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ARTICLE

Advances in Molecular Cytogenetics for the Evaluation of Mental Retardation

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Recent years have witnessed rapid advances in molecular cytogenetics and its impact in studying mental retardation (MR). We review new molecular cytogenetic methods, including interphase fluorescence in situ hyrbridization (FISH), comparative genomic hybridization (CGH), multicolor karyotyping, telomere FISH, primed in situ labeling (PRINS), genotyping , microdissection, and microarray for the evaluation of MR. These new methods are very useful in two major aspects: further characterization of chromosome abnormalities as detected with routine banding analysis, including additions, duplications, deletions, translocations, markers, or complex aberrations; and screening for ''hidden'' chromosome aberrations in patients with an apparently normal karyotype. These new methods have great diagnostic potential in prenatal, postnatal, and preimplantational settings. Although powerful, at this point, they are primarily research tools in nature. It is essential that these new methods be used in conjunction with standard methods in order to maximize obtainable information for better management of patients with MR. © 2003 Wiley-Liss, Inc.

KEY WORDS: mental retardation; molecular cytogenetics; interphase FISH; CGH; multicolor karyotyping; telomere FISH; PRINS; genotyping; microdissection; microarray

Mental retardation (MR) is a variable and heterogeneous manifestation of central nervous system dysfunction. It is characterized by significantly subaverage intellectual functioning, existing concurrently with related limitations in two or more of the following adaptive skill areas: community use, self-direction, health and safety, functional academics, leisure, and work [Battaglia et al., 1999]. It is generally divided into three categories: mild MR (IQ of 50–70), moderate MR (IQ of 35–50), and severe MR (IQ of 20–35) [Battaglia et al., 1999]. It is estimated that mild MR occurs with an incidence of 20–30 per 1,000 and is 7–10 times more common than moderate or severe MR, which occurs at 3–4 per 1,000 [Schaefer and Bodensteiner, 1992; Harper, 1998].

CHROMOSOMAL ABNORMALITIES ASSOCIATED WITH MR

The etiology of MR is complex; the cause remains unknown in about 50% of cases. Mendelian disorders, chromosomal abnormalities, or environmental factors can act as a single cause or work in combination. It is difficult to give an accurate estimate of the contribution of cytogenetic abnormalities to MR because reports vary in parameters such as clinical criteria of selecting subjects and detection sensitivity of cytogenetic methods. Nevertheless, data on 16 worldwide published series show that chromosomal abnormalities are found in 4–34.1% (averaging 16.1%, 3,906/24,245) of individuals with MR (see Table I). Table II lists some well-known chromosomal anomalies/syndromes associated with MR.

MOLECULAR CYTOGENETIC APPROACHES TO MR

For the past decade, molecular cytogenetics has played an increasingly important role in the research and diagnosis of MR. We present a brief review on the use of advanced molecular cytogenetics in the evaluation of MR.

Interphase FISH

Interphase fluorescence in situ hybridization (FISH) has several advantages over metaphase FISH. It can be used to score a large number of cells, thus increasing the likelihood of detecting a chromosomal aberration when present at a low-level mosaic state. Interphase FISH is also the method of choice for detection of a chromosomal aberration that is preferentially present in uncultured cells. Isochromosome 12p [i(12p)], for example, is the cytogenetic hallmark of Pallister-Killian syndrome. i(12p) is preferentially present in fibroblasts and is less frequently found in peripheral lymphocytes [Speleman et al., 1991]. Traditional metaphase analysis of cultured samples, especially bloods, can miss this anomaly. Interphase FISH using a chromosome

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TABLE I. Frequency of Chromosomal Abnormalities in Patients With MR*

Reference	No. of patients	Patient type	Country	No. (%) of chromosomal abnormality
Bourgeois and Benezech [1977]	600	MR (psychiatric hospital)	France	54 (9)
Kodama [1982]	197	Severe MR	Japan	8 (4)
Opitz et al. [1982]	168	Severe MR	United States	42 (25)
Rasmussen et al. [1982]	1,905	MR	Denmark	359 (18.8)
Wuu et al. [1984]	470	MR	Taiwan	38 (8.1)
Gustavson et al. [1987]	171	Mild MR	Sweden	20 (11.9)
Srsen et al. [1989]	324	MR	Czechoslovakia	92 (28.4)
Wuu et al. [1991]	1,323	MR	Taiwan	
			IQ 50–75	104 (7.87)
			IQ <50	231 (17.5)
Curry et al. [1997]	1,314	MR	India	311 (23.7)
Schwartz [1988]	350	MR/DD	United States	42 (11.9)
Phelan et al. [1996]	4,485	MR	United States	538 (12)
Hou et al. [1998]	11,892	Intellectually disabled children	Taiwan	1,889 (15.9)
Felix et al. [1998]	202	MR	Brazil	69 (34.1)
Hong et al. [1999]	604	Child psychiatric patients	Korea	69 (11.4)
Battaglia et al. [1999]	120	MR/DD	Italy	18 (15.0)
Cora et al. [2000]	120	MR	Turkey	23 (19)
Total	24,245			3,906 (16.1)
				Ranging 4–34.1%

*This is based on Table IV of Curry et al. [1997] with data from 1998 to 2000 added.

12-specific alpha satellite probe, a whole chromosome paint, or a microdissection paint specific to a band on 12p has been used to detect i(12p) from fibroblast cells, uncultured lymphocytes, and buccal mucosal cells [Speleman et al., 1991; Ohashi et al., 1993]. Of particular interest, Ohashi et al. [1993] found that direct buccal smear preparations yielded the highest proportion (53–68%) of positive cells in comparison to phytohemagglutinin-stimulated lymphocytes, cultured skin fibroblasts, or directly harvested T and B cells. Using interphase FISH, Reddy and Mak [2001] reported that buccal mucosal cells showed a higher proportion of cells than lymphocytes with each of three de novo mosaic unbalanced structural aberrations involving 13q22q33, 14q31qter, and 3p26pter. These observations suggest that epithelial cells of buccal mucosa are more likely to retain certain chromosomal abnormalities. Interphase FISH on direct buccal smear preparations is particularly attractive because it can provide a rapid, effective, and noninvasive confirmation of a chromosomal syndrome.

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A set of five Food and Drug Administration (FDA)-approved probes specific to chromosomes 21, 13, 18, X, and Y is commercially available. Interphase FISH on uncultured amniocytes or chorionic villus sampling cells has made it possible to shorten the turnaround time from a routine 7- to 14-day analysis to a 24-hr test. A rapid prenatal interphase detection of these common aneuploidies is a “standard” practice in many diagnostic labs and serves as a shining example of how molecular cytogenetics can make a significant difference in prenatal management and genetic counseling for high-risk pregnancies. In addition, FISH on blastomeres biopsied from embryos at the 7–10 cell stage from in vitro fertilization has been attempted to select chromosomally normal/balanced embryos for preimplantation in a familial translocation carrier [Pierce et al., 1998].

Chromatins in interphase nuclei are less condensed and are ideal materials for FISH analysis of microdeletions/duplications of loci that are physically close together. Gersh et al. [1997] developed an interphase FISH method for the differential diagnosis of 5p deletions that have subtle physical differences but profound prognostic effects. Distal 5p

TABLE II. Selected Well-Known Chromosomal Abnormalities/Syndromes Associated With MR

Trisomy 21/Down syndrome	
Fragile X syndrome	
Unbalanced translocations	
Duplications	
Deletions (interstitial, terminal)	
Extra structurally abnormal chromosomes (markers)	
Diploid/triploid mosaicism[a]	
Submicroscopic aberrations at breakpoints in apparently balanced rearrangements	
Subtelomere rearrangements	
Cryptic deletions	
del(1)(p36.3)	Monosomy 1p
del(4)(p16)	Wolf-Hirschhorn
del(5)(p15)	Cri du chat
del(7)(q11.23q11.23)	William syndrome
del(8)(q24.1q24.1)	Langer-Giedion syndrome
del(11)(p13p13)	WAGR[b] syndrome
del(15)(q11q13)pat	Prader-Willi syndrome
del(15)(q11q13)mat	Angelman syndrome
del(16)(p13.3)	Rubinstein-Taybi syndrome
del(17)(p11.2p11.2)	Smith-Magenis syndrome
del(17)(p13.3)	Miller-Dieker syndrome
del(20)(p11.23p11.23)	Alagille syndrome
del(22)(q11.2q11.2)	VCF[b]/DiGeorge
Uniparental disomy	
UPD(14)mat	IUGR[b], developmental delay, precocious puberty, short stature, small hands and feet
UPD(14)pat	Polyhydramnios, facial anomalies, severe neurologic involvement, skeletal anomalies and growth retardation
UPD(15)mat	Prader-Willi syndrome
UPD(15)pat	Angelman syndrome

[a]Most of the reported cases with a normal karyotype in blood but diploid/triploid mosaicism in the cultured fibroblasts (van de Larr et al., 2002).
[b]WARG, Wilm's tumor, aniridia, genitourinary malformation and retardation of growth and development; VCF, velocardiofacial; IUGR, intrafnerinc growth rerardation.

deletion is associated with cri-du-chat syndrome; however, the phenotype varies depending on the location of deletion breakpoints. A deletion involving a critical region at 5p15.2 is associated with distinct facial features as well as severe MR and developmental delay, whereas a deletion involving a more distal portion at 5p15.3 is associated with the characteristic cat-like cry with a much better prognosis. Cosmid probes were isolated specific to each of these two regions and were able to easily differentiate between deletions related to the cri-du-chat syndrome phenotype and deletions associated only with the isolated cat-like cry. Likewise, interphase FISH has also been successfully used to detect microduplications, such as dup(X)(q22q22) (Pelizaeus-Merzbacher disease) and dup(17)(p12p12) (Charcot-Marie-Tooth disease type 1A) [Lupski et al., 1991; Shaffer et al., 1997; Inoue et al., 1999].

Using microdissection-based multicolor FISH banding of chromosome 5, recently Lemke et al. [2002] made an important discovery that the length of the axis of the interphase chromosome is comparable to that of the corresponding metaphase chromosome at 600-band resolution and the banding pattern is similar between the interphase and metaphase chromosome. This finding opens exciting possibilities of interphase studies of the MR-causing structure aberrations (e.g., deletion, duplication, unbalanced translocation) in nondividing cells.

CGH

Comparative genomic hybridization (CGH) provides a genome-wide screening for chromosomal imbalance in a single hybridization directly from DNA samples without requiring the sample material to be mitotically active [Kallioniemi et al., 1992]. While used mostly as a research tool in cancer cytogenetics, CGH has been applied to constitutional karyotyping of postnatal [Levy et al., 1998; Breen et al., 1999], prenatal [Bryndorf et al., 1995; Lestou et al., 2000; Tabet et al., 2001], and preimplantational [Wilton et al., 2001] cases. The resolution power of CGH to detect chromosome imbalances is in the range of 2–10 Mb [Bentz et al., 1998; Ghaffari et al., 1998; Kirchhoff et al., 1999; Joly et al., 2001]. CGH has two major uses in analysis of MR:

1. Further characterization of unbalanced karyotypes as identified by G-banding and other methods. These applications include the delineation of chromosomal additions, duplications, deletions, unbalanced translocations, or markers [Ghaffari et al., 1998; Levy et al., 1998; Breen et al., 1999]. Breen et al. [1999] provided an excellent example using CGH to define breakpoints. They reported two cases of deletions in the long arm of chromosome 11, each having three possible breakpoints that could not be resolved using G-banding analysis, i.e., del(11)(q13.5q21), (q14.2q22.2), or (q21q23.1). CGH identified del(11)(q21q23.1) in one case and del(11)(q14.2q22.2) in the other. The ability of CGH to detect a mosaic marker depends on the proportion of abnormal cells, the size and makeup of the marker, and the restrictive threshold of fluorescence

ratio [Ghaffari et al., 1998]. The minimum proportion of abnormal cells that can be detected by CGH has yet to be determined but has been reported to be in the range of 15–50% [Kallioniemi et al., 1994; Ghaffari et al., 1998].

2. Screening for small chromosome rearrangements. In comparison with telomere FISH screening, CGH has its advantage in being able to detect "hidden" duplications/deletions in not only the telomere region but also interstitial regions, as well as in the detection of extra chromosomes in a mosaic state. The detection rate in chromosomally normal subjects ranges from 60% (3/5) [Ghaffari et al., 1998] to 29% (5/17) [Joly et al., 2001] in small series, and 11% (16/144) in a large series [Kirchhoff et al., 2001]. Kirchhoff et al. [2001] reported that among 25 dysmorphic and mentally retarded patients with apparently balanced de novo translocations, four were detected with CGH to have deletions at translocation breakpoints and two deletions elsewhere in the genome.

The main disadvantages of CGH include its inability to detect a balanced rearrangement or regions consisting of highly repetitive sequences such as pericentromeric and heterochromatic regions as well as p-arms of acrocentric chromosomes. In addition, the overall rate of detecting very small markers is quite low because these markers consist primarily of centromeric repeat sequences, and the fluorescence ratio at centromeric regions shows great fluctuation. It is often difficult to identify the chromosome origin of a euchromatic region located on small markers.

Multicolor Karyotyping

Two alternative multicolor karyotyping technologies, i.e., fluorochrome-specific optical filter-based multiplex (M-) FISH [Speicher et al., 1996] and interferometer-based spectral imaging or karyotyping (SKY) [Schrock et al., 1996], can simultaneously visualize all 24 chromosomes in a single experiment and have proven to be a powerful tool in clinical cytogenetics [Ried et al., 1998]. The 24-color karyotyping system has two general applications in cytogenetics: refining cytogenetic anomalies, including markers, translocations, and complex rearrangements; and screening for hidden aberrations in patients with a normal karyotype who are, nevertheless, suspected to have a chromosome syndrome [Schrock et al., 1997; Uhrig et al., 1999; Bayani and Squire, 2001]. Ried et al. [1998] reported that a patient with an indication of Wolf-Hirschhorn syndrome and a normal karyotype, upon subsequent SKY and high-resolution G-banding analysis, was identified as having a der(4)(4;8). Similarly, a comprehensive analysis with M-FISH and other methods by Uhrig et al. [1999] revealed that among 20 patients with MR and dysmorphic features who had a normal karyotype, 2 (10%) had unbalanced terminal translocations, i.e., a der(18) t(18;20)(q21;p11.2) and a der(1)t(1;12) (q43;p13), respectively.

SKY and M-FISH, however, have some major limitations. They cannot determine the exact band origin of a marker nor the breakpoints on intrachromosome rearrangements. Detection sensitivity is estimated to be in the range of 1–2.6 Mb [Schrock et al., 1996; Fan et al., 2000; Holinski-Feder et al., 2000]. Fan et al. [2000] assessed the sensitivity of SKY based on 10 small translocations as identified with G-banding analysis at the 550- to 850-band level. They found that SKY missed 4 of 20 possible segments; therefore, a normal screening result by multicolor karyotyping should not be interpreted as a definite exclusion of a rearrangement. Combining multicolor karyotyping with other methods, including high-resolution G-banding, telomere FISH, or CGH, is needed in order to maximize obtainable information.

Another inherent mechanic-based problem is blending color by fluorescence flaring at interface of the translocated segments [Azofeifa et al., 2000; Lu et al., 2000; Lee et al., 2001]. This fluorescence flaring effect can obscure or distort the fluorescence pattern of adjacent chromatin, leading to misinterpretation of observations; for example, false insertion at the interface of translocated segments or misclassification of small insertions or translocations [Lee et al., 2001]. Caution should be exercised in the interpretation of such findings.

Screening for Subtelomeric Aberrations in Idiopathic MR

Chromosome ends are characterized by a lack of distinctive G-bands. Small rearrangements at these regions can be missed in routine karyotyping analysis at the 450- to 500- (even at the 550- to 800-) band level. Recent studies showed that subtelomere regions are gene rich [Saccone et al., 1992], and thus rearrangements involving these regions are very likely to have clinical consequences. Telomere regions have the highest recombination rate and are prone to aberrations resulting from illegitimate pairing and crossover. Molecular cytogenetics approaches have revealed that many cases of unexplained MR have cryptic subtelomeric aberrations. At least six methods—FISH with a complete set of subtelomere probes, M-FISH/SKY, multiple amplifiable probe hybridization (MAPH), CGH, primed in situ labeling (PRINS), and genotyping—have been used to screen for telomeric abnormalities in patients with idiopathic MR (Table III).

It is difficult to get an accurate estimate of the prevalence of subtelomere aberrations in patients with idiopathic MR because of the limited number of reports, the unequal representation of reports on different screening methods, variations in factors such as banding levels (450–650) for karyotyping, clinical criteria for inclusion of the patients, sample size, and detection sensitivity of the methods utilized. Nevertheless, the overall rate of subtelomeric anomalies in idiopathic MR, based on the summarized data of 21 studies (Table III), averages 4.6% (114/2,490), ranging from 0.0–29.4%. Of 102 informative cases, 50 (49.0%) were found to have deletions, 46 (45.1%) unbalanced translocations, 4 (3.9%) duplications,

TABLE III. Screening for Subtelomere Aberrations Associated With Idiopathic MR

Reference	MR patients	No. (%) of aberrations
Telomere FISH screening		
Viot et al. [1998] (T)	17	4 (23.0%)
Vorsanova et al. [1998] (T)	209	8 (3.8%)
Lamb et al. [1999] (T)	43	1 (2.3%)
Knight et al. [1999] (T)	284 moderate/ severe MR	21 (7.4%)
	182 mild MR	1 (0.5%)
Uhrig et al. [1999] (M)	20	2 (10.0%)
Joyce et al. [2001] (T)	200	0 (0.0%)
Rossi et al. [2001] (T)	200	13 (6.5%)
Hersh et al. [2001] (T)	44	3 (6.8%)
Fan et al. [2001] (T + S)	150	6 (4.0%)
Riegel et al. [2001] (T)	254	13 (5.1%)
Sismani et al. [2001] (T + MAPH)	70	1 (1.4%)
Anderlid et al. [2002] (T)	111	10 (9.0%)
Baker et al. [2002] (T)	250	9 (3.6%)
Clarkson et al. [2002] (T + S)	50	2 (4%)
CGH screening		
Joly et al. [2001]	17	5 (29.4%)
Kirchhoff et al. [2001]	144	4 (2.8%)
PRINS screening		
Bonifacio et al. [2001]	65	2 (3.0%)
Genotyping screening		
Flint et al. [1995]	99	3 (3.0%)
Slavotinek et al. [1999a]	27	2 (7.5%)
Colleaux et al. [2001]	24	2 (8.3%)
Borgione et al. [2001]	30	2 (6.7%)
Total	2490	114 (4.6%), ranging 0.0–29.4%

T, telomere FISH; T+S, a combination of telomere FISH and SKY; M, M-FISH; T+MAPH, a combination of telomere FISH and multiplex amplifiable probe hybridization.

and 2 (2.0 %) recombinant chromosomes. In the cases in which parental studies were done, 94% (33/35) of deletions were de novo, 84% (37/44) of those with unbalanced translocations were derived from familial balanced translocations, and 75% (3/4) of duplications were de novo. The largest series by Knight et al. [1999], based on telomere FISH screening, showed that subtelomeric aberrations account for 7.4% (21/284) of children with moderate to severe MR and 0.5% (1/182) of children with mild MR.

Some subtelomeric rearrangements are associated with specific phenotypes. For example, children with a 1p36 deletion have growth and mental retardation; seizures; visual problems; large anterior fontanelle; asymmetrical, low-set, dysplastic ears; deep-set eyes; a depressed nasal bridge; a pointed chin; and fifth finger clinodactyly [Riegel et al., 1999; Slavotinek et al., 1999b]. 22qter deletion is associated with hypotonia, developmental delay, absence of speech in the child, and overgrowth [Doheny et al., 1997; Precht et al., 1998; de Vries et al., 2000]. In addition, subtelomeric defects are also found to be associated with prenatal onset of growth retardation [de Vries et al., 2001].

FISH and genotyping are major methods used in subtelomeric studies. FISH using a complete set of subtelomere specific probes is most commonly used for subtelomere screening. The probe set is commercially available and consists of YAC, BAC, or P1 clones that are about 100–200 kb in size and ~300 kb in physical distance from the chromosome ends [National Institute of Health and Institute of Molecular Medicine Collaboration, 1996; Knight and Flint, 2000; Knight et al., 2000]. This technique has the advantage of instant detection of deletions and balanced or unbalanced translocations. One potential concern is that 11 probes can cross hybridize with other chromosome regions (e.g., 8p with 1p and 3q, 11p with 17 p) and that the 2q probe shows polymorphism [Knight et al., 2000]. Thus, this method emphasizes the necessity of follow-up studies on parents or other relatives for clinical interpretation of positive FISH findings [Shaffer et al., 1999]. Fauth et al. [2001] recently developed a new telomere FISH strategy that combines two different probe sets, i.e., microdissection subtelomeric probes (each with 5–10 Mb in size) and the second generation of subtelomeric PAC, P1, and BAC-clones [Knight et al., 2000]. This new approach is reportedly able to improve efficiency for subtelomere screening.

Genotyping analysis uses polymorphic markers (e.g., microsatellite) and requires DNA samples from the proband and both parents [Wilkie, 1993; Slavotinek et al., 1999a; Colleaux et al., 2001]. Markers have to be very informative for this strategy to be efficient. This method has the advantage of being able to detect not only deletions and duplications, but also parental origin of aberrations and uniparent disomy. The cost of genotyping analysis is estimated to be 3–4 times less than that of FISH methods. Advances in human genome sequencing and automation are likely to make this strategy even more sensitive

and affordable. This approach can also lead to the identification of imprinted regions.

In contrast to almost all other studies, Joyce et al. [2001] reported that the telomere FISH screening, following high-resolution G-banding analysis, identified no anomalies in 200 MR patients, but did find anomalies in 2 of 150 normal subjects in the control group. They concluded that "true cryptic telomeric rearrangements were not a significant cause of idiopathic MR." However, Biesecker [2002] argued that an "alternative conclusion is that cytogenetics is highly variable in quality, and if done very well, detects most but not all rearrangements." Further technology advancement and accumulation of more data will help us understand better the clinical significance of subtelomeric aberrations.

PRINS

PRINS is an alternative to, as well as a complementary approach for, FISH for chromosome studies. PRINS is featured by its fast turnaround time (1–4 hr), ability for high specificity, and cost-effectiveness. Since the first report on PRINS by Koch et al. [1989], there have been many developments in its methodology and uses. Single-, double-, and multicolor PRINS have been developed and can be used to study several sequences simultaneously [Koch et al., 1989; Wilkens et al., 1997; Yan et al., 2001]. Depending on the primers used, PRINS is able to detect repetitive sequences, such as centromere specific alpha satellite sequences [Koch et al., 1995; Pellestor et al., 1995a], telomere repeats [Krejci and Koch, 1998; Bonifacio et al., 2001], Alu sequences [Coullin et al., 2002], and single-copy genes [Cinti et al., 1993, 2002; Kadandale et al., 2000, 2002; Harrer et al., 2001; Tharapel et al., 2002].

Several lines of evidence demonstrate that PRINS is a promising approach to MR studies. Koch et al. [1995] constructed a panel of PRINS primers specific to each human chromosome except 6, 19, and 20. These primers have great potential in the rapid detection of aneuploidies (e.g., trisomy 21) or markers in metaphase and interphase cells from postnatal, prenatal, or preimplantation samples [Pellestor et al., 1995b; Petit et al., 2000]. Bonifacio et al. [2001] used PRINS and telomere specific TTAGGG repeats as the primer for quick screening of subtelomeric deletions in children with idiopathic MR. They found 2 of 65 subjects with telomere deletions. Recently, Tharapel et al. [2002] reported the use of disease-specific primers and PRINS in the diagnosis of microdeletions associated with the Prader Willi/Angelman and DiGeorge/Velocardiofacial syndromes. Their results are remarkable. Since every gene of known sequence is a potential priming target, PRINS could now be used in the diagnosis of many single-copy gene diseases. This approach is especially advantageous when a rapid result is needed and workable FISH probes are not readily available.

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Microdissection

Chromosome microdissection is featured by its ability to generate DNA sequences specific for a chromosome, an arm, or a region. It has been widely used for many purposes, including genomic mapping and gene isolation [Kao and Yu, 1991; Cannizzaro, 1996], characterization of double minutes and homogeneous staining regions in cancer specimens [Guan et al., 1994a; Taguchi et al., 1997], and generation of chromosome, arm, or region specific paints for FISH analysis and multicolor FISH banding [Meltzer et al., 1992; Guan et al., 1994b, 1995; Chudoba et al., 1999; Lemke et al., 2002]. In molecular cytogenetic analysis of MR, microdissection has been used in the characterization of markers, rings, additions, deletions, and complex rearrangements [Engelen et al., 1996; Chen et al., 1997, 2001; Xu et al., 2000].

Microdissection is the best way to delineate markers that are small, mosaic, and complex, and are thus very difficult or impossible to identify by other methods (e.g., CGH). This method typically requires only 5–10 copies of a target chromosome for marker identification and is the most sensitive method for identification of markers present in a very low proportion of cells. This method is capable of identifying markers of any origin, including the centromeric region [Ostroverkhova et al., 1999] and the short arms of acrocentric chromosomes [Coelho et al., 1996]. The latter contain highly repetitive sequences, which are usually suppressed and thus cannot be identified by using other methods (e.g., SKY/M-FISH or CGH). Microdissection has been used in prenatal [Muller-Navia et al., 1995] and postnatal diagnosis. Using microdissection in conjunction with SKY and FISH, an extra ring chromosome consisting of the entire 10p was identified in a patient with MR and other multiple congenital anomalies [Chen et al., 2001]. This case represents another new cytogenetic mechanism leading to the formation of pure complete trisomy 10p. Furthermore, microdissection has the potential to make a diagnosis on long-term stored specimens. Using this technique on several-years-old Carnoy-fixed preparations, Chinen et al. [1997] successfully identified two previously undetected chromosome abnormalities.

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Microarray

Microarray is an emerging molecular cytogenetic approach to genome-wide analysis of copy number changes of genomic DNA and gene expression. In cancer genetics, the combination of cDNA, tissue, and CGH microarrays has been used for studies of amplification and overexpression of genes associated with tumor initiation and progression [Pinkel et al., 1998; Pollack et al., 1999; Monni et al., 2001]. In constitutional cytogenetics, genomic microarray can potentially provide accurate diagnosis of deletions/duplications of a chromosome (e.g., trisomy 21), a chromosome segment, or disease-specific sequences (e.g., deletion 22q11 in DiGeorge/Velocardiofacial syndrome) [Pollack et al., 1999; Antonarakis, 2000]. Mohammed et al. [2001] developed a high-resolution CGH genomic microarray with BAC clones spaced at ~3-Mb intervals on average throughout the genome. In addition, a higher density of BAC clones was arrayed in clinically significant genomic regions such as in subtelomeres and in regions associated with well-known microdeletion syndromes. This array allowed the correct identification of cytogenetically detectable gains/losses of partial chromosome arms, including a small deletion of 1p36, cryptic deletions of 4p16.3, 11p11.2, and 22q11.2, and a double segment imbalance in a case with deletion of 1p36 and duplication of 9q34. This array is equivalent to more than 1,000 individual FISH tests and has great potential for rapid screening for genomic imbalances associated with genetic diseases, including MR.

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CONCLUSIONS

The list of new molecular cytogenetic methods with potential applications in diagnosis and prognosis of MR is increasing, thanks to rapid advances in the Human Genome Project and its related areas. Although powerful, these new methods are primarily research tools in nature; it takes time to develop and validate these techniques. It is essential that follow-up confirmation on the majority of cases tested with these techniques be performed with standard methods. In addition, these advanced methods are not accessible to all diagnostic services and remain relatively costly. Technical success rates are variable, and interpretation of clinical implications is not always straightforward. The clinician's decision as to what test(s) to order and the cytogeneticist's decision as to what technical algorithm to use are no longer a simple task. The choices will depend on many factors, including clinical indications, medical and family history, degree of clinical urgency, accessibility to technical approaches, and tolerance of suboptimal results.

The choices will depend on many factors, including clinical indications, medical and family history, degree of clinical urgency, accessibility to technical approaches, and tolerance of suboptimal results.

In general, a routine chromosome analysis should be used as a starting point for any cytogenetics investigation of MR. Depending on clinical indications, additional chromosome counts may be needed to rule out mosaicism, and appropriate band levels should be reached to detect small aberrations in targeted regions. Based on family history and clinical phenotype, subsequent workups can follow various pathways for a diagnosis. For example, a patient with the clinical features of a microdeletion syndrome should start with a karyotyping analysis. A normal result should warrant subsequent FISH analysis with selected microdeletion probe(s). In the absence of a positive FISH finding, a comprehensive analysis combining screening and other methods may be further considered. An abnormal karyotype with, for instance, a de novo marker can be further characterized by methods such as microdissection, CGH, or FISH with selected probes to determine the exact chromosome origin of the marker.

More effective methods for diagnosis and prognosis of MR will continue to emerge in the future. It is always a challenge to transfer new technology from a research setting to clinical applications. We believe that active communication among physicians, molecular cytogeneticists, and genetic counselors is essential for the effective management of patients with MR.

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