the authors stated “there is insufficient evidence to recommend introduction of this test into routine clinical practice.”

Wong et al. (2005) conducted a case series to evaluate the feasibility of using CGH as a routine clinical tool for identifying telomere rearrangements in patients with unexplained mental retardation (n=102). Previous G-banded karyotype and FISH analysis were normal. One CGH array was developed by the authors (41 BAC or P1-derived artificial chromosome [PAC] clones), and the second array used was the Genosensor Array 300 (Abbott Vysis, Inc., Downers Grove, IL). Representing 100% sensitivity, CGH detected all of the abnormalities previously identified by FISH analysis and identified an additional two imbalances (duplications) not identified by FISH. The abnormalities included four unbalanced translocations with monosome and trisomy, eight terminal deletions, four duplications and one interstitial deletion. Consistent with karyotype and FISH normal results, 84 individuals did not show any imbalance with CGH. The authors noted that further studies were indicated to prospectively evaluate CGH against G-banding and FISH to estimate sensitivity, specificity, and technological time to determine if CGH should be routinely used in diagnostic genetic testing. A specific genetic disorder was not linked to the abnormalities identified by CGH, nor was patient management discussed.

#### **Literature Review – Studies involving mixed patient populations (i.e., Autism Spectrum Disorders (ASD), Intellectual Disability, Developmental Delay, and Congenital Anomalies)**

Gekas et al. (2011) prospectively collected and analyzed 376 samples to assess the relevance of karyotyping after first-tier aCGH in patients with unexplained intellectual disability (ID) or developmental delay (DD), dysmorphic features, unique or multiple malformations, growth disorder, behavior disorder and/or autism. Age ranged from one day to 49 years, median 4.8 years. Using aCGH, abnormalities were found in 28.7% of cases of which 7.4% were inherited (likely benign), 11.7% were de novo and 9.6% were undetermined (parental samples were unavailable). Out of 288 patients who underwent parallel karyotyping, 69.8% showed results similar to aCGH and the majority of the results were normal. For patients with a chromosome imbalance detected by aCGH, 7.9% showed similar results with karyotyping. Thirteen of 20 patients showed dissimilar results compared to aCGH analysis. Four balanced rearrangements and nine additional chromosomal anomalies were not seen with aCGH.

Coulter et al. (2011) conducted a retrospective chart review (n=1792) of patients with developmental delay (DD), intellectual disability (ID), multiple congenital anomalies (MCA) and ASD to determine what portion of cases an abnormal CGH impacted recommendations for clinical action. Out of the 1792 patients, 131 (7.3%) had an abnormal variant (i.e., known microdeletion/microduplication syndromes, deletion of genes known to be associated with disease by loss of one copy and large deletions and/or duplications involving many genes) (7.3%) and 104 (5.8%) had a variant of possible significance (VPS). A total of 194 patients were included in this analysis (n=121 abnormal variant; n=73 VPS). For the purpose of this study, a recommendation for clinical action was defined as a “specialist referral, imaging study, diagnostic test or medication prescription by the patient’s physician” specifically because of the CGH results. ASD was the indication for testing in 26 patients with an abnormal variant and 23 patients with a VPS. DD/ID was the indication for testing in 53 patients with an abnormal variant and 21 patients with a VPS. Congenital anomalies were the indication for testing in 19 patients with an abnormal variant and 12 patients with a VPS, and seven percent of patients with an abnormal variant were referred for CGH to confirm a previously identified genetic abnormality. A total of 356 other tests (e.g., karyotype, Fragile X CGG repeat analysis, Prader-Willi/Angelman syndrome methylation, plasma amino acids, urine organic acid) were performed on the 194 patients in the study before or at the same time as CGH. In 25 cases, other testing identified a cause for the patient’s

condition, but the cause identified was not different from what was identified by CGH. Compared to VPS patients, the rate of recommendation for clinical action in patients with abnormal variants was significantly higher ( $p=0.01$ ). In the 65 patients with abnormal variants, 67 specialist referrals, 25 imaging studies and 20 laboratory tests were recommended. In the 25 patients with VPS, 11 specialist referrals, 9 imaging studies and 18 laboratory tests were recommended. Twelve patients had a duplication or deletion known to be associated with ASD and DD/ID, two cases of Angelman syndrome, one case of Prader-Willi syndrome and seven cases of sex chromosome aneuploidy were newly identified. Patients younger than age two years were significantly more likely to have congenital anomalies as an indication for testing compared to patients older than age 2 years ( $p=0.001$ ) and had significantly more abnormal variants compared to VPS ( $p=0.02$ ). Compared to ASD patients, there were significantly more DD/ID patients with an abnormal variant ( $p=0.02$ ). Author-noted limitations of this study included: the retrospective study design, small heterogeneous patient population from a single institution, lack of a control group, many different physicians were involved in the care of the patients, and the rate of abnormal physical findings (e.g., dysmorphic features) may have been underreported. In this study, CGH patients who had VPS had significantly more clinical actions taken. However, the findings of these actions were not reported and the impact on clinical management was not discussed.

Miller et al. (2010) conducted a systematic review ( $n=33$  studies; 21,698 patients) of postnatal testing of patients with unexplained developmental delay/intellectual disability (DD/ID), autism spectrum disorders (ASD), dysmorphic features, and/or multiple congenital anomalies (MCA) tested by CGH compared to G-banded karyotyping to determine if CGH should be used as a first tier test. Studies included case series and cohort studies that used bacterial artificial chromosome (BAC) or oligonucleotide CGH arrays. Diagnostic yield was defined as the “number of patients with abnormal variants divided by the total number of patients tested and was derived directly from each original study.” Compared to G-banded karyotyping, a 15%–20% higher yield of abnormal variance was seen with CGH (about 3%, excluding Down syndrome and other recognizable chromosomal syndromes), about 10% more than G-banded karyotyping. Truly balanced rearrangements and low-level mosaicism ( $<1\%$ ) were generally not detectable by CGH. Limitations of the study include: heterogeneous patient populations, inability of CGH to detect non-copy number variants abnormalities, various array types were utilized, and lack of systematically collected data on variants of uncertain clinical significance in each study.

**Professional Societies/Organizations:** The 2013 American College of Medical Genetics (ACMG) guideline revision for genetic evaluation for autism spectrum disorders (ASDs) (Schaefer and Mendelsohn, 2013) lists CMA (oligonucleotide array-comparative genomic hybridization or single-nucleotide polymorphism array) as a first tier diagnostic test for the evaluation of ASDs. If the individual has a recognizable syndrome firmly documented as associated with ASDs (e.g., Angelman syndrome, Fragile X syndrome), further investigation into the etiology is not necessary. For genetic conditions that have been reported in association with ASDs for which the reported association is not convincing, ACGM recommends that an etiologic evaluation of the ASD be conducted, including CGH.

In the 2010 guidelines on array-based technology, ACGM (Manning, et al., 2010) recommended the following:

1. “CMA testing for CNV is recommended as a first-line test in the initial postnatal evaluation of individuals with the following:
  - A. Multiple anomalies not specific to a well-delineated genetic syndrome
  - B. Apparently nonsyndromic DD/ID [developmental delay/intellectual disability]
  - C. Autism spectrum disorders
2. Further determination of the use of CMA testing for the evaluation of the child with growth retardation, speech delay, and other less well-studied indications is recommended, particularly by prospective studies and aftermarket analysis.
3. Appropriate follow-up is recommended in cases of chromosome imbalance identified by CMA, to include cytogenetic/FISH studies of the patient, parental evaluation, and clinical genetic evaluation and counseling.”

ACGM noted that “clinicians ordering the test need to be aware of the different clinical platforms (e.g., BAC versus oligo, targeted versus whole genome, and SNP), the variation in resolution among arrays and the information each provides,” and reminded clinicians of the limitations of aCGH including the inability to “identify balanced chromosomal rearrangements, such as translocations or inversions, or differentiate free trisomies from unbalanced Robertsonian translocations;” some aneuploidies and marker chromosomes may be missed; the accuracy of detecting low levels of mosaicism has been questioned; interpretation of the significance of a rare copy number

change can be incomplete and triploidy will not be detected by some forms of microarray. According to ACMG, “the clinician should understand what type of follow-up tests will be performed, and on whom, in the event of abnormal results. Further, for deletions and duplications, parental studies (by fluorescence in situ hybridization [FISH] or metaphase preparations, if possible) should be conducted to rule out the presence of a chromosomal rearrangement such as an insertion or inherited duplication.”

The 2011 American Academy of Pediatrics (AAP) guidelines (McCandless, et al., 2011) on the supervision of children with Prader-Willi syndrome (PWS) do not specifically discuss aCGH for the diagnosis of this syndrome. According to AAP, diagnostic testing for PWS should begin with methylation analysis. When only a maternal methylation pattern is seen, PWS is confirmed but additional testing by karyotyping and FISH to determine the specific cause is recommended. If conventional genotyping is negative (i.e., no evidence of deletion or balanced rearrangement), blood from the parents and child should be obtained for molecular analysis for uniparental disomy (UPD). “If biparental inheritance is discovered in the face of abnormal methylation and normal FISH results, then, by process of elimination, the cause is assumed to be an imprinting defect. The possible role of testing for defects of the HBII-85 small nucleolar RNA cluster remains to be elucidated.”

The most current guidelines on ASD, mental retardation and developmental delay are as follows. In their 2007 guidance for the identification and evaluation of autism, the AAP stated that microarray aCGH is a “promising tool that may become standard of care in the future, but this technique has not been evaluated systematically in children with ASDs” (Johnson, et al., 2007).

The 2006 AAP guidance for the clinical genetic evaluation of children with mental retardation and developmental delays, stated that due to the insufficient published reports, “the use of microarray comparative genomic hybridization in the evaluation of children with developmental delays/mental retardation might be considered best as emerging technology” and there is insufficient evidence to support its use in the evaluation of children with developmental delay and mental retardation (Moeschler, et al., 2006).

American Academy of Neurology (AAN)/Child Neurology Society (CNS) (AAN/CNS) (2011) conducted a systematic review to determine the diagnostic yield of genetic and metabolic evaluation of children with global developmental delay or intellectual disability (GDD/ID). Twenty-three Class III studies (n=6559) reported that microarray was diagnostic in 7.8% (range 0%–50%) of subjects. A Class III study was defined as “a sample of patients studied during the course of the condition. Some patients undergo the intervention of interest. The outcome, if not objective, is determined in an evaluation by someone other than the treating physician”. In 18 studies (n=1524) of patients with facial dysmorphism, congenital anomalies or neurologic symptoms the diagnostic yield was 10.2%. Regarding G-banded karyotype studies, nine Class III studies reported 4.2% of patients had an abnormal karyotype. Subjects with GDD/ID had an abnormality in at least 4% of subjects and in 18.6% of patients with syndromic features. Thirty seven Class III subtelomeric fluorescence in situ hybridization (StFISH) studies (n=18,583) found abnormalities in 3.5% (0%–20%) of patients with either GDD/ID or multiple congenital anomalies. StFISH testing was abnormal in at least 4.2% of patients with syndromic features, 0.5% with mild impairment, and 7.4% of those with moderate/severe impairment. The authors noted that few studies had directly compared genome-wide testing to conventional testing. In their recommendations for future research, AAN/CNS noted that “research is sorely lacking on the medical, social, and financial benefits of having an accurate etiologic diagnosis” in this population and “the ability to rate diagnostic tests on the basis of factors other than diagnostic yield, such as the availability of effective treatment, would have a positive influence on clinical practice”.

In a 2011 guidance document on autism, the National Institute for Health and Clinical Excellence (United Kingdom) (NICE) noted that more genetic abnormalities in autism are being identified, but their causal role in autism is not clear. Currently, the yield of abnormal genetic results using CGH array is reported to be higher in individuals with dysmorphic features and/or intellectual disability. NICE stated that “before extending CGH array testing to a wider population, it is important to have a better understanding of its diagnostic yield. It is also essential to identify any negative consequences that may result from routine testing”.

### **Preimplantation Testing**

Preimplantation genetic diagnosis (PGD) is a diagnostic procedure developed with the intent of providing an alternative to traditional prenatal genetic diagnosis (e.g., amniocentesis and chorionic villus sampling [CVS]) for fertile couples at reproductive risk of transmitting an inherited disease to their offspring. It is a technique that allows embryos to be tested for genetic disorders and deselected before entering the uterus and prior to pregnancy. PGD

has the potential to avoid the need to terminate an affected pregnancy through the identification and transfer of unaffected embryos.

Traditionally, embryos have been screened for chromosome abnormalities using conventional cytogenetic testing such as fluorescence in situ hybridization (FISH). However, FISH has some technical limitations. The test can only detect a limited number of chromosomes. FISH is dependent on fixation/spreading of a single cell onto a microscope slide which can lead to overlapping or split signals, and a high percentage of informative cases can be achieved only if removal of a blastomere or polar body can be accomplished in 3–5 minutes, preferably less to prevent damage to the embryo. Therefore, microarray CGH has been proposed for comprehensive analysis for embryo screening due to its proposed efficacy, level of detection and time of analysis. CGH allows for the analysis of all 24 chromosomes types (23 autosomes and X and Y) in one test without the need of cell fixation (Rubio, et al., 2013; Simpson, 2012; Fiorentino, et al., 2011b; Wells, et al., 2008).

There is insufficient evidence in the peer-reviewed literature to support CGH microarray for preimplantation screening and assessment. Studies are primarily in the form of case reports, case series and retrospective reviews. Data is needed to compare implantation rates and pregnancy rates following microarray testing compared to conventional testing. It has yet to be determined which microarray will provide the optimal accuracy and speed, and the impact of microarray testing on embryo cryopreservation.

### **Genetic Counseling**

It is recommended that individuals undergoing genetic testing for the indications discussed herein should have both pre- and post-test genetic counseling with a board-certified or board-eligible medical geneticist or a licensed or certified genetic counselor. Pretest counseling precedes genetic testing. The genetic counselor interviews the patient, assembling a large amount of information about family medical history, ethnicity, the patient's concerns and expectations of genetic testing, and potential future consequences. Post-test counseling involves providing the results of genetic testing to the person(s) tested. The counselor presents a full explanation of test results and implications for further testing and management. Information is discussed regarding the implications for family members. The genetic counseling should be provided by an independent, board-certified or board-eligible medical geneticist or a licensed or certified genetic counselor that is unaffiliated with the genetic testing lab performing the test.

### **Summary**

Evidence in the published peer-reviewed literature and professional societies support microarray comparative genomic hybridization (CGH), or chromosomal microarray analysis (CMA), when conventional cytogenetic testing (e.g., karyotyping) is normal and abnormal fetal anatomic findings are present and pathognomonic of a genetic abnormality, or in the case of fetal demise in the presence of congenital anomalies. CGH has evolved into an established test for evaluating genetic abnormalities in children, age 13 years or under, diagnosed with autism spectrum disorder, non-syndromic development delay or intellectual disability in which the phenotypic characteristics of a specific genetic disorder are absent. GCH may also be indicated for a child with multiple congenital anomalies not specific to a well-delineated genetic syndrome.

There is insufficient evidence in the published peer-reviewed literature to support microarray CGH for preimplantation genetic diagnosis (PGD). Studies are primarily in the form of case reports, case series and retrospective reviews. Data are needed to compare implantation rates and pregnancy rates following microarray testing compared to conventional testing and the microarray(s) that provide the optimal accuracy and speed has yet to be determined. The impact of microarray testing on embryo cryopreservation is unknown.

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## **Coding/Billing Information**

**Note:** 1) This list of codes may not be all-inclusive.

2) Deleted codes and codes which are not effective at the time the service is rendered may not be eligible for reimbursement.

**Covered when used to report comparative genomic hybridization testing:**

<b>CPT* Codes</b>	<b>Description</b>
81228	Cytogenetic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number variants (e.g., Bacterial Artificial Chromosome [BAC] or oligo-based comparative genomic hybridization [CGH] microarray analysis)
81229	Cytogenetic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants for chromosomal abnormalities.
83890	Molecular diagnostics; molecular isolation or extraction, each nucleic acid type (ie, DNA or RNA) (Code deleted 12/31/2012)
83891	Molecular diagnostics; isolation or extraction of highly purified nucleic acid, each nucleic acid type (ie, DNA or RNA) (Code deleted 12/31/2012)
83892	Molecular diagnostics; enzymatic digestion, each enzyme treatment (Code deleted 12/31/2012)
83894	Molecular diagnostics; separation by gel electrophoresis (eg, agarose, polyacrylamide), each nucleic acid preparation (Code deleted 12/31/2012)
83896	Molecular diagnostics; nucleic acid probe, each (Code deleted 12/31/2012)
83897	Molecular diagnostics; nucleic acid transfer (eg, Southern, Northern), each nucleic acid preparation (Code deleted 12/31/2012)
83900	Molecular diagnostics; amplification, target, multiplex, first 2 nucleic acid sequences (Code deleted 12/31/2012)
83901	Molecular diagnostics; amplification, target, multiplex, each additional nucleic acid sequence beyond 2 (List separately in addition to code for primary procedure) (Code deleted 12/31/2012)
83912	Molecular diagnostics; interpretation and report (Code deleted 12/31/2012)
88384	Array-based evaluation of multiple molecular probes; 11 through 50 probes (Code deleted 12/31/2012)
88385	Array-based evaluation of multiple molecular probes; 51 through 250 probes (Code deleted 12/31/2012)
88386	Array-based evaluation of multiple molecular probes; 251 through 500 probes (Code deleted 12/31/2012)

<b>HCPCS Codes</b>	<b>Description</b>
S3870	Comparative genomic hybridization (CGH) microarray testing for developmental delay, autism spectrum disorder and/or mental retardation

\*Current Procedural Terminology (CPT®) © 2012 American Medical Association: Chicago, IL.

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