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Technical and clinical assessment of fluorescence in situ hybridization: An ACMG/ASHG position statement. I. Technical considerations

Test and Technology Transfer Committee

The initial evaluation of fluorescence in situ hybridization (FISH) in prenatal diagnosis by the American College of Medical Genetics (ACMG) indicated several unresolved analytical and clinical issues concerning this technology.[1] During the intervening 5 years, the importance of many of the analytical questions has waned and issues relating to its clinical use have been clarified. Since that first ACMG position statement on FISH,[1] significant aspects of technical standardization have been achieved leading to consensus guidelines for the analysis of both metaphase chromosomes and interphase nuclei.[2,3] These guidelines address assay standardization, appropriate controls, analytical validation and, in specific areas such as interphase FISH in prenatal diagnosis, clinical validation as well. The first multicenter quality assurance reviews have appeared[4–6] and ongoing interlaboratory FISH quality assurance comparison programs for FISH testing are available through the College of American Pathologists (CAP)/ACMG Laboratory Accreditation Program. These activities, principally through the efforts of professional organizations, have contributed significantly to the recent successful Food and Drug Administration (FDA) review and approval of commercial FISH testing systems. Still, a number of analytical and clinical considerations must be faced.

In this statement, we separate analytical from clinical validity and broaden the clinical considerations of FISH to include not only prenatal diagnosis, but also uses in testing for constitutional abnormalities related to birth defects and/or mental retardation and more general uses that are commonly thought of as adjunctive in complementing other technologies. The discussion will focus only on the use of FISH in the human genome and not on its application to infectious disease testing. The application of FISH testing to acquired abnormalities in cancer will be addressed in a separate statement.

ANALYTICAL CONSIDERATIONS

The spectrum of intended probe use

Genetic testing technologies have a broad range of uses, each with a significant clinical impact on those undergoing testing. The identical test reagent may be used, diagnostically, both pre- and postnatally, in identifying heritable disease in affected individuals, or for detecting carrier states, as well as in the clinical staging of acquired nonheritable conditions such as cancer. Hence, it is critical to distinguish between the analytical capability of the technology and its intended use. While determination of analytical validity has been reasonably straightforward, the lack of "gold standard" tests against which to compare FISH-based tests has limited their transfer and integration into clinical practice.

FISH probes are commonly used to detect the presence of specific DNA sequences either when DNA is condensed into metaphase chromosomes or dispersed in nondividing interphase cells. The fact that hybridization of probes to metaphase chromosomes is visualized in two dimensions while interphase targets are three dimensional has implications for both validation of assays and the development of baseline reference ranges. Metaphase applications generally yield clear yes/no answers while interphase applications commonly require reportable reference ranges before interpreting of results. In addition to determining the presence or absence of particular sequences in the genome, FISH is useful in assessing gene copy number in some disorders (e.g., Charcot-Marie-Tooth 1A[7] and breast cancer.[8,9] Depending on the size of a probe and/or the combination of sequences present, a particular probe or probe set may be amenable to metaphase and/or interphase analysis.

Analytical uncertainty over DNA probe assays also may stem from issues related to inherent population variation. The use of some repeat-sequence probes has been discontinued because

This guideline is designed primarily as an educational resource for medical geneticists and other health care providers to help them provide quality medical genetic services. Adherence to this guideline does not necessarily ensure a successful medical outcome. This guideline should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed toward obtaining the same results. In determining the propriety of any specific procedure or test, the geneticist should apply his or her own professional judgment to the specific clinical circumstances presented by the individual patient or specimen. It may be prudent, however, to document in the patient's record the rationale for any significant deviation from this guideline.

of inability to detect targeted sequences in individuals who possess very few repeats, leading to insufficient probe label in the targeted region which precludes visualization of the signal.[10] Such probes have been eliminated as clinical discriminators. However, rare individuals in the population may still harbor polymorphisms leading to possible false positive or negative results.

The variety of levels of regulatory "approval/clearance"

A broad array of probes are available both commercially or as reagents developed in-house (home brew). Commercially available products are labeled "research use only" (RUO) or "investigational use only" (IUO). Other probes, whose uses have been reviewed by the FDA may be cleared as in vitro diagnostic devices (IVDs). A recently added device classification, which includes nucleic acid probes, is referred to as analyte-specific reagents (ASRs). Among the requirements are that the FDA be notified of a manufacturer's intent to sell ASRs for incorporation into locally developed testing systems and that these reagents only be sold to laboratories qualified as "high complexity" under CLIA'88. A complication associated with ASRs is that their marketing is independent of specific intended uses (see above). Therefore, all tests utilizing ASRs are required to prominently display the following (or similar) disclaimer (21 CFR S809.30(e))[11]:

> **This test was developed, and its performance characteristics were determined by [laboratory name]. It has not been cleared or approved by the U.S. Food and Drug Administration.** The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. Pursuant to the requirements of CLIA '88, this laboratory has established and verified the test's accuracy and precision. [Language in bold must be used. Subsequent language may be used to clarify the required language, if desired.]

Such a statement forces laboratories to promulgate a perception of less than complete confidence in the test. This situation exists since the FDA has no intention, at present, to require "premarket review" of the ASR category of devices. However, many reimbursement agencies demand FDA approval as evidence of the general acceptability of the test before satisfying provider claims. The ASR category of devices clearly eliminates the issue of FDA approval of a test as a requirement for reimbursement since formal FDA review is not intended. The ASR regulations defer to the CLIA'88 demands for analytic validation of any new test prior to its introduction by a laboratory. Therefore, in the absence of an established regulatory mechanism to determine clinical validity, it becomes increasingly important for professional organizations to offer guidance to practitioners as to accepted clinical uses of these reagents.

CLINICAL CONSIDERATIONS

The clinical applications of fluorescence in situ hybridization (FISH) -based testing are broad. The use may be quite generic and adjunctive when used to determine the extent of genomic imbalance originally identified by another testing methodology. In this case, the choice of probes will be dictated by the increasingly accurate and scientifically based map of the human genome. Probe use may also be highly specific, as in microdeletion testing. Probe use may be considered adjunctive when confirming or characterizing an abnormality detected by routine cytogenetics or other molecular methods. They also may be used in diagnostic stand-alone assays when microdeletions are submicroscopic.

As DNA-probe tests and technologies evolve, it is important to understand that one test may have several intended uses or target populations. However, the requisite knowledge for each of these uses is not likely to develop at the same pace. The demonstration of scientific/clinical validity will be reasonably straightforward and the demonstration of the analytical validity of the test system used should be fairly direct. Nonetheless, establishing the relationship of a gene and its mutations or variations to a particular disease does not establish the magnitude or strength of the relationship. Not infrequently, fundamental issues arise concerning the utility of a given FISH probe (i.e., its clinical validity) and cost/benefit considerations relative to other available clinical or laboratory approaches to diagnosis and follow-up.

We will now consider the clinical uses of FISH in three main areas: diagnosis of individuals with birth defects and mental retardation, prenatal diagnosis and screening, and identification and monitoring of acquired chromosome abnormalities in leukemia/cancer. In each area, the critical consideration remains a clear understanding of the capabilities and limitations of a test to provide useful information.

I. Birth defects and mental retardation

Traditional cytogenetic analysis, detecting deletions, duplications, rearrangements and the identification of unknown material of marker or derivative chromosomes, in individuals with birth defects and/or mental retardation has led to an understanding of the etiology of a number of syndromes. The clinical utility and limitations of these tests are both general and disease specific.

Constitutional genetic disorders can arise from a variety of mutation mechanisms, only some of which are identifiable by FISH methods. For any entity in which FISH testing is appropriate, the yield (or clinical sensitivity of the test) is a function of the frequency at which FISH-detectable abnormalities are found among affected individuals. In some disorders, deletions are rare events (e.g., Rubinstein Taybi syndrome in which 11–12% of cases arise deletion)[12] or, it may account for as many as 70% of cases in other disorders (e.g., Prader-Willi and Angelman syndromes).[13,14] In the majority of FISH-diagnosed conditions, the important issues are the phenotypic indications for testing and at what stage of the work-up FISH analysis

is employed. These decisions are complicated by the fact that the disorders diagnosable by FISH are relatively rare. Since the identification of structural abnormalities as the cause of a syndromic phenotype provides a diagnostic test of as well as a tool in risk assessment in families, it is reasonable to utilize a test that may not detect all individuals, since no other alternatives exist.

Regarding disorders associated with dysmorphology/birth defects and/or mental retardation, a number of applications of FISH exist, including microdeletion or microduplication detection, identifying derivative and marker chromosomes, or determining ploidy states. For the most part, FISH testing is based on clinical indications justifying the ancillary test and are not considered part of routine testing in the absence of appropriate clinical indications. The following are conditions in which FISH testing is the standard of practice for the identification of particular mutations.

A. Microdeletion/microduplication syndromes

Syndromes in which submicroscopic genomic gain or loss are among the disease causing mutations pose both technical and clinical limitations. Technical considerations include the fact that, in many of the "contiguous gene syndromes,"[15] no given probe derives from a single disease-related gene. Rather, the probes used are from the smallest region of overlap of those deletions identified in the greatest number of patients. The abnormalities present are commonly identified in metaphase chromosomes, although probe characteristics may allow their detection in interphase nuclei as well. The advantages of metaphase analysis lies in those patients whose deletion is secondary to a gross chromosome rearrangement, but which is detectable when appropriate probe combinations are used or when routine cytogenetic testing is done in conjunction with FISH testing. Clinical limitations primarily relate to variability between disorders of the proportions of affected individuals with deletions and varies with the level of clinical suspicion.

Table 1 includes some of the most commonly utilized FISH-based tests for the detection of microdeletions and microduplications but is not intended to be comprehensive. This table reflects those disorders in which deletion or duplication are frequent mutational mechanisms. However, many other disorders have been reported for which microdeletion is but a minor cause of mutation (e.g., Rubinstein-Taybi and Alagille syndromes). In such situations, although valid, FISH testing may have reduced clinical utility until other, more common, etiologies are considered. In some situations, the clinical diagnosis may be highly suggestive of a syndrome in which microdeletions are common and FISH will be the primary diagnostic test. In other situations in which clinical phenotypes are less specific or deletion a less common cause of the disorder, routine cytogenetics may be the primary test and FISH used as the follow-up test. Lastly, situations arise in cytogenetic testing in which "private" and de novo rearrangements pose additional complications.

Detection of microduplications by FISH also has technical limitations related to the proximity of the duplicated probe targets and the replication of targets during the cell cycle. In this case, testing should be performed on the decondensed DNA of interphase nuclei. However, additional molecular methods may be comparably effective in diagnosing such duplications.

B. Marker and derivative chromosomes

The ability of FISH methods to identify the origin of genetic material is a function of the degree to which complementary

Table 1
Some commonly used FISH-based tests

Disorder	Abnormality	Reference
Microdeletions: stand alone with FISH being primary		
Williams S.	del(7)(q11.2q11.2) [Elastin]	Ewart et al., 1993
Prader-Willi S.	del(15)(q11.2q11.2)	Cassidy and Schwartz, 1998
Angelman S.	del(15)(q11.2q11.2)	Cassidy and Schwartz, 1998
Miller-Dieker S./lissencephaly	del(17)(p13.3p13.3)	Pilz et al., 1998; Kuwano et al., 1991
HNPP (hereditary neuropathy with liability to pressure palsies)	del(17)(p11.2p11.2)	Shaffer et al., 1997
Velocardiofacial S.[a]	del(22)(q11.2q11.2)	Scambler et al., 1992
Microdeletions: stand alone with FISH secondary to chromosome studies		
Wolf-Hirschhorn S.	del(4)(p15)	Altherr et al., 1991
Cri-du-chat S.	del(5)(p15)	Gersh et al., 1995
Smith-Magenis S.	del(17)(p11.2p11.2)	Juyal et al., 1995
Microduplication		
Charcot-Marie-Tooth 1A	dup(17)(p11.2p11.2)	Shaffer et al., 1997
Pelizaeus-Merzbacher	dup(X)(q22q22)	Woodward et al., 1998

[a]Includes DiGeorge S., conotruncal anomaly face S., conotruncal heart defects, and multiple subphenotypes of these disorders.

site-specific DNA sequence is present in the probe or probe set. As whole chromosome or chromosome arm paint probes are developed in parallel with the increasingly dense physical map, probes will be further refined. However, it is important to understand the limitations imposed by both the quantity of material to be accounted for and the possibility that the target sequence is under-represented or absent in the probe set. Although marker chromosomes are commonly assessed using pericentromeric repeat sequence probes,[22] it is critical to consider that not all marker chromosomes may contain these sequences (e.g., chromosome 8p markers).[23]

Some FISH assays are already generally accepted such as (1) the determination of the origin (X vs. Y chromosome) of markers replacing one of the sex chromosomes in which the finding of Y chromosome derived material increases risks of gonadoblastoma. (2) Similarly, when a sex chromosome marker is identified in prenatal testing, FISH assays for the presence of XIST may inform as to the risks of severe mental retardation. An additional use of FISH, as applied to marker chromosomes, is the (3) assessment of supernumerary inverted duplication chromosome 15s. These marker chromosomes are commonly characterized as to the presence of coding sequences which may reflect likely phenotypic consequences. For such determinations, D15S10 or SNRPN are commonly used FISH probes. Another marker chromosome derived from chromosome 18 is the i(18p) which is usually suspected by G-banding and are commonly confirmed by FISH.[24] Suspicion that a marker is derived from chromosome 22 will generally come from a combination of classical cytogenetics and clinical diagnosis of Cat-eye syndrome and is commonly confirmed using FISH probes.[25] Although markers derived from nearly every human chromosome have been reported,[22] few have specific utility. However, as the clinical phenotype of patients with markers from the pericentromeric regions of specific chromosomes becomes available, there clinical utility will improve.

The utility of FISH in the identification of chromosomal rearrangements and their derivatives depends on a resultant detectable genomic imbalance and the availability of informative probe(s) which reflect the rearrangement. The development of informative probe sets to detect recurring rearrangements in cancer is a common use of FISH assays. Individualized probe sets have also been utilized in preimplantation genetic analyses of couples with known chromosome rearrangements.[26] However, in the future, it is likely that such individualized assays will be replaced by more generic tests using subtelomeric probes, which will inform as to the presence of the specific telomere in question.

FISH tests may also have clinical value in determining whether chromosomal rearrangements are balanced and assessing their boundaries. It is important to appreciate that defining the molecular breaksites of an unbalanced rearrangement may also sharpen the focus of clinical follow-up (e.g., marker 15s). Because most familial rearrangements have unique breakpoints, probes are unlikely to be prequalified by a regulatory body. Rather, they will be defined as informative only within a given family and should be analytically validated and used on that basis. This situation is similar to the identification of cryptic rearrangements, which may have only been partially identified or suspected by routine cytogenetics but were subsequently either confirmed or established by FISH. Such scenarios offer the opportunity to provide individually tailored genetic tests and should be considered no less valid than an assay which has broader population applicability.

C. Chromosome enumeration and ploidy states

FISH testing has been widely used to identify aneuploid and polyploid individuals complying with analytical performance characteristics that are commonly quite high. The main analytical concern is that the probe or probe sets have the ability to concentrate significant signal within a small domain in the genome (e.g., repeat sequence probes, large genomic probes (30–200 kb), or cocktails of unique sequence probes from a particular chromosomal subregion). Hence, such assays only reflect the presence of the specific targeted domains and may not identify rearrangements which are not apparent or detectable. Mosaicism and chimeric states pose additional problems which increase the analytical requirements of tests to deal with relatively rare situations, depending on the specific disorder. Because patients undergoing testing are usually suspected of having a specific disorder, the clinical significance of a FISH test result may provide sufficient diagnostic information for individual patient management but insufficient information of mutational mechanism to allow follow-up of at-risk family members. Lastly, FISH probes are applicable to the assessment of individuals with particular sex chromosomes disorders, including the determination of the presence of Y chromosome material in Turner syndrome, XX males, and others.

II. Prenatal applications

Prenatal applications of FISH testing include both screening tests and diagnostic tests.[27] Again, technical issues are few, and clinical utility raises questions as to the intended use of FISH in testing.

A. Prenatal screening

The application of FISH to prenatal screening for common autosomal trisomies and sex chromosome anomalies is becoming increasingly common. The primary considerations involve differing clinical sensitivity between the abnormalities detected by classical cytogenetics versus those detected by FISH-based assays. Although speed of diagnosis is a distinct advantage, the increased cost of FISH must be reduced considerably before the benefit of high throughput screening would offset the reduced rate of abnormality detection for this screening test. An important consideration continues to be the particular type of abnormality for which an individual may be at risk. Since aneuploidy is the primary risk which increases with maternal age, existing FISH tests will have their greatest clinical sensitivity and utility among the oldest individuals in the population to be screened. However, in younger age groups, among whom the vast majority of Down syndrome offspring

are born, there is an increasing proportion of cytogenetically normal or nonaneuploid results versus aneuploid results. Similarly, proportions of aneuploids may vary with other indicators for testing (i.e., ascertainment of abnormalities by ultrasound vs. by prenatal biochemical screening). Based on data from several large series of prenatal diagnostic cases,[27–30] an expected and/or empiric rate of the various abnormalities detected prenatally by routine cytogenetics can be assessed as to the likelihood that a prenatal FISH test using probes from chromosomes X, Y, 13, 18, and 21 would detect the abnormality can be determined. Among patients presenting with advanced maternal age (AMA) $>$ 35 years, clinical sensitivity approaches 80%. In this context, sensitivity is defined as the detection of abnormalities known to be associated or possibly associated with abnormalities in liveborns. However, if one considers those cases ascertained by abnormal ultrasound or triple marker prenatal screening, a group which now includes individuals of younger ages, test sensitivity is reduced. For all patients tested prenatally for all indications, approximately 65–70% of all clinically significant cytogenetic abnormalities would be detected by the FISH test. The detection rate rises with increasing age (80%) due to the increased risk for nondisjunction. Although some abnormalities not detectable by FISH (e.g., inherited balanced translocations or some marker chromosomes) may have no clinical relevance, their identification, nonetheless, is critical in determining those rearrangements carrying the greatest risk of leading to abnormal liveborns. It is important to identify de novo balanced rearrangements and marker chromosomes due to their association with clinically significant abnormalities. Either significantly lower costs and/or technical enhancements to available probe sets would shift this balance in the favor of FISH utilization as the initial study. Lastly, as in other prenatal testing, decisions to act on laboratory test information should be supported by two of the three possible pieces of information, i.e., (1) FISH results, (2) routine chromosome analysis, and (3) clinical information.

B. Phenotype driven testing

Among cases ascertained via ultrasonographically identified fetal anomalies, some may be conclusive for a syndromic diagnosis and may be approached by a "diagnostic" FISH test. Families in which subtle or submicroscopic chromosomal abnormalities, detectable by FISH, are known to segregate will benefit greatly from prenatal FISH studies. These situations parallel those discussed above in the context of postnatal testing of individuals with birth defects and mental retardation. Lastly, when proportions of normal versus abnormal cells or the distribution of normal/abnormal cells in fetal and extraembryonic membranes is an important determinant of abnormality in the liveborn, FISH testing affords greater statistical accuracy because of larger cell samples, scorable in much short times when compared with traditional cytogenetic analyses.

SUMMARY

FISH technologies provide highly analytically accurate test systems. The tests are standardizable and controllable. The majority of questions about the tests arise when considering the appropriate circumstance in which to use the tests and the clinical utility of the test information in that setting. Both laboratorians and clinicians should be aware of the analytical sensitivity and specificity, including false-positive and -negative rates, of these tests.

At this time, the American College of Medical Genetics recommends that:

1. FISH testing be considered a highly useful and accurate test for the diagnosis of microdeletions and for the identification of unknown material in the genome. In disorders in which FISH testing provides results not possible from standard cytogenetic testing, the testing is standalone and should be accepted as such.
2. Microduplication analysis can be a useful test for disorders in which tandem duplication is among the mutation types. When validating such assays to establish reportable reference ranges, additional attention should be paid to background rates of target replication in controls. (Exclusive licensing agreements that monopolize or significantly reduce the sources of testing may prevent external quality assurance.[3])
3. Those requesting interphase FISH testing for prenatal diagnosis or screening should be fully aware of what these focused tests can and cannot do (clinical sensitivity and specificity) relative to the predicate "gold standard" of chromosome analysis, including false-positives and false-negatives. Regardless of intended clinical use, it is clear that the high analytical sensitivity and specificity of these tests provides a highly accurate result for those abnormalities detectable by these tests.
   a. For management of the fetus, it is reasonable to report positive FISH test results. Clinical decision-making should be based on information from two of three of the following: positive FISH results, confirmatory chromosome analysis, or consistent clinical information.
   b. For management of reproductive risks in families in which a fetus is identified as positive by FISH, positive results should be further characterized using traditional chromosome analysis to determine mutational mechanism accounting for the FISH detected abnormality.

Approved by the American College of Medical Genetics Board of Directors on March 7, 2000.

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References

1. American College of Medical Genetics. Prenatal interphase fluorescence in situ hybridization: policy statement. *Am J Hum Genet* 1993;2:526–527.
2. American College of Medical Genetics. Standards and guidelines: clinical genetic laboratories, Supplement 1. Bethesda, Md: American College of Medical Genetics, 1996.
3. American College of Medical Genetics. Standards and guidelines: clinical genetic laboratories, 2nd ed. Bethesda, Md: American College of Medical Genetics, 1999.
4. Dewald GW, Schad CR, Christensen ER, Law ME, Zinmeister A, Stalboerger PG, Jalal JM, Ash RC, Jenkins RB. Fluorescence in situ hybridization with X and Y chromosome probes for cytogenetic study on bone marrow cells after opposite sex transplantation. *Bone Marrow Transplant* 1993;12:149–154.
5. Dewald GW, Stallard R, Bader PI, Chen K, Zenger-Hain J, Harris CJ, Higgins R, Hirsch B, Hsu W-T, Johnson E, Kubic V, Kurczynski TW, Malone JM, McCorquodale DJ, Meilinger K, Meisner LF, Moore JW, Schwartz S, Siembieda S, Storto PD, Vance G, Van Tuinen P, Wiktor A, Yung JF. Toward quality assurance for metaphase FISH: a multicenter experience. *Am J Med Genet* 1996;65:1–8.
6. Dewald GW, Stallard R, Al Saadi A, Arnold S, Bader PI, Blough R, Chen K, Harris CJ, Higgins R, Hoeltge GA, Hsu W-T, Kubic V, McCorquodale DJ, Micale MA, Moore JW, Phillips RM, Scheib-Wixted S, Schwartz S, Siembieda S, Strole K, Van Tuinen P, Vance GH, Wiktor A, Wise L, Yung JF, Zenger-Hain J, Zinmeister A. A multicenter investigation with interphase fluorescence in situ hybridization using X- and Y-chromosome probes. *Am J Med Genet* 1998;76:318–26.
7. Shaffer LG, Kennedy GM, Spikes AS, Lupski JR. Diagnosis of CMT 1A duplications and HNPP deletions by interphase FISH: implications for testing in the cytogenetics laboratory. *Am J Med Genet* 1997;69:325–331.
8. Pauletti G, Godolphin W, Press M, Slamon DJ. Detection and quantitation of HER-2/neu gene amplification in human breast cancer archival material using fluorescence in situ hybridization. *Oncogene* 1996;13:63–72.
9. Masood SM, Bui MM, Yung JF, Mark HFL, Wong EY, Birkmeier JM, Yang S-J, Hsu P. Reproducibility of LSI HER-2/neu SpectrumOrange and CEP 17 SpectrumGreen dual color deoxyribonucleic acid probe kit. *Ann Clin Lab Sci* 1998;28:215–223.
10. Bossuyt PJ, Van Tienen M-N, DeGruyter L, Smets V, Dumon J, Wauters JG. Incidence of low-fluorescence alpha-satellite region on chromosome 21 escaping detection of aneuploidy at interphase by FISH. *Cytogenet Cell Genet* 1995;68:203–206.
11. Food and Drug Administration. Medical devices: classification/reclassification; restricted devices; analyte specific reagents. *Federal Register* 1996;61:10484–10489.
12. Wallerstein R, Anderson CE, Hay B, Gupta P, Gibas L, Ansari K, Cowchock PS, Weinblatt V, Reid C, Levitas A, Jackson L. Submicroscopic deletions of 16p13.3 in Rubinstein-Taybi syndrome: frequency and clinical manifestations in a North American population. *J Med Genet* 1997;34:203–206.
13. Ledbetter DH, Greenberg F, Hohn VA, Cassidy SB. Conference report: Second Annual Prader-Willi Scientific Conference. *Am J Med Genet* 1987;28:779–790.
14. Cassidy SB, Schwartz S. Prader-Willi and Angelman syndromes: disorders of genomic imprinting. *Medicine* 1998;77:140–151.
15. Schmickel RD. Contiguous gene syndromes: a component of recognizable syndromes. *J Pediatr* 1986;2:231–241.
16. Ewart AK, Morris CA, Atkinson D, Jin W, Sternes K, Spallone P, Stock AD, Leppert M, Keating MT. Hemozygosity at the elastin locus in a developmental disorder, Williams syndrome. *Nat Genet* 1993;5:11–16.
17. Pilz DT, Macha ME, Precht KS, Smith ACM, Dobyns WB, Ledbetter DH. Fluorescence in situ hybridization analysis with LIS1 specific probes reveals a high deletion rate in isolated lissencephaly. *Genet Med* 1998;1:29–33.
18. Scambler P, Kelly D, Lindsay E, Williamson R, Goldberg R, Shprintzen R, Wilson DI, Goldship JA, Cross IE, Burn J. Velo-cardio-facial syndrome associated with chromosome 22 deletions encompassing the DiGeorge locus. *Lancet* 1992;339:1138–1139.
19. Altherr MR, Bengtsson U, Elder FF, Ledbetter DH, Wasmuth JJ, McDonald ME, Gusella JF, Greenberg F. Molecular confirmation of Wolf-Hirschhorn syndrome with a subtle translocation of chromosome 4. *Am J Hum Genet* 1991;49: 1235–1242.
20. Gersh M, Goodart SA, Pasztor LM, Harris DJ, Weiss L, Overhauser J. Evidence for a distinct region causing a cat-like cry in patients with 5p deletions. *Am J Hum Genet* 1995;56:1404–1410.
21. Juyal RC, Greenberg F, Mengden GA, Lupski JR, Trask BJ, van den Engh G, Lindsay EA, Christy H, Chen K-S, Baldini A, Shaffer LG, Patel PI. Smith Magenis syndrome deletion: a case with equivocal cytogenetic findings resolved by fluorescence in situ hybridization. *Am J Med Genet* 1995;58:286–291.
22. Callen DF, Eyre HJ, Yip M-Y, Freemantle CJ, Haan EA. Molecular cytogenetic and clinical studies of 42 patients with marker chromosomes. *Am J Med Genet* 1992;43: 709–715.
23. Ohashi H, Wakui K, Ogawa K, Okano T, Niikawa N, Fukushima Y. A stable acentric marker chromosome: possible existence of an intercalary ancient centromere at distal 8p. *Am J Hum Genet* 1994;35:1202–1208.
24. Callen DF, Freemantle CJ, Ringenbergs ML, Baker E, Eyre HJ, Romain D, Haan EA. The isochromosome 18p syndrome: confirmation of cytogenetic diagnosis in nine cases by in situ hybridization. *Am J Hum Genet* 1990;47:493–498.
25. Magenis RE, Sheehy RR, Brown MG, McDermid HE, White BN, Zonana J, Weleber R. Parental origin of the extra chromosome in the cat-eye syndrome: evidence from heteromorphisms and in situ hybridization analysis. *Am J Med Genet* 1988;29:9–19.
26. Weier HU, Munne S, Fung J. Patient-specific probes for preimplantation genetic diagnosis of structural and numerical aberrations in interphase cells. *J Assist Reprod Genet* 1999;16:182–191.
27. Klinger K, Landes G, Shook D, Harvey R, Lopez L, Locke P, Lerner T, Osathanondh R, Leverone B, Houseal T, Pavelka K, Dackowski W. Rapid detection of chromosome aneuploidies in unclutured amniocytes by using fluorescence in situ hybridization (FISH). *Am J Hum Genet* 1992;51:55–65.
28. Evans MI, Ebrahim SAD, Berry SM, Holzgreve W, Isada NB, Quintero RA, Johnson MP. Fluorescent in situ hybridization utilization for high-risk prenatal diagnosis: a trade-off among speed, expense, and inherent limitations of chromosome-specific probes. *Am J Obstet Gynecol* 1991;1055–1057.
29. Lewin P, Kleinfinger P, Bazin A, Mossafa H, Szpiro-Tapia S. Defining the efficiency of fluorescence in situ hybridization on uncultured amniocytes on a retrospective cohort of 27,407 prenatal diagnoses. *Prenat Diagn* 2000;20:1–6.
30. Eiben B, Trawicki W, Hammons W, Goebel R, Pruggmayer M, Epplen JT. Rapid prenatal diagnosis of aneuploidies in uncultured amniocytes by fluorescence in situ hybridization. Evaluation of >3,000 cases. *Fetal Diagn Ther* 1999;14:193–197.
31. Kuwano A, Ledbetter SA, Dobyns WB, Emanuel BS, Ledbetter DH. Detection of deletions and cryptic translocations in Miller-Dieker syndrome by in situ hybridization. *Am J Hum Genet* 1991;49:707–714.
32. Woodward K, Kendall E, Vetrie D, Malcolm S. Pelizaeus-Merzbacher disease: identification of Xq22 proteolipid-protein duplications and characterization of breakpoints by interphase FISH. *Am J Hum Genet* 1998;63:207–217.