

# THE USE OF GENOMIC MICROARRAYS TO STUDY CHROMOSOMAL ABNORMALITIES IN MENTAL RETARDATION

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Mental retardation affects 2 to 3% of the US population. It is defined by broad criteria, including significantly subaverage intelligence, onset by age 18, and impaired function in a group of adaptive skills. A myriad of genetic and environmental causes have been described, but for approximately half of individuals diagnosed with mental retardation the molecular basis remains unknown. Genomic microarrays, also called array comparative genomic hybridization (array CGH), represent one of several novel technologies that allow the detection of chromosomal abnormalities, such as microdeletions and microduplications, in a rapid, high throughput fashion from genomic DNA samples. In one early application of this technology, genomic microarrays have been used to characterize the extent of chromosomal changes in a group of patients diagnosed with one particular type of disorder that causes mental retardation, such as deletion 1p36 syndrome. In another application, DNA samples from individuals with idiopathic mental retardation have been assayed to scan the entire genome in attempts to identify chromosomal changes. Genomic microarrays offer both a genome-wide perspective of chromosomal aberrations as well as higher resolution (to the level of approximately one megabase) compared to alternative available technologies.

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Mental retardation is one of the most common forms of mental illness, affecting approximately 2 to 3% of the US population. Mental retardation is defined by three criteria [American Association on Mental Retardation, 1992]. First, an individual with mental retardation has significantly subaverage intellectual functioning, often measured as an intelligence quotient (IQ) below 70 or 75. Second, impairment occurs with related limitations in 2 or more of 10 adaptive skill areas (communication, self-care, home living, social skills, community use, self-direction, health and safety, functional academics, leisure, and work). Third, the onset of symptoms occurs at age 18 or younger; this criterion distinguishes mental retardation from other causes of decreased intelligence such as dementia. Overall this definition of mental retardation is broad and involves four dimensions: intellectual functioning and adaptive skills, psychological and emotional considerations, etiology, and environmental considerations [American Association on Mental Retardation, 1992].

The subject of this review is recent molecular approaches to identify the etiology of mental retardation. We can describe four major categories of causes, including both genetic and environmental bases (Fig. 1). (1) Single gene disorders, that is, genetic disorders caused primarily by a mutated gene. The most common cause of mental retardation in this category is Fragile X syndrome, resulting from a mutated *FMR1* gene at Xq27.3 with a repeated CGG triplet in its 5'-untranslated region [Verkerk et al., 1991]. (2) Complex disorders, which are caused by mutations in multiple genes. For example, autism is characterized by a triad of symptoms involving repetitive movements, impaired reciprocal communication, and impaired language skills [American Psychiatric Association (APA) 1994]. Approximately 70% of individuals with autism are diagnosed with mental retardation [Piven and Folstein, 1994]. Current evidence suggests that multiple genetic factors are the causative determinants of the majority of cases of autism [Rutter et al., 1994; Veenstra-Vanderweele et al., 2004]. Mental retardation is sometimes associated with other kinds of mental illness that are likely to be complex disorders, including schizophrenia, personality disorders, and affective disorders. (3) Genomic disorders, that is, disease states caused by chromosomal changes such as deletions, duplications, inversions, and rearrangements. The most common genetic cause of mental retardation is Down syndrome [Epstein, 1995], caused by a trisomy of chromosome 21 and affecting 1 in 700 live births. Subtelomeric rearrangements (described below) are another common cause. (4) Environmental causes, including infectious disease, in utero exposure to teratogenic agents, childhood lead poisoning, malnutrition of pregnant mothers and/or newborns, and traumatic brain injury.

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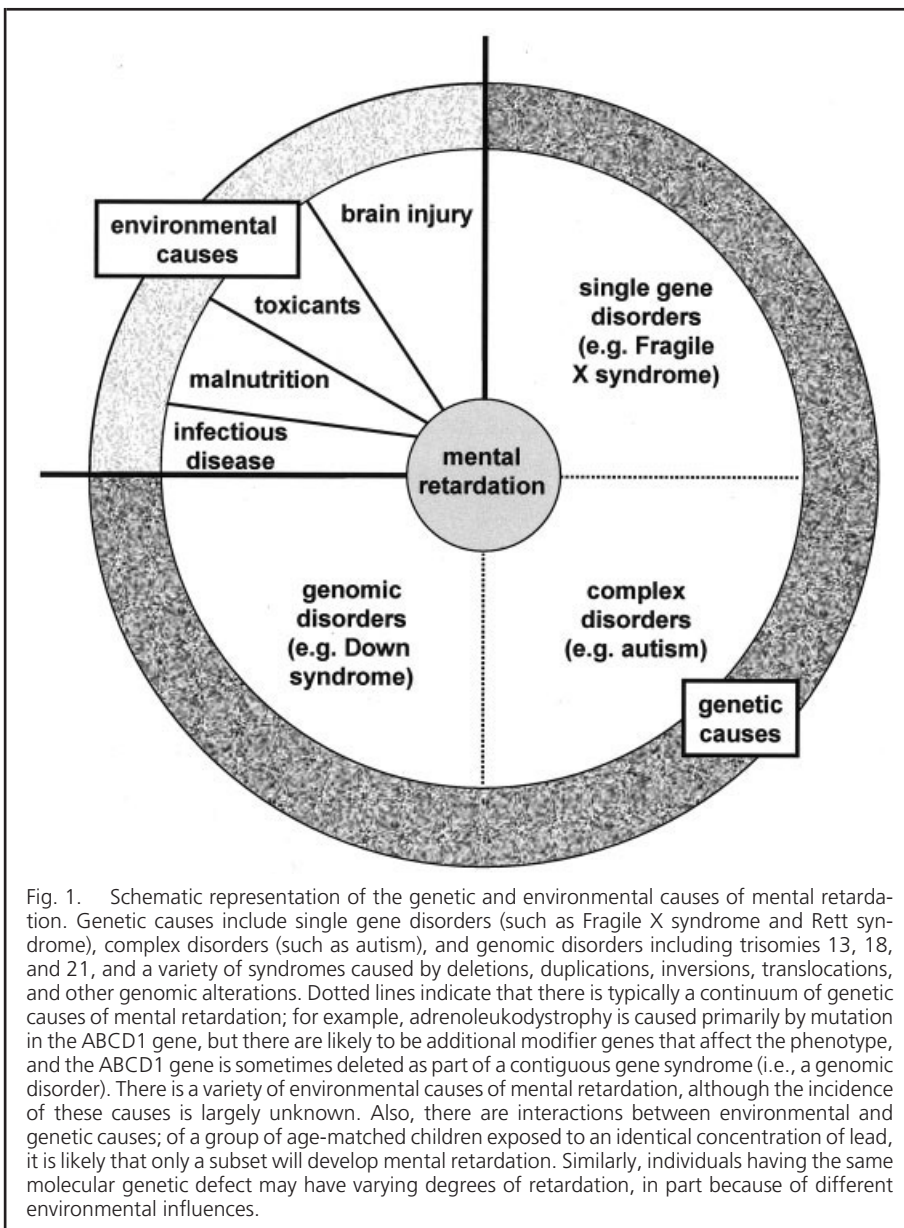


Fig. 1. Schematic representation of the genetic and environmental causes of mental retardation. Genetic causes include single gene disorders (such as Fragile X syndrome and Rett syndrome), complex disorders (such as autism), and genomic disorders including trisomies 13, 18, and 21, and a variety of syndromes caused by deletions, duplications, inversions, translocations, and other genomic alterations. Dotted lines indicate that there is typically a continuum of genetic causes of mental retardation; for example, adrenoleukodystrophy is caused primarily by mutation in the ABCD1 gene, but there are likely to be additional modifier genes that affect the phenotype, and the ABCD1 gene is sometimes deleted as part of a contiguous gene syndrome (i.e., a genomic disorder). There is a variety of environmental causes of mental retardation, although the incidence of these causes is largely unknown. Also, there are interactions between environmental and genetic causes; of a group of age-matched children exposed to an identical concentration of lead, it is likely that only a subset will develop mental retardation. Similarly, individuals having the same molecular genetic defect may have varying degrees of retardation, in part because of different environmental influences.

Given so many known causes, it is clear that mental retardation is a heterogeneous condition that involves diverse kinds of insults to the brain. Nonetheless, it is important to define the broad phenotype and its various causes, including genetic defects, to provide better classification, diagnosis, and treatment strategies. This article reviews recent microarray-based approaches to defining the chromosomal basis of mental retardation.

#### APPROACHES TO DEFINING THE MOLECULAR BASIS OF MENTAL RETARDATION

Upon clinical diagnosis of mental retardation, clinical genetics laboratories routinely perform karyotyping to search for chromosomal abnormalities. Giemsa staining of metaphase chromosomes (G-

banding), at resolution levels from as few as 450 to as many as 850 bands, yields a chromosome-specific pattern of light- and dark-stained bands. Analysis of such patterns allows the detection of deletions and duplications as small as approximately 3 megabases (millions of base pairs). Chromosomes range in size from 245 megabases (chromosome 1, with 2,610 genes) to 46 megabases (chromosome 21, with 337 genes). Thus, a deletion of 3 megabases could span as little as 1.2% or as much as 6.5% of an entire chromosome.

Since the 1970s, G-banding has been the primary tool for diagnosing chromosomal aberrations such as changes in overall chromosome copy number (aneuploidy), changes in copy number over a region of a chromosome (segmen-

tal aneuploidy), rearrangements (e.g., translocations), and microdeletions and microduplications. Improved resolution became available with the introduction of fluorescent in situ hybridization (FISH) in the 1980s. Large fragments of DNA, such as bacterial artificial chromosome (BAC) clones of about 200,000 base pairs (0.2 megabases), are labeled with a fluorescent dye and hybridized to spreads of either metaphase or interphase chromosomes. BACs assigned to defined chromosomal locations can be used as probes to detect and/or confirm chromosomal changes such as rearrangements, deletions, inversions, or the formation of ring chromosomes [Irons, 2003]. Due to the specificity of BAC probe hybridization, the resolution of FISH studies is less than 0.5 megabases. FISH can confirm the existence of cryptic deletions, that is, chromosomal deletions (or other genomic changes) that are not detectable at the light microscopic level by banding. Largely based on these approaches, several dozen syndromes associated with mental retardation have been described (Table 1).

A variety of additional technologies exist for the detection of chromosomal changes [Armour et al., 2002; Salman et al., 2004; Rooms et al., 2005]. These include polymerase chain reaction (PCR)-based approaches allowing qualitative or quantitative estimations of genomic DNA copy numbers. A technology of particular note is comparative genomic hybridization (CGH), in which deletions, duplications, and amplifications are detected in chromosomes from diseased versus normal individuals by simultaneous, competitive hybridization to normal metaphase spreads [Kallioniemi et al., 1992]. CGH offers a rapid, genome-wide technique for detecting chromosomal imbalances and it has been applied to cases of mental retardation [Kirchhoff et al., 2000, 2004]. However, its resolution is typically limited to approximately 10 megabases [Kirchhoff et al., 1999].

#### ARRAY CGH: ARRAY-BASED APPROACHES TO STUDYING CHROMOSOMAL CHANGES IN MENTAL RETARDATION

In conventional CGH, chromosomal aberrations can be detected in a comparison of normal and diseased samples. A further innovation is microarray-based CGH [Oostlander et al., 2004], also called genomic microarrays. Microarrays are solid supports, such as membranes or glass microscope slides, on which macromolecules of known se-

**Table 1. Examples of Syndromes Associated With Mental Retardation, Their Genetic Mechanisms and Chromosomal Loci<sup>a</sup>**

Syndrome	Mechanism	Locus	Gene <sup>b</sup>
Monosomy 1p36	Deletion	1p36	<i>CDC2L1</i>
Wolf-Hirschhorn	Deletion	4p16	<i>HOX7</i>
Cri-du-chat	Deletion	5p15	<i>TERT</i>
Williams-Beuren	Cryptic deletion <sup>c</sup>	7q11.23	<i>CYLN2</i>
Smith-Lemli-Opitiz	Monogenic	11q12-q13	<i>DHCR7</i>
Trisomy 13	Aneuploidy	13	Many
Prader-Willi	Deletion	15q11q13 (paternal deletion)	<i>SNRPN</i>
Angelman	Deletion	15q11q13 (maternal deletion)	<i>UBE3A</i>
Rubinstein-Taybi	Cryptic deletion	16p13.3	<i>CREBBP</i>
Smith-Magenis	Deletion	17p11.2	<i>RAI1</i>
CMT1A/HNPP <sup>d</sup>	Cryptic deletion	17p11.2	<i>PMP22</i>
Neurofibromatosis type I	Monogenic; cryptic deletion	17p11.2	<i>NF1</i>
Miller-Dieker	Deletion	17p	<i>LIS1</i>
Trisomy 18	Aneuploidy	18	Many
Trisomy 21 (Down syndrome)	Aneuploidy	21	Many
DiGeorge/VCFS <sup>d</sup>	Cryptic deletion	22q11.2	<i>TBX1</i>
Fragile X	Monogenic	Xq27	<i>FMR1</i>
Rett	Monogenic	Xq28	<i>MECP2</i>
Incontinentia pigmenti	Cryptic deletion	Xq28	<i>IKBK</i>

<sup>a</sup>Diagnostic laboratories commonly screen DNA samples from patients with mental retardation in an attempt to identify these and other disorders. <sup>b</sup>For deletion syndromes, many genes may be affected; the symbol for one representative gene (but not necessarily the most significant or relevant to mental retardation) from that region is listed. <sup>c</sup>Cryptic deletions tend to be undetectable by conventional cytogenetic analysis. <sup>d</sup>CMT1A, Charcot-Marie-Tooth disease type 1A; HNPP, hereditary neuropathy with liability to pressure palsies; VCFS, velocardiofacial syndrome.

quence are deposited in a regular array. Because of Watson-Crick base pairing, nucleic acids in solution (e.g., derived from a patient sample) can hybridize to their complementary probes immobilized on a microarray. This hybridization is a highly regular and specific event that can be controlled by temperature and solution composition.

There have been many applications of microarray technology, but two have been prominent in the past decade: microarrays for gene expression profiling and genomic microarrays. Gene expression microarrays provide information on the absolute level of up to thousands of RNA transcripts in a sample. Gene expression is regulated by the tissue type, developmental stage, physiological state, and disease state. Given the context-dependent, dynamic nature of gene expression and given the limited availability of postmortem (or premortem) human brain samples, there have been no published studies specifically devoted to gene expression changes in brain samples of individuals diagnosed with mental retardation.

In contrast, genomic microarrays provide information about the number of copies of genomic DNA (rather than RNA). These arrays allow one to assess physical changes in readily accessible chromosomal DNA in samples from individuals with mental retardation compared to normal controls. In the early development of genomic microarray technology, copy number changes in hu-

man cancers have been characterized [Albertson et al., 2003]. The significance of genomic microarray technology to the understanding of mental retardation is that many cases of idiopathic mental retardation are caused by unbalanced chromosomal abnormalities that result in chromosome copy number changes, and genomic microarrays in particular are useful to identify such changes. This identification provides a description of the molecular defect that underlies a given case of mental retardation, including the genes that may be present at an abnormal dosage. Many cases of individuals with idiopathic mental retardation and apparently normal karyotypes have chromosomal aberrations that can be identified using genomic microarrays (discussed below). A description of the molecular defect in different cases of mental retardation is expected to lead to better classification and diagnosis, and ultimately it may lead to strategies for therapeutic intervention.

The procedure for performing genomic array experiments, outlined in Fig. 2, resembles that of conventional CGH. A genomic microarray typically consists of BAC or other DNA clones (of size 50 kilobases to 250 kilobases) deposited robotically in a regular array on a solid surface such as a glass microscope slide. Genomic DNA is purified from both a test sample (e.g., a lymphoblastoid cell line derived from a patient with mental retardation) and a reference sample (e.g., a corresponding cell line from a normal individual

or a pool of normal individuals). The genomic DNA is fragmented (usually by sonication or by digestion with a restriction endonuclease), differentially labeled with fluorescent dyes, and hybridized to the genomic array. Repetitive DNA sequences are blocked by the addition of Cot-1 DNA. After washing and image analysis, the fluorescence ratio is determined. Most regions of genomic DNA maintain a 1:1 ratio, but deletions and duplications are detected as larger ratios (e.g., 3:2) that are identified as statistically significant (Fig. 2).

Relative to conventional cytogenetic approaches such as FISH, genomic microarrays offer the advantages of high coverage (typically spanning the entire genome), high resolution (often spanning less than 1 megabase intervals), and speed (a typical genomic microarray experiment requires only several days to perform). Genomic microarrays allow the detection of aneuploidies (e.g., gain or loss of entire chromosomes or segments of chromosomes), interstitial or terminal deletions, duplications, unbalanced translocations, or markers such as ring chromosomes.

We will next consider the application of genomic microarrays to three aspects of mental retardation: previously known syndromes, subtelomeric deletions, and idiopathic disease.

### Genomic Microarrays for Characterization of Known Mental Retardation Syndromes

One basic application of genomic microarray technology is the character-

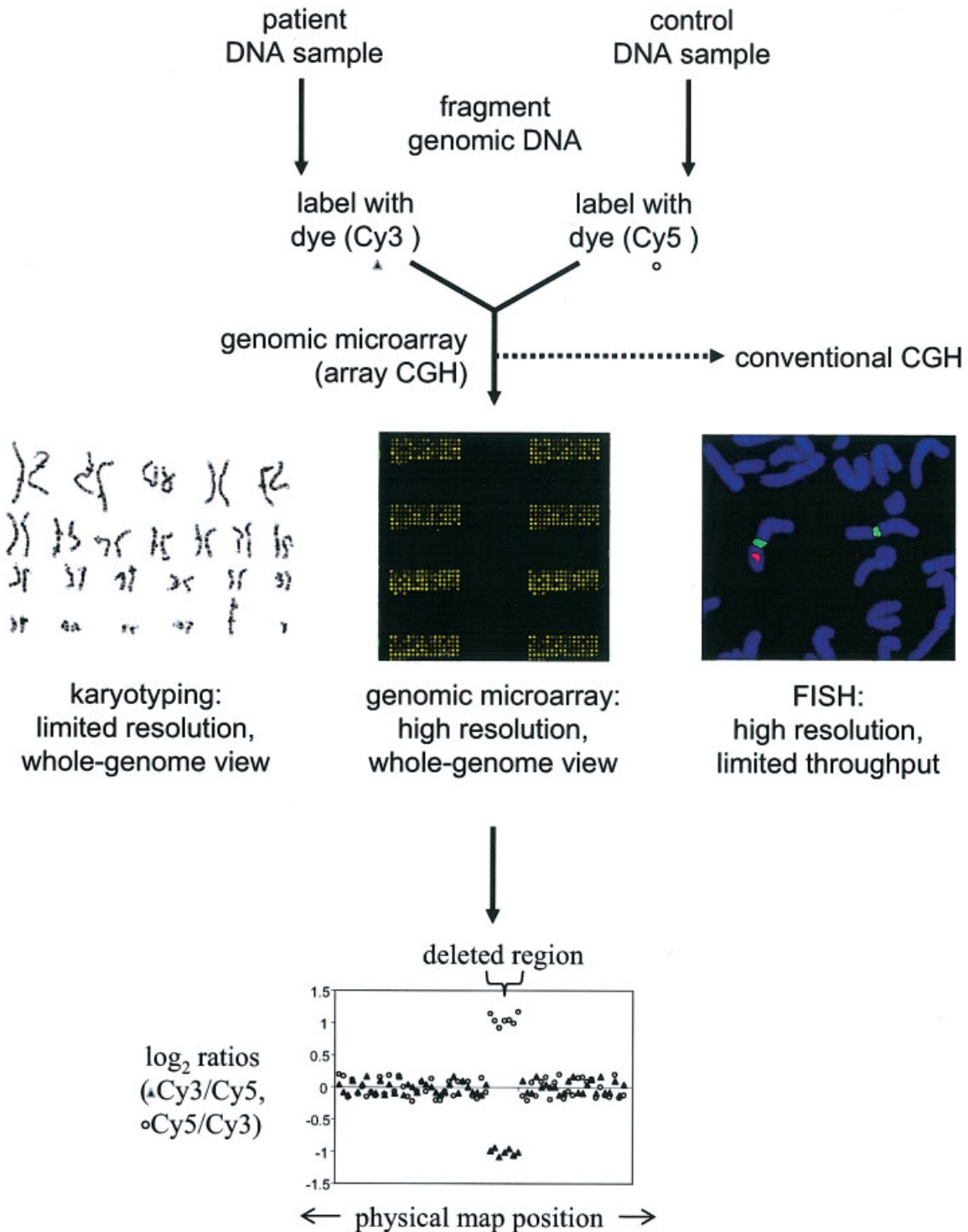


Fig. 2. Genomic microarray (array CGH) methodology in comparison to related techniques. Genomic DNA is isolated from blood (or other) cells from a patient with mental retardation or from a normal control. The DNA is purified, fragmented, and labeled with a fluorescent dye that is visualized as green (Cy3 dye) or red (Cy5 dye). The Cy3- and Cy5-labeled DNA fragments are hybridized simultaneously (competitively) on a genomic microarray. Typically, this array is a glass microscope slide on which several thousand BAC clones of known chromosomal location are robotically spotted and then immobilized. After the slide is washed, the fluorescent signals are detected by a scanner; each signal, in the red or green channel, is proportional to the amount (copy number) of genomic DNA present in each patient or control sample. A plot can then be generated showing the relative signal intensities of one dye to another (e.g., Cy3/Cy5) (y-axis) versus the physical map position of each BAC along each chromosome, in units of megabases (x-axis). Most data points show a 1:1 ratio ( $\log_2 = 0$ ), corresponding to regions in which there are no chromosome copy number changes. A deleted region of the genome is indicated by several contiguous spots (indicated on the plot) with decreased ratios (e.g. 1:2, or  $\log_2 = -1$ ) in one form of the experiment, and with contiguous spots with increased ratios (e.g., 3:2, or  $\log_2 = +1$ ). Genomic microarrays provide the whole-genome perspective of conventional karyotyping with the high resolution (approximately 1 megabase) of FISH. They also provide increased resolution relative to conventional CGH (see dashed arrow), which is performed by hybridizing probes to metaphase spreads. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

ization of known deletion or duplication syndromes associated with mental retardation. There are several reasons why this approach is useful. First, the extent of a duplicated or deleted region can be determined in a high throughput manner with better resolution than is available by karyotyping. For example, Yu et al. [2003] examined genomic DNA samples from 25 previously diagnosed patients with 1p36 deletions, a commonly occurring mental retardation syndrome. They used a microarray that included 97 BAC/PAC clones spanning 10.5 megabases of the distal 1p36 region, and their analyses revealed deletions ranging from approximately 1.5 to 10.5 megabases. With this relatively precise delineation of the deleted region, it is possible to attempt correlations between genotype and phenotypic severity of the disease.

A similar microarray-based approach has been taken to study microdeletions in 112 cases of Sotos syndrome, which is associated with craniofacial dysmorphism and mental retardation [Kurotaki et al., 2003]. Other studies have focused on genomic rearrangements in particularly unstable regions that are prone to rearrangement and genomic disease, including chromosome 15q11-q13 [Locke et al., 2004] and proximal chromosome 17p [Shaw et al., 2004].

A second motivation for applying genomic microarrays to mental retardation syndromes is that additional, unanticipated chromosomal changes may be detected. For example, Gunn et al. [2003] described a patient with a deletion of a portion of chromosome 18q based on FISH analysis. Genomic microarrays further revealed a gain of 3.7 megabases on chromosome 4q, a finding that was subsequently confirmed by FISH as a translocation distal to the 18qter breakpoint. In another example, Prescott et al. [2005] described a patient with features typical of 22q11 deletion syndrome, in which analysis of genomic microarrays revealed a 6-megabase deletion on chromosome 5q11.2—the first deletion described in this region.

### **Genomic Microarrays for Characterization of Subtelomeric Deletions**

Insight into the molecular basis of mental retardation has come from the finding that approximately 5 to 10% of previously unexplained cases can be attributed to submicroscopic, subtelomeric deletions [Flint et al., 1995; Knight et al., 1999; Biesecker, 2002; De Vries et al., 2003]. Human telomeres are characterized by tracts of TTAGGG units repeated

up to several thousand times [Moyzis et al., 1988]. The adjacent subtelomeric regions tend to have additional repetitive DNA sequences as well as a high density of protein-coding genes. Because subtelomeric regions have repetitive DNA regions, they are prone to rearrangements that may be associated with diseases including mental retardation (see below).

Unbalanced chromosomal abnormalities in subtelomeric regions may be particularly difficult to visualize cytogenetically by G-banding. A variety of FISH-based studies using specific subtelomeric probes have revealed such deletions. In 1995, Flint et al. used subtelomeric FISH probes to screen 99 individuals having idiopathic mental retardation and suggested that subtelomeric deletions were responsible in 6% of the cases. Other high throughput FISH studies have suggested that cryptic rearrangements and deletions can be found in subtelomeric regions in about 7 to 16% of cases [Anderlid et al., 2002; Engels et al., 2003; Nair-Miranda et al., 2004; Novelli et al., 2004; Rodriguez-Revenge et al., 2004]. Rooms et al. [2004] have recently used a PCR-based technique called multiplex ligation-dependent probe amplification to screen for subtelomeric deletions and found four such changes in a group of 75 patients (6%).

FISH-based approaches are limited by the need for high-quality metaphase spreads and the relatively small number of chromosomal loci that can be screened in a single reaction. Application of array CGH, in comparison, has the advantages of high-resolution screening and data acquisition on a genome-wide scale. Veltman et al. [2002] used array CGH to examine 20 patients with known subtelomeric imbalances and found not only concordance with the cytogenetic diagnosis but also additional telomere rearrangements in 3 of these 20 patients.

### **Genomic Microarrays for Characterization of Idiopathic Mental Retardation**

Some cases of mental retardation can be explained by the presence of gross chromosomal abnormalities (such as a trisomy). For perhaps half of mental retardation cases, the underlying genetic cause is unknown. Several groups have used conventional (nonarray) CGH to study patients diagnosed with mental retardation and dysmorphic features (suggestive of chromosomal aberrations) who also have an apparently normal karyotype. For example, Joly et al. [2001] identified chromosomal changes in 7 of 17 such

patients, while Ness et al. [2002] found deletions or insertions in 5 of 50 patients.

Genomic microarrays have also been used to search for chromosomal aberrations in patients having mental retardation, karyotypes that are apparently normal, and dysmorphic features that are suggestive of chromosomal abnormality. Visser et al. [2003] examined 20 such patients and identified 3 cases with microdeletions and 2 microduplications; these were independently confirmed by FISH. Similarly, Shaw-Smith et al. [2004] identified copy number abnormalities in 12 of 50 patients. As array CGH is a relatively recent innovation, all of these reports include validation studies. For example, Schoumans et al. [2004] selected 10 cases with a variety of known cryptic aberrations and found that genomic arrays (but not complementary DNA arrays) were useful in detecting all of the anomalies at a resolution of about 1 megabase.

### **WHY DELETIONS AND DUPLICATIONS OCCUR: MECHANISMS OF GENOMIC DISEASE**

Genomic microarrays have proven particularly useful for the study of cryptic chromosomal aberrations from about one megabase up to tens of megabases in size. Why do deletions, duplications, inversions, and rearrangements occur? The mechanisms underlying these changes are based largely on repetitive DNA elements found in the human genome. Approximately 5% of the genome consists of segmentally duplicated regions, that is, tracts of DNA greater than 1,000 base pairs in length and sharing at least 90% nucleotide identity [Lander et al., 2001]. Duplications may occur within a chromosome (intrachromosomally) or between chromosomes (interchromosomally). These duplicated regions, also called duplicons [Eichler, 1998], have been found to flank numerous regions that are susceptible to deletions and other chromosomal rearrangements [Ji et al., 2000]. When homologous duplicons recombine, chromosomal segments may become deleted, duplicated, or inverted. Lupski and colleagues have defined genomic disorders as conditions from DNA rearrangements due to regional genomic architecture [Lupski, 1998; Inoue and Lupski, 2003; Stankiewicz et al., 2003].

### **FUNCTIONAL CONSEQUENCES OF CHROMOSOMAL ABERRATIONS**

One basic question about chromosomal gains and losses is whether they

cause a particular disease phenotype or instead represent variations that are found in the normal population. In most published genomic microarray studies, experiments comparing different control DNA samples were performed, and some small number of copy number variations (also called copy number polymorphisms) were observed. These gains and losses are usually reported as false positive (or false negative) results. However, it is also possible that they reflect copy number polymorphisms that occur in the general population. Using genomic microarrays, extensive large-scale copy number variations that involve gains or losses of several kilobases to hundreds of kilobases have recently been detected throughout the normal human genome [Iafate et al., 2004; Sebat et al., 2004]. Variant segmental duplications have been identified by bioinformatic approaches

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*It is clear that, once regions of chromosomal aberrations are identified, a far greater challenge is to elucidate the connections between such genotypic changes and the phenotype of mental retardation.*

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[Mehan et al., 2004], and sequencing of human chromosomes has also revealed regions of extensive polymorphism [Martin et al., 2004].

Once disease-causing chromosomal aberrations are detected, an important but challenging goal is to establish the functional consequences. Duplications and deletions are likely to lead to gene dosage-dependent changes in gene expression in the affected region. Additional pathological or compensatory changes in gene expression are also possible. The levels of RNA transcripts can be assessed using DNA microarrays for gene expression profiling. Such studies in the human brain are especially challenging because of the limited availability of postmortem or premortem samples [Colantuoni et al., 2000; Mirnics and Pevsner, 2004].

Gene expression profiling has been applied to many disease conditions. In general, the purpose of performing such studies is to identify regulated genes that serve as biomarkers or that reflect cellular pathways that have been perturbed. We have shown that fetal brains from trisomy 21 cases have an elevated expression of genes assigned to chromosome 21 [Mao et al., 2003]. Other groups have combined array CGH studies with gene expression measurements in cancer cell lines and tumor samples [Ulger et al., 2003; Mahlamaki et al., 2004; Melendez et al., 2004; Myers et al., 2004]. Such combined genomic DNA and gene expression studies are likely to be useful to help define the consequence of a chromosomal aberration on gene expression in a particular cell or tissue type.

## CONCLUSIONS AND PROSPECTS

Genomic microarrays have not replaced FISH or other low or high throughput approaches to detecting chromosomal changes in mental retardation. Instead, microarray-based technologies offer a complementary approach with advantages such as rapidity, genome-wide coverage, and high resolution. One limitation of genomic microarrays is the occasional occurrence of false negatives or false positive results due to cross hybridization between sample DNA and multiple BAC clones. It also remains necessary to verify microarray results with FISH or other independent tests.

In the near future, other array-based technologies such as single nucleotide polymorphism (SNP) microarrays (commonly known as "snip chips") are likely to provide alternative high throughput approaches to detecting chromosomal abnormalities [Rauch et al., 2004]. High-density SNP chips allow the detection of regions of homozygosity that reflect hemizygous deletion. Also, in duplicated or deleted regions, changes in the intensity of SNP signal intensities may be analyzed to identify chromosomal copy number changes. This provides another example of the trend toward technologies that maximize coverage of the genome while offering increased resolution (potentially to the level of just dozens or hundreds of nucleotides) and relative ease of data collection. It is clear that, once regions of chromosomal aberrations are identified, a far greater challenge is to elucidate the connections between such genotypic changes and the phenotype of mental retardation. ■

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## REFERENCES

- Albertson DG, Collins C, McCormick F, et al. 2003. Chromosome aberrations in solid tumors. *Nat Genet* 34:369–376.
- American Association on Mental Retardation. 1992. *Mental Retardation: Definition, Classification, and Systems of Supports*. Washington, DC: American Association on Mental Retardation.
- American Psychiatric Association. 1994. *Diagnostic and Statistical Manual of Mental Disorders*. Washington, DC: APA Press.
- Anderlid BM, Schoumans J, Anneren G, et al. 2002. Subtelomeric rearrangements detected in patients with idiopathic mental retardation. *Am J Med Genet* 107:275–284.
- Armour JA, Barton DE, Cockburn DJ, et al. 2002. The detection of large deletions or duplications in genomic DNA. *Hum Mutat* 20:325–337.
- Biesecker LG. 2002. The end of the beginning of chromosome ends. *Am J Med Genet* 107: 263–266.
- Colantuoni C, Purcell AE, Bouton CM, et al. 2000. High throughput analysis of gene expression in the human brain. *J Neurosci Res* 59:1–10.
- De Vries BB, Winter R, Schinzel A, et al. 2003. Telomeres: a diagnosis at the end of the chromosomes. *J Med Genet* 40:385–398.
- Eichler EE. 1998. Masquerading repeats: paralogous pitfalls of the human genome. *Genome Res* 8:758–762.
- Engels H, Ehrbrecht A, Zahn S, et al. 2003. Comprehensive analysis of human subtelomeres with combined binary ratio labelling fluorescence in situ hybridisation. *Eur J Hum Genet* 11:643–651.
- Epstein CJ. 1995. Down syndrome (trisomy 21). In: Scriver CR, Beaudet AL, Sly WS, et al., editors. *The metabolic and molecular bases of inherited disease*. New York: McGraw Hill. p 749–794.
- Flint J, Wilkie AO, Buckle VJ, et al. 1995. The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. *Nat Genet* 9:132–140.
- Gunn SR, Mohammed M, Reveles XT, et al. 2003. Molecular characterization of a patient with central nervous system dysmyelination and cryptic unbalanced translocation between chromosomes 4q and 18q. *Am J Med Genet* 120:127–135.
- Iafate AJ, Feuk L, Rivera MN, et al. 2004. Detection of large-scale variation in the human genome. *Nat Genet* 36:949–951.
- Inoue K, Lupski JR. 2003. Genetics and genomics of behavioral and psychiatric disorders. *Curr Opin Genet Dev* 13:303–309.
- Irons M. 2003. Use of subtelomeric fluorescence in situ hybridization in cytogenetic diagnosis. *Curr Opin Pediatr* 15:594–597.
- Ji Y, Eichler EE, Schwartz S, et al. 2000. Structure of chromosomal duplicons and their role in mediating human genomic disorders. *Genome Res* 10:597–610.
- Joly G, Lapiere JM, Ozilou C, et al. 2001. Comparative genomic hybridization in mentally retarded patients with dysmorphic features

- and a normal karyotype. *Clin Genet* 60:212–219.
- Kallioniemi A, Kallioniemi OP, Sudar D, et al. 1992. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* 258:818–821.
- Kirchhoff M, Gerdes T, Maahr J, et al. 1999. Deletions below 10 megabasepairs are detected in comparative genomic hybridization by standard reference intervals. *Genes Chromosomes Cancer* 25:410–413.
- Kirchhoff M, Pedersen S, Kjeldsen E, et al. 2004. Prospective study comparing HR-CGH and subtelomeric FISH for investigation of individuals with mental retardation and dysmorphic features and an update of a study using only HR-CGH. *Am J Med Genet* 127A:111–117.
- Kirchhoff M, Rose H, Maahr J, et al. 2000. High resolution comparative genomic hybridization analysis reveals imbalances in dyschromosomal patients with normal or apparently balanced conventional karyotypes. *Eur J Hum Genet* 8:661–668.
- Knight SJ, Regan R, Nicod A, et al. 1999. Subtle chromosomal rearrangements in children with unexplained mental retardation. *Lancet* 354:1676–1681.
- Kurotaki N, Harada N, Shimokawa O, et al. 2003. Fifty microdeletions among 112 cases of Sotos syndrome: low copy repeats possibly mediate the common deletion. *Hum Mutat* 22:378–387.
- Lander ES, Linton LM, Birren B, et al. 2001. Initial sequencing and analysis of the human genome. *Nature* 409:860–921.
- Locke DP, Segraves R, Nicholls RD, et al. 2004. BAC microarray analysis of 15q11–q13 rearrangements and the impact of segmental duplications. *J Med Genet* 41:175–182.
- Lupski JR. 1998. Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. *Trends Genet* 14:417–422.
- Mahlamaki EH, Kauraniemi P, Monni O, et al. 2004. High-resolution genomic and expression profiling reveals 105 putative amplification target genes in pancreatic cancer. *Neoplasia* 6:432–439.
- Mao R, Zielke CL, Zielke HR, et al. 2003. Global up-regulation of chromosome 21 gene expression in the developing Down syndrome brain. *Genomics* 81:457–467.
- Martin J, Han C, Gordon LA, et al. 2004. The sequence and analysis of duplication-rich human chromosome 16. *Nature* 432:988–994.
- Mehan MR, Freimer NB, Ophoff RA. 2004. A genome-wide survey of segmental duplications that mediate common human genetic variation of chromosomal architecture. *Hum Genomics* 1:335–344.
- Melendez B, Diaz-Uriarte R, Cuadros M, et al. 2004. Gene expression analysis of chromosomal regions with gain or loss of genetic material detected by comparative genomic hybridization. *Genes Chromosomes Cancer* 41:353–365.
- Mirnic K, Pevsner J. 2004. Progress in the use of microarray technology to study the neurobiology of disease. *Nat Neurosci* 7:434–439.
- Moyzis RK, Buckingham JM, Cram LS, et al. 1988. A highly conserved repetitive DNA sequence, (TTAGGG)<sub>n</sub>, present at the telomeres of human chromosomes. *Proc Natl Acad Sci USA* 85:6622–6626.
- Myers CL, Dunham MJ, Kung SY, et al. 2004. Accurate detection of aneuploidies in array CGH and gene expression microarray data. *Bioinformatics* 20:3533–3543.
- Nair-Miranda K, Murch A, Petterson B, et al. 2004. An investigation into sub-telomeric deletions of chromosome 22 and pervasive developmental disorders. *Am J Med Genet* 125B:99–104.
- Ness GO, Lybaek H, Houge G. 2002. Usefulness of high-resolution comparative genomic hybridization (CGH) for detecting and characterizing constitutional chromosome abnormalities. *Am J Med Genet* 113:125–136.
- Novelli A, Ceccarini C, Bernardini L, et al. 2004. High frequency of subtelomeric rearrangements in a cohort of 92 patients with severe mental retardation and dysmorphism. *Clin Genet* 66:30–38.
- Oostlander AE, Meijer GA, Ylstra B. 2004. Microarray-based comparative genomic hybridization and its applications in human genetics. *Clin Genet* 66:488–495.
- Piven J, Folstein S. 1994. The genetics of autism. In: Bauman ML, Kemper TL, editors. *The neurobiology of autism*. Baltimore: The Johns Hopkins University Press. p 18–44.
- Prescott K, Woodfine K, Stubbs P, et al. 2005. A novel 5q11.2 deletion detected by microarray comparative genomic hybridization in a child referred as a case of suspected 22q11 deletion syndrome. *Hum Genet* 116:83–90.
- Rauch A, Ruschendorf F, Huang J, et al. 2004. Molecular karyotyping using an SNP array for genome-wide genotyping. *J Med Genet* 41:916–922.
- Rodriguez-Revena L, Badenas C, Sanchez A, et al. 2004. Cryptic chromosomal rearrangement screening in 30 patients with mental retardation and dysmorphic features. *Clin Genet* 65:17–23.
- Rooms L, Reyniers E, Kooy RF. 2005. Subtelomeric rearrangements in the mentally retarded: a comparison of detection methods. *Hum Mutat* 25:513–524.
- Rutter M, Bailey A, Bolton P, et al. 1994. Autism and known medical conditions: myth and substance. *J Child Psychol Psychiatry* 35:311–322.
- Salman M, Jhanwar SC, Ostrer H. 2004. Will the new cytogenetics replace the old cytogenetics?. *Clin Genet* 66:265–275.
- Schoumans J, Nordgren A, Ruivenkamp C, et al. 2004. Genome-wide screening using array-CGH does not reveal microdeletions/microduplications in children with Kabuki syndrome. *Eur J Hum Genet*.
- Sebat J, Lakshmi B, Troge J, et al. 2004. Large-scale copy number polymorphism in the human genome. *Science* 305:525–528.
- Shaw CJ, Shaw CA, Yu W, et al. 2004. Comparative genomic hybridisation using a proximal 17p BAC/PAC array detects rearrangements responsible for four genomic disorders. *J Med Genet* 41:113–119.
- Shaw-Smith C, Redon R, Rickman L, et al. 2004. Microarray based comparative genomic hybridization (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disabilities/mental retardation and dysmorphic features. *J Med Genet* 41:241–248.
- Stankiewicz P, Inoue K, Bi W, et al. 2003. Genomic disorders: genome architecture results in susceptibility to DNA rearrangements causing common human traits. *Cold Spring Harb Symp Quant Biol* 68:445–454.
- Ulger C, Toruner GA, Alkan M, et al. 2003. Comprehensive genome-wide comparison of DNA and RNA level scan using microarray technology for identification of candidate cancer-related genes in the HL-60 cell line. *Cancer Genet Cytogenet* 147:28–35.
- Veenstra-Vanderweele J, Christian SL, Cook EH Jr. 2004. Autism as a paradigmatic complex genetic disorder. *Annu Rev Genomics Hum Genet* 5:379–405.
- Veltman JA, Schoenmakers EF, Eussen BH, et al. 2002. High-throughput analysis of subtelomeric chromosome rearrangements by use of array-based comparative genomic hybridization. *Am J Hum Genet* 70:1269–1276.
- Verkerk AJ, Pieretti M, Sutcliffe JS, et al. 1991. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 65:905–914.
- Vissers LE, de Vries BB, Osoegawa K, et al. 2003. Array-based comparative genomic hybridization for the genome-wide detection of submicroscopic chromosomal abnormalities. *Am J Hum Genet* 73:1261–1270.
- Yu W, Ballif BC, Kashork CD, et al. 2003. Development of a comparative genomic hybridization microarray and demonstration of its utility with 25 well-characterized 1p36 deletions. *Hum Mol Genet* 12:2145–2152.