



## ARRAY COMPARATIVE GENOMIC HYBRIDIZATION FOR THE IDENTIFICATION OF CHROMOSOMAL ABNORMALITIES

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### Overview

Chromosomal abnormalities are frequently identified as the cause of many neurodevelopmental disorders. For many well-defined syndromes, the type and location of the chromosomal abnormality has been established and conventional methods of chromosome analysis, such as G-banded karyotype (karyotyping) and fluorescence in situ hybridization (FISH) can identify the genetic etiology for a clinical diagnosis. These conventional methods have a very low diagnostic yield (i.e., the proportion of tested patients with clinically relevant genomic abnormalities) in patients with clinical features that do not clearly fit into one of the classically defined syndromes.

Array comparative genomic hybridization (array CGH), also known as chromosome microarray analysis, has emerged as a way to identify copy number variations (CNVs), i.e., chromosomal amplifications, gains, losses, and deletions, in some individuals, that are undetectable by conventional chromosome analysis. Array CGH makes it possible to compare chromosomes from two differentially labeled genomes and identify CNVs. The two genomes, a reference (or control) and a test (or patient) are cohybridized onto a solid support (usually a glass microscope slide) on which cloned or synthesized DNA fragments have been immobilized.

### Evidence-based medicine

On one hand it is tempting to apply the latest technology in clinical diagnostics. On the other hand, such an application will generate results that are so new that we do not have an understanding of their clinical significance. More studies with large sample sizes and functional genetic experiments are required to answer these questions. Evidence-based medicine is defined as the conscientious, explicit, and judicious use of current best evidence, combined with individual clinical expertise and patient preferences and values, in making decisions about the care of individual patients.

The National Institutes of Health-Department of Energy Working Group on Ethical, Legal and Social Implications of Human Genome Research Task Force (<http://www.genome.gov/10001733>) strongly recommends that all of the following criteria be satisfied before a genetic test is made available to the public.

1. Analytical sensitivity and specificity of a genetic test must be determined before it is made available in clinical practice.
2. The genotypes to be detected by a genetic test must be shown by scientifically valid methods to be associated with the occurrence of a disease. The observations must be independently replicated and subject to peer review.

3. Data to establish the clinical validity of genetic tests (clinical sensitivity, specificity, and predictive value) must be collected under investigative protocols. In clinical validation, the study sample must be drawn from a group of subjects' representative of the population for whom the test is intended. Formal validation for each intended use of a genetic test is needed.
4. Before a genetic test can be generally accepted in clinical practice, data must be collected to demonstrate the benefits and risks that accrue from both positive and negative results.

It is the opinion of the Task Force that no clinical laboratory should offer a genetic test whose clinical validity has not been established, unless it is collecting data on clinical validity under either an IRB-approved protocol or conditional premarket approval agreement with FDA.

The CDC's Office of Public Health Genomics established and supports the ACCE Model Project, which developed the first publicly-available analytical process for evaluating scientific data on emerging genetic tests. <http://www.cdc.gov/genomics/gtesting/ACCE/> The ACCE framework has guided or been adopted by various entities in the United States and worldwide for evaluating genetic tests. ACCE, takes its name from the four main criteria for evaluating a genetic test — alytic validity, clinical validity, clinical utility and associated ethical, legal and social implications.

1. Analytic validity (analytical sensitivity and specificity) refers to the accuracy with which a particular genetic characteristic is identified by a given test. This accuracy does not imply any clinical significance, such as diagnosis.
2. Clinical validity (clinical sensitivity and specificity) refers to the accuracy with which the test distinguishes affected and unaffected populations including a determination of the probability of being affected, i.e., genotype-phenotype correlation (if a genotype does not predict a disease phenotype, the test will not support appropriate management decisions)
3. Clinical utility is the likelihood that there will be an improved outcome for the patient as a result of testing (presuming effective interventions are available). Clinical utility is the degree to which a test is predictive of treatment benefit, and hence is a critical foundation for the use of a test in clinical decision making.

#### Analytical validity of Array CGH

Array CGH is commercially available from several clinical laboratories as laboratory developed tests. Laboratory developed tests do not require FDA approval for marketing. Each of the commercially available Array CGH tests vary considerably in technology, resolution, and likelihood of producing results of unknown significance. Each of these facets of microarray technology has important implications. There are two basic levels of Array CGH testing: targeted and whole-genome. Targeted arrays focus on genomic regions of known clinical significance. They may be designed to study a specific chromosome or chromosomal segment. Whole-genome microarrays provide an extensive, albeit arbitrary coverage, across the entire genome. Arrays have been built with a variety of platforms that include oligonucleotides and bacterial artificial chromosomes (BACs).

The performance of microarray analysis in detecting chromosomal abnormalities is dependent on the resolution of the array and the distance between those sequences naturally located on the chromosome. Studies evaluating the analytical validity of microarrays using BAC or oligonucleotide probes have demonstrated that Array CGH provides a chromosomal evaluation at a much high resolution than standard karyotype or FISH with the ability to detect CNVs as small as 50 kilobases (kb) in length. (Hayes GTE Report, 2010)

As indicated above, the sensitivity of Array CGH varies from lab to lab. The EMArray Cyto6000™ (manufactured by Agilent Technologies, Santa Clara, CA) is used by a consortium of academic and commercial laboratories throughout the U.S. The EMArray Cyto6000™ is an oligonucleotide platform. EMArray Cyto6000™ demonstrated 100% concordance with previous karyotyping and/or FISH results (proven analytic validity). This array routinely detects genomic copy number changes of >500 Kb, in the genomic backbone. The resolution is significantly better for targeted regions and imbalances as small as 50 Kb can be efficiently detected. Thus the resolution of Array CGH analysis using this oligonucleotide array is at least 10-fold greater than a conventional 550-band karyotype. (Baldwin et al., 2008)

#### Clinical validity of Array CGH

Generally, Array CGH is highly sensitive, approaching 100%, for the detection of known CNVs previously identified by conventional methods. Studies that have evaluated the sensitivity of aCGH in patients with developmental delay, mental retardation and autism spectrum disorders, suspected of having a chromosomal abnormality, but who are negative by conventional cytogenetic evaluation, cite rates of detection of clinically relevant CNVs ranging from 5.4% to 12.1% for targeted arrays and between 7.5% and 15.6% for whole-genome arrays. Although seemingly small, this represents a doubling of the rates of detection prior to the application of this technique. To determine the clinical relevance of CNVs, Array CGH results must be interpreted in the context of other clinical and laboratory findings.<sup>1</sup> For the same population, benign CNVs were found in up to 15% of patients and CNVs of unknown clinical significance were found in up to 4.2% of patients. The clinical sensitivity of Array CGH varies depending on whether target or whole genome array is performed, the platform used, and the population being evaluated (patients with dysmorphic features versus patients with no dysmorphic features, etc.). (Shaffer et al. 2006; Lu et al., 2007; Aston, et al. , 2008; Shao et al., 2008; Shevell et al. 2008; Miller et al., 2010, Shen et al., 2010)

Like all genetic testing, array-CHG has limitations that are important to understand. Array CGH will not detect:

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<sup>1</sup> Each laboratory must establish a protocol that allows for confirmation of abnormal or ambiguous Array CGH results. This may include karyotyping, FISH, polymerase chain reaction, or other comparable methods. Parental studies or studies of other family members may be indicated after identification of some chromosome abnormalities. In some cases, follow-up testing is performed to exclude a balanced parental rearrangement or the possibility that a parent has a deletion or duplication. In other cases, parental testing is needed when CNVs are detected by a-CGH in regions of the genome with unclear clinical significance. In these cases, parental studies may be indicated to distinguish between a familial variant and a *de novo*, possibly clinically relevant alteration. (Shaffer et al., 2007)

- Balanced chromosome rearrangements, such as balanced translocations or inversions
- Deletions or duplications that are smaller than 50 kb or point mutations within genes
- Mosaicism at a level lower than 20%
- Some types of polyploidy, such as triploidy
- Alterations in chromosome structure at areas of the genome not covered by the array

Another key limitation of Array CGH is the identification of CNVs of undetermined significance whose contributions to genetic variation and disease are not yet understood.

#### Clinical utility of Array CGH

Array CGH is recommended in the evaluation of individuals with non-syndromic global developmental delay, mental retardation and autism spectrum disorders by the American College of Medical Genetics (Manning et al., 2010) and the American Society of Human Genetics (Miller et al., 2010). Clinical utility is determined by the impact of a genetic etiology on outcomes that matter to the patient. Studies of the clinical utility of array CGH infer that the diagnosis of a chromosomal abnormality will lead to improved outcomes for the patient and family through the avoidance of additional diagnostic tests, more accurate genetic counseling and risk reduction, more informed reproductive decision-making, and improved access to services. Few published studies address the clinical utility of Array CGH. Saam et al., (2008) published a survey of 14 physicians and 48 patients with developmental delay who had CNVs detected by Array CGH. The authors reported that 70.8% of patients had management changes most commonly physicians provided patients' families with a recurrence risk for subsequent pregnancies. Therefore it is not possible to come to any evidence based conclusions regarding the clinical utility of Array CGH.

With respect to the evaluation of children with global developmental delay or mental retardation, the AAP Clinical Report (Moeschler et al., 2006) finds *"The use of microarray comparative genomic hybridization in the evaluation of children with developmental delay or mental retardation might be considered best as "emerging technology." There are currently insufficient published reports on the use of this technology in the evaluation of the child with developmental delay or mental retardation."*

With respect to the evaluation of children with autism spectrum disorders, the American Academy of Pediatrics (AAP) Clinical Report (Johnson et al., 2007 and reaffirmed September 2010) does not support an extensive etiology work-up of children with autism spectrum disorders in the absence of coexisting dysmorphic features, family history, severe global developmental delay or mental retardation. The AAP position on Array CGH is that *"Comparative 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."*

#### Ethical and legal considerations

In Massachusetts, General Law C 111, § 70G requires written consent prior to genetic testing. The consent form must be signed by the person who is the subject of the test,

or if that person lacks the capacity to consent, signed by the person authorized to consent for such person. There are numerous issues relating to genetic testing of children, who are unable to give informed consent. The American Society of Human Genetics (ASHG) and the American College of Medical Genetics (ACMG) recommend that a “timely medical benefit to the child should be the primary justification for genetic testing in children and adolescents.” (ASHG/ACMG Report, 1995) Although sympathetic to the considerable difficulties inherent in living with uncertainty about the health status of a child, these difficulties do not warrant foreclosing the child’s right to make an independent decision in regard to genetic testing in adulthood. Because genetic testing has potential for both great benefit and great harm, providers of genetic services play increasingly important roles in counseling families about the suitability of genetic testing for their children. Evidence is needed that the potential benefits of genetic testing in children truly exist and that they outweigh the putative harms.

### Definitions

**Copy-number variations (CNVs)** - a form of genomic structural variation, CNVs are alterations of the DNA of a genome that results in the cell having an abnormal number of copies (i.e., insertions, deletions and duplications) of one or more sections of the DNA. CNVs may either be inherited or caused by *de novo* mutation. Many of structural variants are associated with genetic diseases, however more are not. CNVs have been identified in some individuals with neurodevelopmental disorders, but the full etiologic role is unknown.

**Genetic test** -The analysis of human DNA, RNA, chromosomes, proteins, and certain metabolites in order to detect heritable disease-related genotypes, mutations, phenotypes or karyotypes for clinical purposes. Such purposes include predicting risk of disease, identifying carriers and establishing prenatal and clinical diagnosis or prognosis. Prenatal, newborn and carrier screening, as well as testing in high-risk families, are included. <http://www.genome.gov/10002405>

### Policy

Array CGH has dramatically increased the rate of identification of chromosomal abnormalities among individuals with neurodevelopmental disorders including but not limited to global developmental delay, mental retardation and autism spectrum disorder. However, it is not clear what impact this information has on outcomes that matter to the plan member. Array CGH is considered not medically necessary for the identification of chromosomal abnormalities in individuals with neurodevelopmental disorders including but not limited to global developmental delay, mental retardation and autism spectrum disorder.

Array CGH is considered not medically necessary for the identification of chromosomal abnormalities in prenatal diagnosis.

### Coding

Codes	Number	Description
CPT	88384	Array-based evaluation of multiple molecular probes; 11 through 50 probes
	88385	Array-based evaluation of multiple molecular probes; 51 through 250 probes

Codes	Number	Description
	88386	Array-based evaluation of multiple molecular probes; 251 through 500 probes
HCPCS	S3870	Comparative genomic hybridization (CGH) microarray testing for developmental delay, autism spectrum disorder and/or mental retardation

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### Products to Which This Policy Applies

- ⊕ FCHP Direct & Select Care
- ⊕ Fallon Preferred Care
- ⊕ Major Medical
- ⊕ MassHealth
- ⊕ Commonwealth Care
- ⊕ Companion Care
- ⊕ Fallon Senior Plan
- ⊕ Navicare
- ∅ Summit Elder Care® PACE (With the exception of emergency care, all services for Summit ElderCare® PACE participants must be authorized and arranged by the Summit ElderCare (SE) Interdisciplinary Team (IDT) overseeing the care for that participant. The applicable IDT can be determined by the HCO code on the participant ID card. A Summit ElderCare clinician is always on call and can be reached by dialing any of the site telephone numbers.)

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**Committee Review Dates:**

Technology Assessment Committee: 05/04/2011

**IMPORTANT NOTE**

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