Comparative Genomic Hybridization (CGH)

Aetna considers comparative genomic hybridization (CGH) medically necessary for the following indications:

- Evaluating fetuses with structural abnormalities detected on fetal ultrasound or fetal magnetic resonance imaging; or
- For evaluating histologically equivocal Spitzoid melanocytic neoplasms (Spitz nevus and atypical Spitz tumors); or
- Analyses of stillbirths with congenital anomalies or in stillbirths in which karyotype results cannot be obtained.

Aetna considers CGH medically necessary for diagnosing genetic abnormalities in children with multiple congenital anomalies when the following criteria are met:

I. If warranted by the clinical situation, biochemical testing for metabolic diseases has been performed and is negative; and
II. Targeted genetic testing, if or when indicated by the clinical and family history, is negative; and
II. Targeted genetic testing, (for example: FMR1 gene analysis for Fragile X), if or when indicated by the clinical and family history, is negative; and

III. The member’s clinical presentation is not specific to a well-delineated genetic syndrome*;

IV. The results for the testing have the potential to impact the clinical management of the member.

* CGH is considered not medically necessary when a diagnosis of a disorder or syndrome is readily apparent based on clinical evaluation alone.

Chromosomal microarray analysis is considered experimental and investigational in all other cases of suspected genetic abnormality in children with developmental delay/intellectual disability or autism spectrum disorder except when criteria above are met. Only one CGH is considered medically necessary per lifetime.

Aetna considers comparative genomic hybridization (CGH) experimental and investigational for any other indications
including the following (not an all-inclusive list) because of insufficient evidence of its effectiveness:

- Detection of balanced rearrangements
- Evaluation of autoimmune lymphoproliferative syndrome
- Evaluation of short stature
- Evaluation of unexplained epilepsies
- Screening for prenatal gene mutations in fetuses without structural abnormalities, such as in advanced maternal age, positive maternal serum screen, previous trisomy, or the presence of "soft markers" on fetal ultrasound
- Testing products of conception
- Diagnosis of melanoma.

See also CPB 0140 Genetic Testing (../100_199/0140.html), CPB 0189 Genetic Counseling (../100_199/0189.html), and CPB 0358 Invasive Prenatal Diagnosis of Genetic Diseases (../300_399/0358.html).

Notes: The Oligo HD Scan is a type of array CGH. The CombiMatrix DNArray is a CGH test for developmental delay.

**Background**

Comparative genomic hybridization (CGH), also referred to as chromosomal microarray analysis (CMA), and array CGH (aCGH), is a method of genetic testing that may identify small deletions and duplications of the subtelomers, each pericentromeric region and other chromosome regions. It is being investigated for the screening, diagnosis and treatment of congenital anomalies, autism spectrum disorder (ASD), developmental delays (DD), idiopathic mental retardation (MR) in newborns or children, and screening for prenatal gene mutations.

Karyotype analysis by chorionic villus sampling (CVS) or amniocentesis has been the standard method for prenatal cytogenetic diagnosis since the 1970s. Although highly reliable, limitations cited in the literature include: the requirement for cell culture, a time delay from 10 to 14 days between sampling and results, limited resolution, interpretation that relies on subjective analysis, and limited ability to detect submicroscopic deletions, duplications or other rearrangements. This category of structural genomic variants is
an increasingly recognized cause of genetic disorders, and has been estimated to be associated with approximately 17% of syndromic and nonsyndromic MR (Peng et al., 2009).

Fluorescence in-situ hybridization (FISH) and more recently, quantitative fluorescent polymerase chain reaction (QF-PCR), are used for the fast detection of clinically relevant major aneuploidies; however, they have limitations as a general prenatal diagnostic tool when there are nonspecific findings because they do not provide a genome wide screen for unexpected imbalances.

Comparative genomic hybridization combines chromosome and FISH analyses to allow detection not only of aneuploidies, but also of all known microdeletion and microduplication disorders, including telomere rearrangements. As in karyotype analysis or FISH, CHG requires an invasive procedure (CVS or amniocentesis) to obtain the fetal sample. In CHG, the nucleus of the embryo is labeled with a fluorescent dye and a control cell is labeled using another color (i.e., red or green). The embryo and the control cell are then cohybridized onto a control metaphase spread, and the ratio between the two colors is compared. If the chromosomal analysis shows an excess of red, the embryo nucleus contains an extra chromosome. If an excess of green is apparent, then the embryo nucleus is missing one of these chromosomes. Currently, this technique takes 72 hours, and embryo cryopreservation is necessary to provide the time necessary to undertake the diagnosis.

More than 70 disorders are known to be associated with birth defects or developmental problems that are caused by deletion or duplication of genomic material, which are called "copy number variants" or "CNVs". Comparative genomic hybridization is intended to increase the chromosomal resolution for detection of CNVs, and as a result, to increase the diagnostic yield and the genomic detail beyond that of conventional methods. It is intended to combine the speed of DNA analysis with a large capacity to scan for genomic abnormalities in a single assay. Comparative genomic hybridization may be ordered when conventional results are negative. It is not known, however, whether the diagnostic benefit gained from being able to test for a large number of
genomic disorders and other chromosomal defects with CGH outweighs the risks of detecting CNVs of uncertain clinical significance.

Targeted arrays for CGH, designed to optimize coverage of disease-associated CNV regions (CNVRs), are intended to reduce the chance of detecting polymorphic CNVRs of unknown or uncertain clinical significance. Researchers have used targeted arrays using CGH to screen for DD, and/or MR, dysmorphic features, multiple congenital anomalies, seizure disorders, and autistic, or other behavioral abnormalities (Shao et al, 2008). Such CGH platforms readily detect trisomies, monosomies, microscopic or submicroscopic unbalanced chromosomal rearrangements and deletions and duplications associated with known genomic disorders. The reported detection rate for clinically significant chromosomal abnormalities has reported to vary between 6.9 % to 9 %, approximately twice that of conventional cytogenetic analysis, although most of these cases were evaluated postnatally and prenatal experience has been limited (Peng et al, 2009). However, a large multi-center trial that will evaluate aCGH in several thousand prenatal samples is currently underway.

In a prospective study, Sahoo and colleagues (2006) analyzed 98 DNA samples from pregnant women who had undergone invasive prenatal testing for standard indications on a targeted bacterial artificial chromosome (BAC) array. There was complete concordance between the karyotype and array results on all 56 amniotic fluid and 42 CVS samples, including 5 cases with chromosomal abnormalities. This study also showed that using whole-genome amplification for amniotic fluid samples, and some CVS samples with limiting amounts of fetal DNA, allowed the results to be available within one week, in contrast to the average 10 to 14 days needed for a standard karyotype analysis.

Shen et al (2007) reported the design and validation of a focused oligonucleotide-array CGH assay for clinical laboratory diagnosis of genomic imbalance. They selected greater than 10,000 60-mer oligonucleotide features from Agilent's eArray probe library to interrogate all subtelomeric and pericentromeric regions and 95 additional clinically relevant regions for a total of 179 loci. Sensitivity and specificity were
measured for 105 patient samples, including 51 with known genomic-imbalance events, as detected by BAC-based array CGH, FISH, or multiplex ligation-dependent probe amplification. Focused array CGH detected all known regions of genomic imbalance in 51 validation samples with 100% concordance.

Subramonia-lyer et al (2007) reviewed the literature on the ability of CGH to detect chromosomal abnormalities in individuals with learning disabilities. The authors included seven studies (462 patients) in their review and found an overall diagnostic yield of causal abnormalities of 13%. The false-positive yield of non-causal abnormalities ranged from 5% to 67%, although this range was only 5% to 10% in 6 of the studies. The authors concluded, "[a]lthough promising, there is insufficient evidence to recommend the introduction of CGH testing into routine clinical practice. A number of important technical questions need answering, such as optimal array resolution, which clones to include, and the most appropriate platforms. A thorough assessment of clinical utility and cost-effectiveness compared with existing tests is also needed."

Shevell et al (2008) utilized CGH testing to assess potential etiologic yield on children with previously un-diagnosed non-syndromal global DD. The children were drawn from a previously reported consecutive series of children with well-defined global developmental delay (GDD). Almost all subjects had undergone prior karyotyping and neuroimaging studies with non-diagnostic results. Array-based CGH was undertaken using the SignatureChip(R) (1,887 BACs representing 622 loci) with abnormalities verified by subsequent FISH analysis and testing of parents to distinguish between pathogenic and familial non-pathogenic variants. On CGH analysis, 6 of 94 children (6.4%) had a causally related pathogenic copy number changes (CNC). Three were sub-telomeric in location. An analysis of a variety of clinical factors revealed that only the presence of minor dysmorphic features (less than 3) was predictive of etiologic yield on CGH analysis (4/26 versus 2/68, p = 0.05). Severity of delay was not found to be predictive. The authors reported that in children with non-syndromal GDD, CGH has an etiologic yield of 6.4%, suggesting that this emerging technology may be of diagnostic value when applied subsequent to detailed history, physical examination, and
targeted laboratory testing and may merit consideration as a first-tier test in the context of a child with un-explained GDD.

Lu et al (2008) reported the frequency of genomic imbalances in neonates with birth defects with CGH. Between March 2006 and September 2007, 638 neonates with various birth defects were referred for CGH. Three consecutive chromosomal microarray analysis versions were used: bacterial artificial chromosome-based versions V5 and V6 and bacterial artificial chromosome emulated oligonucleotide-based version V6 Oligo. Each version had targeted but increasingly extensive genomic coverage and interrogated more than 150 disease loci with enhanced coverage in genomic re-arrangement-prone peri-centromeric and sub-telomeric regions. Overall, 109 (17.1 %) patients were identified with clinically significant abnormalities with detection rates of 13.7 %, 16.6 %, and 19.9 % on V5, V6, and V6 Oligo, respectively. All of these abnormalities were verified by a secondary independent laboratory test, FISH and/or high-resolution retrospective partial karyotype analyses. Of the 109 patients with clinically significant genomic imbalance or pathogenic CNVs, 16 (14.7 %) had numerical anomalies including trisomy 21 (8), trisomy 18 (3), trisomy 13 (3), trisomy 22 (1), and monosomy X (1). The authors reported that "[t]he remaining 93 (85.3 %) patients had genomic imbalances that may not be detected by standard cytogenetic studies, including 37 (33.9 %) with common micro-deletions or micro-duplications involving 22q11.2 (13), 5p15.2 (6), 3p26.3 (4), 4p16.3 (4), and other chromosomal regions (10); 44 (40.4 %) with genomic imbalances at relatively rare disease loci; and 12 (11.0 %) with chromosomal mosaicism." Of the neonates who were referred for possible chromosomal abnormalities, 66.7 % were found to have genomic imbalances with CGH testing, a threefold increase from previous cytogenetic studies. The authors concluded that CGH is a valuable clinical diagnostic tool that allows precise and rapid identification of genomic imbalances and mosaic abnormalities as the cause of birth defects in neonates and that it allows for timely molecular diagnoses and detects many more clinically relevant genomic abnormalities than conventional cytogenetic studies.

A review article by Shinawi and Cheung (2008) explained that CGH is a comprehensive tool in disease gene discovery and is
sheding light on the abundance of CNVs of unclear significance that are scattered throughout the human genome, "though more intensive research is needed to understand their involvement in human diseases." In addition, the review article stated, "[t]argeted aCGH enables the detection of all clinically relevant genomic imbalances but has limitations in the detection of polyploidy and balance translocations. Whole-genome higher density arrays significantly increase the sensitivity of the method and are important for the discovery of new genomic syndromes, but complicate the clinical interpretation of copy number variants of unclear significance."

The Genetics Committee of the Society for Gynaecology and Obstetrics of Canada (Duncan, et al., 2011) summarized the current literature on array genomic hybridization in prenatal diagnosis, and outlined recommendations regarding the use of this new technology with respect to prenatal diagnosis. The quality of evidence was rated using the criteria described in the Report of the Canadian Task Force on Preventive Health Care. The Genetics Committee concluded that 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) 2. The Genetics Committee concluded that 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) 3.

The Committee recommended that 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).

Wapner et al (2012) concluded that, in the context of prenatal diagnostic testing, chromosomal microarray analysis identified additional, clinically significant cytogenetic information as
compared with karyotyping and was equally efficacious in identifying aneuploidies and unbalanced rearrangements but did not identify balanced translocations and triploidies. Samples from women undergoing prenatal diagnosis at 29 centers were sent to a central karyotyping laboratory. Each sample was split in 2; standard karyotyping was performed on one portion and the other was sent to 1 of 4 laboratories for chromosomal microarray. The investigators enrolled a total of 4,406 women. Indications for prenatal diagnosis were 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 %). In 4,340 (98.8 %) of the fetal samples, microarray analysis was successful; 87.9 % of samples could be used without tissue culture. Microarray analysis of the 4,282 non-mosaic samples identified all the aneuploidies and unbalanced rearrangements identified on karyotyping but did not identify balanced translocations and fetal triploidy. In samples with a normal karyotype, microarray analysis revealed clinically relevant deletions or duplications in 6.0 % with a structural anomaly and in 1.7 % of those whose indications were advanced maternal age or positive screening results.

An accompanying editorial (Dugoff et al, 2012) stated that the results of the study by Wapner et al (2012) support the use of microarray analysis instead of conventional karyotyping when fetal structural anomalies have been identified on ultrasonography. The editorialist stated that, although the copy-number variant will be of uncertain clinical significance in a small percentage of these cases (which presents a real problem in counseling the couple), this disadvantage is outweighed by the higher percentage of well-characterized copy-number variants that are found. The editorialist stated, in contrast, that the incremental value of microarray analysis as compared with conventional karyotyping alone is not clearly established when amniocentesis is performed for diagnostic purposes in cases of advanced maternal age or a positive screen for Down’s syndrome. The editorialist stated that, in such cases, the proportion of well-characterized copynumber
variants as opposed to uncharacterized copy-number variants is much closer, and it and it is possible that the counseling conundrum may outweigh the value of the incremental information provided by the microarray analysis. The editorialist noted, in addition, the increased cost associated with microarray -- including the cost of parental studies in some cases -- as compared with the cost of conventional karyotyping may be significantly out of proportion to the information gained in these lower-yield cases.

An Agency for Healthcare Research and Quality (AHRQ) Technical Brief (Sun, et al., 2015) collected and summarized information on genetic tests clinically available in the United States to detect genetic markers that predispose to developmental disabilities, and also identified existing evidence addressing the tests’ clinical utility. The assessment primarily focused on patients with idiopathic or unexplained developmental disabilities, particularly intellectual disability, global developmental delay, and autism spectrum disorder. Several better-defined developmental disability syndromes, including Angelman syndrome, fragile X syndrome, Prader-Willi syndrome, Rett syndrome, Rubinstein-Taybi syndrome, Smith-Magenis syndrome, velocardiofacial syndrome, and Williams syndrome were also included. Patient-centered health outcomes (e.g., functional or symptomatic improvement) and intermediate outcomes (e.g., changes in clinical decisions or family reproductive decisions, the tests’ diagnostic accuracy and analytic validity) were examined. The investigators sought input from nine Key Informants to identify important clinical, technology, and policy issues from different perspectives. The investigators searched the National Center for Biotechnology Information’s Genetic Testing Registry (GTR) to identify genetic tests. A structured search of studies published since 2000 was performed to identify available evidence that address genetic tests’ clinical utility. The investigators reported that their search of the GTR database identified 672 laboratory-developed tests offered by 63 providers in 29 States. They also identified one test cleared by the U.S. Food and Drug Administration. Common genetic testing methods used included array
comparative genomic hybridization, microarray, DNA sequencing (the Sanger method or next-generation sequencing), and polymerase chain reaction. The investigators did not identify any studies that directly assessed the impact of genetic testing on health outcomes. Most of the clinical studies identified for indirect assessment of clinical utility are case series reporting on a test’s diagnostic yield.

An assessment by the BlueCross BlueShield Association Technology Evaluation Center (BCBSA, 2015) reviewed the evidence for chromosomal microarrays for the genetic evaluation of patients with global developmental delay, intellectual disability, and autism spectrum disorder. The report found that data supporting analytic validity are readily available only for the Affymetrix CytoScan Dx assay; however, laboratories meeting CLIA standards but using other platforms would be expected to achieve adequate technical performance. The assessment found consistent evidence that the diagnostic yield obtained from chromosomal microarray testing is higher than with karyotyping in children with global developmental delay, intellectual disability, or autism spectrum disorder, with or without congenital anomalies. The report stated, however, that establishing the pathogenicity of detected copy number variants relies on evidence, informatics, and genetics expertise. The report stated that a particular challenge when considering the evidence and methods used to determine variant pathogenicity is that, outside the more common syndromes, diagnoses include a large number of rare disorders a clinician, even a specialist, might not encounter during a lifetime. The investigators were unable to identify case reports of incorrect diagnoses, and stated that it is unclear how often they might occur. The report posited 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 (3) affect reproductive planning for parents and potentially the affected patient. The investigators identified 5 retrospective studies and 1 database analysis that examined a potential impact
of chromosomal microarray results on clinical decisions. Collectively, the studies found 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.

Evidence-based guidelines by the National Collaborating Centre for Women's and Children's Health (NICE, 2011) noted the need for a better understanding of CGH before its widespread adoption in autism spectrum disorder. The guideline noted that the causal relationship between many of the genetic abnormalities detected by CGH and autism spectrum disorder is not clear. In addition, the guideline noted the need for a better understanding of the diagnostic yield of CGH, and the negative consequences of routine CGH testing in ASD.

The American Academy of Pediatrics (AAP) has issued the following position statement on the use of CGH for children with MR and DDs: "The use of microarray comparative genomic hybridization in the evaluation of children with DD/MR might be considered at best as 'emerging technology.' This methodology promises to detect abnormal copy numbers of DNA sequences - deletions and duplications of very small segments of the entire chromosomes. Some clinical geneticists have begun to take advantage of this testing technique in patients with undiagnosed DD/MR because it is an efficient method for subtelomere testing and can be used to confirm clinical suspicion on certain diagnoses (e.g., Williams syndrome). It appears that this method will increase the clinician's ability to determine the cause of DD/MR, particularly in cases with minor anomalies. There are currently insufficient published reports of the use of this technology in the evaluation of the child with DD/MR."

Regarding its use in ASD, the AAP guidelines for the identification and evaluation of children with ASDs (Johnson et al, 2007) noted that "[c]omparative genomic hybridization-microarray analysis 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." The American Academy of Pediatrics clinical report on the identification and evaluation of children with autism spectrum disorders was reaffirmed in September 2010.

The American College of Medical Genetics published practice guidelines for the clinical genetics evaluation in identifying the etiology of ASD (Schaefer et al, 2008). The guidelines state: "Currently, array comparative genomic hybridization (aCGH) has emerged as a powerful new tool that promises further revolution of clinical genetic testing. The technology of assessing submicroscopic re-arrangements is evolving at a mind-boggling rate. New platforms are being developed at rates faster than clinical studies can define their use. The availability of multiple platforms further complicates the ability to compare studies from various sites. Relatively few studies have been published that provide an actual estimate of the diagnostic yield of aCGH in evaluating patients with autism." The guidelines note that it can be estimated that current aCGH platforms can identify abnormalities on the order of 10% beyond what would be identified with standard chromosomal testing. They noted that, "Until definitive, large-scale studies provide confirmation of the use of aCGH, its role in the evaluation of autism spectrum disorders may not be fully appreciated."

The BlueCross BlueShield Association Technology Evaluation Center (BCBS TEC, 2009) assessment of CGH for the genetic evaluation of patients with DD/MR and ASD reported that current CGH services using higher-resolution BAC arrays or high resolution oligo CGH arrays achieve 100 % sensitivity for known chromosomal abnormalities, inconsistent false-positive rates, and a diagnostic yield between 5 % to 16.7 % in DD/MR patients and from 3.4 % to 11.6 % in ASD patients. The assessment stated that CGH arrays have the advantage of greatly improved resolution and more exact locus definition of conventionally detectable abnormalities; however, the results are conceptually similar to those obtained by conventional
methods. The assessment found that, while the diagnostic yield represents a vast improvement in identifying a genetic etiology for these patients, very few studies have addressed the impact of testing on patient outcomes; thus, it is not possible to draw evidence-based conclusions regarding the clinical utility of aCGH genetic evaluation. However, the same may be said of conventional cytogenetic testing. The assessment stated: "Some have called for broader efforts to standardize protocols, define quality criteria for successful analysis, and develop reporting guidelines; in addition, a national multicenter trial to address accuracy, indications, and efficacy has been suggested."

The American College of Obstetricians and Gynecologists (ACOG, 2007) has issued this statement about the use of CGH: "Comparative genomic hybridization (CGH) is an evolving method that identifies submicroscopic chromosomal deletions and duplications. This approach has proved useful in identifying abnormalities in individuals with developmental delay and physical abnormalities when results of traditional chromosomal analysis have been normal. The use of CGH in prenatal diagnosis, at present, is limited because of the difficulty in interpreting which DNA alterations revealed through CGH may be normal population variants. Until there are more data available, use of CGH for routine prenatal diagnosis is not recommended."

An article by Moeschler et al (2009), described the experience of clinical genetics practices in Northern New England (Vermont, New Hampshire, and Maine). The genetic practices formed a learning collaborative with the purpose of improving genetic health care and outcomes. The aim was to improve the rate of etiological diagnosis of those with DDs referred to each genetics center by improving the processes of care. Four of 5 sites also evaluated the impact of CGH laboratory testing of such patients and found significant site-to-site variation in the rate of new diagnoses by CGH with the average new diagnosis rate of 11.8% (range of 5.4 to 28.8%). One source of variation noted was the type of array utilized. Some sites utilized targeted BAC arrays and others utilized the oligonucleotide
arrays. However, the authors stated, "[w]ithout process and outcomes data to contribute to the discussion of how the group might improve the application of such technology, we can only speculate on possible explanations for the variation. This would be particularly informative and this represents an opportunity for future clinical improvement activities and, perhaps, an impetus to accelerate implementation in the clinical sites."

Sagoo et al (2009) updated the diagnostic and false-positive yields of CGH testing from a previously reported systematic review and meta-analysis on patients with learning disability and congenital anomalies in whom conventional cytogenetic analyses had proven negative (Subramonia-lyer et al, 2007). Nineteen studies (13,926 patients) were included of which 12 studies (13,464 patients) were published since their previous analysis. The overall diagnostic yield of causal abnormalities was 10 % (95 % confidence interval [CI]: 8 to 12 %). The overall number needed to test to identify an extra causal abnormality was 10 (95 % CI: 8 to 13). The overall false-positive yield of non-causal abnormalities was 7 % (95 % CI: 5 to 10 %). The authors stated that this updated meta-analysis provides new evidence to support the use of CGH in investigating patients with learning disability and congenital anomalies in whom conventional cytogenetic tests have proven negative; however, given that this technology also identifies false positives at a similar rate to causal variants, caution in clinical practice should be advised. An evaluation of this meta-analysis by the Center for Reviews and Dissemination (2009) stated that the reliability of the conclusions of this meta-analysis "is uncertain due to some unclear reporting, potential publication bias, and failure to appropriately consider study quality."

A review article on the clinical utility of CGH for the detection of chromosomal imbalances associated with mental retardation and multiple congenital anomalies reported that a number of different array CGH platforms have emerged and are being used as an adjunct test to standard karyotype analysis. The authors noted that the current limitations of the technology include the inability to detect balanced chromosome rearrangements and
the equivocal nature of copy number alterations of unknown significance that may be identified (Edelmann and Hirschhorn, 2009).

Shen et al (2010) evaluated genetic testing in a combined cohort of 933 patients with ASD. Clinical genetic testing included G-banded karyotype, fragile X testing, and CMA to test for submicroscopic genomic deletions and duplications. Karyotype yielded abnormal results in 19 of 852 patients (2.23 % [95 % CI: 1.73 % to 2.73 %]), fragile X testing was abnormal in 4 of 861 (0.46 % [95 % CI: 0.36 % to 0.56 %]), and CMA identified deletions or duplications in 154 of 848 patients (18.2 % [95 % CI: 14.76 % to 1.64 %]). CMA results for 59 of 848 patients (7.0 % [95 % CI: 5.5 % to 8.5 %]) were considered abnormal, which includes variants associated with known genomic disorders or variants of possible significance. Chromosomal microarray analysis results were normal in 10 of 852 patients (1.2 %) with abnormal karyotype due to balanced rearrangements or unidentified marker chromosome. Chromosomal microarray analysis with whole-genome coverage and CMA with targeted genomic regions detected clinically relevant copy-number changes in 7.3 % (51 of 697) and 5.3 % (8 of 151) of patients, respectively, both higher than karyotype. With the exception of recurrent deletion and duplication of chromosome 16p11.2 and 15q13.2q13.3, most copynumber changes were unique or identified in only a small subset of patients. The authors concluded that (i) CMA had the highest detection rate among clinically available genetic tests for patients with ASD, (ii) interpretation of microarray data is complicated by the presence of both novel and recurrent copy-number variants of unknown significance, and (iii) despite these limitations, CMA should be considered as part of the initial diagnostic evaluation of patients with ASD.

The ACOG’s Committee Opinion on array CGH in prenatal diagnosis (2009) stated that the widespread use of array CGH for the diagnosis of genomic re-arrangements in children with idiopathic mental retardation, developmental delay, and multiple congenital anomalies has spurred interest in applying
array CGH technology to prenatal diagnosis. The use of array CGH technology in prenatal diagnosis is currently limited by several factors, including the inability to detect balanced chromosomal re-arrangements, the detection of copy number variations of uncertain clinical significance, and significantly higher costs than conventional karyotype analysis. Although array CGH has distinct advantages over classic cytogenetics in certain applications, the technology is not currently a replacement for classic cytogenetics in prenatal diagnosis. Furthermore, the Committee Opinion stated that targeted array CGH may be useful as a screening tool; however, further studies are needed to fully determine its utility and limitations.

A statement by the American College of Medical Genetics (ACMG) that recommended the use of CGH in autism spectrum disorders (Manning, et al., 2010) appears to be based upon consensus, as its conclusions are not supported by a systematic evidence review with evidence grading.

There is a lot of interest in CGH as a promising tool that allows for the genome-wide detection of DNA CNV such as deletions or duplications; however, current guidelines do not recommend CGH for the diagnosis of congenital anomalies, ASD, DDs, or idiopathic MR in newborns or children, or for screening for prenatal gene mutations. The technology is still evolving and wide variation in the type of array utilized exists. The extent of genomic CNV and phenotypes associated with specific CNVs is also still incomplete. How prenatal management will be altered by these findings remains unclear and the optimal type of array for use in prenatal diagnosis has yet to be defined. Further studies are needed to determine parameters for the interpretation and implementation of this technology.

Roesser (2011) reviewed genetic testing guidelines in the evaluation of children with ASDs. The clinical report published by the AAP recommended individualizing the work-up, including karyotype and specific DNA testing for fragile X syndrome. A recent publication reported higher rates of abnormalities on CGH microarray (CMA) testing on children
with ASD. The medical records of 507 children seen through the Kirch Developmental Services Center were abstracted for genetic testing and factors associated with this testing. Abnormalities were found on karyotype in 2.3% and in DNA for fragile X in 0.04%. The author concluded that the diagnostic yield of the genetic testing was low in this population. Furthermore, their findings supported the theory that CMA can be considered as part of the initial genetic screening in children with ASD in most situations. They stated that prospective studies will need to be performed to evaluate children in a standard fashion.

There are currently insufficient published reports on the use of CGH in recurrent miscarriage. Available guidelines for genetic evaluation and counseling of couples with recurrent miscarriage (Laurino et al, 2005) stated: "The use of specialized chromosomal studies such as comparative genome hybridization, subtelomeric studies, interphase studies on sperm and assays for skewed X-inactivation patterns are not warranted at this time, as their clinical utility has yet to be determined."

Bartnik et al (2012) noted that there are only few studies of the role of CNVs in epilepsy and genetic etiology in the vast majority of cases remains unknown. These researchers applied whole-genome exon-targeted oligonucleotide array CGH to a cohort of 102 patients with various types of epilepsy with or without additional neurodevelopmental abnormalities. Chromosomal microarray analysis revealed 24 non-polymorphic CNVs in 23 patients, among which 10 CNVs are known to be clinically relevant. Two rare deletions in 2q24.1q24.3, including KCNJ3 and 9q21.13 are novel pathogenic genetic loci and 12 CNVs are of unknown clinical significance. The authors concluded that these findings further support the notion that rare CNVs can cause different types of epilepsy, emphasize the efficiency of detecting novel candidate genes by whole-genome array CGH, and suggest that the clinical application of array CGH should be extended to patients with unexplained epilepsies. These results need to be validated by well-designed studies.
Comparative Genomic Hybridization (CGH) for Histologically Equivocal Spitzoid Melanocytic Neoplasms (Spitz Nevus and Atypical Spitz Tumors):

Bauer and Bastian (2006) stated that cancer typically results in loosened control over genomic integrity, resulting in alterations of the genome of cancer cells. Comparative genomic hybridization (CGH) is a method that can be used on DNA extracted from routinely fixed tissue to assess the entire genome for the presence of changes in DNA copy number. CGH analysis has revealed that melanoma differs from melanocytic nevi by the presence of frequent chromosomal aberrations. In contrast, melanocytic nevi typically show no chromosomal aberrations, or have a restricted set of alterations with basically no overlap to melanoma. These marked differences between aberration patterns in melanomas and melanocytic nevi can be exploited diagnostically to classify melanocytic tumors that are ambiguous based on histopathologic assessment. In addition to potential diagnostic applications, detailed analyses of recurrent aberrations can lead to the identification of genes relevant in melanocytic neoplasia.

Ali et al (2010) stated that melanocytic neoplasms with spitzoid features including spitz nevi, spitz tumors and spitzoid melanomas are commonly encountered in the practice of dermatopathology. Although many cases can be accurately diagnosed on the basis of morphology and histology, a significant number of cases may be difficult to accurately classify. Several studies have now shown that chromosomal copy number aberrations are typical of melanoma while present in only a small percent and to a limited degree in spitz nevi. In this study, these researchers correlated the clinical, histologic and array CGH findings of 10 spitzoid melanocytic neoplasms. This study showed that the clinical and histologic changes correlate well with the molecular findings by array CGH. Further that array CGH can be used to help classify and predict behavior of spitzoid melanocytic neoplasms. A limited variety of copy number aberrations including gains of 11p and much more rarely 7q may be seen in spitz nevi. Additionally in
this report these investigators presented the first case of a typical spitz nevus with copy number gains involving both 7q and 11p. Conversely, melanomas with spitzoid features typically have multiple chromosomal copy number aberrations involving a variety of loci. A smaller number of chromosomal aberrations, possibly a single aberrant locus, may be present in spitz tumors, but their presence may predict more aggressive behavior.

Ahmadi et al (2010) stated that melanocytic proliferations with Spitz differentiation present a difficult clinicopathologic dilemma, as their spectrum ranges from benign to malignant. Distinct entities include Spitz nevus, atypical Spitz nevus, and Spitzoid melanoma. Their histopathologic differentiation can be challenging, and cases of Spitzoid melanoma initially diagnosed as benign Spitz nevi were reported in the literature. The goal of this study was to discuss the diagnostic tools (including CGH), which may be helpful in differentiating benign Spitz nevi from malignant melanoma with Spitzoid features, and to propose an appropriate management strategy for each entity. Medical records of patients referred for suspicious nevi were reviewed. Data regarding demographics, site, pathology reports, and treatment were reviewed. A total of 4 patients with 3 distinct diagnoses involving Spitz differentiation were identified. The pathologic interpretation of these biopsies was difficult and multiple dermatopathologists were involved. All 4 patients underwent excision with or without sentinel node biopsy. The authors concluded that otolaryngologists, plastic surgeons and dermatopathologists will encounter patients who have melanocytic lesions with Spitz differentiation at some point in their career. The management of these patients is significantly impacted by the histopathologic diagnosis, and should not be undertaken until it is confirmed, possibly with CGH. In the authors’ experience, it is not unusual to have multiple independent pathologic examinations. They believed that a team approach between the surgeon and the dermatopathologist is crucial when diagnosing and managing patients with Spitz lesions.

Busam et al (2014) noted that Spitz tumors represent a group of
melanocytic neoplasms that typically affect young individuals. Microscopically, the lesions are composed of cytologically distinct spindle and epithelioid melanocytes, with a range in the architectural display of the cells, their nuclear features, and secondary epidermal or stromal changes. Recently, kinase fusions have been documented in a subset of Spitz tumors, but there is limited information on the clinical and pathologic features associated with those lesions. These investigators reported a series of 17 patients (9 males, 8 females) with spitzoid neoplasms showing ALK fusions (5 Spitz nevi and 12 atypical Spitz tumors). The patients' ages ranged from 2 years to 35 years (mean of 17 years; median of 16 years). Most lesions were located on the lower extremities and presented clinically as polypoid nodules. All tumors were compound melanocytic proliferations with a predominant intra-dermal growth. Tumor thickness ranged from 1.1 to 6 mm (mean of 2.9 mm; median of 2.5 mm). The most characteristic histopathologic feature of the tumors (seen in all but 2 lesions) was a plexiform dermal growth of intersecting fascicles of fusiform melanocytes. All but 2 tumors were amelanotic. All tumors were strongly immunoreactive for ALK. The ALK re-arrangements were confirmed in all cases by fluorescence in situ hybridization (FISH), and the fusion partner was determined by quantitative polymerase chain reaction as TPM3 (tropomyosin 3) in 11 cases and DCTN1 (dynactin 1) in 6 cases. None of the 8 tumors that were analyzed by FISH for copy number changes of 6p, 6q, 9p, or 11q met criteria for melanoma. Two patients underwent a sentinel lymph node biopsy, and in both cases melanocyte nests were found in the subcapsular sinus of the node. Array CGH of these 2 tumors revealed no chromosomal gains or losses. The authors concluded that the findings of this study revealed that Spitz nevi/tumors with ALK re-arrangement showed a characteristic plexiform morphology and that ALK immunohistochemistry and FISH enable the accurate identification of this morphologic and genetic distinct subset of spitzoid neoplasms.

An UpToDate review on “Spitzoid melanocytic neoplasms (Spitz nevus and atypical Spitz tumors)” (Barnhill and Kim, 2015)
states that “Comparative genomic hybridization -- Whole genome analysis for DNA gains or losses by comparative genomic hybridization (CGH) revealed amplification of chromosome 11p (a genome area that contains the HRAS gene) in approximately 20% of Spitz tumors. The 11p amplification is characteristic of Spitz tumors and is not commonly seen in melanoma. A study of 16 atypical Spitz tumors with array CGH showed that almost all of the chromosomal aberrations present in these lesions were not those commonly seen in conventional melanoma. These findings provide support for atypical Spitz tumor as a group of lesions distinct from both conventional Spitz tumors and conventional melanoma .... Histopathologic diagnosis -- The initial step in diagnosis is histopathologic examination of the excised tumor to determine whether a clear-cut Spitz tumor or melanoma is present. Atypical lesions should be systematically evaluated for histopathologic and immunohistochemical features. For ambiguous lesions, investigation of genetic aberrations by comparative genomic hybridization or fluorescence in situ hybridization may be performed if available .... Immunostaining may be helpful in the evaluation of ambiguous lesions. Ki-67 labeling in the deepest part of lesion and/or a labeling index > 30% favors melanoma, although considerable overlap exists between atypical Spitz tumors and melanoma. Molecular analysis with comparative genomic hybridization or fluorescence in situ hybridization can be performed if the diagnosis remains uncertain. The absence of genetic aberrations or the demonstration of an isolated copy gain in chromosome 11p favors a diagnosis of benign lesion. In contrast, the demonstration of chromosomal alterations characteristic of conventional melanoma, including deletions in 9p, 10q, gains in chromosome 7, and BRAF mutations suggest malignancy”.

Furthermore, National Comprehensive Cancer Network’s clinical practice guideline on “Melanoma” (Version 3.2015) states that “Considers use of comparative genomic hybridization (CGH) or fluorescence in situ hybridization (FISH) for histologically equivocal lesions“.
Evaluation of Short Stature:

UpToDate reviews on “Diagnostic approach to children and adolescents with short stature” (Rogol, 2016a), “Causes of short stature” (Rogol, 2016b) and “Growth hormone treatment for idiopathic short stature” (Rogol, 2016c) do not mention comparative genomic hybridization as a management tool.

Appendix

DSM-5 Diagnostic Criteria for Autism Spectrum Disorders is available at the following website:


DSM-5 Diagnostic Criteria for Intellectual Disability is available at the following website: http://aaidd.org/intellectual-disability/definition (http://aaidd.org/intellectual-disability/definition)

DSM-5 defines global developmental delay (GDD) as occurring in children less than five years of age who fail to meet expected developmental milestones in multiple areas of functioning. Developmental milestones are available at the following website: http://www.cdc.gov/ncbddd/actearly/milestones/index.html (http://www.cdc.gov/ncbddd/actearly/milestones/index.html)

Major defects are structural abnormalities that affect the way a person looks and require medical and/or surgical treatment. Minor defects are abnormalities that do not cause serious health or social problems. When multiple birth defects occur together and have a similar cause, they are called syndromes. If two or more defects tend to appear together but do not share the same cause, they are called associations.

Major congenital malformations include: absent digits; absent or hypoplastic clavicles; absent or limb deficiencies; ambiguous
genitalia; anencephaly; anophthalmia; cleft lip; cleft palate; colobomas (iris, retina); cystic hygroma; ectodactyly; encephalocele (occipital, frontal); gastrochisis; holoprosencephaly; hydrocephaly; hypoplasia or coarctation of the aorta; hypoplastic left heart; interrupted aortic arch type B; macro or microglossia; meningomyelocele; micrognathia severe (Robin sequence); microphthalmia; microtia severe; omphalocele; pectus excavatum; polydactyly, complete syndactyly, polysyndactyly; spina bifida; Tetralogy of Fallot; total anomaly of pulmonary venous return; transposition of the great vessels; truncus arteriosus; and ventricular or atrial septal defect.

Minor congenital malformations include: abnormal hair whorls (absence, more than 2); anteverted nostrils; bifid uvula; branchial sinuses; bridge crease; cubitus valgus; cup-shaped ear; dimples over major joints; ear lobe: attached, creases, notches, or bifid; ear tags; epicanthal folds; extra nipples; fifth finger clinodactyly; flat bridge; flat occiput; frontal bossing; hemangioma; hypertelorismm hypo or hyperpigmented macules; hypotelorism; lop ear; macrostomia; metopic fontanel; micrognathia; microstomia; minor hypospadias; multiple frenula; nail hypoplasia; nevi; overlapping digits; partial syndactyly between 2-3 toes; persistent finger pads (fetal pads); philtrum long, short, flat; plagiocephaly; preauricular sinuses; prominence of the heels; protruding ear; ptosis; redundant skin; sacral dimple; shawl scrotum; short neck; short palpebral fissures; single transverse plamar crease; small ears; synophrys; tapered fingers; upslanting or downsousing palpebral fissures; vaginal tags; and webbing.

<table>
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<th>CPT Codes / HCPCS Codes / ICD-10 Codes</th>
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<td>Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by &quot;+&quot;:</td>
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<td>CPT codes covered if selection criteria are met:</td>
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**HCPCS codes covered if selection criteria are met:**

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<tr>
<td>S3870</td>
<td>Comparative genomic hybridization (CGD) microarray testing for developmental delay, autism spectrum disorder and/or intellectual disability</td>
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**ICD-10 codes covered if selection criteria are met:**

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<th>Code</th>
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<tr>
<td>F01 - F99</td>
<td>Mental, behavioral and neurodevelopmental disorders</td>
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<tr>
<td>D22.0 - D22.9</td>
<td>Melanocytic nevi [Spitzoid melanocytic neoplasms]</td>
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<tr>
<td>O35.0xx0 - O35.0xx9</td>
<td>Maternal care for (suspected) central nervous system malformation in fetus</td>
</tr>
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<td>O35.1xx0 - O35.1xx9</td>
<td>Maternal care for (suspected) chromosomal abnormality in fetus</td>
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<tr>
<td>O35.3xx0 - O35.3xx9</td>
<td>Maternal care for (suspected) damage to fetus from viral disease in mother</td>
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<td>O35.4xx0 - O35.4xx9</td>
<td>Maternal care for (suspected) damage to fetus from alcohol</td>
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<td>O35.5xx0 - O35.5xx9</td>
<td>Maternal care for (suspected) damage to fetus by drugs</td>
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<td>Maternal care for (suspected) damage to fetus by radiation</td>
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<td>O35.8xx0 - O35.8xx9</td>
<td>Maternal care for other (suspected) fetal abnormality and damage</td>
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<td>O35.9xx0 - O35.9xx9</td>
<td>Maternal care for (suspected) fetal abnormality and damage, unspecified</td>
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<tr>
<td>Q00.0 - Q99.9</td>
<td>Congenital malformations, deformations and chromosomal abnormalities [for evaluating fetuses with structural abnormalities detected on fetal ultrasound or fetal MRI]</td>
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<tr>
<td>Z37.1</td>
<td>Single stillbirth</td>
</tr>
<tr>
<td>Z37.3</td>
<td>Twins, one liveborn and one stillborn</td>
</tr>
<tr>
<td>Z37.4</td>
<td>Twins, both stillborn</td>
</tr>
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</table>
Z37.7 | Other multiple births, all stillborn

**ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):**

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<td>C43.0 - C43.9, D03.0 - D03.9</td>
<td>Malignant melanoma of skin</td>
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<tr>
<td>D89.82</td>
<td>Autoimmune lymphoproliferative syndrome [ALPS]</td>
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<td>G40.001 - G40.919</td>
<td>Epilepsy and recurrent seizures</td>
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<td>O02.81 - O02.9</td>
<td>Other abnormal product of conception</td>
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<td>O09.511 - O09.529</td>
<td>Supervision of elderly primigravida or multigravida</td>
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<tr>
<td>O35.2xx0 - O35.2xx9</td>
<td>Maternal care for (suspected) hereditary disease in fetus</td>
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<td>O35.7xx0 - O35.7xx9</td>
<td>Decreased fetal movements, second trimester</td>
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<tr>
<td>O73.1</td>
<td>Retained portions of placenta and membranes, without hemorrhage</td>
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<td>Z13.4</td>
<td>Encounter for screening for certain developmental disorders in childhood</td>
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<td>Z13.71 - Z13.79</td>
<td>Encounter for screening for genetic and chromosomal anomalies</td>
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<td>Z13.810 - Z13.89</td>
<td>Encounter for screening for other specified diseases and disorders</td>
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<td>Z13.9</td>
<td>Encounter for screening, unspecified</td>
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<tr>
<td>Z36</td>
<td>Encounter for antenatal screening of mother</td>
</tr>
</tbody>
</table>

The above policy is based on the following references:


18. American College of Obstetricians and Gynecologists


27. Edelmann L, Hirschhorn K. Clinical utility of array CGH


29. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Special report: Array comparative genomic hybridization (aCGH) for the genetic evaluation of patients with developmental delay/mental retardation and autism spectrum disorder. TEC Assessment Program. Chicago, IL: BCBSA; April 2009; 23(10).


68. BlueCross BlueShield Association (BCBSA), Technology Evaluation Center (TEC). Chromosomal microarray for the genetic evaluation of patients with global developmental delay, intellectual disability, and autism spectrum disorder. TEC Special Report. Chicago, IL: BCBSA; August 2015;30(2).


72. Ahmadi N, Davison SP, Kauffman CL. Melanocytic nevi with Spitz differentiation: Diagnosis and management. Laryngoscope. 2010;120(12):2385-2390.


78. Rogol AD. Diagnostic approach to children and adolescents with short stature. UpToDate Inc., Waltham, MA. Last reviewed July 2016a.
80. Rogol AD. Growth hormone treatment for idiopathic short stature. UpToDate Inc., Waltham, MA. Last reviewed July 2016c.
Amendment to
Aetna Clinical Policy Bulletin Number: 0787
Comparative Genomic Hybridization

There are no amendments for Medicaid.