Further Section

Cytogenet Genome Res 1995;69:110-126

Abstracts of the 32nd Annual American Cytogenetics Conference

Held on April 23–26, 1994 at the Wintergreen Resort, Virginia

W.	Wendy	Golden
J.	James	Mascarello
L.	Linda	Cannizzaro
S.	Susanne	Gollin
P.	Patricia	Howard-Peebles
N.	Nagesh	Rao
D.	Debra	Saxe
S.	Stuart	Schwartz
U.	Urvashi	Surti
J.	John	Wiley

Recipients of Student Travel Awards Sue Ann Berend Robert Chin Derek Latour Josh Weerasinghe

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Program

SUNDAY, APRIL 24, 1994

8:00–8:15 Opening remarks Wendy Golden

8:15–9:15 INVITED SPEAKER: Peter L. Pearson, “From chromosome to gene: the
shrinking
world of the cytogeneticist”

Session 1: Human Constitutional Cytogenetics A

Moderators: P.N. Howard-Peebles and CM. Moore

9:15–9:30 High-resolution mapping of 10 unique DNA sequences to chromosome 3 subregions by in situ hybridization L. Atchison,* M.L. Atchison, and L.A. Cannizzaro

9:30–9:45 Heritability estimates and heteromorphic distribution of isoschizomers Aibol and Sai/3AI B. Huang,* J.M. Meyer and C. Jackson-Cook

9:45–10:00 FISH analysis of a Shprintzen clinical series J.B. Ravnan,* E. Chen, M. Golabi, R.R. Flandermeyer, M.S. Golbus, and R.V. Lebo

10:30–10:45 Molecular characterization of de novo secondary trisomy 13 L.G. Shaffer,* C. McCaskill, J.-Y. Han, D.M. Cutillo, A.E. Donnenfeld, L. Weis, and D.L. Van Dyke

10:45–11:00 Fluorescent in situ hybridization for diagnosis of Prader-Willi/Angelman syndrome S.L. Wenger* and J.H. Cummins

11:00–11:15 A jumping Robertsonian translocation: characterization of chromosome segregation and chromosome structure V.M. Park,* S. Gross, A.T. Tharapel, O.P. Phillips, and L.P. Shulman

11:15–11:30 Jumping translocations and the association of interstitial telomeric sequences L.S. Jenkins,* S. Cobberly, L. Bros, and B. Blumberg

11:30–11:45 It is time to abandon the cytogenetic fragile X test! P.N. Howard-Peebles*

Session 2: Human Constitutional Cytogenetics B

Moderators: L.S. Jenkins and S.A. Schonberg

2:00–2:15 Supernumerary inv dup(15) in a patient with Angelman syndrome and del(15)(q11q13) N.B. Spinner,* E. Zackai, R. Kline, S. de Cheng, and J. Knoll

2:15–2:30 Deletion of 15q11–>q13 in a mother and son without features of Prader-Willi or Angelman syndrome M.C. Phelan,* R.C. Michaelis, J.C. Tarleton, T.A. Donlon, and S.A. Skinner

2:30–2:45 Identification of an unbalanced cryptic translocation using fluorescent in situ hybridization P.A. Mowery-Rushton,* A. Estop, K. Cieply, S.J. Kochmar, E. McPherson, and U. Surti

2:45–3:00 Identification of de novo translocations involving 1p36 L.M. Pasztor* and D.J. Harris

3:00–3:15 Duplication within medial 5q R. Stallard,* V. Zurcher, J. Viront, L. Ko, and S. Schwartz

3:15–3:30 Paracentric inversion of chromosome 1 inherited from a mosaic father J.L. Zackowski,* A. Newlin, L.G. Leichtman, and R.L. Neu

3:30–3:45 Familial pericentric inv(12)(p11.2q15) V.R. Potluri,* C. Malin, E. Uus, and S. Nair

3:45–4:00 A complex de novo rearrangement involving three chromosomes in a child with multiple anomalies and unusual hematological findings R.S. Muneer,* D.J. Hopcus, A.D. Garnica, and C.L. Sexauer

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Session 3: Human Constitutional Cytogenetics C

Moderators: N.B. Spinner and P.B. Jacky

4:30–4:45 Association of a heritable fragile site at 2q13 with fetal demise P.B. Jacky,* T.L. Berry, S.M. Freese, R.A. Foxley, O.A. Lamb, M.I. Langlois, K.L. Meagher, C.L. Olson, J.S. Rubinstein, J.A. Reiss, F.F. Weeks, and M.J. Yoshitomi

4:45–5:00 Mosaicism for X-chromosomal aneuploidy in women who experience recurrent pregnancy loss evaluated by fluorescence in situ hybridization F. Grass,* R.S. Ostrowski, J. Norton, R. Krishna, S. Jenkins, and M. Stegar

5:00–5:15 Confined placental mosaicism for trisomy 12 in CVS with varying outcomes

F.Z. Bischoff,* J. Zenger-Hain, D.L. Van Dyke, and L.G. Shaffer

5:15–5:30 The risk of chromosomal aneuploidy and the sensitivity of ultrasound following an

elevated maternal serum alpha-fetoprotein EC. Lambert, J.W. Larsen, and V.M. Barnabei*

5:30–5:45 Mosaicism for trisomy 20 and a marker 20 detected prenatally and studied by FISH

D.A.S. Batista,* B.K. Welty, M.S. Gibson, C. Escallon, K. Blakemore, and G. Stetten

5:45–6:00 Prenatal diagnosis of a complex chromosome rearrangement involving four chromosomes CM. Moore,* B.R. DuPont, V.L. Mattern, and K. Higby

6:00–6:15 Fluorescence in situ hybridization with a new approach for identification of trisomy 21

in interphase amniocytes using different cosmid probes I.V. Soloviev,* F. Fayet, P. Malet, Y.B. Yurov, and S.G. Vorsanova

MONDAY, APRIL 25, 1994

8:00–9:00 INVITED SPEAKER: Terry Magnuson, “Genetics of early embryo survival in the mouse”

Session 4: Animal and Experimental Cytogenetics A

Moderators: J.E. Wiley and I.F. Greenbaum

9:00–9:15 Ribosomal gene location polymorphism in North Carolina populations of the toad,

Bufo terrestris J.E. Wiley,* D.L. Foote, and T.D. Lupton, Jr.

9:15–9:30 Cytogenetic analysis of the neo-XY sex-chromosomal system of collared lemmings

S.A. Berend,* D.W. Hale, M.D. Engstrom, and I.F. Greenbaum

9:30–9:45 How common are common fragile sites? I.F. Greenbaum,* B.F. McAllister, and P.F. Dahm

9:45–10:00 Comparative analysis of fragile sites in two species of *Peromyscus* J.K.

Weerasinghe* and I.F. Greenbaum

10:00–10:15 Pre-meiotic nondisjunction as a source of aneuploid meiocytes, gametes, and offspring

D.W. Hale*

10:15–10:30 Gene mapping in *Peromyscus* using fluorescence in situ hybridization Z. Wang,

S.R. Young,* L. Liu, and W. Dawson

Session 5: Animal and Experimental Cytogenetics B

Moderators: B.K. Vig and M.M. Cohen

11:00–11:15 The X chromosome of the Indian muntjac: subunit repeats or multiple centromeres?

D. Latour* and B.K. Vig

11:15–11:30 Minor satellite in mouse is not associated with centromere activity B.K. Vig* and D. Latour

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11:30–11:45 Aneuploidy in offspring of female mouse chimeras transmitting a male genotype J.T. Mascarello,* S.K. Bronson, and O. Smithies

11:45–12:00 Mouse Chromosome 6 in Rb translocations: segregation and sex-ratio distortions

I.P. Aranha* and P.A. Martin-DeLeon

12:00–12:15 Replication analysis of X-linked genes in females by fluorescence in situ hybridization (FISH) B.S. Torchia,* L.M. Call, and B.R. Migeon

12:15–12:30 A rapid method to amplify DNA fragments for DNA analysis from cells fixed with Carnoy S. Li,* CM. Tuck-Muller, Q. Yan, W. Wertlecki, and H. Chen

TUESDAY, APRIL 26, 1994

8:00–9:00 INVITED SPEAKER: Jaclyn Biegel, “Pediatric brain tumors: the ‘p’s’ and ‘q’s’”
Session 6: Cytogenetics of Neoplasia A

Moderators: U. Surti and S.M. Gollin

9:00–9:15 Chromosome analysis of nine endocrine neoplasms of the pancreas C.A. Griffin,* P.P. Long, R.H. Hruban, R. Lo, C.J. Yeo, and L.A. Morsberger

9:15–9:30 Comparative classical and molecular cytogenetic analysis of oral squamous cell carcinoma CM. Lese,* K.M. Rossie, J.T. Johnson, and S.M. Gollin

9:30–9:45 Further definition of 20q deletion in acute myeloid leukemia using multicolor FISH

P.N. Rao,* R. Hayworth, A.J. Carroll, D.W. Bowden, and M.J. Pettenati

9:45–10:00 An abnormal clone with monosomy 7 and trisomy 21 in the bone marrow of a child with congenital agranulocytosis (Kostmann disease): possible evolution towards myelodysplastic syndrome after treatment with granulocyte colony-stimulating factor

S. Shekhter-Levin,* L. Penchansky, M. Wollman, M.E. Sherer, N. Wald, and S.M. Gollin

10:00–10:15 Structural abnormalities of chromosome 14 in meningiomas: evidence for a progression gene B.F. Schneider,* S.R. VandenBerg, K.W. Sudduth, C. von Kap-herr, V. Shashi, and W.L. Golden

Session 7: Cytogenetics of Neoplasia B

Moderators: A.R. Brothman and P.N. Rao

10:45–11:00 Cytogenetic findings in posttransplant lymphoproliferative disorder C.J. Alvares,* J.L.C. McKnight, R.B. Herberman, and S.M. Gollin

11:00–11:15 Detection of numerical chromosome abnormalities in interphase cells of ovarian carcinomas using fluorescence in situ hybridization J.K. Brock,* W.H. Liu, ST. Smith, and S.R. Young

11:15–11:30 Simultaneous detection of numerical chromosome abnormalities and affected cell lineage R. Chin,* B. Kaplan, and J. Felix

11:30–11:45 Some of the questions generated by FISH studies: answers, theories, and more questions A.R. Brothman,* E. Jones, and L.J. Brothman

11:45–12:00 Cytogenetic analysis of familial paragangliomas A.L. Zaslav,* D. Myssiorek, C. Mucia, and J.E. Fox

12:00–12:15 Tamoxifen induces hepatic aneuploidy and mitotic spindle disruption after a single in vivo administration to female Sprague-Dawley rats L. Sargent,* Y. Dragan, N. Bahnub, J.

Wiley, C. Sattler, P. Schroeder, G. Sattler, V.C. Jordan, and H. Pitot

12:15–12:30 Closing remarks W. Golden and J. Mascarello

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Abstracts of the 32nd Annual American Cytogenetics Conference

From chromosome to gene: the shrinking world of the cytogeneticist

P.L. Pearson

The Johns Hopkins University, Baltimore, MD

Clinical cytogenetic practice has always been technology driven. The major contributions of the last four decades have arisen from “break-through” technologies and resulted in rapid changes in community expectations on the types of services offered by clinical cytogenetics laboratories. However, in many instances, the introduction of a new technique has not replaced but simply added to the number of procedures carried out on a single sample. Central to this dilemma is “how can the extra procedures be paid for?” The most recent example is the introduction of FISH, which highlights many of the tensions experienced when new methods of analysis become available. The result is that it is becoming increasingly difficult for lab directors to file reports of unidentified marker chromosomes or possible translocations without including FISH analysis to specify the abnormality further, despite the fact that state and federal authorities are still concerned with imposing standards based on 20-year-old technology. There is also a high level of competitive pride among labs on the level of FISH service offered. The availability of painting, the number and size of repetitive and unique sequences detected, competitive hybridization, and number of wavelengths used are now the battleground on which leading labs try to distinguish themselves from the rest, and the gap between what is expected and what is legally necessary is only going to increase further.

Detection of microdeletions and the specification of breakpoints to a much higher level of resolution than that offered by banding pattern analysis will become an expected part of clinical cytogenetic analysis. Further, cytogenetic analysis offers valid alternatives, and frequently some advantages, to molecular genetics procedures for the detection of single-gene deletion or duplication disorders. The continual shift of cytogenetic analysis from the chromosome towards the gene mirrors the progress in gene mapping. We can expect that chromosome banding standards will be supplemented, and in some situations replaced, by a raster of mapped DNA markers. However, implementation will be a gradual process, and cytogenetic laboratories will play a major role in the development and acceptance of such a system.

One of the major problems now facing all medical genetics centers is how to access information efficiently. Questions such as which probes are needed to characterize a breakpoint, which structural abnormalities are associated with a particular syndrome, or which center specializes in the analysis of which chromosome region will be provided by information services over the Internet. Meaningful integration of molecular cytogenetic data into the totality of medical genetics information has become one of the major challenges of the coming years. Perhaps boards in medical informatics, in addition to those in clinical cytogenetics and molecular genetics, should become de rigueur for cytogeneticists!

High-resolution mapping of 10 unique DNA sequences to chromosome 3 subregions by in situ hybridization

L. Atchison,¹ M.L. Atchison,² and L.A. Cannizzaro³

¹ Biology/Molecular Biology Department, Chestnut Hill College, Philadelphia, PA; ²

Department of Animal Biology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA; and ³ Department of Pathology, Albert Einstein College of Medicine, Bronx, NY

A series of single-copy DNA sequences were previously isolated and characterized from human chromosome 3-specific genomic libraries (LA03NS01-ATCC; Atchison et al., *Genomics* 6:441–450, 1990). By Southern blot analyses these probes gave clear, single band, hybridization signals with EcoRI-digested DNA isolated from cell hybrids containing human chromosome 3. No signal was detected with DNA isolated from controls lacking human chromosome 3. In order to precisely define the subchromosomal localization of these DNA

sequences, in situ molecular hybridization was performed using a method described previously (Cannizzaro et al., *Cancer Res* 51:3 818–3 820, 1991). Briefly, genomic single copy DNA probes in plasmid pUC 18 were tritium labeled with [³H]dCTP by nick translation to a specific activity of 2–4 x 10⁷ cpm/μg DNA. For each analysis, between 60 and 275 metaphase chromosomes from normal male peripheral lymphocytes were analyzed and between 100 and 400 grains were counted. Based on the percentages of grains lying over specific sites on chromosome 3, the following assignments were made. Seven sequences were assigned to 3q subregions (D3S72: 3q22; D3S74: 3q12→q13; D3S75: 3q; D3S83: 3q27→q28; D3S88: 3q22→q26; D3S173: 3q13.3; D3S176: 3q27). Two sequences were assigned to 3p subregions (D3S76: 3p23→p21; D3S77: 3p21). One single-copy DNA probe (D3S78) was assigned to the centromeric region of chromosome 3 by Southern blot analysis of DNA isolated from a somatic cell hybrid containing chromosome 3 centromere sequences. These DNA clones mapped by in situ hybridization can provide useful landmarks for various disease specific loci on chromosome 3. They can also be useful for ordering the localization of other clones to generate physical and genetic maps. Heritability estimates and heteromorph distribution of the isoschizomers Mbol and Sau3AI B. Huang,¹ J.M. Meyer,² and C. Jackson-Cook²

¹ National Center for Human Genome Research, National Institutes of Health, Bethesda, MD, and ² Medical College of Virginia/Virginia Commonwealth University, Richmond, VA The heritability estimates and heteromorphism distributions of the chromosome banding variants induced by the isoschizomers Mbol/Sau3I were assessed in 25 monozygotic (MZ) and 25 dizygotic (DZ) twin pairs (100 individuals). The banding variants induced by Mbol or Sau3AI were found to have the same chromosomal locations, with both restriction enzymes (REs) inducing banding patterns that resembled C-bands. Collectively, a total of 36 heteromorphic variants (18 for each RE) were identified, being located in the pericentromeric region of chromosomes 3, 4, 7, 9, 10, 12, and 18; the pericentromeric (c) and satellite (s) regions of chromosomes 13, 14, 15, 21, and 22 and the distal long arm of the Y chromosome. The most heteromorphic variants were those on chromosomes 13s {Mbol} and 3 {Sau3M}, while the least heteromorphic were those seen on chromosomes 9 {Mbol} and 14s {Sau3AI}. The correlations of the variant “scores” between MZ twin pairs (ranging from 0.58 to 0.97) were found to be higher than the correlations between DZ twins (-0.01 to 0.79) for both Mbol and Sau3AI variants. For all but 1 of the 32 variants with distributions adequately characterized by a genetic model, the heritability was estimated to be at least 60%, with the majority of the heritability estimates ranging from 80% to 90%. When the variant “scores” seen with Mbol were compared to those noted with Sau3AI, 13/16 variants were found to be significantly correlated due to the influence of common genetic factors (such as a shared DNA recognition sequence). However, the variants induced by Mbol, compared to Sau3AI, were not perfectly correlated (r values ranging from 0.10 to 0.74), indicating that other factors, such as different sensitivities to DNA methylation, may also play a role in their presentation. In summary, Mbol and Sau3AI appear to induce chromosomal variants that are highly heteromorphic and heritable. Furthermore, genetic factors seem to be the major components leading to the observed covariation in banding patterns.

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FISH analysis of a Shprintzen clinical series

J.R. Ravnan, E. Chen, M. Golabi, R.R. Flandermeyer, M.S. Golbus, and R.V. Lebo

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Deletions in chromosome band 22q11.2 have been associated with Shprintzen (velocardiofacial) syndrome and DiGeorge sequence. Probes mapped to the disease gene region by positional cloning studies have been used to classify patients according to the presence or absence of a deletion, and these results have subsequently been compared to clinical characteristics. At this writing, we have completed studies on 32 patients with features of Shprintzen syndrome who were referred for chromosomal analysis. Fluorescence in situ hybridization (FISH) using the Oncor DiGeorge critical region probe and our hybridization protocols gave clearly interpretable results. Ten (31%) of the 32 patients were found to have a deletion of D22S75 in one chromosome 22. A retrospective study by FISH of 10 patients originally studied by prometaphase GTG-banding found five patients with a deletion; only one of these patients had a deletion visible by GTG-banding. Clinical findings were compared between the group of Shprintzen patients with a deletion of D22S75 and those with no detectable deletion. We found that the most significant finding associated with the deletion was the presence of ventriculoseptal heart defects. We are continuing our study of patients with features of Shprintzen syndrome and are currently studying family members of deletion patients to determine the percentage of deletions that are inherited. Even more robust tests are anticipated as the molecular and clinical characterization of this chromosomal region defines additional probes unique to each disease phenotype.

Molecular characterization of de novo secondary trisomy 13

LG. Shaffer,¹ C. McCaskill,¹ J.-Y. Han,¹ D.M. Cutillo,² A.E. Donnenfeld,² L. Weiss,³ and D.L. Van Dyke³

¹ Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX; ² Section of Genetics, Pennsylvania Hospital, Philadelphia, PA; and ³ Medical Genetics and Birth Defects Center, Henry Ford Hospital, Detroit, MI

Unbalanced Robertsonian translocations are a significant cause of mental retardation and fetal wastage. The majority of homologous rearrangements of chromosome 21 in Down syndrome have been shown to be isochromosomes. Aside from chromosome 21, very little is known about other acrocentric homologous rearrangements. Unbalanced translocations associated with Patau syndrome (trisomy 13) occur in approximately 1 in 25,000 live births (Hook, 1981) with the majority of the rearrangements occurring as Robertsonian translocations. In one survey of spontaneous abortions, 26% of the trisomy 13 cases were the result of a Robertsonian translocation.

In the current study, four cases of de novo secondary trisomy 13 are presented. Of the four cases, one was a newborn referred for a chromosome analysis because of dysmorphic features, one case was identified after amniocentesis and chromosome analyses for abnormal ultrasound findings, and two cases were identified after chromosome analysis of a spontaneous miscarriage.

Fluorescence in situ hybridization (FISH), performed using D13Z1/D21Z1 (Oncor) and two plasmids containing the 28S and 18S ribosomal genes, showed all four rearrangements to be dicentric and devoid of ribosomal genes. Eleven microsatellite markers from chromosome 13 were used to characterize the rearrangements to identify the four possible outcomes; isochromosomes or Robertsonian translocations of either maternal or paternal origin. Of the possible outcomes, a maternal Robertsonian translocation, a paternal isochromosome, and a maternal isochromosome were observed. One case is pending. A double recombination was observed in the rob(13q13q). No recombination was detected in either i(13q). Our results

demonstrate the occurrence of both isochromosomes and true Robertsonian translocations forming in secondary trisomy 13.

Fluorescent in situ hybridization for diagnosis of Prader-Willi/Angelman syndrome

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Seventy percent of Prader-Willi syndrome (PWS) patients have deletions of 15q11—>q13, diagnosed by high-resolution chromosomal analysis or RFLP analysis. Recently, DNA probes have become commercially available (Oncor) for the detection of the deleted area using fluorescent in situ hybridization (FISH) analysis. Using these probes (A and B), we have analyzed 28 clinically documented cases of PWS, two clinically documented cases of Angelman syndrome (AS), and 25 cases with a questionable diagnosis of either PWS or AS. Of the 28 known cases of PWS, 12 had deletions detected by FISH, three of which were reported as cytogenetically normal. The two AS cases were cytogenetically deleted and also had a molecular deletion detectable by FISH. Among the 25 cases with a questionable diagnosis, two of the PWS cases had a molecular deletion, of which one was cytogenetically normal. The remaining 23 ambiguous cases were normal both cytogenetically and when probed with FISH. Our results suggest that FISH analysis using the PWS/AS probes can facilitate diagnostic evaluation by eliminating the necessity of high-resolution chromosome analysis.

A jumping Robertsonian translocation: characterization of chromosome segregation and chromosome structure

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We report a patient with mosaicism for multiple Robertsonian translocations. Cytogenetic analysis of lymphocyte cultures revealed the following rearrangements: 45, XX, t(21q22q)/46, XX,-21,+t(21q21q)/45, XX, t(14q21q) (97%/2%/1%). Thus, the proband carries a balanced translocation in most of her cells, with low-level mosaicism for an unbalanced cell line containing a different Robertsonian rearrangement. Several similar cases have been reported (see Wells et al., *Prenat Diag* 11:875, 1991, and references therein). The proband's pregnancy history suggests that the 21/21 rearrangement may be more abundant in germ cells than it is in blood. She has two offspring who both carry the 21/21 rearrangement in unbalanced form [46,-21,+t(21q21q)]. Cord blood cultures show that the second child exhibits low-level mosaicism for a normal cell line. This suggests continued instability of the 21/21 rearrangement inherited from the mother. In addition, the mother has had two spontaneous abortions. FISH with alphoid probes indicated that both the 21/21 rearrangement and the 21/22 translocation are dicentric and that each long arm segment retains its appropriate centromere. Thus, the 21/22 translocation exhibits positive hybridization with an alphoid probe for chromosomes 13 plus 21 and also with an alphoid probe for chromosomes 14 plus 22. The 21/21 rearrangement exhibits two closely spaced signals when probed with chromosomes 13 plus 21.

FISH studies are in progress to further characterize the centric regions of the rearranged chromosomes. In addition, preliminary results of segregation studies using microsatellite polymorphisms suggest that the 21/21 rearrangement is an isochromosome. Although several mechanisms can be invoked to explain these unusual findings, we favor a model in which the i(21q) is the progenitor rearrangement. It is unstable and participates in nonreciprocal rearrangements characteristic of a jumping translocation.

Jumping translocations and the association of interstitial telomeric sequences

L.S. Jenkins, S. Cobberly, L. Bros, and B. Blumberg

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We present two independent cases in which translocations of specific chromosomal regions have produced mosaic genotypes. The term "jumping translocation" has been used to describe these mobile segments, suggesting that they jump from one chromosome to another during the cell cycle.

In case 1, an amniocentesis performed for maternal age, the common donor breakpoint appeared to be in the pericentromeric region of chromosome 22. The terminal regions of a variety of chromosomes served as the acceptors for the chromatin from chromosome 22. The three cell lineages comprising the mosaicism in the amniocytes were detected again after a repeat amniocentesis. A PUBS detected yet another lineage. Parental chromosomes were normal. In case 2, a therapeutic abortion performed because of anencephaly detected on ultrasound, the common breakpoint appeared to be in the pericentromeric region of chromosome 14.

Translocation of chromatin from chromosome 14 to a variety of other chromosomes again yielded a complex mosaic karyotype. Parental chromosomes were normal. In situ hybridization was performed to determine the presence of interstitial telomeric sequences at the junction points involved in the jumping translocations.

dup(15) karyotype. The marker chromosome in this patient was relatively small, and on molecular and molecular cytogenetic studies only a centromeric probe (D15Z1) was duplicated, while the distal loci D15S18, D15S9, D15S11, D15S63, D15S10, GABRB3, and D15S12 were all absent. On molecular cytogenetic studies, one of the apparently normal 1's was found to contain a deletion for probes D15S9, GABRB3, and D15S12. Therefore it is likely that the etiology for this patient's Angelman syndrome lies in the deletion for these loci. Deletion of these loci is well known to be associated with Angelman syndrome. This is the second reported case of a patient with an inverted dup(15) and Angelman syndrome. One previous patient was identified who demonstrated paternal uniparental disomy for chromosome 15, and the supernumerary inverted dup(15) was assumed to be unrelated to the etiology of Angelman syndrome.

The relationship between the inv dup(15) and the deletion of 15q11 → q13 is unknown.

However, it does not appear that the supernumerary marker is related to the Angelman phenotype. At this time, deletion of 15q11 → q13 and uniparental disomy for this region of chromosome 15 are the only known mechanisms for the etiology of Angelman syndrome.

A review of the literature suggests there may be an increased frequency of inv dup(15)'s in the Prader-Willi population, compared with the general population, and it has been suggested that patients with inv dup(15)'s may be at increased risk for uniparental disomy. Taken together, these findings suggest that patients with an inv dup(15) should be studied to rule out additional chromosome 15 abnormalities that may result in the Prader-Willi or Angelman phenotypes.

It is time to abandon the cytogenetic fragile X test!

P.N. Howard-Peebles

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Direct DNA testing for fragile X (fraX) was developed in mid-1991. Multiple studies since that time have shown such testing to be valid, allowing classification of normal, premutation, and full-mutation genotypes. These studies have also shown the limits of the cytogenetic test in both

sexes, i.e., error rates as well as the high percentage of carrier females that test negative. DNA testing is cheaper and faster, as well as significantly more accurate.

The usual argument for cytogenetic testing is that affected individuals screened for fraX should also have a cytogenetic study, since 95–97% of them will be negative for fraX. Most geneticists would agree with this policy, including the author. Our center has used a protocol (Appel Cytogenet, 1994, in press) allowing both to be accomplished. Over the last 2½ years (as of March, 1994), we have screened 321 individuals (as of February 14, 1994) with this protocol; five fraX full mutations were identified (four males and one female). Thus, less than 2% were positive for the fraX, which is lower than the incidence usually reported (3–5%).

Supernumerary inv dup(15) in a patient with Angelman syndrome and del(15)(q11 q13)

N.B. Spinner,¹ E. Zackai,¹ R. Kline,¹ S. de Cheng,² and J. Knoll²

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Inverted dup(15)'s are one of the most common supernumerary marker chromosomes, having been demonstrated in phenotypically normal individuals; individuals exhibiting mental retardation, seizures, and behavioral problems; and in a few patients with the Prader-Willi syndrome. Comparison of the extent of duplicated material with phenotype shows that patients with mental retardation and other anomalies have larger duplications than do individuals with a normal phenotype.

We have studied a patient with Angelman syndrome and a 47, XY,+inv

Deletion of 15q11 → q13 in a mother and son without features of Prader-Willi or Angelman syndrome

M.C. Phelan,¹ R.C. Michaelis,¹ J.C. Tarleton,¹ T.A. Donlon,² and S.A. Skinner¹

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D.H. was a 12-year-old white male with mild mental retardation and dysmorphic facial features, including down-slanting palpebral fissures, a prominent nose, broad forehead, small chin, midface hypoplasia, and large ears. Initial chromosome studies were performed in 1988 and revealed an interstitial deletion of chromosome region 15q11 → q13. Examination of the parental chromosomes showed the mother to have the same deletion. She had normal intelligence and no phenotypic abnormalities. Molecular DNA analysis of the patient was consistent with a deletion of markers detected by probes pIRIO-1 (D15S12) and LS6-1 (D15S113). Due to the lack of phenotypic features of Prader-Willi or Angelman syndrome, the patient and his mother were recently restudied. Fluorescent in situ hybridization was performed using the Prader-Willi/Angelman syndrome cosmids obtained from Oncor (P5130). Both the patient and his mother were deleted for the Region B probe (G ABRB3) but were not deleted for the Region A probe (D15S11). The linear order of probes mapping to 15q11 → q13 is cen-D15S11-D15S13-D15S113-D15S10-GABRB3-D15S12-tel. The Angelman syndrome critical region has been localized to an interval containing the loci D15S113, D15S10, and GABRB3. The deletion in our patient most likely overlaps the Angelman syndrome critical region. Analysis of additional markers is underway to further characterize the deletion. Delineation of this deletion will aid in the further refinement of the Angelman syndrome critical region.

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We report a case of an unbalanced cryptic telomeric translocation, $t(9;17)(q34;p13)$, in a boy with dysmorphic features and developmental delay as a result of partial monosomy of 17p and partial trisomy for 9q. The proband had intrauterine growth retardation and postnatal short stature with mild microcephaly. Dysmorphic features included a flat occiput, epicanthal folds, down-turned upper lip, low-set posteriorly rotated ears, cryptorchidism, inguinal hernia, deep sacral dimple, and clinodactyly of the fifth fingers. MR1 showed incomplete myelination, but no evidence of lissencephaly. Cytogenetic analysis of the boy showed a small deletion of 17p. His mother's karyotype was initially interpreted as normal. Fluorescent in situ hybridization (FISH) with a Miller-Dieker specific cosmid probe did not detect a deletion for that area. Further analysis with a 17p telomere-specific probe (Oncor) indicated that the boy had an unbalanced telomeric translocation. This probe corresponds to D17S34 which has been reported to be deleted in certain types of pediatric brain tumors (Cogen et al., Am J hum Genet 50:584, 1992). The same probe was used to determine the presence of an apparent balanced translocation, $t(9;17)(q34;p13)$, in the mother. Direct confirmation of the presence of the 9q telomere on the terminus of 17p could not be made due to the lack of a 9q telomere-specific probe. Two phenotypically normal half-sibs were also examined, and both were found to be balanced translocation carriers. The mother also reported a history of two spontaneous abortions. The maternal grandmother had nine spontaneous abortions and two apparently normal children, in addition to the proband's mother. Neither the grandmother nor the maternal aunt and uncle were available for testing.

The incidence of cryptic translocations is not known, but they may represent a major contributor to human pathology. Recently, several other cases of cryptic translocations associated with an abnormal phenotype have been described in the literature (Lamb et al., Lancet 2:819, 1989; Overhauser et al., Am J hum Genet 45:296, 1989; Kuwano et al., Am J hum Genet 49:707, 1991). These cases represent a major challenge to clinical cytogenetics laboratories and require careful analysis of high-resolution-banded prometaphase chromosomes to identify the abnormal chromosome and confirmation by molecular genetic or cytogenetic techniques.

Duplication within medial 5q

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The medial long arm of chromosome 5 displays a sequence of three dark bands of remarkably similar shading and size by GTG-banding. Even at high resolution, the segment is little differentiated by changes in shading or by subdivision of bands. Consequently, assigning unambiguous breakpoints for deletions, inversions or duplications within the segment has proven difficult. Fluorescent in situ hybridization (FISH) with libraries and cosmids offers the possibility of precise identification and positioning of abnormalities within the segment. We report our experience with two independent cases.

Case 1. An 8-year-old male with mild mental retardation, poor motor coordination, and hyperactivity, but with a normal MRI and without striking dysmorphism, was referred for fragile-X testing. The fragile X was not demonstrated with stress testing. However, the medial segment of the long arm of one chromosome 5 exhibited additional material. Paternal and maternal chromosomes were normal, excepting that one maternal chromosome 5 was

indistinguishable from that of her son. The mother was described as slow, although her history included graduation from high school and four normal sibs. Assessment has been complicated by a seriously disturbed psychosocial environment.

Case 2. A newborn female was referred for microcephaly, small cloudy pupils, segmented gums, dislocated hips, and hypoplastic toenails. She was born weighing 2,700 g with a length of 48 cm following a 40-wk gestation complicated by oligohydramnios and a breech presentation.

Brothers (3 $\frac{1}{2}$ and 1 $\frac{1}{2}$ yr old) and two adult half-sibs were reported as normal. Her karyotype was normal except that the long arm of one chromosome 5 exhibited additional material medially.

Parental karyotypes have not yet been determined.

From observation, the abnormal chromosomes 5 were interpreted as presenting an addition equal to about 12% of the long arm of chromosome 5, including band 5q21 and indeterminate amounts of each of the two adjacent bands, 5q15 and 5q22.1. Fluorescent in situ hybridization with Coatasome 5 (Oncor) confirmed that the addition originated from chromosome 5. Karyotypes were designated as 46, XY, dup(5)(q15q22.1)mat for case 1 and as 46, XX, dup(5)(q15q22.1) for his mother and for case 2. In situ studies with more specific cosmid probes mapped to the possibly duplicated segment are in progress.

We are unaware of published cases with karyotypes identical to these, and we believe case 1 to be the first report of a familial duplication of medial 5q. All cytogenetically similar cases should be examined with FISH for appropriate groupings and comparisons.

Identification of de novo translocations involving 1p36

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We report cytogenetic studies on two children with dysmorphic features and congenital heart disease. L.H., the female product of a 38-wk gestation, has down-slanting palpebral fissures, wide fontanelles, anteverted nares, posteriorly rotated ears, heart disease, and dislocated hips. Her growth has been at the 5th percentile for length but below the 5th percentile for both head circumference and weight. Cytogenetic studies in 1991 found a dark band at the distal end of 1p. Using G-banding, C-banding, NOR-staining, and FISH with a cosmid probe for p58, we reanalyzed her karyotype and found 46, XX, -1,+der(1)t(1;D or G)(p36.1;p12). Physical examination of J.S., the female product of a 37-wk gestation, revealed slight separation of the sagittal sutures and a soft, flat anterior fontanelle. Her ears were small, low set, and posteriorly rotated. Her palpebral fissures were small and downward slanting. A mild-to-moderate tricuspid regurgitation was detected on echocardiogram, and a CT scan showed agenesis of the corpus callosum with secondary enlargement of the lateral ventricles. Initial chromosome analysis demonstrated additional chromatin on the distal portion of 1p. G-banding and FISH studies revealed the karyotype 46, XX,-1,+der(1)t(1;7)(p35;q33). The significance of terminal deletions of 1p will be discussed in relation to the role of the CDC-related protein kinase p58.

Paracentric inversion of chromosome 1 inherited from a mosaic father

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Although reports of documented or inferred parental mosaicism exist, the proportion of parents who are mosaic for chromosome aberrations seen in their offspring is unknown. We report an 8-year-old boy who was referred for genetic consultation because of global developmental delay, growth retardation, and dysmorphic features. Cytogenetic analysis revealed a 46, XY, inv(1)(p31.2p34.3) karyotype. Results of fluorescence in situ hybridization with a total

chromosome 1 probe (Imagenetics) were consistent with the interpretation that it was an intrachromosomal rearrangement. Subsequent cytogenetic analysis of the phenotypically normal father revealed that 7 of 25 cells had the same inversion; the remaining cells were 46, XY. These findings reinforce the need for caution in routine cytogenetic analysis, wherein generally only 5 or 6 cells of 20 are completely analyzed.

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Familial pericentric inv(12)(p11.2q15)

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A 34-year-old primagravida underwent an amniocentesis for low maternal serum alpha-fetoprotein that conferred on her an increased risk for trisomy 21 of 1/152. Her family history revealed that her mother had three miscarriages. Her maternal first cousin had trisomy 21.

Cytogenetic analysis on amniocytes showed a chromosome constitution of 46, XX, inv(12)(p11.2q15). Parental chromosome studies revealed that this inversion was of maternal origin. To our knowledge, this is the first reported pericentric inversion of chromosome 12 with breakpoints at p11.2 and q15.

A complex de novo rearrangement involving three chromosomes in a child with multiple anomalies and unusual hematological findings

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A 3-year-old white male was referred to our institution for easy bruising confined to the lower extremities since infancy. The family history is unremarkable, with healthy parents and six normal siblings. Physical examination revealed a height of 92 cm (25%), weight 151 gm (50%), and FOC 53 cm (95%). Major findings included a large head with prominent forehead; widely spaced, deep-set eyes; a thin, sharp nose with a pointed tip; a small chin; short thumbs with convex nails; dysplastic toenails; and abnormal elbow and kneejoints. He was also noted to have speech delay. Hematological evaluation revealed PT/PTT values in the normal range with a prolonged bleeding time exceeding 15 min.

Because of the abnormal elbow and kneejoints, a Mitomycin C stress test was performed to rule out Fanconi anemia (FA). The chromosome breakage frequency was found to be within the normal range for both the patient and the control. Thus, the diagnosis of FA was ruled out.

However, chromosomal analysis revealed a complex rearrangement between chromosomes 4, 6, and 21 with an apparent balanced carrier male karyotype: 46, XY, t(4;6;21)(p16; p12.1;q21). Both parents have normal karyotypes.

bearing chromosome. Rather, what appears to be intrinsically different about translocation rearrangements and derivative chromosomes involving a fragile site is that chromosome structural fragility may both predispose to chromosome rearrangement and add an additional mosaic dimension to an abnormal karyotype involving a derivative chromosome. The common classes of heritable fragile sites will be described, including folate-sensitive and BrdU-inducible, and methods for eliciting their specific expression discussed. Fragile sites as possible candidates for apparent de novo chromosome rearrangements or deletions will be discussed prospectively. Mosaicism for X-chromosomal aneuploidy in women who experience recurrent pregnancy loss evaluated by fluorescence in situ hybridization

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Cytogenetic analysis of couples referred because of recurrent abortions often reveals X-chromosome aneuploidy in a small number of cells. Confirmation of low-level mosaicism for sex-chromosome aneuploidy by standard techniques is labor intensive. Molecular probes allow for rapid evaluation of large numbers of interphase cells. We report the initial results of a controlled study in which interphase cells from women who have had multiple spontaneous abortions were examined. Controls were women of known fertility who had experienced no abortions. Evaluation was by FISH using X-chromosome-specific satellite probes (Oncor). A maximum of 300 cells per subject were scored from 31 patients and 21 controls. Results support previous observations that habitual aborters have a significantly different frequency of low-level X-chromosome aneuploidy in peripheral blood compared to controls. Specifically, the mean number of cells with two signals was 237 for patients versus 282.0 for controls; the mean number of cells with only one signal (45, X) was 39.8 for patients versus 10.9 for controls. A mean of 20.8 cells in patients and 3.7 cells in controls had no signals. Differences between patients and controls were significant ($P \leq 0.001$ by the Wilcoxin rank sum test) for zero, one, and two signals but not for signal numbers in excess of two. These initial study results support anecdotal reports of low-level mosaicism for sex-chromosome aneuploidy in women with recurrent spontaneous abortions.

Association of a heritable fragile site at 2q13 with fetal demise

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Heritable fragile sites have now been described on most human chromosomes. With the exception of the fragile site at Xq27.3, which is associated with a common form of familial intellectual handicap, few, if any, of these other fragile sites have been clearly associated with any physical abnormality or reproductive liability. We describe here an association between a familial folate-sensitive fragile site at 2q13 and its apparent involvement in chromosome rearrangement in a fetal demise at 10 wk gestation: 46, XY,-18, +der(18)t(2;18)(q13;p11.3). For normal individuals carrying the fra(2)(q13), variation in their chromosome 2 constitution can be demonstrated in peripheral blood lymphocytes cultured under conditions appropriate for eliciting the fragile site. These include whole or partial trisomies for chromosome 2. The inherent reproductive liability of a fragile site does not, however, appear to lie in its ability to produce partial monosomies or trisomies of the fragile site-

Confined placental mosaicism for trisomy 12 in CVS with varying outcomes

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Trisomy 12 observed in chorionic villus sampling (CVS) is often confined to the placenta (CPM). CPM has been observed at different stages of embryonic development and is associated with variable pregnancy outcomes, including spontaneous abortions, intrauterine growth retardation (IUGR), and chromosomally normal births. In this study, we report three cases of trisomy 12 observed in CVS with a normal diploid outcome. Seven dinucleotide repeat polymorphisms for chromosome 12 were used to delineate the mechanism(s) that gave rise to the trisomy and to determine the chromosome 12 origins in the diploid fetus or child. In one case,

trisomy 12 was observed in all cells analyzed, and in two cases trisomy 12 mosaicism was seen. In all three cases, molecular markers revealed normal biparental inheritance in the diploid cell line with varying pregnancy outcomes. Two of the three pregnancies resulted in a normal liveborn. In the third pregnancy, a child was born by Cesarean section at 37 wk because of IUGR and oligohydramnios which resulted in neonatal death. In one case, the molecular results suggest an

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apparent duplication of one paternal chromosome 12 in the CVS. Although predicting the outcome of pregnancies involving CPM remains difficult, molecular techniques are valuable tools for distinguishing uniparental from biparental disomy and may be useful in predicting pregnancy outcomes.

The risk of chromosomal aneuploidy and the sensitivity of ultrasound following an elevated maternal serum alpha-fetoprotein

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Current obstetrical practice has been to offer amniocentesis to women with elevated midtrimester maternal serum alpha-fetoprotein (AFP) to evaluate amniotic fluid AFP level and fetal karyotype. The advent of high-resolution ultrasonography has led some clinicians to question the value of routine amniocentesis for these patients. The rate of chromosomal aneuploidy following an elevated maternal serum AFP with a normal sonogram has been estimated in previous studies to be 0.6%. Our review of the current literature suggests that this rate is as high as 0.89%. This is based on a review of five studies with a total of 1,351 amniocenteses and 12 chromosomal abnormalities. Maternal age was not reported in any of these studies but is assumed to be less than 35 yr for the majority of women being screened for maternal serum analytes. This rate of 0.89% is considerably higher than the well-established risk of 1/270 (0.37%) for a 35-year-old woman to carry a fetus with trisomy 21. Most chromosomal abnormalities were diagnosed in patients who had normal levels of amniotic fluid AFP, suggesting that the anomalies were not associated with birth defects characteristic for elevated maternal serum AFP. We also reviewed the literature on the sensitivity of ultrasound to rule out neural-tube defects (NTD) and other anomalies in patients with an elevated maternal serum AFP level and found the misdiagnosis rate to range from 0% to 1.17% for NTDs and 0.6% to 1.85% for all abnormalities. Investigators that routinely scanned for cranial abnormalities (lemon and banana signs) had lower misdiagnosis rates for NTDs, but they continued to miss non-NTD anomalies. We feel that elevated maternal serum AFP continues to be an indication for amniocentesis, even after a normal sonogram, since the aneuploidy risk is greater than that of a 35-year-old woman (1/270). Mosaicism for trisomy 20 and a marker 20 detected prenatally and studied by FISH
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Additional marker chromosomes occur at a frequency of about 1-2/1,000 prenatal cases and 0.3/1,000 newborns. Today, FISH is being used to identify these markers and provides the possibility of establishing phenotype-karyotype correlations. Chromosomal mosaicism is found in about 2.5/1,000 amniocenteses, with trisomy 20 being one of the most frequently detected. In the majority of cases the trisomy 20 line is confined to extraembryonic tissues but in some cases it is present in specific fetal tissues as well. The carriers are usually phenotypically normal.

We report a prenatal case referred for maternal age in which both trisomy 20 and a marker were found. Chorionic villi, amniocytes, and fibroblasts showed three cell lines: 46, X^Y/47, X^Y,+20/47, X^Y,+mar. Cord blood showed only two cell lines: 46, XY/47, XY,+mar. Parental blood karyotypes were normal. The marker appeared to be a small ring and was identified with FISH using a chromosome 20 library as being derived from this chromosome. In addition to containing a functional centromere, pericentric heterochromatin was shown to be present by C-banding. The baby was born at 35 wk gestation with generalized hypotonia and dysmorphic features, including facial asymmetry. At 16 mo he is failing to thrive and requires a percutaneous gastrostomy tube for feeding.

Several explanations for this rare combination of two chromosomal anomalies in one individual exist. It seems highly unlikely that the two abnormal lines are unrelated. The presence of the mar(20) in one of the parents, undetectable in lymphocytes, could have led to gametic or postzygotic nondisjunction of chromosomes 20. This would imply that the zygote had 48 chromosomes, including three chromosomes 20 plus the marker. It is also possible that the line with the marker developed from the trisomic line when one chromosome 20 was partially deleted, leaving only the centromere and pericentric sequences.

Prenatal diagnosis of a complex chromosome rearrangement involving four chromosomes
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The clinical use of fluorescent in situ hybridization (FISH) has become of significant value in the detection and identification of chromosome translocations and marker chromosomes for which standard banding techniques have been uninformative; the identification of de novo chromosome rearrangements pre- or postnatally; the identification of cryptic deletions; the rapid identification of sex and ploidy in metaphase spreads and interphase nuclei; and, as in the case presented here, the confirmation of complex rearrangements that standard techniques did not completely define. The mother, a 33-year-old known to have a complex chromosome rearrangement involving four chromosomes, 46, XX, t(10;11;14;22), and six previous spontaneous abortions, was referred at 35 wk gestation for evaluation of severe oligohydramnios and intrauterine growth retardation.

An ultrasound examination at 9 wk gestation had shown a fetus with a large cystic hygroma. Saline was injected into the uterus both for visualization of the fetus for possible abnormalities and for fetal blood sampling. After this, saline containing fetal cells was withdrawn. Both the blood and cells were cultured for chromosome analysis. FISH was carried out using whole chromosome and sequence-specific probes. Examination of the fetal chromosomes revealed an unbalanced complement: 46, XX, -11,-14,-22,+rec(11)(11pter→

11q23.3::22q11.1→22qter),+rec(14)(14pter→14q32.3::11q12.2→11qter), +der(22)t(10;11;14;22)(10pter→10q22.1::11q23.3→11qter; 11pter→

11q12.2::10q22.1→10qter; 14pter→14q32.3::11q12.2→11q23.3::22q11.1→22qter; 22pter→22q11.1::14q32.3→14qter)mat. This resulted in trisomy for the interstitial segment 11q12.2→q23.3.

A stillborn infant was delivered at term with the following features: severe IUGR, microcephaly, hypertelorism, flat nasal bridge, low-set ears with malformed helix, an extra chin fold, excess tissue around the neck, increased inter nipple distance, shortened forearms, and overlapping toes bilaterally. An autopsy was denied.

Fluorescence in situ hybridization with a new approach for identification of trisomy 21 in interphase amniocytes using different cosmid probes

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Fluorescence in situ hybridization (FISH) with chromosome 21-specific cosmid clones was used after cytogenetic analysis to confirm the presence of trisomy 21 in amniotic cells from 18 patients. Two new, original region-specific chromosome 21 cosmid clones that map to bands 21q22 and 21qter and a chromosome 21-specific cosmid contig (Zheng et al., 1992) were utilized to accurately diagnose trisomy 21 in interphase amniocytes. The probes differed in their efficacy of hybridization. The number of copies of

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chromosome 21 was correctly identified in 65–75% of trisomic cells. We then compared the efficacy of FISH analysis in interphase cells before and after replication. When cells showing paired hybridization signals on each interphase chromosome in the postreplication period were analyzed, the percentage of informative nuclei increased to 95–97%. This approach permits analysis of cells with overlapping chromosomes, polyploid cells, cells showing a lack of hybridization or excessive background hybridization, and artificial double hybridization signals on separate chromatids in interphase chromosomes. Application of a new set of cosmid probes and specific analysis of hybridized nuclei at different periods of the cell cycle may be helpful for accurate prenatal diagnosis of trisomy 21.

Genetics of early embryo survival in the mouse

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The morphogenetic movements leading to vertebrate gastrulation are well characterized in organisms such as *Xenopus*, the chick, and the mouse. Due to the accessibility and ease of in vitro and in vivo manipulation of the *Xenopus* embryo, a number of molecules with potential roles in mesoderm induction have been identified. However, in most cases, it is unknown whether these molecules are part of an endogenous pathway or if they simply simulate the normal embryonic induction mechanism. The fate map of the early mouse gastrula is similar to that of *Xenopus* and the chick, and homologs of molecules implicated in *Xenopus* mesoderm induction are also temporally and spatially expressed prior to and during mouse gastrulation. Thus, the underlying mechanism leading to gastrulation is likely to be conserved.

We have used a combination of genetic and embryological analyses of radiation-induced deletions to define three regions on mouse Chromosome 7 containing genes needed during early development. Embryos homozygous for deletions that remove the first region, *exed* (extraembryonic ectoderm development), show abnormal growth and differentiation of the extraembryonic ectoderm and are unable to initiate mesoderm production. Deletion of the second region, *msd* (mesoderm deficiency), affects the process of mesoderm induction. The third region, *eed* (embryonic ectoderm development), results in an anterior primitive-streak defect when removed. Molecular analyses have focused on the two adjoining regions, *eed* and *exed*, and have determined that they are less than 165 and 20 kb in size, respectively. Probes from both regions have led to the isolation of candidate cDNAs.

Ribosomal gene location polymorphism in North Carolina populations of the toad, *Bufo terrestris*

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Some eastern North Carolina populations of the southern toad *Bufo terrestris* have been found that display ribosomal gene locations in addition to the normal site on chromosome 1. All toads examined from Nags Head Woods on the Outer Banks had extra sites, but the majority of animals on the mainland did not. The origin of the extra ribosomal gene locations is unknown, but there are a number of possible mechanisms.

Cytogenetic analysis of the neo-XY sex-chromosomal system of collared lemmings

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Karyological studies of collared lemmings, *Dicrostonyx groenlandicus*, have indicated an extraordinary level of chromosomal variation and differentiation. These chromosomal phenomena range from individual mosaicism to interpopulational differences and include a neo-XY sex-chromosomal system. This unusual sex-chromosomal condition is hypothesized to be the result of a centric fusion of each of the homologs of chromosome pair 23 to the X and Y chromosomes, respectively. To test this hypothesis, we have examined synaptonemal complexes at pachynema and meiotic chromosomes at diakinesis and metaphase I. In pachytene nuclei, we observed a variety of sex-chromosomal configurations, including heteromorphic X-Y bivalents and apparent X and Y univalents. The presence of X and Y univalents at pachynema could indicate that these lemmings retain the typical microtubule pattern of asynaptic and achiasmatic sex chromosomes at prophase I and metaphase I. However, the association of the X and Y chromosomes as a chiasmatic sex-bivalent at diakinesis and metaphase I suggests that the presence of X and Y univalents at pachynema is an artifact of structural weakness at or near the sites of chromosomal fusion. These data, therefore, are consistent with the hypothesized neo-XY sex-chromosomal condition in collared lemmings.

How common are common fragile sites?

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Hecht (Hum Genet 74:207–208, 1986) proposed classifying common fragile sites as those present at populational frequencies exceeding 50%. Estimating frequencies of fragile sites minimally requires that fragility be uniformly defined as a statistically valid phenomenon detectable for single individuals. The identification of fragile sites has, however, been inconsistent and arbitrary. Previous statistical approaches are theoretically unsound and require the pooling of data among individuals.

We have developed a statistical model designed for the identification of fragile sites from data for single individuals. For aphidicolin-induced chromosomal breakage for 10 deer mice (*Peromyscus*) from a natural population, this procedure identified 31 different fragile sites. Individuals had a range of 7–15 (average, 10) fragile sites. Sixteen sites (52%) were fragile in single individuals. Only eight sites (26%) were fragile in more than half of the individuals, and only two sites (6.5%) were fragile in all individuals. Comparison of these results to those obtained by pooling the data support the hypothesis that chromosomal-breakage data lack statistical independence. Pooled-data analyses yield biased fragile-site identifications and are inappropriate for inferences of the populational frequencies of fragile sites.

Comparative analysis of fragile sites in two species of *Peromyscus*

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Even though fragile sites have become a frontier in clinical cytogenetics, there is very little information on the basic biology or evolutionary significance of these chromosomal regions. For example, there are no reliable data relative to the rate at which fragile sites evolve. Although there have been a few comparisons of fragile sites among species, these studies have involved inconsistent methodologies or inappropriate taxa.

Deer mice (*Peromyscus*) provide an ideal model for studies of the genetics and evolution of fragile sites. In particular, the *maniculatus* species group provides a set of closely related taxa for which all available data indicate a consistent phylogeny. *Peromyscus maniculatus* and *P. polionotus* are the most closely related species in this group and are the only species of *Peromyscus* that can be successfully interbred. Using a consistent methodol-

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ogy and analysis, we compare data on fragile sites of *P. polionotus* (four individuals) to those available for *P. maniculatus*.

Chromosomal breaks were induced with aphidicolin in short-term spleen-lymphocyte cultures and mapped to G-bands. These data were analyzed using a statistical model for the analysis of fragility from data for single individuals. The data indicated a high degree of consistency between the fragile sites in the two species. The majority of the sites which were significantly fragile in *P. polionotus* have been documented to be common fragile sites in *P. maniculatus*. However, at least two sites that are fragile in *P. polionotus* have not been observed to be fragile in *P. maniculatus*. These data suggest that the complement of common fragile sites can and does evolve at the level of single speciation events.

Premeiotic nondisjunction as a source of aneuploid meiocytes, gametes, and offspring

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The identification of cytogenetic and restriction-fragment length polymorphisms associated with particular chromosomes has greatly facilitated determination of the parental origin of meiotic nondisjunctions that ultimately produce aneuploid offspring. The application of cytogenetic and molecular approaches in studies on monosomy and trisomy indicate that the majority of aneuploidies are of maternal origin, and that most nondisjunctions occur at the first meiotic division. However, the possibility that premeiotic nondisjunction events can produce aneuploid meiocytes, gametes, and offspring is not generally considered.

In my ongoing cytogenetic work with deer mice (*Peromyscus*) and laboratory mice, I have generated large samples of pachytene nuclei stained for electron-microscopic visualization of synaptonemal complexes. The high resolution provided by this approach allows for the detection of small numbers of pachytene nuclei containing extra chromosomes. The incidence of pachytene aneuploidy was higher in oocytes than in spermatocytes from laboratory mice. Furthermore, a review of the cytogenetic literature from humans, laboratory mice, foxes, horses, goats, and cattle reveals that monosomic and trisomic primary meiocytes occur at appreciable frequencies in a variety of mammalian species. These data suggest that premeiotic nondisjunction potentially contributes to the production of aneuploid offspring. The occurrence of aneuploid primary meiocytes and their relative frequencies in oogenesis and spermatogenesis

may in part explain (1) the large proportion of maternally derived trisomies, (2) the reduced or increased recombination (relative to normally disjoined chromosomes) observed in chromosomes that have apparently undergone nondisjunction, and (3) the excess of trisomy 21 males attributable to paternal first-division nondisjunction.

and to map the genes *Tp53* and *Tk-1* in this species. While there are eight known linkage groups, no gene/chromosome map locations are known in *Peromyscus*. We were also curious to learn whether the homologous *Tp53* and *Tk-1* loci were linked in the deer mouse as they are in mouse, rat, and human. A cosmid library containing 2,016 clones of *leucopus* (white-footed mouse) genomic DNA was obtained courtesy of Dr. Robert Baker (Texas Tech University), and 20-kb *p53* and 9-kb *TK-1* clones were selected. These clones were biotin labeled by nick translation and applied to chromosome preparations using standard FISH technology. Sites of hybridization were scored by detection of a paired fluorescent signal on the chromosome. Photographs of the FISH chromosomes were compared with G-banded photographs previously made of the very same preparation. The results showed the *Tp53* and *Tk-1* probes to hybridize at chromosome 13.2, with 71–1 being slightly more distal. Our findings show that FISH technology can easily and effectively be applied to *Peromyscus* gene-mapping studies. Further, this is the first report of chromosomal assignment of linked unique sequence genes in *Peromyscus*.

The X chromosome of the Indian muntjac: subunit repeats or multiple centromeres?

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The centromeres of the X chromosome and chromosome 1 of the Indian muntjac are extraordinarily long structures. Generally, these centromeres exhibit an antikinetochores antibody reaction along their entire length. This has led to the suggestion that several subunits of a continuous DNA fiber make up the centromere (Zinkowsky et al., *J Cell Biol* 113:93, 1992). We analyzed the two centromeres by using antikinetochores antibody and by extending the centromere with 5-bromodeoxyuridine or bisbenzimidazole. An analysis using antikinetochores antibody reveals that these centromeres are made up of an array of several tandemly arranged segments; a similar observation has been reported for a rat centromere complex which evolved in the test tube (Pawletz et al., *Cancer Genet Cytogenet* 42:75, 1989). These unit centromeres are interrupted by noncentromeric DNA which stays condensed after treatment with bisbenzimidazole. The interphase centromere shows even more subunits. Electron microscopy reveals at least three distinct trilamellar kinetochores structures in the X chromosome. We propose that at least some centromeres of the Indian muntjac genome are compound structures made up of multiple centromeres. Species like the mouse, rat, and human do not display subunits of the centromere-kinetochores region.

Gene mapping in *Peromyscus* using fluorescence in situ hybridization

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The deer mouse (*Peromyscus maniculatus*) represents a subfamily (Sigmodontinae) of murid rodents, native exclusively to the Western hemisphere, whose ancestry diverged from that of Eurasian rodents, represented by *Mus* and *Rattus* (subfamily Murinae), at least 16 million years ago. All *Peromyscus* species have $2n = 48$, with known chromosomal variation entirely attributable to pericentric inversions and heterochromatic additions (Stangl and Baker, 1984). A karyotype standard for the genus is currently under development (IF. Greenbaum, personal

communication). A partial recombination map for the deer mouse contains eight linkage groups with 23 marker loci (Dawson and Rogers, 1993).

The objective of our study was to apply the newly developed fluorescence in situ hybridization (FISH) technology to *Peromyscus* gene mapping efforts

Minor satellite in mouse is not associated with centromere activity

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Minor satellite in mouse has been implicated to be the centromere DNA and is found linked to the telomeres. We report that in CUD ceils some chromosomes carry up to three sites of minor satellite but only one centromere per chromosome. Hence, this fraction is not always associated with the centromere. Upon treatment with bisbenzimidazole, the major satellite fraction decondenses, but, apparently, the sites carrying the minor satellite are not affected. The chromosome splits at the latter after the major satellite-carrying region has already split. This might indicate that the two types of satellites replicate at different times, the regions carrying minor satellite replicating the last in the genome. Besides, the minor satellite is not always linked to the telomere in chromosomes exhibiting Robertsonian translocations, nor even in some acrocentric chromosomes. These and other data indicate that the centromeres of various species might not carry "private" centromere sequences and that the centromere owes its properties to some factor (e.g., the stereophysical properties of a few bases underlying the centromere) rather than to the so-called centromere sequences.

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Aneuploidy in offspring of female mouse chimeras transmitting a male genotype

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By injecting male embryonic stem (ES) cells into the inner cell mass of mouse blastocysts having a female genotype, it is possible to produce female chimeras capable of transmitting the (injected) male genotype. We have examined the karyotypes of 15 male and three female mice derived from the XY component of eight transmitting female chimeras. Six of the males were originally ascertained because of sterility. Only 5 of the 13 males had normal karyotypes. Eight exhibited nonmosaic aneuploidy, including four XXY karyotypes, three XYY karyotypes, and one trisomy 3 karyotype. One male appeared to have a low-frequency mosaicism involving an aneuploid cell lineage, and another was diploid, with an i(Y) replacing the normal Y chromosome. All three of the females had normal karyotypes. In the subset of animals for which adequate breeding records were available, nearly half of the males had abnormal karyotypes. Offspring of male chimeras were not included in this study. Nevertheless, the fact that offspring of male chimeras are rarely infertile suggests that sex-chromosome aneuploidy is uncommon in them and that most of the aneuploidy observed in our mice is the result of compromised XY pairing/segregation during female meiosis.

Mouse Chromosome 6 in Rb translocations: segregation and sex-ratio distortions

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Previous studies (Hum Genet 87:278, 1991; Hum Genet 90:79, 1992) have shown that sperm bearing the Rb(6.16) translocation have impaired fertilizing ability, compared to their chromosomally normal reciprocals, particularly when sperm are unaged in sexually active males. Further, the impairment which has been attributed to an alteration on Chromosome 6 is potentiated in X-bearing sperm, leading to a skewed sex ratio. The present study aimed at determining if the distortions are seen in other translocations, with or without Chromosome 6. First-cleavage metaphases, resulting from chromosomally normal females that were mated to sexually active or rested Rb(6.15) or Rb(2.8) male carriers, were sequentially G- and C-banded. For unaged sperm from the Rb(6.15) males the ratio of normal (90) to balanced (37) zygotes deviated significantly ($P < 0.001$) from 1:1, as did the sex ratio, 46 XX:81 XY ($P < 0.01$). The latter distortion was related to a deficiency of X-bearing sperm with the translocation (8X:29Y). Both segregation (69:50) and sex-ratio (51 X:68 Y) distortions disappeared for aged sperm, and a contingency table showed that segregant type was related to sperm age ($P < 0.05$). For the Rb(2.8) there was a 1:1 ratio of normal to balanced (65:46) zygotes from unaged sperm, and although the ratio from aged sperm was 62:39, there was no relationship between segregant type and sperm age ($P > 0.05$). There was also no sex-ratio distortion for either unaged or aged sperm ($P > 0.05$). The results suggest that the presence of Chromosome 6 in Rb translocations specifically affects sperm maturation and sex-ratio distribution.

Replication analysis of X-linked genes in females by fluorescence in situ hybridization (FISH)
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The time a gene is replicated during DNA synthesis seems to influence or be influenced by its transcriptional status. Active autosomal genes replicate early, whereas inactive genes frequently replicate later (Goldman et al., Science 224:686, 1984). Although the inactive X chromosome replicates later than its homolog, little is known about the replication of specific X-linked genes. We have used a modification of the FISH assay recently described by Seligetal. (EMBO J 11:1127, 1992) to study replication of X-linked genes in female fibroblast cells. In this assay, a replicated locus is seen as a double signal (D), and an unreplicated locus is seen as a single hybridization signal (S). The percentage of doublets is used as a measure of relative time of replication. The loci studied include: F8C, normally not expressed in fibro-blasts; HPRT and FMR1, two housekeeping genes expressed only from an active X; and XIST, which is expressed only from an inactive X chromosome. Three replication patterns are possible. The homologous loci could be: (1) early replicating on both chromosomes (D/D); (2) late replicating on both chromosomes (S/S); or (3) asynchronous, with one locus replicating early and the other late (D/S). We did not observe a predominant D/D pattern with any loci studied. At the F8C locus most cells (72%) were S/S, indicating that the locus replicates late on both active and inactive chromosomes. At the FMR1 and HPRT loci, the predominant pattern (61% and 68%, respectively) was asynchronous (D/S). At the XIST locus, the predominant pattern was also asynchronous (65–71%). Although our assay cannot distinguish the active X from the inactive X, we can draw conclusions based on our previous studies of males (active X only). As FMR1 and HPRT replicate early in males, the replicated locus in females must also be on the active X. However, because XIST replicates very late in males, the replicated locus in females is on the inactive X chromosome. Interestingly, even when the locus was silent on both chromosomes (F8C), a moderate degree of asynchrony was still observed (25%), indicating that small differences between the X chromosomes remain. Current studies are focusing on the replication

of these loci on active, inactive, and reactivated X chromosomes isolated in hybrids, to provide insights into the relationship between replication and transcription.

A rapid method to amplify DNA fragments for DNA analysis from cells fixed in Carnoy

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The need for molecular analysis is often not appreciated until tissue specimens have already been fixed and the patient is no longer available. Thus, the development of techniques that allow retrospective analysis of archived tissues is clearly warranted. We report a method for fast and efficient polymerase chain reaction (PCR) amplification of specific target DNA sequences from cells fixed by routine cytogenetic methods in 3:1 methanol:acetic acid (Carnoy's fixative). The fixed cells had been maintained at -20° C from a few weeks to 6 yr. Primer sets corresponding to different loci on an autosome (Rb1), as well as the X (DMD) and Y (SRY) chromosomes, were used. Sizes of the amplified products ranged from 251 to 609 bp. No differences in amplification products were found between DNA obtained from fresh tissues and cells fixed for varying lengths of time in Carnoy's fixative. This technique has the following advantages: (1) it allows the retrospective study of genetic disorders from archived specimens, (2) it requires only a limited number of cells, (3) it is rapid and simple, and (4) it avoids the contamination problems inherent in multistep procedures for extraction of the DNA.

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Pediatric brain tumors: the "p's" and "q's"

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Pediatric brain tumors are the second most common group of malignancies in childhood, and yet little is known regarding the genetic basis for tumor development or progression. We have undertaken a combined approach, including analysis of detailed family history and cytogenetic, molecular, and FISH studies of primary tumors, in an effort to define regions of the genome that may contain disease genes. Cytogenetic studies of pediatric brain tumors have demonstrated nonrandom abnormalities in several different histologic subtypes. The most common malignant brain tumors in children are primitive neuroectodermal tumors (PNETs), or medulloblastomas. At least one third of PNETs contain an i(17q), and an additional 20% of cases demonstrate deletions of chromosome 17. Molecular and FISH studies have confirmed the cytogenetic findings and have helped narrow down the region of chromosome 17 that is deleted to the distal portion of 17p13.3. These studies therefore implicate the presence of a tumor-suppressor gene in that region. Several other chromosomes have also been shown to be nonrandomly involved in PNETs, including chromosomes 6, 7, and 11 and the X and Y; it is therefore possible that we will eventually be able to subgroup PNETs based on the cytogenetic findings.

Two other types of malignant brain tumors, rhabdoid tumors and glioblastoma multiforme, appear to have nonrandom cytogenetic abnormalities involving chromosomes 22 or 7, respectively. Children with glioblastoma or rhabdoid tumors have a worse prognosis than those with PNETs, and it is important to treat them very aggressively. Since it is often hard to distinguish among these tumors by light microscopy alone, cytogenetic studies have become a useful aid in diagnosis. A summary of our recent cytogenetic and molecular studies in pediatric brain tumors will be presented.

Comparative classical and molecular cytogenetic analysis of oral squamous cell carcinoma
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Oral squamous cell carcinoma (OSCC), the sixth most common cancer worldwide, is known to be caused by tobacco and alcohol use. Frequent numerical alterations in chromosomes 1,3,7, and 11 have been reported in head and neck cancer. We are investigating OSCC using genetic techniques, including karyotyping and fluorescence in situ hybridization (FISH). PCI: SCC-029B was a moderately differentiated SCC of the buccal mucosa in an 84-year-old man who smoked cigarettes and chewed tobacco. Direct harvests were carried out on biopsies of tumor (DHT) and nearby margin (characterized histologically as severe epithelial dysplasia, DHM). Passage 2 tumor cells (P2CH) were harvested 8 wk after culture initiation. Classical cytogenetic analysis of cultured cells showed a complex karyotypic pattern, with hypotriploid and hypotetraploid subpopulations. Dual-color FISH was performed on cells from the three harvests using α -satellite probes to chromosomes 1, 3, 7, and 11 (Oncor).

Interphase FISH revealed diploid complements of these chromosomes in 85% of cells from the DHM, 27% of cells from the DHT, and 27% of P2CH. FISH on the harvests of direct and cultured tumor cells with probes to chromosomes 1 and 3 showed two subpopulations, one with three signals each and the other with four chromosome 1's and three chromosome 3's. Probes to chromosomes 7 and 11 showed six and four signals, respectively. These FISH data correspond well to the composite karyotypes of the cultured tumor cells. In addition, they illustrate that (1) little numerical karyotypic evolution of these chromosomes transpired from direct harvest to passage 2 in culture, (2) clonal selection has not occurred, and (3) interphase FISH accurately reflects the actual karyotypes of the cells.

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Chromosome analysis of nine endocrine neoplasms of the pancreas

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Endocrine neoplasms of the pancreas differ from the more common adenocarcinomas of the pancreas, not only in histologic appearance but also in clinical presentation and biologic behavior. Chromosomes were analyzed from nine fresh pancreatic endocrine neoplasms. Clonal chromosomal abnormalities were found in five; all were malignant neoplasms. One showed only a loss of the Y chromosome, and another had a small triploid population of cells in addition to a normal mainline, with a karyotype of 61-66, XX,-X,-1,-2,-3,-4,+5,-6,+7,-11,-14,+17,+18,+20,+M1x2,+M2inc. Three neoplasms had near-haploid clones. One neoplasm had a composite karyotype of 31-36 < n > X,+1,+3,+5,+7,+9,+10,+17,+18,+mar. Two were from the same patient, who had the autosomal dominant syndrome MEN-1. The same clone, described as 29 < n > X,+add(1)(p12),+5,+7,+8,+18,+19, was found in both the primary pancreatic neoplasm and the metastatic tumor. To our knowledge, this is the first report of a haploid clone in both a primary and metastatic solid tumor and suggests that the near-haploid state is at least compatible with metastasis. These data, combined with the limited reports of cytogenetic data from endocrine pancreatic neoplasms, suggest that at least half of such neoplasms will have an abnormal karyotype.

Further definition of 20q deletion in acute myeloid leukemia using multicolor FISH

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Interstitial deletions of the long arm of chromosome 20 have been one of the consistent abnormalities in myeloid disorders and have led to suggestions that the deletion results in a loss of a tumor-suppressor gene(s) that could provide a proliferative advantage to myeloid cells. Through in situ hybridization mapping, several investigations have delineated the region of deletion to be between 20q11.2 and 20q12 and flanked on the proximal side by RPN2 and distally by D20S17. There was a consistent loss of ADA and SRC (Roulston et al., Blood 82:3424, 1993). We have been actively involved in constructing a genetic map of chromosome region 20q12→q13.1 and mapping the location of the locus for maturity-onset diabetes of the young (MODY). To further define the 20q deletion, we used FISH to identify the presence or absence of the probes HCK, SRC, ADA, TOP1, PLC1, and 20S17 in five AML patients with 20q-. The probes were labeled with biotin or digoxigenin and hybridized individually or in combination.

At least 20–30 metaphase and/or interphase cells were analyzed from each slide. All probes were found to be deleted in the abnormal chromosome 20 in each patient, with the exception of HCK, which mapped pericentromeric 20q11 and was not deleted. Deletions of SRC, ADA, and 20S17 confirms previous observations. The loss of PLC1 (phospholipase C) and TOP1 (topoisomerase 1) in AML is reported for the first time. Of interest is PLC1, an enzyme that catalyzes inositol phospholipids to diacylglycerol and inositol triphosphate. These then act as second messengers and directly affect cell division and growth. Both PLC1 and TOP1 are known to be involved in cell

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replication and proliferation, and loss of either or both may play a vital role in the progression of the myeloid leukemia.

tent cell line of 45, XX, der(1), der(7), del(14q), der(16),-22. In all three cases, the breakpoint on chromosome 14 appears to be in the region q23→q24. This may designate an area of the genome important in progression from benign to malignant meningiomas.

An abnormal clone with monosomy 7 and trisomy 21 in the bone marrow of a child with congenital agranulocytosis (Kostmann disease): possible evolution towards myelodysplastic syndrome after treatment with granulocyte colony-stimulating factor

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Congenital agranulocytosis, or Kostmann disease (KD), is a rare autosomal recessive condition characterized by severe neutropenia, maturation arrest of neutrophil precursors at the promyelocytic stage, and frequent infections leading to death in the first year of life. Cytogenetic analyses of a few patients were normal. Pharmacological doses of human granulocyte colony-stimulating factor (G-CSF) increase the number of neutrophils, decrease the number of infections, and prolong survival in KD patients, implying that the basic defect could be associated with G-CSF itself or with G-CSF receptors. We present a case of KD diagnosed in an

infant born in 1981 with severe neutropenia. He had a normal bone marrow karyotype in 1989 prior to initiation of long-term maintenance therapy with G-CSF. Five years later, at age 12 years, he was evaluated because of the recent onset of anemia, thrombocytopenia, progressive splenomegaly, and trilineage dyspoiesis in the bone marrow. Analysis of GTG-banded bone marrow metaphase cells revealed an abnormal mosaic pseudodiploid karyotype: 46, XY,-7,+21[11]/ 46, XY[7]. The emergence of an abnormal clone with monosomy 7 and trisomy 21, in combination with trilineage dyspoiesis, suggests the diagnosis of myelodysplastic syndrome, which could be secondary to treatment with G-CSF or it could be a natural evolution toward a preleukemic state in KD with prolonged survival. Further followup of this patient and similar cases are essential for understanding this observation.

Cytogenetic findings in posttransplant lymphoproliferative disorder

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Posttransplant lymphoproliferative disorder (PTLD) consists of a spectrum of Epstein-Barr virus (EBV)-related, predominantly B-cell lymphoproliferations that occur at an increased frequency in organ transplant recipients as a complication of immunosuppression. Trisomies 3, 9, 11, 12, and 14 have been reported as nonrandom cytogenetic anomalies in a few PTLDs. Our prior preliminary data suggested a high frequency of nonclonal cytogenetic abnormalities in PTLDs. From 42 transplant recipients, we have now accrued 65 specimens, including fresh tumors and spontaneous outgrowth lymphoblastoid cell lines derived from PTLD lesions or peripheral blood lymphocytes. Metaphase cells were obtained from 39 specimens (60%) from 19 patients. Specimens from five patients (27%) had normal karyotypes, five expressed nonclonal abnormalities only, and nine had both clonal and nonclonal karyotypic aberrations. Thus, nonclonal abnormalities were seen in 14 of 19 patients (74%). Numerical changes were more frequent than structural aberrations, with three patients each expressing +3, +13, or +19 and two patients each expressing +12 or partial trisomy 6. Trisomies 9 and 14 occurred together as a clonal finding in one case. Chromosomes 1, 8, and 14 were most frequently involved in structural aberrations. Specifically, the breakpoint 14q32 was involved in translocations in 4/19 (21%) patients, two of whom had a clonal t(8;14)(q24;q32). In addition, 8q24 was involved in three nonclonal translocations in two patients. Collectively, 8q24 and/or 14q32 were rearranged in 6/19 (32%) patients. Nonclonal chromosome abnormalities and rearrangements of 8q24 and 14q32 appear to play key roles in the development and progression of PTLD.

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Structural abnormalities of chromosome 14 in meningiomas: evidence for a progression gene

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Most meningiomas are benign tumors that arise from the arachnoid covering of the brain. Only 10–20% are atypical or malignant. Loss of chromosome 22 is a consistent finding in typical (benign) meningiomas and may play a role in the initiation of benign tumors. However, in our cytogenetic analyses (as well as others') of atypical and malignant meningiomas, chromosome 14 is frequently lost or structurally abnormal. Here we report three cases of meningiomas with structural abnormalities of chromosome 14.

Case 1 is from a 52-year-old male with two apparently separate tumor foci. One tumor, located superficially in the frontal cortex, revealed a karyotype of 42, X,-Y,-10,-14,-22. A second tumor located deeper in the frontal cortex revealed a karyotype of 48, X,-Y, der(1)t(1;6),+5, der(6)t(1;6),+11,+12,-13, der(14)t(13;14),+20. Pathologic diagnosis was consistent with malignant meningioma. Case 2 is a meningioma from a 79-year-old female. Although there was focal nuclear atypia on histopathologic examination, this was thought to be due to preoperative embolization and thus was diagnosed as a typical meningioma. Thirteen of 16 cells analyzed were abnormal. Consistent abnormalities included +der(1), der(1), del(4p), der(6), der(7), add(8q), add(9p), add(11q), der(12), del(14q), der(17), add(17p),-19,+20,-22. Case 3 is a tumor from a 41-year-old female and was diagnosed as an atypical meningioma. Cytogenetic analysis of 26 cells revealed a consis-

Detection of numerical chromosome abnormalities in interphase cells of ovarian carcinomas using fluorescence in situ hybridization

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For the past 30 years cytogenetic studies of various cancers have shown that recurrent chromosome abnormalities can be used for basic diagnostic purposes. In addition, some of these specific chromosome rearrangements have been useful in identifying genes involved in the development and progression of neoplasia. Recently, advances in molecular cytogenetic techniques have been instrumental in the identification of complex chromosome rearrangements from metaphase cultures, as well as the analysis of certain chromosome aberrations directly from interphase cells. We have initiated a study to identify recurrent chromosome changes in ovarian cancers using fluorescence in situ hybridization (FISH). Both fresh and frozen tissue samples are enzymatically disaggregated, and interphase cells are analyzed by FISH using α -satellite centromere sequences for direct chromosome enumeration. In addition, short-term cultures are initiated from fresh samples for subsequent metaphase chromosome studies.

To date, we have analyzed the centromere copy number of 20 primary ovarian carcinomas using α -satellite probes to the centromeres of chromosomes 1-4, 6-12, and 16-18 and the X chromosome. Twenty-five percent

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(5/20) of these samples demonstrate specific monosomy for one or a few specific chromosomes. The most frequent of these monosomies include centromeres 4, 17, and 18. Another 60% (12/20) of samples show a general increase in ploidy (three to four copies) for most or all the centromeres examined. In a subset of these samples (3/12), the centromere of chromosome 8 was found at increased copy number relative to other centromeres tested. In the remaining samples (3/20), no apparent changes in copy number were found for any of the tested centromeres. From cultured cells, G-banded karyotypes and chromosome specific painting probes are being used to examine specific chromosome aberrations as well as amplification of the cellular oncogenes *Her1/neu* and *c-myc*, and loss of the tumor-suppressor gene *p53*. We are also compiling family histories of patients in order to correlate specific genetic changes with possible familial or heritable ovarian cancer syndromes.

Simultaneous detection of numerical chromosome abnormalities and affected cell lineage

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To identify marrow-cell lineages containing numerical chromosome abnormalities, we have developed a method that combines immunophenotyping and fluorescence in situ hybridization (FISH). Nucleated cells were separated from marrow on Ficoll gradients. These cells were fixed and applied to slides according to methods specific for the cell lineage of interest. Slides were then probed with commercially available α -satellite probes labeled with digoxigenin. The presence of lineage-specific antibodies was detected with a biotin-avidin-FITC system, and probe hybridization was detected with a rhodamine anti-digoxigenin system. Cells bearing the targeted antibody exhibited green fluorescent cytoplasm and red nuclear signals corresponding to the number of copies of the targeted chromosome. In addition to allowing one to score the chromosome number in a specific cell lineage, the elimination of a cell-culture step in this method helps avoid artifacts attributable to discrepant in vitro growth rates for the various cell lineages.

Some of the questions generated by FISH studies: answers, theories, and more questions

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The use of fluorescence in situ hybridization (FISH) techniques as a supplement to cytogenetic studies has recently been accepted by most clinical and research cytogenetic laboratories. We are in a stage of rapid data collection, yet interpretation of results can sometimes be less than straightforward. While many of the commercially available chromosome- or locus-specific probes generate easy answers to previously difficult questions, sometimes data obtained by either metaphase or interphase analyses pose new potential dilemmas regarding clinical interpretation. Several examples will be presented where the FISH results were discordant with classical cytogenetic results.

In a series of prostate tumors, cells were evaluated at various stages of culture, and FISH results indicated that large abnormal populations were present that were missed during classical cytogenetic evaluation. Cytogenetic changes, however, were obviously not detected by FISH when probes at the affected loci were not used. In an initial study of peripheral blood from patients with trisomy 18 with a "stigmata phenotype," one patient showed interphase cells that did not correspond to metaphase data: up to 30% of interphase cells revealed disomy, while 100% of metaphase cells were trisomic. In a male sex-mismatched bone-marrow transplant patient in relapse, XX cells were only detected in metaphase preparations, whereas interphase cells showed predominantly XY cells. Our laboratory has more than 300 cases using FISH technology, most of which gave clear and meaningful results that complemented cytogenetic studies. In addition to interphase analyses, FISH has proven to be an invaluable method for the detection of cryptic deletions and translocations. The few discordant examples noted certainly do not detract from the decipherable majority, yet should not be disregarded. The combination of FISH and classical cytogenetic studies thus has raised questions that could have wide-spread implications regarding cell-culture conditions, clonality, and mosaicism. The value of combining these procedures is enormous, yet caution must be given prior to interpretation.

Cytogenetic analysis of familial paragangliomas

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Familial paragangliomas (FPs) of the head and neck are slow-growing tumors that originate from neural-crest cells. Between 7% and 9% of these tumors are inherited, with an autosomal dominant mode of inheritance with incomplete penetrance. Familial paragangliomas exhibit genomic imprinting. Individuals that inherit the gene for FPs from a male express the phenotype, whereas individuals with maternal inheritance do not. The gene has been postulated to be located at chromosome region 1 lq23—> qter. The original first-generation carrier in our large three-generation family was a male that transmitted the gene (with two separate spouses) to 8 of 16 offspring. Approximately 20 individuals in the third generation are at a high risk of developing tumors.

To our knowledge, there has been only one report on the chromosome complement of FPs of the head and neck. That study yielded only normal karyotypes. We have begun a cytogenetic analysis of lymphocytes and solid tumors from three affected individuals to identify chromosome abnormalities particularly of the 1 lq23 region. Tumor cells were cultured using a modification of Zaslav et al. (1991). All lymphocytes and two of the three tumor cell cultures were chromosomally normal. Tumor cells from the third patient yielded normal karyotypes and nonrandom abnormalities affecting chromosomes 5 and 7.

The growth of tumor cells in culture is a formidable task. We have developed a culture system for the successful growth of FPs. Our study is in its preliminary stages, and we are in the process of modifying culture techniques to further optimize tumor growth. We anticipate that future cytogenetic analyses will yield significant data and contribute to the localization of this gene. Tamoxifen induces hepatic aneuploidy and mitotic spindle disruption after a single in vivo administration to female Sprague-Dawley rats

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Tamoxifen has found extensive use in the treatment of all stages of human breast cancer. The efficacy of tamoxifen treatment for the prevention of second primary tumors and its chemosuppressive action in animal models has led to initiation of clinical trials to test the efficacy of tamoxifen for prevention of this disease in humans.

Recently, tamoxifen has been shown to induce hepatocellular carcinomas in rats. To address the mechanism of induction of these tumors and to assess the possibility of risk of cancer development from tamoxifen treatment, female Sprague-Dawley rats (five rats per treatment) were administered

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tamoxifen at doses ranging from 0.3 to 35 mg/kg. One day after treatment, the rats were sacrificed, and the hepatocytes were isolated and cultured for 50 h. Colcemid was added 3 h prior to harvest, and the hepatocytes were prepared for karyotypic evaluation. One hundred metaphase spreads were examined per animal. Tamoxifen treatment resulted in the induction of aneuploidy in approximately 70% of the examined hepatocytes. In addition, premature condensation (2–10%) and endoreduplication (5–10%) were observed in hepatocytes of rats treated with tamoxifen. Furthermore, exchanges between chromosomes, as well as chromosome breakage,

were observed. Examination of the cultured hepatocytes from rats treated with tamoxifen by electron microscopy demonstrated both unipolar spindles and incompletely elongated spindles. Exposure of rats to a single in vivo dose of tamoxifen produces multiple changes in rat hepatocytes, including clastogenic damage at doses comparable to that administered to humans. The demonstration of aneuploidy induction, premature condensation, chromosome breakage, and improper mitotic spindle formation indicates that the risk-versus-benefit ratio of tamoxifen treatment should be carefully evaluated.