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# Special Report: aCGH for the Genetic Evaluation of Patients with Developmental Delay/Mental Retardation or Autism Spectrum Disorder

## Executive Summary

### Background

Children with signs of neurodevelopmental delays or disorders in the first few years of life may eventually be diagnosed with mental retardation or autism syndromes, serious and lifelong conditions that present significant challenges to families and to public health. A large proportion of cases of developmental delay/mental retardation and of autism are associated with genetic abnormalities. Current guidelines for these patients recommend cytogenetic evaluation to look for certain kinds of chromosomal abnormalities that may be causally related to their condition. Most commonly these abnormalities are deletions and duplications of large segments of genomic material, which are called “copy number variants,” or CNVs. For many well-described syndromes, the type and location of the chromosomal abnormality have been established with the study of a large number of cases and constitute a genetic diagnosis; for others, only a small number of patients with similar abnormalities may exist to support a genotype-phenotype correlation. Finally, for some patients, cytogenetic analysis will discover entirely new chromosomal abnormalities that will require additional study to determine their significance.

Conventional methods of cytogenetic analysis, including karyotyping (e.g., G-banded) and fluorescence in situ hybridization (FISH), have relatively low resolution and a low diagnostic yield (i.e., proportion of tested patients with clinically relevant genomic abnormalities), leaving the majority of cases without identification of a chromosomal abnormality associated with the child’s condition. Array comparative genomic hybridization (aCGH) is a newer cytogenetic analysis method that increases the chromosomal resolution for detection of CNVs, and, as a result, increases the diagnostic yield and the genomic detail beyond that of conventional methods. Array CGH results are clinically informative in the same way as results derived from conventional methods and thus aCGH represents an extension of standard methods with increased resolution. aCGH may be ordered when conventional results are negative, although some believe it will eventually replace conventional technology.

There are two types of CGH arrays. The first kind, targeted CGH arrays, provide high-resolution coverage of the genome primarily in areas containing known, clinically significant CNVs. The second kind, whole-genome arrays, provide high resolution coverage of the entire genome. Thus, in addition to detecting known CNVs like targeted arrays, whole genome arrays also promote discovery of new CNVs. Such discoveries have resulted in the characterization of several new genetic syndromes by aCGH, with other potential candidates currently under study. However, the whole-genome arrays also have the disadvantage of potentially high numbers of apparent false-positive results, because benign CNVs are found in phenotypically normal populations; both benign and pathogenic CNVs are continuously cataloged and made available in public reference databases to aid in clinical interpretation. Additionally, some new CNVs are neither known to be benign nor causal; these CNVs may require



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detailed family history and family genetic testing to determine clinical significance, and/or may require confirmation by subsequent accumulation of similar cases and so, for a time, may be considered a CNV of undetermined significance (some may eventually be confirmed true positives or causal, others false positives or benign).

This Special Report will summarize the current status of aCGH, including what is known about how the technology improves diagnostic yield and outcomes in patients with clinically diagnosed developmental disability/mental retardation (DD/MR) or autism spectrum disorder (ASD), but normal results by conventional cytogenetic evaluation.

### Search Strategy

An initial MEDLINE® (via PubMed) search strategy of (“Developmental Disabilities”[MeSH®] OR “Mental Retardation”[MeSH®] OR “Autistic Disorder”[MeSH®]) AND (“Genetic Screening”[MeSH®] OR “Genetic Predisposition to Disease”[MeSH®] OR “Genetic Phenomena”[MeSH®] OR “Genetic Techniques”[MeSH®]), limited to human subjects, English language, and published within the last 3 years, yielded 2,172 citations. Specific searches for studies of the clinical outcomes of cytogenetic testing in DD/MR or ASD were also conducted. Relevant, earlier articles were selected from bibliographies of recent publications.

### Selection Criteria

Included studies were case reports, case series, or cohort studies published in peer-reviewed journals that enrolled patients with clinical diagnoses of developmental delay/mental retardation or of autism with known or suspicion of genetic abnormalities, but negative results by conventional cytogenetic evaluation, and that conducted molecular karyotyping by aCGH on enrolled patients. Studies were also included if they examined management decisions and/or patient outcomes based on genetic evaluation results, either by conventional methods or by molecular karyotyping.

### Results

**Question 1A: What is the clinical sensitivity of molecular karyotyping by aCGH for known chromosomal abnormalities? What is the false-positive rate in normal controls? (Clinical Validity).** Several studies have conducted aCGH on samples with known chromosomal abnormalities by standard karyotyping. In general, currently available aCGH clinical services using higher-resolution bacterial artificial chromosome (BAC) arrays or high-resolution oligo CGH arrays achieve 100% sensitivity for known chromosomal abnormalities. False-positive rates (i.e., CNVs of undetermined clinical significance) were inconsistently reported and are not summarized here. However, it should be noted that as array resolution increases, the likelihood of indeterminate CNV results also increases. This is somewhat mitigated by the continual addition of new abnormalities, as well as new benign polymorphic CNVs to databases available for consultation.

**Question 1B: What is the diagnostic yield of molecular karyotyping by aCGH for patients suspected of having a chromosomal abnormality, but who are negative by conventional cytogenetic evaluation? (Clinical Validity).** Several studies reported the diagnostic yield (i.e., proportion of patients with clinically relevant genomic abnormalities) of aCGH in patients with normal standard karyotype. Overall, diagnostic yield ranged from 5–16.7% in DD/MR patients and from 3.4–11.6% in patients with ASD. Although seemingly small, these yields represent significant improvements in identifying a genetic etiology for these patients. Based on the diagnostic yields reported, the number needed to test (NNT) by aCGH to detect 1 clinically relevant abnormality ranges from 6 to 25. The estimate depends on the resolution of the array used and on the stringency of the patient selection criteria.

Increasing array resolution is accompanied by increasing potential for CNVs of uncertain clinical relevance. To determine clinical relevance in individual cases, the following actions are taken:

- CNVs are confirmed by another laboratory method.
- CNVs detected are checked against databases of benign and pathogenic CNVs.

- The laboratory may establish a size cutoff (e.g., 500 kb to 2 Mb); potentially pathogenic CNVs are likely to be larger than benign polymorphic CNVs.
- Parental studies are indicated when CNVs of appropriate size are detected and not found in available databases; CNVs inherited from a clinically normal parent are assumed to be benign, whereas those appearing de novo are likely pathogenic.

The final genetic assessment may not be definitive in all cases, but instead may report a CNV of undetermined significance. An attempt was made to abstract the rate of aCGH results requiring parental testing and of CNVs of undetermined significance for the studies included for this question; however, reporting was often unclear and dissimilar across studies and comparable rates could not be determined.

**Question 2: How are management decisions changed and outcomes improved by the results of molecular karyotyping by aCGH? (Clinical Utility).** Clinical utility is determined by the impact of a genetic diagnosis on outcomes that matter to the patient and family. Several outcomes are regarded as important in the setting of DD/MR or ASD, including estimation of recurrence rate for reproductive decision-making, avoidance of additional diagnostic tests and specialist consultations, and early and improved access to behavioral and educational services. However, neither standard cytogenetic analysis nor aCGH have been systematically studied for impact on these kinds of outcomes. Rather, clinical utility of genetic testing is primarily inferred based on the value of knowledge to the family, estimation of recurrence risk, and on the importance of early detection and early intervention.

A few published studies address aspects of clinical utility. The authors of one study interviewed 14 physicians (2 neurologists, 12 medical geneticists) regarding management changes as a result of positive aCGH testing results from the University of Utah Cytogenetics Laboratory for 48 patients with DD or MR and normal karyotypes. Only 29 percent of patients had no management changes reported. For significant proportions of patients, the diagnostic odyssey was ended, recurrence risk evaluation was possible in about one-third of families, and patients had improved access to services. However, this study was only a survey and did not attempt to quantitate the diagnostic tests avoided, determine the impact of improved access to services on patient outcomes, or assess the effect of recurrence risk on reproductive planning. Therefore the results are only suggestive of, but do not confirm, clinical utility.

The authors of another published report studied the reproductive decisions of women from 38 families with X-linked MR. Most of the women in these families spent many years knowing that they were at some risk of being carriers and of having a boy with MR. Prior to the availability of pathogenic mutation analysis, the birth rate for these families was well below average for the district (United Kingdom-New South Wales). After pathogenic mutation status was determined, both carriers and noncarriers (previously thought to be at risk) of the mutation had children at the same rate, with 74% of carriers choosing prenatal genetic evaluation. While the results of this study are suggestive, they do not show that knowledge of recurrence risk directly affected reproductive decisions.

A recent review of studies of early intervention for autism notes that while the evidence suggests that early intervention programs are beneficial, the evidence is generally not of the highest quality and whether early improvements in behavior and reductions in symptom severity lead to greater independence and social functioning in adulthood is unknown. Few randomized trials have been conducted and described interventions differ considerably, indicating that the field is still early in researching the elements of effective early intervention. Nevertheless, guidelines such as those published by the American Academy of Pediatrics and the American Academy of Neurology emphasize the importance of early diagnosis and intervention.

For DD/MR or ASD syndromes diagnosed by aCGH, it may be possible to predict the patient prognosis and the elements of care that will be most important to improving outcomes. However, the outcomes of prognosis and treatment planning have not been systematically studied.

**Question 3: What is the evidence that the technical performance of clinically available aCGH assays accurately detects clinically significant CNVs and any other chromosomal abnormalities included in the array, and perform in a robust manner over time? (Analytic Validity).** Molecular karyotyping is commercially available from several Clinical Laboratory Improvements Amendments (CLIA) -licensed laboratories as a laboratory-developed test (LDT). U.S. Food and Drug Administration (FDA) clearance for marketing is not required for LDTs; none of the commercially offered tests has been voluntarily submitted to the FDA for review. Clinically available molecular karyotyping tests currently differ considerably in technology, resolution, and likelihood of results with unknown significance. No laboratories were found that post a validation summary on their website. Some laboratories have published a summary of their assay validation procedure; examples highlight the complexity of designing and validating CGH arrays, as well as their analysis and interpretation.

#### **Author's Comments and Conclusions**

Current guidelines for early assessment of DD/MR and for ASD recommend genetic evaluation for those cases that cannot be readily diagnosed from clinical characteristics or other specific tests. Conventional cytogenetic analysis (e.g., G-banded karyotype, specific FISH assays, and subtelomeric screening) has been routinely used for many years but has low resolution and low diagnostic yield. aCGH specifically detects CNVs, which account for the majority of genomic abnormalities currently detectable by conventional cytogenetic testing. aCGH does not detect balanced translocations or inversions, which account for a minority of clinically important genomic abnormalities. However, aCGH has shown that a substantial proportion of apparently balanced translocations by conventional methods contain submicroscopic deletions (i.e., are actually unbalanced).

CGH arrays have the advantage of greatly improved resolution, which allows detection of smaller, clinically significant genomic abnormalities not detectable by conventional assays (improving diagnostic yield), and more exact locus definition of conventionally detectable abnormalities (improving information for genotype-phenotype correlation). While aCGH technology is relatively new, the results are conceptually similar to those obtained by conventional methods, and should be evaluated as an extension of those methods.

The results of neither conventional cytogenetic evaluation nor of aCGH evaluation have been systematically studied for impact on patient outcomes other than diagnostic yield, which is an intermediate outcome. Impact of testing on the kinds of outcomes that matter to the patient and family has been directly addressed in very few studies. Thus, it is not possible to draw evidence-based conclusions regarding the clinical utility of aCGH genetic evaluation. The same may also be said of conventional cytogenetic evaluation.

Expert consensus and clinical guidelines state that genetic information is of value because it establishes a causal explanation that is helpful to families. It is suggested that such genetic information avoids additional consultations and various types of diagnostic tests, assists with early and improved access to community services that may ameliorate or improve behavioral and cognitive outcomes, provides estimates of recurrence rates to better guide reproductive decision-making, and enables an understanding of prognosis and future needs. However, little evidence supports these outcomes.

aCGH technology is rapidly evolving and different kinds of arrays with different capabilities of detecting genomic abnormalities are clinically available from different laboratories; it is up to the ordering physician to know the limits of the particular technology employed by the laboratory.

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. Currently, a consortium of scientists from academic cytogenetic laboratories have agreed to develop a uniform, evidence-based “Molecular Karyotype” and shared national database to accumulate data on pathogenic versus benign deletions and duplications in the human genome. Such cooperative efforts should lead to more comparable results across platforms, more complete databases to aid in individual results interpretation, more uniform reporting, and more rapid accumulation of genotype-phenotype correlation information for future reference.

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## Objective

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Children with signs of neurodevelopmental delays or disorders in the first few years of life may eventually be diagnosed with mental retardation or autism syndromes, serious and lifelong conditions that present significant challenges to families and to public health. A diagnostic evaluation seeks to determine the etiology of the condition. A large proportion of cases of developmental delay/mental retardation (DD/MR) and of autism spectrum disorders (ASD) are associated with genetic abnormalities, likely involving many different genes. Current guidelines for these patients recommend cytogenetic evaluation to look for certain kinds of chromosomal abnormalities that may be causally related to their condition. Most commonly these abnormalities are deletions and duplications of large segments of genomic material, which are called “copy number variants,” or CNVs. For many well-described syndromes, the type and location of the associated chromosomal abnormality has been established by studying a large number of cases and constitutes a genetic diagnosis; for others, only a small number of patients with similar abnormalities may exist to support a genotype-phenotype correlation. Finally, for some patients, cytogenetic analysis will discover entirely new chromosomal abnormalities that will require additional study to determine their significance.

Conventional methods of cytogenetic analysis, including karyotyping (e.g., G-banded) and fluorescence in situ hybridization (FISH), have relatively low resolution and a low diagnostic yield (i.e., proportion of tested patients with clinically relevant genomic abnormalities), leaving the majority of cases without identification of a chromosomal abnormality associated with the child’s condition. Array comparative genomic hybridization (aCGH) is a newer cytogenetic analysis method that increases the chromosomal resolution for detection of CNVs, and as a result increases the diagnostic yield and the genomic detail beyond that of conventional methods. Array CGH results are clinically informative in the same way as results derived from conventional methods and thus aCGH represents an extension of standard methods with increased resolution. aCGH may be ordered when conventional results are negative, although some believe it will eventually replace conventional technology.

This Special Report will summarize the current status of aCGH, including what is known about how the technology improves diagnostic yield and outcomes in patients with clinically diagnosed DD/MR and/or ASD, but normal results by conventional cytogenetic evaluation.

This Special Report will not evaluate the use of molecular karyotyping by aCGH for prenatal evaluation. Rather, the initial focus will be evaluation of the evidence in the context of clinical signs and symptoms of a diagnosable medical problem, as this is the setting for the majority of the currently available evidence.

## Background

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*Note: For genetic terminology clarification and definitions, please see the Glossary of Genetic Terms.*

### Developmental Delay/Mental Retardation

The diagnosis of DD is reserved for children less than 5 years of age who have significant delay in two or more of the following developmental domains: gross or fine motor, speech/language, cognitive, social/personal, and activities of daily living (Moeschler 2008). The diagnosis implies developmental delays that may be significant and may predict life-long disability, although not all children diagnosed with DD will later be diagnosed with MR.

MR, also termed cognitive or intellectual disability, is a life-long disability diagnosed at or after age 5 when intelligence quotient (IQ) testing is considered valid and reliable. The Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association (DSM-IV, 1994) defines patients with MR as having an IQ less than 70, onset during childhood, and dysfunction or impairment in more than two of areas of adaptive behavior or systems of support.

The prevalence of both DD and MR in the general population has been estimated at 1–3%; mild MR (IQ 50–70) is more prevalent than moderate or severe MR (Shaffer 2005). DD/MR is influenced by genetic, environmental, infectious, and perinatal factors. The results from several studies estimate that 4 to 40% of all cases of MR have a genetic etiology (Burton 2006), with variability in this estimate likely due to patient selection criteria and available laboratory technology.

A significant proportion of children with DD/MR have multiple congenital anomalies (e.g., facial dysmorphism, cerebral palsy, muscle weakness, spasticity, epilepsy, microcephaly). Etiology can be determined in about 20–40% of DD/MR patients based on clinical history and physical examination alone (reviewed in Moeschler 2008). In other patients with less specific characteristics, history and examination may suggest differential diagnoses that can be verified with laboratory tests specific for these diagnoses, or by imaging studies.

For those cases of DD/MR for whom etiology or a specific syndrome is not clinically evident, additional diagnostic testing, including genetic evaluation, is recommended in a tiered approach, under the guidance of a medical specialist (see “Statements from Professional Societies,” following). Genetic evaluation is recommended whether anomalies are present or not; for example, Graham and Selikowitz (1993) reported that 4 of 10 patients with MR and a detectable chromosomal abnormality had no dysmorphic features.

Genetic testing for DD/MR consists of standard cytogenetic analyses (see following) and/or tests targeted to specific syndromes suggested by differential diagnosis based on clinical presentation. For example, the Fragile X syndrome, a relatively common cause of inherited MR, is associated with an excessive repetition of a trinucleotide gene sequence (i.e., CGG) in the *FMR1* gene on the X chromosome, resulting in a decrease or complete loss of Fragile X mental retardation 1 protein expression, important for normal neural development. Specific assays can detect and characterize the Fragile X mutation; diagnostic yield is estimated at 2% of males with MR in screening studies, with higher yield when limited to moderate to profound MR (van Karnebeek et al. 2005).

### Autism Spectrum Disorders

According to the DSM-IV, pervasive developmental disorders (PDD) encompass five conditions: autistic disorder, Asperger disorder, pervasive developmental disorder—not otherwise specified (PDD-NOS), childhood disintegrative disorder, and Rett syndrome. While the term “autism spectrum disorder” (ASD) is not mentioned in the DSM-IV, it is now accepted to include the first 3 in this list. However, ASD, PDD, and autism are often used interchangeably (Caronna et al. 2008). These conditions are

characterized by varying degrees of restrictions in communication and social interaction and atypical behaviors. The prevalence of ASD in the U.S. was reported to be about 6.6 per 1,000 children aged 8 years, or 1 in 151, in U.S. regions sampled from 14 states in 2002 by the Centers for Disease Control and Prevention (MMWR 2007).

Some children present with features of both DD/MR and of autism. For example, Yeargin-Allsopp et al. (2003) reported that nearly 70% of children with a validated diagnosis of ASD, sampled from 5 metropolitan Atlanta counties, had cognitive impairment. The evaluation pathway depends on the pediatrician, consulting specialists, and their consensus on the primary neurodevelopmental diagnosis.

The initial evaluation of suspected ASD includes an audiogram, electrophysiologic studies if clinical history suggests a seizure disorder, and clinical evaluation conducted by trained professionals using objective, validated evaluation tools. Metabolic tests and imaging studies may be considered depending on patient presentation.

A variety of evidence supports a genetic etiology in ASD. Recurrence risk (i.e., the risk of ASD in a child born to a family with an already affected child) is about 25-fold higher than population prevalence. Twin studies show a concordance of at least 70% in monozygotic twins but an estimate of only 3–25% in dizygotic twins (reviewed in Schaefer and Mendelsohn 2008a, 2008b; Abrahams and Geschwind 2008). ASD genetic determinants may be heritable or may occur de novo (Zhao et al. 2007). Therefore, evaluation of suspected ASD generally includes a genetic component. Exceptions are clinically recognizable syndromes known to be strongly associated with autistic behaviors, where an additional genetic evaluation for autism may not be needed (recommendations summarized by Schaefer and Mendelsohn 2008a).

High-resolution cytogenetic analysis (see following text) of indicated ASD patients, and testing for Fragile X, strongly associated with autism, are both recommended early in the genetic evaluation process. Individual targeted FISH and other specific gene mutation studies may be used to confirm clinically suspected monogenic conditions; a few examples are summarized in Table 1. Rare mutations in several other single genes have been associated

**Table 1.** Examples of Specific Genes Associated with Disorders that Include Autistic Behaviors

Gene (Syndrome)	Patient Selection	Yield (%)	Reference
<i>FMR1</i> (Fragile X)	unselected autism	3–10	Schaefer and Mendelsohn 2008b
<i>PTEN</i>	autism patients with macrocephaly	≤17	Butler et al. 2005
<i>MECP2</i> (Rett syndrome)	female children with nonsyndromic autism, mental retardation, and cerebral palsy	3–13	Schaefer and Mendelsohn 2008b

with autism, although clinical testing may not yet be available for all. Examples are described in Appendix Table A.

### Expected Outcomes of Genetic Testing

For children with clear, syndromic DD/MR or ASD, diagnoses are based primarily on clinical history and physical examination, and then confirmed with genetic testing. However, for children who do not present with an obvious syndrome, who are too young for full expression of a suspected syndrome, or who may have an atypical presentation, genetic testing is the primary basis for establishing a diagnosis.

In 2006, the Cambridge Genetics Knowledge Park published a report resulting from the “Learning Disability & the Interface with Genetics Project” (Gogarty 2006), an interdisciplinary project that sought to develop expertise and services for the genetic investigation of learning disability. The project employed a collaborative process that involved families affected by learning disability; the report summarized the parent/caregiver perspective. The parents’ most commonly cited reason for pursuing diagnostic testing was “to know,” to establish a medical cause for their child’s condition, and to end the uncertainty of not knowing. Several additional reasons and expected outcomes of establishing a diagnosis were to:

- understand prognosis and future needs;
- improve response (be taken seriously) by medical and educational service providers;
- gain improved and early access to educational and social services and support;
- test other offspring with questionable symptoms; and
- make future reproductive decisions based on estimated recurrence rate.

The United Kingdom Genetic Testing Network, which advises the National Health Service on

genetic testing, requested a Working Group to evaluate the use of aCGH for investigating etiological factors in learning disability. The Working Group was composed of clinical and public health experts who provided insight regarding the clinical utility of aCGH. The final report (Burton 2006) listed the clinical benefits of achieving a specific genetic diagnosis from the clinical viewpoint, as follows:

- end the diagnostic odyssey and allay parents’ fears about other causes;
- refer patients to an appropriate specialist;
- determine possible prognosis;
- guide optimal management and surveillance, e.g., of associated comorbidities;
- advise on risk of recurrence in future offspring or in extended family;
- increase knowledge about precise genotype-phenotype correlation; and
- provide potential insight into disease mechanisms and eventual development of therapeutic interventions.

### Statements from Professional Societies

The American Academy of Neurology (AAN) has published practice parameters for evaluating children with global DD (Shevell et al. 2003) and for the screening and diagnosis of autism (Filipek et al. 2000) on the AAN website (<http://www.aan.com/>). The American College of Medical Genetics (ACMG) published a practice guideline on the cytogenetic evaluation of patients with developmental delay or mental retardation (Shaffer 2005). All three documents discuss the use of conventional cytogenetic evaluation, but not molecular karyotyping by aCGH (see “Standard Cytogenetic Analyses and Molecular Karyotyping by Array Comparative Genomic Hybridization,” following).

The American Academy of Pediatrics (AAP) published a guidance document for the clinical genetic evaluation of children with mental retardation or developmental delays

(Moeschler et al. 2006). The ACMG published a practice guideline on the clinical genetics evaluation of ASD (Schafer and Mendelsohn 2008a). Both documents outline a tiered approach to diagnosis, with clinical evaluation and specialist expertise (primarily neurologists and medical geneticists) directing the early selection of tests to those most likely to yield a diagnosis for the individual patient. The 2006 AAP guidance on DD/MR evaluation mentions aCGH as “emerging technology” that can efficiently replace screening subtelomeric regions for microdeletions, confirm clinical suspicion on certain diagnoses, and likely will increase diagnostic yield. At the time of the AAP guidance publication, aCGH was not widely available clinically. The 2008 ACMG guidance (ASD) suggests aCGH as “second tier” testing (i.e., following negative standard cytogenetic evaluation) although a footnote suggests that advances in technology “will likely elevate aCGH to a first tier study in the near future.”

ACMG also published a general practice guideline regarding the use of aCGH in medical genetics (Manning and Hudgins 2007). The guideline discusses certain “concerns,” such as the extent to which detected CNVs can be distinguished as normal genetic variation versus disease-causing abnormalities, and the current “lack of standardization for array production, analysis, and reporting of results.”

### Standard Cytogenetic Analyses

As previously stated, the purpose of a cytogenetic evaluation is to look for certain kinds of chromosomal abnormalities that may be causally related to the patient’s condition. Most commonly these abnormalities are CNVs, i.e., deletions and duplications of large segments of genomic material. Less commonly, other kinds of chromosomal abnormalities, like balanced translocations (i.e., exchanges of equally sized DNA loci between chromosomes) may be pathogenic. For many well-described syndromes, the type and location of the associated chromosomal abnormality have been established by studying a large number of cases and constitute a genetic diagnosis; for others, only a small number of patients with similar abnormalities may exist to support a genotype-phenotype correlation. Finally, for some patients, cytogenetic analysis will discover entirely new chromosomal abnormalities that will require additional study to determine their significance.

The first step in conventional cytogenetic analysis is usually karyotyping, which was first developed for clinical use in 1959 and involves preparing chromosomes from a patient’s cultured cells at a stage when chromosomes are highly condensed. Very large gains or losses in chromosomal material can be microscopically visualized. Resolution (detectable size of chromosomal abnormality) was increased by standard G-banding, developed in the late 1960s, for which chromosomes are prepared with Giemsa stain to yield a series (400–550) of light and dark bands on each chromosome. Gains or losses in DNA that delete specific bands or add new ones compared to a normal karyotype are recorded. High-resolution G-banding can detect changes as small as 3–5 megabases (Mb) in size by staining chromosomes before they are fully condensed, approximately doubling the resolution of standard G-banding. Other stains and techniques can be used to incrementally improve information and resolution. Because all chromosomes are evaluated at the same time, banded chromosomal analysis does not depend on having a differential diagnosis to direct the evaluation toward known abnormalities in specific chromosomal locations. In children with DD/MR, chromosomal abnormalities were detected by G-banded karyotyping in 10% on average (range: 2–50%) in a systematic review of studies (van Karnebeek et al. 2005). High-resolution karyotyping has identified chromosomal abnormalities in up to 5% of ASD cases (Stuart et al. 2007).

When karyotype analysis is negative, molecular cytogenetic techniques, initially developed in the 1980s, can be used to detect small, submicroscopic chromosomal alterations. Fluorescence in situ hybridization (FISH) is one type of molecular technique for which the differential diagnosis must first be decided so that individual, chromosome location-specific (“targeted”) FISH assays can be selected to address known chromosomal abnormalities associated with the potential diagnoses. When a differential diagnosis and targeted analyses are not possible, a screening examination of subtelomeric regions is recommended. The subtelomere is the chromosomal region just proximal to the terminal sections of each chromosome, is gene-rich, and is believed to be prone to rearrangements (Shaffer 2005). In children with DD/MR, subtelomeric abnormalities may be second to Down syndrome as the most common cause of mental retardation

(Moeschler et al. 2006); the diagnostic yield is about 6% (Moeschler 2008). The yield for subtelomere assays is much lower in ASD (i.e., less than 1%; Stuart et al. 2007) and is not recommended (Schaefer and Mendelsohn 2008a).

### **Molecular Karyotyping by Array Comparative Genomic Hybridization**

**Copy Number Variants.** Copy number variants (CNVs) are duplications or deletions of stretches of DNA about 1kb and up to several megabases (Mb) in size. Large CNVs can be routinely identified by G-banded karyotyping and smaller, previously characterized CNVs can be detected by targeted FISH assays. Specific CNVs have been associated with various diseases including many DD/MR syndromes and ASD.

Interestingly, however, the majority of CNVs in the human genome appear to be polymorphisms that contribute to normal genetic variation. For example, Sebat et al. (2004) studied 20 phenotypically normal individuals and found 211 total and 76 unique CNVs. Similarly, Iafrate et al. (2004) studied 39 healthy people and found 255 CNVs. While some of the CNVs identified in these studies contained genes known to be associated with disease (other than DD/MR or ASD) presumably not yet expressed in these healthy individuals, most did not. Thus, when CNVs are identified in the course of a pediatric diagnostic evaluation, it is necessary to distinguish between those that are benign and those that may be causal to the patient's condition (see "Clinical Context for Molecular Karyotyping," following).

**Array Comparative Genomic Hybridization.** Molecular karyotyping methods greatly increase resolution and diagnostic yield beyond that of banded karyotyping. One such method, CGH, detects CNVs by comparing a normal genomic sequence ("control") with the corresponding patient sequence. Patient and control samples are each labeled with a different fluorochrome so that they can be distinguished, and both are competitively co-hybridized to a sample of a specific reference (also normal) DNA fragment of known genomic locus. If the patient sequence is missing part of the normal sequence (i.e., deletion) or has the normal sequence plus additional genomic material within that genomic location (e.g., a duplication of the same sequence), the sequence imbalance is detected as a difference in bound fluorescence intensity between patient and control

samples. CGH cannot detect balanced CNVs (equal exchange of material between chromosomes) or sequence inversions (same sequence is present in reverse base pair order) because the fluorescence intensity would not differ between patient and control.

Array CGH (aCGH) uses thousands of cloned or synthesized DNA fragments of known genomic locus immobilized on a glass slide (microarray) to conduct thousands of CGH reactions at the same time; prepared patient and control DNA are hybridized to the fragments on the slide, and CNVs are identified by computer analysis of the array patterns and intensities of the fluorescent hybridization signals. Array resolution is limited only by the average size of the fragment used and by the chromosomal distance between loci represented by the reference DNA fragments on the slide. Reference DNA fragments may be relatively large sequences mass-produced by cloning in bacterial artificial chromosomes (BAC clones; 80–200 kilobase [kb]). Alternatively, much smaller synthesized oligonucleotide fragments may be used (oligo; 25–80 base pairs [bp]) to increase resolution. Finally, arrays using oligos that specifically detect single nucleotide polymorphisms (SNP) can detect changes as small as a single nucleotide substitution and as large as chromosome aneuploidy (Bejjani and Shaffer 2008). Oligo and SNP arrays also have the advantage of being easier to standardize and manufacture in large quantities. As with karyotyping, the patient differential diagnosis does not need to be determined in advance because a single array assay detects all genomic variants represented on the array.

There are two types of CGH arrays. The first kind, targeted CGH arrays, provide high resolution coverage of the genome primarily in areas containing known, clinically significant CNVs. For example, diGeorge syndrome is associated with a submicroscopic deletion on one copy of chromosome 22 and can be detected with targeted FISH. DNA marker fragments to detect deletions in this region would be included on a targeted CGH array along with markers of many other known abnormalities.

The second kind of aCGH array, whole-genome arrays, provide high resolution coverage of the entire genome. Thus, in addition to detecting known CNVs like targeted arrays, whole genome arrays also promote discovery of new

CNVs, and allow clinical investigation by genotype first, rather than the traditional phenotype first (Figure 1). For example, the 17q21.3 microdeletion syndrome, which may account for as many as 1% of all cases of mental retardation, was reported by four different groups using whole genome aCGH (Aradhya and Cherry 2007). Whole genome arrays include a “backbone” of DNA fragments representing the genome at regular intervals (e.g., 500–1000 kb or less), and usually include increased density (e.g., 50 kb) in areas where pathogenic abnormalities have already been described (whole genome/targeted array). Such improvements in resolution across the entire genome have resulted in the characterization of several new syndromes by aCGH, with other potential candidates currently under study (reviewed in Slavotinek 2008).

In addition to DNA fragments used to detect CNVs, targeted probes to detect non-CNV types of known genomic abnormalities associated with specific heritable pediatric syndromes may be added to the CGH array. Thus, molecular karyotyping arrays may screen for many genetic abnormalities simultaneously. Array platforms, the combination of array type, assay protocol, and analysis system (Wordsworth et al. 2007), vary from laboratory to laboratory in terms of targeted versus whole genome, resolution, and targeted abnormalities; a given laboratory may offer a variety of aCGH services, depending on clinical need. Clinicians ordering such tests should understand the details of the aCGH service and the kind of results that will be provided before ordering.

#### **Clinical Context for Molecular Karyotyping**

The clinical context for molecular karyotyping is under debate and in flux, as new information is being rapidly acquired. aCGH is typically ordered in the postnatal setting of DD/MR and, more recently, of ASD, when high-resolution karyotyping and targeted genetic studies are not or insufficiently informative. However, some have suggested that aCGH should be considered the primary test in this clinical situation (Shevell et al. 2008). Possible results of aCGH analysis include:

- Identify pathologic genomic abnormalities in patients with idiopathic DD/MR/ASD and normal conventional cytogenetic studies;
- Identify pathologic genomic abnormalities in patients with clinical characteristics suggestive of but atypical for a recognizable syndrome;
- With identification of well-characterized genetic abnormalities, provide information on prognosis, and specific recommendations for clinical referrals and management;
- Identify new genetic abnormalities (parental studies may be necessary);
- Provide more accurate identification and resolution of karyotype abnormalities and correlate genotype with phenotypic variants;
- Investigate genes associated with genomic abnormalities to determine causal associations.

Note that the second set of bullets have a lower likelihood of immediate impact on the case at hand, but with additional accumulated information may eventually aid the original patient or other patients in the future.

An interpretive complication of aCGH, particularly high resolution whole genome arrays, is differentiating pathogenic from benign CNVs that are found in phenotypically normal individuals (see Background, “Molecular Karyotyping by Array Comparative Genomic Hybridization, Copy number variants,” previous; see also Review of Evidence, Question 1B). Efforts to catalog both pathogenic and benign CNVs into publicly available reference databases, used to aid in interpreting clinical aCGH results, are ongoing and accumulating data rapidly (Table 2). Clinical laboratories with large aCGH case loads also maintain internal CNV databases, which have the advantage of greater CGH array consistency, to inform interpretation of future cases.

**FDA Status.** Array CGH is commercially available from several laboratories as a laboratory-developed test. Laboratory-developed tests performed by laboratories licensed for high complexity testing under the Clinical Laboratory Improvement Amendments (CLIA) do not require U.S. Food and Drug Administration (FDA) clearance for marketing.

**Figure 1.** Genetic Evaluation of Children with Developmental Delay/Mental Retardation/Autism Spectrum Disorder (Summarized from Shaffer et al. 2007).

### A. Phenotype first

**Syndromic DD/MRI/ASD** – Characterized by clinical history and physical examination; patients with similar constellation of characteristics grouped together (syndrome); cytogenetic basis initially unknown

→ **Example: DiGeorge syndrome;** characteristic signs and symptoms may include congenital heart disease, defects in the palate, learning disabilities, mild facial dysmorphology, and recurrent infections

**Early karyotyping:** Uniform chromosome staining allowed detection of aneuploidies and large structural rearrangements (deletions, duplications, translocations)

→ Features constituting DiGeorge's syndrome associated with the absence of one entire chromosome 22

**Standard G-banded karyotyping:** Giemsa stain yields a series of lightly and darkly stained bands (400–550 per chromosome) — changes in banding patterns allow increased resolution of chromosomal abnormalities

→ Family with 4 diGeorge children led to identification of 22q11 monosomy as likely cause

**High-resolution G-banded karyotyping:** ~double the resolution; detect balanced and unbalanced abnormalities as small as 3–5 Mb in size

→ High-resolution G-banding and FISH confirmed and further delineated 22q11.2 deletion; present in 95% of diGeorge patients in large studies

**Fluorescence in situ hybridization (FISH):** detects specific submicroscopic chromosomal alterations using probes targeted to known chromosomal sequences

### B. Genotype first

**Non-syndromic DD/MR/ASD** – Specific syndrome not clinically evident; physical anomalies may/may not be

**Screen for genome-wide abnormalities** with standard and/or high-resolution G-banded karyotyping

→ Approximately 10% of DD/MR and 5% of ASD patients positive for abnormality; positive results followed with targeted FISH assays

**Screen subtelomeric regions** at a finer level for genomic abnormalities with FISH or multiplex ligation-dependent probe amplification (MLPA)

→ Approximately 6% of DD/MR patients positive for abnormality in telomeric region (poor yield in ASD; not recommended)

**Example:** 22q13 deletion characterized by subtelomeric analysis; patients with this deletion most frequently have global developmental delay, generalized hypotonia, absent or severely delayed speech, and normal to advanced growth

**Array comparative genomic hybridization (aCGH):**

– **Targeted:** screens many specific chromosomal regions where clinically significant chromosomal abnormalities have been documented, including subtelomeres; may also include a 'backbone' of more widely-spaced markers along the full genome

→ **Example:** the 22q11.2 region, deleted in diGeorge syndrome, is included in targeted CGH arrays

**New abnormalities** may be detected by 'backbone' genomic markers but require additional genetic evaluation and parental studies to determine likely pathogenicity

– **Whole-genome:** includes targeted areas, and provides broader and more detailed coverage of the entire genome

→ Detects previously described abnormalities

**Best method for discovery of new abnormalities;** as above, requires additional investigation

**Example:** de novo microdeletions of 17q21.31 identified initially in 3 and eventually in at least 12 reported patients with mild to moderate developmental delay, growth delay, severe neonatal hypotonia, and characteristic facies

**Table 2.** Databases that Catalog Human CNVs

Database, URL	Focus	Comment
Database of Genomic Variants <a href="http://projects.tcag.ca/variation/">http://projects.tcag.ca/variation/</a>	All reported structural variation identified in healthy control samples	As of March 2007, 6,482 CNVs; As of November 11, 2008, 19,792 CNVs entered
Chromosome Anomaly Collection <a href="http://www.ngrl.org.uk/Wessex/collection/">http://www.ngrl.org.uk/Wessex/collection/</a>	Collection contains examples of unbalanced chromosome abnormalities (UBCAs) without phenotypic effect	~150 entries as of November 11, 2008
DECIPHER <a href="https://decipher.sanger.ac.uk/">https://decipher.sanger.ac.uk/</a>	Collects clinical information about CNVs in order to relate the location of the submicroscopic chromosome imbalance to the phenotype	As of November 11, 2008, 57 syndromes listed
ECARUCA <a href="http://agserver01.azn.nl:8080/ecaruca/ecaruca.jsp">http://agserver01.azn.nl:8080/ecaruca/ecaruca.jsp</a>	Collects and provides cytogenetic and clinical information on rare chromosomal disorders, including microdeletions and microduplications	Contains over 4,000 cases with more than 5,000 aberrations
The 1000 Genomes Project <a href="http://www.1000genomes.org/page.php">http://www.1000genomes.org/page.php</a>	The 1000 Genomes Project will sequence the genomes of at least 1,000 people from around the world and catalog SNPs and CNVs present at a frequency of 1% or more. The goal is to create the most detailed and medically useful picture to date of human genetic variation.*	Raw data made available as soon as possible; summary data including positions of variants in individuals and populations to follow.

\* Beals 2008.

## Methods

### Literature Search

An initial MEDLINE® (via PubMed) search strategy of (“Developmental Disabilities” [MeSH®] OR “Mental Retardation” [MeSH®] OR “Autistic Disorder” [MeSH®]) AND (“Genetic Screening” [MeSH®] OR “Genetic Predisposition to Disease” [MeSH®] OR “Genetic Phenomena” [MeSH®] OR “Genetic Techniques” [MeSH®]), limited to Human, English, and published within the last 5 years yielded 2,172 citations on August 20, 2008.

Specific searches for studies of the clinical outcomes of cytogenetic testing in DD/MR and ASD were also conducted, as follows:

- (“Developmental Disabilities” [MeSH®] OR “Mental Retardation” [MeSH®] OR “Autistic Disorder” [MeSH®]) AND “Karyotyping” [MeSH®] AND (education[tw] OR recurrence[tw] OR intervention[tw] OR “Therapeutics” [MeSH®] OR “therapy” [Subheading] OR “Outcome Assessment (Health Care)” [MeSH®])

- Retardation” [MeSH®] OR “Autistic Disorder” [MeSH®] AND “Karyotyping” [MeSH®] AND (“Diagnosis” [MeSH®] OR “diagnosis” [Subheading])

Titles and relevant abstracts were reviewed; full copies of potentially relevant papers were retrieved for review. In addition, earlier articles were also selected from bibliographies of recent relevant publications.

### Study Selection

Included studies were published in peer-reviewed journals, and:

- were case reports, case series, or cohort studies;
- enrolled patients with clinical diagnoses of DD/MR or of autism;
- enrolled patients with known genetic abnormalities by conventional cytogenetic evaluation or with suspicion of genetic abnormalities but negative results by conventional cytogenetic evaluation;
- conducted molecular karyotyping by aCGH on enrolled patients.

Studies were also included if they examined management decisions and/or patient outcomes based on genetic evaluation results, either by conventional methods or by molecular karyotyping.

### Medical Advisory Panel Review

This Special Report was reviewed by the Blue Cross and Blue Shield Association Medical Advisory Panel (MAP) on December 16, 2008. In order to maintain the timeliness of the scientific information in this report, literature search updates were performed subsequent to the Panel's review (see "Search Methods"). If the search updates identified any additional studies that met the criteria for detailed review, the results of these studies were included in the tables and text where appropriate. No studies were found that would change the conclusions of this Special Report.

## Review of Evidence

### Question 1A: What is the clinical sensitivity of molecular karyotyping by aCGH for known chromosomal abnormalities? What is the false positive rate in normal controls? (Clinical Validity)

Several studies have conducted aCGH on samples with known chromosomal abnormalities by standard karyotyping; results are summarized in Appendix Table B. In general, currently available aCGH clinical services using higher-resolution BAC arrays or high-resolution oligo CGH arrays achieve 100% sensitivity for known chromosomal abnormalities. The few studies that reported sensitivity less than 100% likely used older, BAC-clone arrays that had insufficient resolution.

False-positive rates (i.e., CNVs of undetermined clinical significance) on known normal samples were inconsistently reported and are not summarized here. However, it should be noted that as array resolution increases, the likelihood of indeterminate CNV results also increases. This

feature is somewhat mitigated by the continual addition of new abnormalities as well as new benign polymorphic CNVs to databases available for consultation (see Question 1B).

### Question 1B: What is the diagnostic yield of molecular karyotyping by aCGH for patients suspected of having a chromosomal abnormality but who are negative by conventional cytogenetic evaluation? (Clinical Validity)

Several studies reported the diagnostic yield of aCGH in DD/MR or ASD patients with normal standard karyotype and in several cases normal *FMR1* gene analysis and/or subtelomere FISH screening (Appendix Table C). Overall, diagnostic yield ranged from 5 to 16.7% in DD/MR patients and from 3.4 to 11.6% in patients with ASD, excluding outliers that likely resulted from unusually stringent selection criteria. Although seemingly small, these yields represent significant improvements in identifying a genetic etiology for these patients. For example, Stankiewicz and Beaudet (2007) estimate the following diagnostic yields in DD/MR patients based on numerous reports:

- G-banded karyotype: 3–5%
- Subtelomeric FISH: 5–6% (in those negative by karyotype)
- aCGH: 4–7%<sup>1</sup> (in those negative by karyotype and subtelomeric FISH).

Based on the range of diagnostic yields, number needed to test (NNT) by aCGH to detect 1 clinically relevant abnormality is shown in Table 3.

Diagnostic aCGH yields were reported in studies using CGH arrays that differed in resolution, both in genomic areas known to harbor abnormalities and in whole genome coverage, as technology improvements have allowed rapid gains in resolution over a short time. Results in Appendix Table C were broadly categorized into 3 types of CGH arrays: those targeted to known "hotspots," those that are targeted to hotspots and also contain markers widely spaced across the entire genomic

**Table 3.** Calculated Number Needed to Test (NNT) for Different Average aCGH Diagnostic Yields

If average yield (%) is:	4	7	10	13	16
Then NNT is:	25	14	10	8	6

<sup>1</sup> 4–7% or more depending on resolution.

backbone, and those that cover the entire genome with approximately even density<sup>2</sup>. Minimal resolution (i.e., of the genomic backbone) differs across those arrays with backbone coverage. Finally, patient selection criteria range from none (beyond normal standard genetic studies) to stringent criteria that markedly increase the likelihood of a genetic etiology (e.g., syndromic features or nonsyndromic dysmorphic features and/or multiple congenital anomalies). Therefore, diagnostic yield results cannot be compared across studies on the basis of the type and resolution of the CGH array, or on patient selection criteria, as each is confounded by the other. It should be noted that most if not all, published reports are likely out of date in terms of currently available array resolution, given the rapid advancement of array design, and current diagnostic yields are likely slightly larger than those summarized here.

Whole genome arrays with the smallest minimum resolution (e.g., 35 kb) improve the diagnostic yield, but also increase the detection rate of CNVs of uncertain clinical relevance. To determine clinical relevance in individual cases, the following actions are taken:

- CNVs are confirmed by another method (e.g., FISH, MLPA, PCR).
- CNVs detected are checked against public databases (see Table 2) and, if available, against private databases maintained by the laboratory. Known pathogenic CNVs associated with the same or similar phenotype as the patient are assumed to explain the etiology of the case; known benign CNVs are assumed to be nonpathogenic (Stankiewicz and Beaudet 2007; Rodriguez-Revenga et al. 2007; Vermeesch et al. 2007).
- A pathogenic etiology is additionally supported when a CNV includes a gene known to cause the phenotype when inactivated (microdeletion) or overexpressed (microduplication) (Vermeesch et al. 2007).
- The laboratory may establish a size cutoff; potentially pathogenic CNVs are likely to be larger than benign polymorphic CNVs; cutoffs for CNVs not previously reported typically range from 500 kb to 2 Mb (Stankiewicz and Beaudet 2007; Fan et al. 2007; Baldwin et al. 2008).

- Parental studies<sup>3</sup> are indicated when CNVs of appropriate size are detected and not found in available databases; CNVs inherited from a clinically normal parent are assumed to be benign polymorphisms whereas those appearing de novo are likely pathogenic; etiology may become more certain as other similar cases accrue (Rodriguez-Revenga et al. 2007; Zahir and Friedman 2007).

Determining pathogenicity on the basis of parental studies may be difficult. If a parent harbors the same CNV detected in the child and especially if the child's phenotype is relatively mild, a detailed clinical history of an apparently normal parent, and possibly extended family studies, may be required to reveal a similar parental phenotype. Incomplete penetrance or variable expressivity of a clinically relevant abnormality may result in a milder or undetectable parental phenotype than the child (Rodriguez-Revenga et al. 2007). A causal CNV may be recessive rather than dominant; the heritability of complex CNVs may be difficult to establish. Finally, parents may be unavailable for follow-up studies. Thus, the final genetic assessment may not be definitive in all cases, but instead may report a CNV of undetermined significance. An attempt was made to abstract the rate of aCGH results requiring parental testing and of CNVs of undetermined significance for the studies in Appendix Table C; however, reporting was often unclear and dissimilar across studies and comparable rates could not be determined. Baldwin et al. (2008) reported conducting follow-up parental studies on only 2.4% of all clinically evaluated aCGH patients.

As noted, the selection criteria for patients enrolled in the studies listed in Appendix Table C differed considerably and likely explain the range in diagnostic yield. Several studies required non-syndromic phenotypic abnormalities such as dysmorphic features and/or multiple congenital anomalies to increase diagnostic yield. de Vries et al. (2001) developed a checklist and resulting score cutoff ( $\geq 3$ ) for selecting MR patients for subtelomeric genetic screening; this checklist was also used by Schoumans et al. (2005), Tyson et al. (2005), and Vissers et al. (2003) to select patients for aCGH testing (Appendix Table C). However, Hoyer et al.

<sup>2</sup> Publications were not always clear on the characteristics of the array; studies were categorized as accurately as possible based on the information given.

<sup>3</sup> Parental studies consist of confirmatory analysis (e.g., FISH, MLPA) of the specific abnormality detected rather than aCGH; clinical laboratories may conduct parental analyses without additional charge and parental samples are often collected with the patient sample (e.g., see [http://genedx.com/pdf\\_files/info\\_sheet\\_genomedx.pdf](http://genedx.com/pdf_files/info_sheet_genomedx.pdf)).

(2007) categorized the clinically relevant aCGH results derived from 104 unselected MR patients by their de Vries score and found that 2 of 10 patients would have been excluded from testing. de Vries et al. (2005) reported that all 10 confirmed de novo aberrations detected had scores 3 or greater; but of 5 candidate de novo aberrations (parents not available for follow-up), 2 had scores less than 3. Thus, the de Vries checklist may not be a good predictor. Current guidelines do not recommend such criteria and note that genetic abnormalities may be detected in the absence of dysmorphic features (Moeschler et al. 2006).

**Question 2: How are management decisions changed and outcomes improved by the results of molecular karyotyping by aCGH? (Clinical Utility)**

Diagnostic yield is an intermediate outcome. Clinical utility is determined by the impact of a genetic diagnosis on outcomes that matter to the patient and family. Several outcomes are regarded as important in the setting of DD/MR or ASD (see Background, “Outcomes of Genetic Testing”). However, neither standard cytogenetic analysis nor aCGH have been systematically studied for impact on these kinds of outcomes (Subramonia-Iyer et al. 2007; Moeschler et al. 2006); Schaefer and Mendelsohn (2008a) acknowledge that a genetic diagnosis “typically will not change interventions for the [autism] patient.” Rather, clinical utility of genetic testing is primarily inferred based on the value of knowledge to the family, estimation of recurrence risk, and on the importance of early detection and early intervention

(Moeschler et al. 2006). The few studies found that address aspects of the clinical utility of aCGH are summarized in this section.

A genetic diagnosis might end the diagnostic odyssey of time-consuming, disruptive, costly, and repetitive diagnostic screening at various institutions in search of a causative explanation of the patient’s condition (Vermeesch et al. 2007). Saam et al. (2008) interviewed 14 physicians (2 neurologists, 12 medical geneticists) regarding management changes as a result of positive aCGH test results from the University of Utah Cytogenetics Laboratory for 48 patients with DD/MR and normal karyotypes. Results are summarized in Table 4. Only 29 percent of patients had no management changes reported. For significant proportions of patients, the diagnostic odyssey was ended; additionally, recurrence risk evaluation was possible in about one-third of families. However, this study was only a survey and did not attempt to quantify the diagnostic tests avoided. Therefore, the results are only suggestive of, but do not confirm, clinical utility in the sense of ending the diagnostic odyssey.

An expected outcome of genetic diagnosis by any method at ages as young as 2 or younger is early and improved access to medical services, and to community behavioral and educational programs. As aCGH improves diagnostic yield above that of standard cytogenetic screening, more patients should benefit in this way. Access, however, has not been systematically studied. Saam et al. (2008) reported that 14.6% of patients with genetic diagnoses were referred to medical specialists and 25% had

**Table 4.** Changes in Management after Positive aCGH Testing in Patients with DD/MR and Normal Karyotype (Saam et al. 2008)

Management Change	n (48 total)	Percent
Specialist referral (e.g., by neurologist to medical geneticist)	7	14.6
Medical screening for comorbidities associated with genetic diagnosis	8	16.7
Stop medical screening – no longer appropriate based on genetic diagnosis	1	2.1
Avoid other genetic testing	12	25.0
Avoid other diagnostic testing e.g., muscle biopsy, MRI, infectious disease	8	16.7
Recurrence risk evaluation, including testing of parents	17	35.4
Improved access to services, e.g., insurance and educational services	12	25.0
No changes	14	29.2

improved access to insurance and educational services, but the study did not assess the benefits of specialist referrals or screening for comorbidities on patient outcomes, or describe and quantitate the improvement in access to community services.<sup>4</sup>

Recurrence risk is also an intermediate outcome. Knowledge of recurrence risk is expected to lead to improved future reproductive decision-making in families with children affected with DD/MR or ASD. For example, in a family with a child with idiopathic ASD the average sibling recurrence risk is estimated to be 5% (Freitag 2007). However, if a genetic cause is established, the revised estimate for the family may be very different, as shown in Table 5.

Turner et al. (2008) studied the reproductive decisions of women from 38 families characterized by male members with mental retardation and a pattern consistent with chromosome X-linked transmission. Most of the women in these families spent many years knowing that they were at some risk of being carriers and of having a boy with MR. Prior to the availability of pathogenic mutation analysis, the birth rate for these families was below average for the district (United Kingdom-New South Wales), 1 in 27 versus 1 in 11 per year, respectively. Only 2 affected children were born versus an expected 9 based on regional birth rate and the

population risk of the condition. After pathogenic mutation status was determined, both carriers and non-carriers (previously thought to be at risk) of the mutation had children at the same rate, with 74% of carriers choosing prenatal genetic evaluation. While the results of this study are suggestive, they do not show that knowledge of recurrence risk directly affected reproductive decisions. Saam et al. (2008), in the survey described previously (Table 4) reported that recurrence risk evaluation was possible in about one-third of families after positive aCGH results, but did not study the impact of recurrence risk evaluation on reproductive planning.

The clinical utility of early diagnosis in ASD is summarized in the American Academy of Neurology (AAN) practice parameter for the screening and diagnosis of autism (Filipek et al. 2000):

“Identifying children with autism and initiating intensive, early intervention during the preschool years results in improved outcomes for most young children with autism [citations given]. Early diagnosis of autism and early intervention facilitates earlier educational planning, provisions for family supports and education, management of family stress and anguish, and delivery of appropriate medical care and treatment.”

**Table 5.** Revised Sibling Recurrence Risk after Identification of Different Types of Genomic Abnormalities Associated with ASD

Type of Genetic Abnormality	Clinical Example	Sibling Recurrence Risk
Dominant single gene disorder with full penetrance	Tuberous sclerosis: involves abnormalities of the skin, brain, and heart; associated with MR and autism	50% if parent carries the disease-causing variant (i.e., not a de novo mutation)
Recessive single gene disorder	Smith-Lemli-Opitz syndrome: congenital multiple anomaly syndrome; associated with ASD	25%
X-linked single gene disorder	Fragile X: most common cause of mental retardation; associated with ASD	brother: 50% sister: up to 50% will be a carrier, or might be mildly affected
CNV	Prader-Willi syndrome/Angelman syndrome (15q11-q13 duplication)	same as population prevalence if de novo (i.e., not found in parents)

<sup>4</sup> Although not considered evidence, anecdotal reports of clinicians who manage patients with DD/MR and ASD agree that it is easier to obtain services with a genetic diagnosis, rather than with a clinical, non-syndromic diagnosis (personal communication, 2008, Dr. Mehdi Jamehdor, Medical Genetics Laboratories, Southern California Permanente Medical Group; and Dr. Mark Lipson, Medical Genetics, Kaiser Permanente, Sacramento, CA). For example, a family with two children clinically diagnosed with more and less severe autistic symptoms was able to obtain services for only the child with the more severe symptoms; once a genetic diagnosis was established for both children, both had access to services (personal communication, Dr. Mark Lipson).

Similarly, the AAN practice parameter on evaluation of the child with global developmental delay states (Shevell et al. 2003):

“Accumulating evidence also demonstrates the benefits of early intervention through a variety of programs (e.g., Head Start) with respect to short-term outcomes [citation] and suggests that early diagnosis of a child with global delay may improve outcome.”

A recent review of studies of early intervention for autism notes that while the evidence suggests that early intervention programs are beneficial, the evidence is generally not of the highest quality and whether early improvements in behavior and reductions in symptom severity lead to greater independence and social functioning in adulthood is unknown (Rogers and Vismara 2008). Few randomized trials have been conducted and described interventions differ considerably, indicating that the field is still early in researching the critical elements of effective early intervention. Nevertheless, guidelines such as those published by the American Academy of Pediatrics emphasize the importance of early diagnosis and intervention (Myers and Johnson 2007).

For well-characterized genetic syndromes, it is important to incorporate monitoring for co-morbidities known to be associated with the condition. For example, 22q11 microdeletion syndrome (includes diGeorge and velocariofacial syndromes) is associated with development of hearing impairment in a significant proportion of patients and subsequent delayed speech (Digilio et al. 1999). Early diagnosis and monitoring for hearing impairment can ameliorate communication problems. For less specific DD/MR or ASD syndromes diagnosed by aCGH, it may also be possible to predict the patient prognosis and the elements of care<sup>5</sup> that will be most important to improving outcomes. However, the outcomes of prognosis and treatment planning have not been systematically studied.

**Question 3: What is the evidence that the technical performance of clinically available aCGH assays accurately detect clinically significant CNVs and any other chromosomal abnormalities included in the array, and perform in a robust manner over time? (Analytic Validity)**

Molecular karyotyping is commercially available from several CLIA-licensed laboratories (examples listed in Appendix Table D) as a laboratory-developed test (LDT). FDA clearance for marketing is not required for LDTs; none of the commercially offered tests has been voluntarily submitted to the FDA for review. While CLIA requires LDTs to be validated in-house, validation summary data (e.g. required in kit inserts of FDA-cleared diagnostic tests) are not required to be publicly available for LDTs.

Clinically available molecular karyotyping tests currently differ considerably in technology, resolution, and likelihood of results with unknown significance. None of the laboratories listed in Appendix Table D post a validation summary on their website. Some laboratories have published a summary of their assay validation procedure; examples highlight the complexity of designing and validating CGH arrays, as well as their analysis and interpretation (e.g., Shen et al. 2007; Cheung et al. 2005; Baldwin et al. 2008), aspects of which are briefly summarized in Table 6.

A general validation process is to compare the results for samples known to have G-banded karyotype abnormalities with aCGH. As noted in the evidence summary for Question 1A, 100% sensitivity and a low false-positive rate is expected, the latter depending largely on array resolution and data analysis protocols. Signature Genomics Laboratories summarizes detection rates from the literature for a growing list of abnormalities detected by their aCGH tests (see [http://www.signaturegenomics.com/detection\\_rates.html](http://www.signaturegenomics.com/detection_rates.html)). However, such comparisons do not cover all possible abnormalities and cannot substitute for appropriate validation of (often manufactured) components of the array platform as the test is developed in the clinical laboratory.

<sup>5</sup> For example, educational training; speech and language therapy; occupational therapy, physical therapy, or adaptive physical education; behavioral therapy; pharmacotherapy (Aman 2005).

**Table 6.** Aspects of aCGH Design and Validation

- Determine BAC clone/oligo probe density in targeted and backbone regions to ensure detection of known abnormalities and establish minimum backbone resolution
- Ensure quality of BAC clone/oligo probe targets and redundancy of all BAC or a subset of oligo probe targets on the array
- Include clone/probe targets on the array for normalization of each analysis to avoid systematic bias
- Determine sample type, processing, minimum DNA requirements, quality assurance measures
- Establish control samples to be used for comparison (normal/abnormal; male/female; individual/pooled)
- Select software/determine methods for data analysis and CNV identification (methods vary from simple defined thresholds to complex statistical modeling; Carter 2007)
- Establish protocols for verification of detected CNVs

Insufficiently validated aCGH clones/oligo probes can lead to problems. For example, Thorland et al. (2007) compared a commercially available targeted BAC aCGH platform against confirmatory FISH assays and identified false-negative results, as well as problems with mismatched and suboptimal clone performance. Bejjani et al. (2005) constructed an in-house BAC CGH array; initially testing 906 BAC clones and attempting to confirm by FISH. The authors reported that 7% of clones were mismatched, 16% cross-hybridized to other chromosomes, and 12% did not hybridize or showed poor hybridization signals under uniform FISH conditions; as a result, only 589 (65%) were adequate for use in the array. In the case of oligo arrays, the synthesized oligonucleotide probes printed on these arrays cannot be individually validated after array printing; rather, probes should be supported by the array manufacturer with protocols ensuring successful synthesis, and the loci identified by each probe should be appropriately documented.

A common element to aCGH clinical services is the confirmation of CNVs by another method such as FISH or MLPA; this functions as internal quality assurance and helps to minimize the false-positive rate. Excessively high rates of discrepancy between aCGH and confirmatory results should also prompt investigation.

The American College of Medical Genetics has published an “educational resource” guideline that suggests minimum validation procedures for array CGH (Shaffer 2007). Thorland et al. (2007) have suggested the need for validation requirements in addition to those suggested by the ACMG guidelines, especially as regards the “initial and . . . critical comprehensive validation studies that the platform and the probes included on the platform perform appropriately”

and as regards “the roles and responsibilities of the manufacturer providing these platforms.”

## Discussion

Current guidelines for early assessment of DD/MR and for ASD recommend genetic evaluation for those cases that cannot be readily diagnosed from clinical characteristics or other specific tests. Conventional cytogenetic analysis (G-banded karyotype, specific FISH assays, and subtelomeric screening) has been routinely used for many years, but has low resolution and low diagnostic yield. Array CGH detects large genomic deletions and duplications (CNVs), which account for most of the genomic abnormalities currently detectable by conventional cytogenetic analysis. Array CGH does not detect balanced translocations or inversions, which account for a minority of clinically important genomic abnormalities. However, aCGH has shown that a substantial proportion of apparently balanced translocations by conventional methods contain submicroscopic deletions (i.e., are actually unbalanced).

CGH arrays have the advantage of greatly improved resolution, which allows detection of smaller, clinically significant genomic abnormalities not detectable by conventional assays (improving diagnostic yield), and more exact locus definition of conventionally detectable abnormalities (improving information for genotype-phenotype correlation). While aCGH technology is relatively new, the results are conceptually similar to those obtained by conventional methods and should be evaluated as an extension of those methods.

The results of neither conventional cytogenetic evaluation nor of aCGH evaluation of DD/MR or

ASD patients have been systematically studied for impact on patient outcomes other than diagnostic yield, which is an intermediate outcome. Impact of testing on the kinds of outcomes that matter to the patient and family have been directly addressed in very few studies. Thus, it is not possible to draw evidence-based conclusions regarding the clinical utility of aCGH genetic evaluation. The same may also be said of conventional cytogenetic evaluation.

Expert consensus and clinical guidelines state that genetic information is of value because it establishes a causal explanation that is helpful to families. It is suggested that such genetic information avoids additional consultations and various types of diagnostic tests, assists with early and improved access to community services that may ameliorate or improve behavioral and cognitive outcomes, provides estimates of recurrence rates to better guide reproductive decision-making, and enables an understanding of prognosis and future needs. However, little evidence supports these outcomes.

Array CGH technology is rapidly evolving; it is up to the ordering physician to know the limits of the particular technology employed by the laboratory. 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 (Pergament 2007). Currently, a consortium of scientists from academic cytogenetics laboratories have agreed to develop a uniform, evidence-based “Molecular Karyotype” and shared national database to accumulate data on pathogenic versus benign deletions and duplications in the human genome (Ledbetter et al. 2008). Such cooperative efforts should lead to more comparable results across platforms, more complete databases to aid in individual results interpretation, more uniform reporting, and more rapid accumulation of genotype-phenotype correlation information for future reference.

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# Appendix

**Table A.** Possible Monogenic Determinants of Autism

Source	Gene	Description
Jamain et al. 2003	<i>NLGN3</i> <i>NGN4X</i>	Rare children with mutations in neuroligin 3 and neuroligin 4, genes located on the X chromosome, have been found in studies of families with a clear X-linked pattern of neurobehavioral/neurodevelopmental disorders and cognitive deficits
Autism Genome Project Consortium (2007)	<i>NRXN1</i>	A large genome scan found an association between autism and mutations in neurexin 1, a protein involved in glutamate function. Glutamate-related genes had previously been implicated in some cases of autism (Jamain S et al. 2003; Feng J et al. 2006)
Moessner et al. 2007; Durand 2007	<i>SHANK3</i>	Mutations in the SHANK3 gene have been found in 1% of 2 ASD cohorts. SHANK3 binds neuroligins that, together with neurexins, form a complex at glutamatergic synapses.
Strauss KA et al. 2006; Arking et al. 2008; Bakkaloglu et al. 2008; Alarcon et al. 2008	<i>CNTNAP2</i>	Mutations in the CNTNAP2 gene, resulting in functional variant proteins, have been associated with ASD in several studies

**Table B.** Sensitivity of aCGH Testing Conducted in Groups of Patients with Previously Identified Chromosomal Anomalies and False Positive Rates in Normal Controls

Study	n	Array Type	Array Target	Minimum Resolution (kb)	Previous Analysis	Sensitivity (%)
Cheung et al. 2005	25	BAC/PAC	targeted regions/ subtelomeres	NS <sup>1</sup>	G-banded karyotype and/ or FISH	100
Bejjani et al. 2005	36	BAC	targeted regions/ subtelomeres	NS <sup>2</sup>	NS	94
Shearer et al. 2007	48	BAC	targeted regions/ subtelomeres	NS <sup>3</sup>	karyotype	96
Rickman et al. 2006	30	BAC	targeted; whole genome	NS <sup>4</sup> ; 1,000	karyotype	97 73
Lu et al. 2007	117	BAC/PAC	whole genome + targeted regions/ subtelomeres	NS	G-banded karyotype and/ or FISH	92.5
Toruner et al. 2007	15	oligo	whole genome + subtelomere	125 5	karyotype, FISH	100
Shen et al. 2007	51	oligo	whole genome + targeted regions/ subtelomeres	<35 in targeted regions	FISH, MLPA, and/or BAC- aCGH,	100
Baldwin et al. 2008	30	oligo	whole genome	500	G-banded karyotype and/ or targeted or subtelomeric FISH	100
Harada et al. 2004	5	BAC/PAC	subtelomeres	NS	karyotype, FISH	100
Bauters et al. 2005	7	BAC/PAC	X-chromosome	82	“standard cytogenetics”	100
Madrigal et al. 2007	4	BAC	X-chromosome	100	karyotype, FISH	100
Hayashi et al. 2007	4	BAC/PAC	X-chromosome	tiling	karyotype	100
Yu et al. 2003	25	BAC/PAC	chromosome region 1p36	100–300	NS	100

**Abbreviations:** BAC, bacterial artificial chromosome; PAC, plasmid artificial chromosome; MLPA, multiplex ligation-dependent probe amplification; FISH, fluorescent in situ hybridization; PCR, polymerase chain reaction; NS, not specified

<sup>1</sup> Array covered genomic regions implicated in >40 known genomic disorders and 41 clinically relevant human chromosome telomeric regions.

<sup>2</sup> Not including benign, polymorphic CNVs previously reported or seen in several normal controls.

<sup>3</sup> Array designed to cover unique subtelomeric regions, the unique pericentromeric regions, common microdeletion/microduplication syndromes, and other selected areas of the genome.

<sup>4</sup> Custom whole-genome array designed to detect aneuploidy, known microdeletion syndromes, and large unbalanced chromosomal rearrangements.

**Table C.** Case Series (n ≥ 20) of Patients with Suspected Genetic Abnormality but Negative Results by Standard Genetic Evaluation

Study	n	Patient Description	Previous Normal Studies	Array Type	Minimum Array Resolution (kb)	Confirmation	% Diagnostic Yield
<b>Studies of Patients with Clinical Diagnoses of Developmental Delay/Mental Retardation</b>							
Studies using arrays targeted to "hotspot" candidate genome regions							
Shaffer et al. 2006	1,500	consecutive patients with diverse range of DD or MR diagnoses	karyotype (94%), FISH (20%) where prior testing available	BAC (Signature Chip v7)	(no DNA backbone coverage)	FISH	5.6
Ballif et al. 2006	3,600	consecutive cases with diverse range of DD or MR features	NS	BAC (Signature Chip v3)	(no DNA backbone coverage)	FISH	5
Sharp et al. 2006	290	idiopathic MR with or without dysmorphism or multiple congenital anomalies	karyotype, subtelomere FISH (255)	BAC/PAC	(no DNA backbone coverage)	FISH and/or oligo array targeted to same hotspots	5.5
Baliff et al. 2007	6,946	variety of clinical presentations, most commonly DD, dysmorphic features, and/or multiple congenital anomalies	karyotype, subtelomere FISH	BAC	(no DNA backbone coverage)	FISH	2.4 (subtelomeric abnormalities only)
Shevell et al. 2008	94	final diagnosis of DD, <5 years old at diagnosis	high resolution karyotype, FMR1, imaging	BAC (Signature Chip v4)	(no DNA backbone coverage)	FISH	6.4
Studies using whole genome arrays with less dense coverage of DNA backbone regions (minimum resolution) and dense coverage of targeted regions							
Vissers et al. 2003	20	idiopathic MR and dysmorphism; pts scored ≥3 on de Vries checklist (2001)	karyotype	BAC	≤1,000	FISH, aCGH dye-swap replicate	10
Lu et al. 2007	1,726	diverse range of DD/MR, dysmorphic, or multiple congenital anomaly features	G-banded karyotype and/or FISH	BAC/PAC	NS	FISH	5.4

**Table C.** Case Series (n ≥ 20) of Patients with Suspected Genetic Abnormality but Negative Results by Standard Genetic Evaluation (cont'd)

Study	n	Patient Description	Previous Normal Studies	Array Type	Minimum Array Resolution (kb)	Confirmation	% Diagnostic Yield
<b>Studies of Patients with Clinical Diagnoses of Developmental Delay/Mental Retardation (cont'd)</b>							
Studies using whole genome arrays with less dense coverage of DNA backbone regions (minimum resolution) and dense coverage of targeted regions (cont'd)							
Shen et al. 2007	211	idiopathic MR and/or dysmorphism or multiple congenital anomalies	(not selected by prior results)	oligo	(<35 in targeted regions)	FISH, MLPA, BAC-aCGH, aCGH dye-swap replicate	8.1
Baldwin et al. 2008	211	various, including idiopathic DD/MR, dysmorphic features, congenital anomalies, autism or syndromal phenotype	G-banded karyotype ("many")	oligo	300	FISH	15.6
Aradhya et al. 2007	20	DD/MR and either dysmorphic features, congenital anomalies, or growth retardation	G-banded karyotype, FISH	oligo	≤35	FISH	50
Studies using whole genome arrays with ~even coverage across all regions							
Aradhya et al. 2007	20	(as above)	(as above)	BAC	1,000		30
Shaw-Smith et al. 2004	50	idiopathic MR and dysmorphism or other features	karyotype, subtelomere (41)	BAC	1,000	FISH	14
de Vries et al. 2005	100	idiopathic MR	karyotype, subtelomere	BAC	tiling	FISH	10
Schoumans et al. 2005	41	mild to severe idiopathic MR and dysmorphism and/or family history; pts scored ≥3 on de Vries checklist (2001)	spectral karyotype (11), subtelomere (30)	BAC	1,000	FISH	10
Tyson et al. 2005	22	mild to moderate MR and nonsyndromic dysmorphic features; pts scored ≥3 on de Vries checklist (2001)	G-banded karyotype	BAC	1,000	FISH	13.6

**Table C.** Case Series (n ≥ 20) of Patients with Suspected Genetic Abnormality but Negative Results by Standard Genetic Evaluation (cont'd)

Study	n	Patient Description	Previous Normal Studies	Array Type	Minimum Array Resolution (kb)	Confirmation	% Diagnostic Yield
<b>Studies of Patients with Clinical Diagnoses of Developmental Delay/Mental Retardation (cont'd)</b>							
Studies using whole genome arrays with ~even coverage across all regions (cont'd)							
Menten et al. 2006	140	idiopathic MR and multiple congenital anomalies	karyotype, subtelomere (31)	BAC/PAC	1,000	FISH and/or PCR	13.6
Miyake et al. 2006	30	idiopathic MR with some dysmorphic features	G-banded karyotype	BAC	1,400	FISH	16.7
Rosenberg et al. 2006	81	idiopathic MR and congenital anomalies	karyotype	BAC	1,000	FISH	16
Krepischi-Santos et al. 2006	95	syndromic MR or other	karyotype, FMR1 (in some)	BAC	1,000 or 3,000	FISH or MLPA	16
Lugtenberg et al. 2006	40	idiopathic MR, suspicious for X-linked abnormality	karyotype	BAC	100 tiling <sup>1</sup>	MLPA	7.5
Thuresson et al. 2007	48	idiopathic MR and congenital malformations	G-banded karyotype, subtelomere FISH	BAC	1,000	FISH	6
Friedman et al. 2006	100	moderate-severe idiopathic MR/DD with congenital anomalies	karyotype	SNP (Affymetrix 100k)	400–1,000 <sup>2</sup>	FISH	11
Hoyer et al. 2007	104	unselected patients with idiopathic MR	G-banded karyotype	SNP	400–1,000 <sup>2</sup>	FISH	9.1
Wagenstaller et al. 2007	67	idiopathic MR	G-banded karyotype, FISH (42)	SNP	400–1,000 <sup>2</sup>	PCR	16.4
Bruno et al. 2009	117	idiopathic MR and/or congenital malformations	karyotype (400-650 band level)	SNP (Affymetrix 250K array)	NS	FISH, MLPA	15

**Table C.** Case Series (n ≥ 20) of Patients with Suspected Genetic Abnormality but Negative Results by Standard Genetic Evaluation (cont'd)

Study	n	Patient Description	Previous Normal Studies	Array Type	Minimum Array Resolution (kb)	Confirmation	% Diagnostic Yield
<b>Studies of Patients with Clinical Diagnoses of Developmental Delay/Mental Retardation (cont'd)</b>							
Studies using arrays limited to specific chromosomes or chromosomal regions							
Madrigal et al. 2007	54	idiopathic MR; 52 from families c/w X-linked inherited MR; 2 with suspicion of X chromosome deletion	karyotype, FMR1	BAC (X-chromosome)	100	MLPA, PCR	14.8
Froyen et al. 2007	108	suspicious for X-linked MR	karyotype, FMR1	BAC (X-chromosome)	80	PCR	13
Harada et al. 2004	69	idiopathic MR, with or without multiple congenital anomalies	karyotype (400 band level)	BAC/PAC (subtelomeres)	NS	FISH	5.8
<b>Studies of Patients with Clinical Diagnoses of Autism Spectrum Disorder</b>							
Studies using whole genome arrays with less dense coverage of DNA backbone regions and dense coverage of targeted regions							
Lu et al. 2007	146	autistic behavioral features	G-banded karyotype and/or FISH	BAC/PAC	NS	FISH	3.4
Studies using whole genome arrays with ~even coverage across all regions							
Jacquemont et al. 2006	29	syndromic ASD	high resolution karyotype, biochemical tests	BAC/PAC	1,000	FISH	28
Christian et al. 2008	397	non-syndromic autism, subset of AGRE subjects (Roswell Park Cancer Institute)	karyotype	BAC (19K)	tiling <sup>1</sup>	FISH, PCR	11.6

**Table C.** Case Series (n ≥ 20) of Patients with Suspected Genetic Abnormality but Negative Results by Standard Genetic Evaluation (cont'd)

Study	n	Patient Description	Previous Normal Studies	Array Type	Minimum Array Resolution (kb)	Confirmation	% Diagnostic Yield
<b>Studies of Patients with Clinical Diagnoses of Autism Spectrum Disorder (cont'd)</b>							
Studies using whole genome arrays with ~even coverage across all regions (cont'd)							
Sebat et al. 2007	195	non-syndromic autism; majority from AGRE or NIMH Center for Collaborative Genetic Studies on Mental Disorders	karyotype	oligo	35	aCGH dye-swap replicate, other CGH arrays	7.2
Marshall et al. 2008	427	diagnosed ASD	karyotype (13 with known abnormality)	SNP (Affymetrix 500K)	NS	PCR	6.3 (de novo)

**Abbreviations:** AGRE, Autism Genetic Resource Exchange; NIMH, National Institute of Mental Health; FMR1, fragile X mental retardation 1 gene analysis; BAC, bacterial artificial chromosome; PAC, plasmid artificial chromosome; MLPA, multiplex ligation-dependent probe amplification; FISH, fluorescent in situ hybridization; PCR, polymerase chain reaction; NS, not specified; aCGH, array comparative genomic hybridization; SNP, single nucleotide polymorphism; N/A, not applicable

<sup>1</sup> Tiling arrays use DNA fragments that overlap such that the entire genomic sequence is represented; resolution depends on the size of the fragments.

<sup>2</sup> Using criteria of at least 4 adjacent SNPs for a CNV.

<sup>3</sup> Using criteria of 3 adjacent SNPs for a deletion and 6 adjacent SNPs for a duplication.

**Table D.** Examples of Laboratories Offering Whole-Genome Array Molecular Karyotyping for Detecting Genomic Abnormalities Associated with Developmental Delay/Mental Retardation

Institution	Pre- and/or Post-natal?	Test name	Array Description
Baylor College of Medicine, Medical Genetics Laboratories <a href="http://www.bcm.edu/geneticlabs/">http://www.bcm.edu/geneticlabs/</a>	Both	Chromosomal Microarray Analysis	105K oligo array – targeted + whole genome @ ≤30kb interval
Mayo Medical Laboratories <a href="http://www.mayomedicallaboratories.com/index.html">http://www.mayomedicallaboratories.com/index.html</a>	Post-natal	Array Comparative Genomic Hybridization	44k oligo array – targeted + whole genome @ ≤75kb interval
Emory School of Medicine, Genetics Laboratory <a href="http://genetics.web.emory.edu/egl/">http://genetics.web.emory.edu/egl/</a>	Post-natal	Array CGH - EmArray Cyto6000	44k oligo array – targeted + whole genome @ ≤75kb interval
University of Oklahoma, Health Sciences Center, Genetics Lab <a href="http://www.genetics.ouhsc.edu/default.asp">http://www.genetics.ouhsc.edu/default.asp</a>	Both	Chromosomal Microarray Analysis	? (no information on website)
Signature Genomic Laboratories <a href="http://www.signaturegenomics.com/">http://www.signaturegenomics.com/</a>	Both	SignatureChipWG™ SignatureChipOS™ PrenatalChip™	4.6k BAC array – targeted + whole genome @ ? interval 105k oligo array – targeted + whole genome @ ≤35kb interval 1k BAC array, targeted
GeneDx <a href="http://www.genedx.com/index.php">http://www.genedx.com/index.php</a>	Post-natal	GenomeDx High-Resolution Genome-Wide Microarray	105k oligo array – targeted + whole genome @ ≤37kb interval
Combimatrix Molecular Diagnostics <a href="http://www.cmdiagnostics.com/">http://www.cmdiagnostics.com/</a>	Both	BAC HD Scan™ Oligo HD Scan™ Prenatal Scan™	2.4k BAC array – targeted + whole genome @ ≤1Mb interval 105k oligo array – targeted + whole genome @ ≤35kb interval 1.7k BAC array – targeted
Quest Diagnostics, Nichols Institute <a href="http://www.questdiagnostics.com/index.html">http://www.questdiagnostics.com/index.html</a> Specialty Laboratories <a href="http://www.specialtylabs.com/default.htm">http://www.specialtylabs.com/default.htm</a>	Post-natal	Genomic Alterations, postnatal, ClariSure™ CGH	1.3k BAC array – targeted + whole genome @ ≤3Mb interval
Laboratory Corporation of America (LabCorp) <a href="http://www.labcorp.com/index.html">http://www.labcorp.com/index.html</a>	Both	Chromosome Micro Array	1.8 million SNP array – targeted + whole genome

# Glossary of Genetic Terms

These definitions are derived primarily from the Genetics Home Reference (<http://ghr.nlm.nih.gov/glossary>), the National Library of Medicine's web site for consumer information about genetic conditions and the genes or chromosomes related to those conditions, and also from the National Cancer Institute Dictionary of Cancer Terms (<http://www.cancer.gov/dictionary/>), with minor edits or additions.

**Aneuploidy:** The occurrence of one or more extra or missing chromosomes leading to an unbalanced chromosome complement.

**Array:** see *DNA microarray*

**Array comparative genomic hybridization (aCGH):** Conceptually the same as CGH but using an array of DNA segments covering (completely or in segments) a reference genome, to which the patient sample is hybridized. aCGH has increased resolution compared to CGH; the degree of resolution depends on the size of the reference DNA fragments spotted onto the array, fragment distribution across the genome, fragment hybridization specificity, and on the method of signal analysis.

**Bacterial artificial chromosome (BAC):** Large segments of DNA, 100,000 to 200,000 bases, from another species cloned into bacteria. Once the foreign DNA has been cloned into the host bacteria, many copies of it can be made.

**Band, banding:** see *karyotype*

**Base (b):** The bases are the "letters" that spell out the genetic code. In DNA, the code letters are A, T, G, and C, which stand for the chemicals adenine, thymine, guanine, and cytosine, respectively.

**Base pair (bp):** In the DNA molecule, adenine always pairs with thymine, and guanine always pairs with cytosine.

**Biomarker:** see *genetic marker*

**Candidate gene:** Gene selected for study based on existing knowledge about the disease biology.

**Chip:** see *DNA microarray*

**Chromatin:** The material of chromosomes. It is a complex of DNA, histones, and nonhistone proteins found within the nucleus of a cell. Chromatin occurs in two forms during the phase between mitotic divisions: 1) as heterochromatin, seen as condensed, readily stainable clumps; 2) as euchromatin, dispersed lightly staining or nonstaining material. During mitotic division the chromatin condenses into chromosomes.

**Chromosome:** Structure found in the nucleus of a cell, which contains the genes. Chromosomes come in pairs, and a normal human cell contains 46 chromosomes.

**Chromosomal translocation:** see *translocation*

**Coding region:** Sequence of DNA consisting of a series of nucleotide bases (code) giving rise to the mature messenger RNA that will be translated into the specific amino acids of the protein product.

**Comparative genomic hybridization (CGH):** A method of identifying deletions or duplications anywhere in the genome in a single experiment. Patient total genomic DNA is fluorescently labeled and hybridized in equal amounts to a differentially labeled reference sample. Deletions or duplications are seen as regions of inequality in the fluorescent signal. see also *array comparative genomic hybridization*

**Copy number variants (CNVs):** deletions and duplications of DNA segments larger than 1000 bases (1kb) and up to several megabases (Mb) in size that are present in variable copy number compared with a reference genome.

**Deoxyribonucleic acid (DNA):** The molecules inside cells that carry genetic information and pass it from one generation to the next.

**Diagnostic yield:** The proportion of tested patients with clinically relevant genomic abnormalities.

**DNA microarray:** A process that allows thousands of pieces of DNA that are fixed to a glass slide to be analyzed at one time. It is used to identify the genes (pieces of DNA) in specific cells or tissue that are actively used to make RNA, which then may be used to make proteins. It is also used to detect single nucleotide polymorphisms within a population.

**Epigenetics:** Changes in the regulation of the expression of gene activity without alteration of DNA sequence. *Methylation* is one epigenetic mechanism.

**Expression:** see *gene expression*

**Expressivity:** The relative capacity of a gene to affect the phenotype of the organism of which it is a part.

**Fluorescent in situ hybridization (FISH):** A physical mapping approach that uses fluorescein tags to detect hybridization of sequence-specific DNA probes with metaphase chromosomes and with the less-condensed somatic interphase chromatin.

**Fusion gene, protein:** A protein created after two genes are joined together (e.g. as a result of a translocation).

**Gene:** The functional and physical unit of heredity passed from parent to offspring. Genes are pieces of DNA, and most genes contain the information for making a specific protein.

**Genes, tumor-suppressing:** Genes that normally restrain cell growth but, when missing or inactivated by mutation, allow cells to grow uncontrolled.

**Gene expression:** The process by which a gene's coded information is translated into the structures present and operating in the cell (either proteins or RNAs).

**Gene mapping:** Any method used for determining the location of and relative distances between genes on a chromosome.

**Gene sequencing:** Process by which the nucleotide sequence is determined for a segment of DNA.

**Genetics:** The branch of science concerned with the means and consequences of transmission and generation of the components of biological inheritance.

**Genetic marker:** An identifiable segment of DNA with enough variation between individuals that its inheritance and co-inheritance with alleles of a given gene can be traced; used in linkage analysis.

**Genetic profile:** Information about specific genes, including variations and gene expression, in an individual or in a certain type of tissue. A genetic profile may be used to help diagnose a disease or learn how the disease may progress or respond to treatment with drugs or radiation.

**Genetic susceptibility:** Increased susceptibility to a particular disease due to the presence of one or more gene mutations, and/or a combination of alleles (haplotype), not necessarily abnormal, that is associated with an increased risk for the disease, and/or a family history that indicates an increased risk for the disease.

**Genetic testing:** Examining a sample of blood or other body fluid or tissue for biochemical, chromosomal, or genetic markers that indicate the presence or absence of genetic disease.

**Genome:** All the DNA contained in an organism or a cell, which includes both the chromosomes within the nucleus and the DNA in mitochondria.

**Genome-wide association study (GWAS):** A method of searching the genome for single nucleotide polymorphisms (SNPs) that occur more frequently in people with a particular disease than in people without the disease. Each study can look at hundreds or thousands of SNPs at the same time (e.g. using DNA microarray technology). Researchers use data from this type of study to pinpoint genes that may contribute to a person's risk of developing a certain disease.

**Genotype:** see *genetic profile*

**Hereditary mutation:** see *mutation, hereditary*

**Histone:** A type of protein found in chromosomes. Histones bind to DNA, help give chromosomes their shape, and help control the activity of genes.

**Hypermethylation:** see *methylation*

**Intensity ratio (for aCGH):** Array CGH  $\log_2$ -based intensity ratios provide useful information about genome-wide CNVs. In humans, the normal DNA copy number is two for all the autosomes. In an ideal situation, the normal clones would correspond to a  $\log_2$  ratio of zero. The  $\log_2$  intensity ratio of a single copy loss would be -1, and a single copy gain would be 0.58. The goal is to effectively identify locations of gains or losses of DNA copy number.

**Inversion:** A chromosomal rearrangement in which a segment of genetic material is broken away from the chromosome, inverted from end to end, and re-inserted into the chromosome at the same breakage site.

**Karyotype:** A photographic representation of the condensed chromosomes of a single cell, cut and arranged in pairs. After appropriate staining, each chromosome has a characteristic banding pattern that helps to identify them; both chromosomes in a pair will have the same banding pattern. Chromosome bands are named as follows. Each chromosome consists of two arms separated by the centromere. The long arm and short arm are labeled q (for queue) and p (for petit), respectively. At the lowest resolution, only a few major bands can be distinguished, which are labeled q1, q2, q3; p1, p2, p3, etc., counting from the centromere. Higher resolution reveals sub-bands, labeled q11, q12, q13, etc. Sub-sub-bands identified by even higher resolution are labeled q11.1, q11.2, q11.3, etc.

**Kilobase (kb):** A DNA segment that is 1,000 base pairs long.

**Locus (plural, loci):** The physical site or location of a specific gene or DNA sequence on a chromosome. Locus naming conventions at the macroscopic level include the chromosome number (e.g. 10), designation of the short (p) or long (q) chromosomal arm, and the band (e.g. 11) plus sub-band if appropriate (e.g. 23), written as e.g. 10q11.23. See *karyotype*.

**Malignant transformation:** The genetic change(s) that a normal cell undergoes as it becomes malignant.

**Mapping:** see *gene mapping*

**Marker chromosome:** a structurally abnormal, partial chromosome. The clinical significance depends on what genetic material is contained within the marker.

**Megabase (Mb):** A DNA segment that is 1,000,000 base pairs long.

**Messenger RNA (mRNA):** RNA that serves as a template for protein synthesis.

**Methylation:** The attachment of methyl groups to DNA at cytosine bases; correlated with reduced transcription of the gene and thought to be the principal mechanism in X-chromosome inactivation and imprinting.

**Microarray:** see *DNA microarray*

**Mitochondria:** Any of various round or long cellular organelles of most eukaryotes that are found outside the nucleus, produce energy for the cell through cellular respiration, and are rich in fats, proteins, and enzymes.

**Mitochondrial DNA:** Double-stranded DNA of mitochondria. In eukaryotes, the mitochondrial genome is circular and codes for ribosomal RNAs, transfer RNAs, and about 10 proteins.

**Monosomy:** The presence of only one chromosome from a pair.

**Mosaicism:** The post-fertilization occurrence of two or more cell lines with different genetic or chromosomal constitutions within a single individual or tissue.

**Multiplex ligation-dependent probe amplification (MLPA):** A high resolution method to detect copy number variation in specific genomic sequences.

**Mutation:** Any alteration in a gene from its natural state; may be disease causing or a benign, normal variant. See also *polymorphism*.

**Mutation, hereditary:** The presence of an altered gene within the egg and/or sperm (germ cell) such that the altered gene can be passed to subsequent generations.

**Mutation, somatic:** Alterations in DNA that occur after conception. Somatic mutations can occur in any of the cells of the body except the germ cells (sperm and egg) and therefore are not passed on to children. These alterations can (but do not always) cause cancer or other diseases.

**Mutation, sporadic:** Denoting a genetic disorder that occurs for the first time in a family due to a new mutation.

**Nucleic acid:** Any of various acids (as an RNA or a DNA) composed of nucleotide chains.

**Nucleotide:** One of the structural components, or building blocks, of DNA and RNA. A nucleotide consists of a base (one of four chemicals: adenine, thymine, guanine, and cytosine) plus a molecule of sugar and one of phosphoric acid.

**Oncogene:** A gene that normally directs cell growth. If altered, an oncogene can promote or allow the uncontrolled growth of cancer. Alterations can be inherited or caused by an environmental exposure to carcinogens.

**Penetrance:** The probability of a gene or genetic trait being expressed. “Complete” penetrance means the gene or genes for a trait are expressed in all the population who have the genes. “Incomplete” penetrance means the genetic trait is expressed in only part of the population. The percent penetrance also may change with the age range of the population.

**Plasmid:** An extrachromosomal ring of DNA that replicates autonomously and is found especially in bacteria.

**Plasmid artificial chromosome (PAC):** Segments of DNA from another species cloned into a plasmid. Once the foreign DNA has been cloned into the host plasmid, many copies of it can be made.

**Polymerase chain reaction (PCR):** A procedure that produces multiple copies of a short segment of DNA through cycles of: 1) denaturation (heat-induced separation of double-stranded DNA into single strands); 2) annealing (binding of specific primers on either end of the target segment); and 3) elongation (extension of the primer sequences over the target segment with DNA polymerase). The amplified product, doubled each cycle for 30 or more cycles, can then be subjected to further testing. RT-PCR stands for reverse transcriptase PCR, in which the starting material is RNA, which is transcribed by RT into DNA and then the DNA is subjected to PCR.

**Polymorphism:** Difference in DNA sequence among individuals. See also *mutation*.

**Polymorphism, single nucleotide (SNP):** A single nucleotide variation in a genetic sequence that occurs at appreciable frequency in the population. SNPs are archived in the NIH Single Nucleotide Polymorphism Database (dbSNP) of Nucleotide Sequence Variation. SNPs are mapped to a specific location in the human genome DNA sequence; multiple SNPs submitted to the database that map to the same position are called a reference SNP cluster. Each reference SNP cluster is given a unique rs ID number.

**Proteomics:** Field that utilizes protein sequences, expression and structure to determine how proteins relate, interact and function in an organism; includes characterizing and cataloguing proteins and protein libraries, comparing variations in protein expression levels under different conditions, studying protein interactions and functional roles; techniques are performed in an automated, large scale manner; may also involve bioinformatic analysis and storage of data.

**Proto-oncogene:** A gene having the potential for change into an active oncogene.

**Rearrangement:** A structural alteration in a chromosome, usually involving breakage and reattachment of a segment of chromosome material, resulting in an abnormal configuration; examples include inversion and translocation.

**Sequencing:** see *gene sequencing*

**Single nucleotide polymorphism:** see *polymorphism, single nucleotide*

**Somatic mutation:** see *mutation, somatic*

**Sporadic mutation:** see *mutation, sporadic*

**Subtelomere:** The chromosomal region just proximal to the telomere, composed of highly polymorphic repetitive DNA sequences that are typically situated adjacent to gene-rich areas. see also *telomere*

**Susceptibility:** see *genetic susceptibility*

**Telomere:** A terminal section of a chromosome that has a specialized structure and is involved in chromosomal replication and stability. see also *subtelomere*

**Tiling resolution (in aCGH arrays):** Tiling arrays use large-insert clones such as BAC clones uniformly distributed across the genome with very few gaps in coverage.

**Transcription:** The process of copying information from DNA into new strands of messenger RNA (mRNA). The mRNA then carries this information to the cytoplasm, where it serves as the blueprint for the manufacture of a specific protein.

**Translation:** The process of turning instructions from mRNA, base by base, into chains of amino acids that then fold into proteins. This process takes place in the cytoplasm, on structures called ribosomes.

**Translocation:** A chromosome alteration in which a whole chromosome or segment of a chromosome becomes attached to or interchanged with another whole chromosome or segment, the resulting hybrid segregating together at meiosis.

**Tumor-suppressing genes:** see *genes, tumor-suppressing*





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