

Genetics of Childhood Disorders: VI. FISH, FISH, and More FISH

DAVID WARD, PH.D., PATRICIA BRAY-WARD, PH.D., AND PAUL J. LOMBROSO, M.D.

It is perhaps surprising to those not intimately involved in the field of genetics to realize that the total number of human chromosomes was not clearly established until 1956. However, over the past 43 years, cytogenetics has undergone a spectacular evolution involving both cytological and molecular techniques. A major breakthrough was the development in 1970 of the first chromosomal banding technique, called quinacrine banding. This provided the ability to identify and enumerate the 46 individual human chromosomes as well as to detect translocations, inversions, regions of amplification, deletions, rearrangements, insertions, and other chromosomal abnormalities. Very quickly, a number of additional banding techniques were developed. Today, Giemsa banding of metaphase chromosomes is the gold standard for karyotypic analysis.

The advent of recombinant DNA cloning techniques in the mid 1970s provided an approach to identify genes associ-

ated with specific genetic disorders. Cloning made available chromosome-specific repetitive DNA probes and single-copy sequence probes that could be hybridized to chromosomes to detect genetic changes at the molecular level. The development of chromosome-specific libraries by flow cytometry, the introduction of nonisotopically labeled probes, and the advent of methods to suppress hybridization signals from ubiquitous, repetitive sequences, rapidly led to high-throughput gene mapping. Furthermore, the development of interphase cytogenetics permitted the analysis for extra chromosomal copies, loss of heterozygosity, and translocations in interphase nuclei as well as metaphase chromosomes.

One of the steps in isolating a gene of interest is to know exactly where it lies on the human genome. Fluorescence detection methods, which rapidly superseded isotopic detection, are the most sensitive and efficient means for detecting

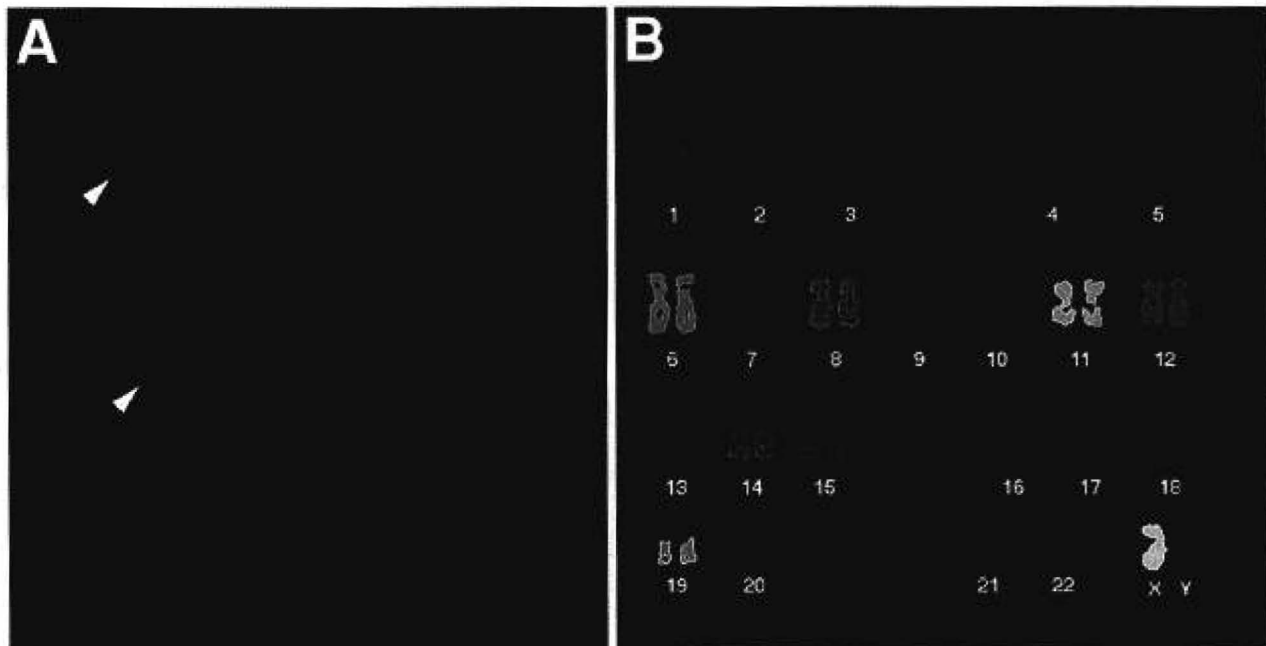


Fig. 1 A: A metaphase spread shows individual chromosomes counterstained blue. Two chromosomes show hybridization signals from a human genomic DNA clone that maps to chromosome 2p (arrows). Note that each chromosome consists of 2 individual chromatids, which explains why there are 4 hybridization signals to the probe that is being used. B: A karyotype of normal male metaphase chromosomes generated by analysis of hybridization with a 24-probe chromosome cocktail (M-FISH). From Speicher M, Ward D (1996), The coloring of cytogenetics. *Nature Medicine* 9:1046-1048. Reprinted with permission.

hybridized probes. Fluorescence in situ hybridization (FISH) of single-sequence probes is used today for clinical testing as well as for research (Fig. 1A). Increasingly, clinical cytogenetic laboratories are using disease-specific FISH probes in confirmational cytogenetic screening for many microdeletion syndromes with recognizable phenotypes, e.g., cri-du-chat, DiGeorge, and Williams syndromes.

FISH has become an essential element in positional cloning strategies by providing a bridge between 2 data sets: the clinical data on patients with characteristic cytogenetic abnormalities and the information on the physical organization of the human genome derived from the human genome project. The approximate position of a chromosomal abnormality in a patient is first described by cytogenetic band or by the distance from the p telomere. Using public databases that are now available at a touch of a personal computer, human genomic DNA clones are identified that have a similar physical location and known genetic linkage association, providing the options of testing patient DNA by analysis of neighboring genetic linkage markers or using genomic DNA clones for further testing of patient chromosomes by FISH.

These technologies have led to an intensive search for characteristic chromosomal changes in individuals with neuropsychiatric disorders. The search for the gene(s) within microdeletion syndrome regions has been successful in several cases. For example, Williams syndrome is now known to result from a deletion on chromosome 7, while Angelman and Prader-Willi syndromes are most often caused by deletions on chromosome 15. It is likely that most neuropsychiatric disorders are due to single-base mutations that are undetectable by in situ techniques. However, even a single translocation case can lead to the identification of the disease-causing gene through the identification of clones mapping to the translocation breakpoint. This permits subsequent mutation analysis of patients with no detectable cytogenetic abnormality. Reported chromosomal changes seen in individuals with autism include small duplications (Xp22.3–22.2), small deletion (1q43), and larger deletions (5q15–q22.3 or 18q). There is an increased incidence of autism in individuals with 15q11.2–q13 duplications (Prader-Willi region). Molecular analysis of such breakpoints has not yet led to the identification of an autism gene, although this is being pursued in several laboratories. Similarly, an increased incidence of schizophrenia has been reported to be associated with abnormalities on X chromosome as well as with a variety of autosomal rearrangements.

Another advantage of FISH is that several spectrally distinct fluorophores can be used simultaneously. By hybridizing sets of chromosome-specific DNA probes, each labeled with a different combination of fluorescent dyes, it is possible to ascribe to each chromosome a unique spectral signature or identifier tag (Fig. 1B). Only 5 fluorophores were needed to distinguish the 24 different human chromosomes. A banding probe labeled

with a sixth fluorophore can be used to give banding profiles similar to Giemsa. Karyotyping by color has been shown to detect chromosomal abnormalities that are difficult or impossible to identify by Giemsa banding.

An alternative use of multiple fluors is that demonstrated by Lignon and coworkers. They developed a probe set for 4 common deletion regions: Prader-Willi and Angelman, Williams, Smith-Magenis, and DiGeorge/velocardiofacial syndromes. Ten of 46 cases were found to have one of these microdeletions.

Recent advances have described the development of telomere-specific probes that can identify previously undetectable rearrangements at the tips of chromosomes, termed *telomeric translocations* or *deletions*. A number of retardation disorders including the ATF-16 syndrome, cri-du-chat, Wolf-Hirschhorn, and Miller-Dieker syndromes may result from telomere translocation or subtelomeric deletions.

The recognition that telomere translocations or deletions might be a frequent cause of mental retardation led to a key study carried out by Flint and colleagues that addressed the question of what percentage of mentally retarded patients with apparently normal karyotypes will show cryptic translocations and whether these translocations are frequent in the non-retarded population. They used 3 dozen variable number tandem repeat (VNTR) probes to examine 28 (of 48 possible) subtelomeric regions. They studied 99 patients with varying degrees of idiopathic mental retardation and apparently normal karyotypes. Three of the patients had telomere monosomy, 1 on chromosome 13 and 2 on chromosome 22. Two of the cases of monosomy resulted from unbalanced translocations, and 1 resulted from an interstitial or terminal deletion. Considering that only half the telomeres were surveyed and that many of the alleles were noninformative, they estimated the percentage of cryptic subtelomeric monosomy in retarded individuals to be 6% (95% confidence limits are 1.2%–17.6%). In a survey to determine the rate of telomere monosomy of chromosome 13 and chromosome 22 telomeres in normal individuals, no rearrangements were found in more than 160 nuclear families with 1,000 individuals tested. Recently, by use of one additional hypervariable polymorphic probe using the same patient population, they identified an additional telomeric deletion, bringing the current estimate of frequency of unbalanced telomeric rearrangements to slightly more than 7%. These observations strongly suggest that cryptic translocations are a major contributor to the mental retardation phenotype, yet this possibility has not been rigorously examined.

The recent development of M-FISH and multiplex telomere screening assays provides a novel opportunity to definitively establish the role of cryptic translocations and subtelomeric deletions in the etiology of mental retardation and other childhood neuropsychiatric diseases. Are there discrete classes of genomic rearrangements associated with certain subpopulations of affected individuals? How many different types of

cryptic translocations or deletions are associated with neuropsychiatric disorders? Are they chromosomally clustered or distributive? Although several hundred genes have been postulated to contribute to the mental retardation phenotype, only a limited number have been actually identified. The new molecular cytogenetic tools that have emerged within the past few years permit the exploration of these patient populations in a fashion previously unattainable.

WEB SITES OF INTEREST

<http://www.ncbi.nlm.nih.gov/disease/>
<http://www.lrc.arizona.edu/webpath2/CYTGHTML/CYTOG024.HTM>
<http://members.aol.com/cdousa/intro.htm>

ADDITIONAL READINGS

Flint J, Wilkie AO, Buckle VJ, Winter RM, Holland AJ, McDermid HE (1995), The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. *Nat Genet* 9:132-140

Lichter P, Tang CJ, Call K et al. (1990), High-resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. *Science* 247:64-69

Lignon AH, Beaudot AL, Shaffer LG (1997), Simultaneous multilocus FISH analysis for detection of microdeletions in the diagnostic evaluation of developmental delay and mental retardation. *Am J Hum Genet* 61:51-59

Schrock E, du Manoir S, Veldman T et al. (1996), Multicolor spectral karyotyping of human chromosomes. *Science* 273:494-497

Speicher MR, Gwyn Ballard S, Ward DC (1996), Karyotyping human chromosomes by combinatorial multi-fluor FISH. *Nat Genet* 12:368-375

Accepted March 17, 1999.

Dr. Ward is Professor, Genetics, Molecular Biophysics and Biochemistry; Dr. Bray-Ward is Research Scientist, Genetics; and Dr. Lombroso is Associate Professor, Child Study Center, Yale University School of Medicine, New Haven, CT.

Correspondence to Dr. Lombroso, Child Study Center, Yale University School of Medicine, 230 South Frontage Road, New Haven, CT 06520; e-mail: paul.lombroso@yale.edu.

To read all the columns in this series, visit the Web site at <http://info.med.yale.edu/chldstudy/plomdevelop/>

0890-8567/99/3809-1200©1999 by the American Academy of Child and Adolescent Psychiatry.

Relation of Circumferences and Skinfold Thicknesses to Lipid and Insulin Concentrations in Children and Adolescents: The Bogalusa Heart Study. David S. Freedman, Mary K. Serdula, Sathanur R. Srinivasan, Gerald S. Berenson

Background: Although body fat patterning has been related to adverse health outcomes in adults, its importance in children and adolescents is less certain. **Objective:** We examined the relation of circumference (waist and hip) and skinfold-thickness (subscapular and triceps) measurements to lipid and insulin concentrations among 2996 children and adolescents aged 5-17 y. **Design:** This was a community-based, cross-sectional study conducted in 1992-1994. **Results:** A central or abdominal distribution of body fat was related to adverse concentrations of triacylglycerol, LDL cholesterol, HDL cholesterol, and insulin; these associations were independent of race, sex, age, weight, and height. These associations were observed whether fat patterning was characterized by using 1) waist circumference alone (after adjustment for weight and height), 2) waist-to-hip ratio, or 3) principal components analysis. Compared with a child at the 10th percentile of waist circumference, a child at the 90th percentile was estimated to have, on average, higher concentrations of LDL cholesterol (0.17 mmol/L), triacylglycerol (0.11 mmol/L), and insulin (6 pmol/L) and lower concentrations of HDL cholesterol (-0.07 mmol/L). These differences, which were independent of weight and height, were significant at the 0.001 level and were consistent across race-sex groups. **Conclusions:** These findings emphasize the importance of obtaining information on body fat distribution, waist circumference in particular, in children. Waist circumference, which is relatively easy to measure, may help to identify children like to have adverse concentrations of lipids and insulin. *Am J Clin Nutr* 1999; 69:308-317.