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LaDonna Immken (Chairperson) Arthur Brothman Susanne Golini James Mascarello Stuart Schwartz Urvashi Surti Doris Wurster-Hill

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FRAGILE X SCREENING VS FAMILY STUDIES (ESPECIALLY IN FEMALES):
LABORATORY PROCEDURES/GUIDELINES. Patricia N. Howard-Peebles, Genetics & IVF Institute, Fairfax, VA & Medical College of Virginia, Richmond.
Testing for fragile X in the clinical laboratory is one of the 3 major frustrating aspects of the fragile X syndrome. Cytogenetic testing is considered to be accurate in affected males when basic guidelines are followed [International Workshop (AMG, in press) & ACT (Karvouniaris 15(6):131–135, 1989)]. However, in females, the test is about 50% accurate. These data were generated with the same culturing techniques used for screening. Recent data from my laboratory indicate that family studies (especially in female family members) should employ extra media/inducers to detect low expression.
Family V09 was ascertained by an 11-month-old affected male. The sister of the proband tested positive (13%) whereas his mother was negative. One normal female (maternal aunt of proband) and one affected female (in a widely separated branch of the family) were found to be low-expressors. Each showed no expression in 4+ media/inducer systems; all positive cells were found in 2 systems, 1 of which was common to both (RPMI1640 + 1 x 10^{-4} FUdR + 600 mg/L thymidine). Preliminary data indicate that daughters of transmitting males do not express using these same systems. Since many factors differ in fragile X families, some families may require the use of other media/inducer systems. Currently, other non-expressing females in this family (3) & other families are being retested utilizing this 3-system protocol.

Because fragile X [fra(X)] chromosome analysis is a labor intensive and time consuming procedure for which a high level of accuracy is necessary, a reliable internal control system to assure successful induction of the fra(X) chromosome would be indispensable. It has been suggested that scoring for other folate sensitive fragile sites could provide such a control.
A total of 57 fra(X) positive individuals (41 males; 16 females) and a total of 139 fra(X) negative randomly selected patients (108 males; 31 females) were compared with regard to other folate sensitive fragile site expression (6q26; Xp22; 3pl4; lp31 and 16q23).
Other folate sensitive fragile sites were found in less than 50% of fra(X) positive patients, and no significant difference in expression of other folate sensitive fragile sites was found in fra(X) positive and negative patients; therefore, the use of the expression of other folate sensitive fragile sites as a control is of no value, further compounds the difficulty of the analysis and should be eliminated.

THE EFFECT OF DIALYZED FETAL BOVINE SERUM ON FRA(X) EXPRESSION AND MITOTIC INDEX; THE EFFECT OF FOLIC ACID ON FUdR-INDUCED FRA(X) EXPRESSION. E.C. Jenkins, C.J. Duncan, H. Gu, M. Genovese, M.S. Krawczun. Institute for
Basic Research, New York State Office of Mental Retardation and Developmental Disabilities, Staten Island.

Short-term whole blood cultures from 5 unrelated male individuals with the fragile X syndrome were exposed to 10-7 M 5-fluorodeoxyuridine (FUdR) during the last 24 hours of a 4 day culture period. Half of the cultures contained 15% fetal bovine serum (FBS)(GIBCO) and half contained 15% dialyzed fetal bovine serum (DFBS). The complete medium included RPMI-1640. After analyzing at least 100 cells per variable, it was clear that the fragile X frequency was higher in all cases where the complete medium had been supplemented with DFBS. In two of the cases, the fragile X frequency more than tripled while in two others, it virtually doubled. In one case, when the fragile X frequency nearly tripled, from 12 to 33%, the mitotic index doubled in the DFBS cultures. In all other cases, although the fragile X frequency increased in the DFBS cultures, the mitotic index decreased. Whether the reduction in mitotic index suggests an inverse correlation between reduced mitotic index and increased fragile X expression will be determined by additional studies. Finally, when DFBS cultures from two other fra(X) individuals were exposed to increasing folic acid concentrations ranging from 2 to 4,000 x 10^-9 M, there was virtually no change in fra(X) expression. In conclusion: (1) medium supplementation with dialyzed fetal bovine serum should be considered when using FUdR for fragile X identification in order to avoid potentially false negative results; (2) there appears to be no direct correlation between increased mitotic index and increased fragile X expression in whole blood cultures; (3) increased folic acid concentrations do not affect fra(X) expression when FUdR fragile X induction is employed; therefore requesting people to refrain from taking vitamins, including folic acid, before fragile X testing appears unnecessary.

APHIDICOLIN INDUCED FRAGILE SITES IN DEER MICE (PEROMYSCUS MANICULATUS). Bryant F. McAllister and Ira F. Greenbaum. Department of Biology, Texas A&M University, College Station, TX 77843.

Although well investigated in terms of their clinical implications in humans, there is little information available relative to the basic biology of chromosomal fragile sites. In order to broaden this perspective, we have recently begun a series of projects using deer mice (Peromyscus) as a model to investigate the population genetic parameters and potential phylogenetic implications of chromosomal fragile sites. To induce chromosomal breakage, spleen lymphocytes from wild-caught P. maniculatus were treated with aphidicolin. The observed gaps were mapped to G-bands referenced to the standardized karyotype for Peromyscus. From preliminary data, the distribution of the breakpoints revealed the apparent existence of fragile sites in these mice. To assess the efficacy of current analytical procedures, alternative statistical approaches were used to test for breakage induction at frequencies significantly in excess of those expected under the assumption of randomness. These approaches were used to examine intraindividual variation for the occurrence of fragile sites and to determine if there is a coincidence between fragile sites and sites of populational and phylogenetic chromosomal rearrangements.

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OCURRENCE OF INDUCED CHROMOSOMAL BREAKS IN EUCHROMATIN VS. HETEROCHROMATIN. Ana M. Dominguez and Ira F. Greenbaum. Department of Biology, Texas A&M University, College Station, TX 77843.

As part of our research into the basic relevance of chromosomal fragile sites, we are investigating the relationship of heterochromatin and induced chromosomal breakage. In order to test for differences in the rate of induced breakage in euchromatin vs. heterochromatin, aphidicolin was added to spleen-lymphocyte cultures from cactus mice (Peromyscus eremicus). This species was chosen because approximately 38% of its karyotype is C-band positive heterochromatin distributed in the short arms of the entirely-biarmed chromosomal complement. To determine (and avoid) any bias from the effects of C-banding on the visualization of chromosomal gaps, metaphases were scored both before and after C-banding. Preliminary data indicate that C-banding does diminish the visibility of chromosomal gaps and that induced breakage occurs at greatly reduced rates in the heterochromatin as compared to the euchromatin of cactus mice.

USE OF CYTOGENETIC AND BIOCHEMICAL MARKERS IN THE GENETIC MANAGEMENT OF A SQUIRREL MONKEY BREEDING AND RESEARCH COLONY. C. M. Moore U, J. L. VandeBerg U, S. Williams-Blangero 2 and C. R. Abee 3. 1Dept. Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX 78284, 2Dept. Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX 78284, 3Dept. Comparative Medicine, University of South Alabama Mobile, AL 36688.

Cytogenetic and biochemical polymorphisms have been identified in a squirrel monkey (Saimiri boliviensis and S. sciureus) breeding and research colony located at the University of South Alabama. These polymorphisms have been used in colony management to confirm the species and subspecies identities of colony members, to identify individuals of mixed ancestry, to maintain accurate pedigrees, to minimize inbreeding, and to maintain genetic variability. Four cytogenetic and sixteen biochemical genetic polymorphisms have been identified thus far. Karyotypic identification of species and subspecies (S. b. boliviensis, S. b. peruviensis and S. s. sciureus) is based upon the position of the centromere in two pairs of homologues. Two species were fixed for alternative forms of a C-band cytogenetic marker and of a biochemical marker. Twelve of the other polymorphic biochemical systems also were useful in discriminating individuals of different species, different subspecies, or mixed ancestry because each system had one or two allelic forms that were restricted to one of the squirrel monkey taxa in the colony. Biochemical markers are useful in detecting hybrids that may be cytogenetically cryptic due to hybridization earlier than the preceding generation; they are also useful in planning matings for maintaining genetic variability in the colony. Pedigree errors have been reduced through changes in management practices based on biochemical and cytogenetic markers. A long-term management goal is to increase reproductive success by genetic strategies. Preliminary data suggest that heterozygosity for a C-band polymorphism may reduce female reproductive success compared to either homozygote.

Supported by NIH Grant RR01254

MONOSOMY X IN A RAT COLONY EXPRESSING PROTEOLIPID PROTEIN DEFICIENCY, T. Casimo, A.M. Willey, A.H. Koeppen 2. 1Nadsworth Center for Laboratories and Research, Albany, NY and 2V.A. Medical Center, Albany, NY
A rat colony expressing the X linked recessive proteolipid protein deficiency in males has on occasion produced affected myelin deficient females. The female md rats show the same neurological features as their diseased male littermates. Routine fibroblast culture methods were utilized to examine cells grown from abdominal skin biopsy from affected females. Biopsies from normal male and female littermates were also analysed. Compared to the normal rat karyotype of 42, XX or 42, XY the karyotype of the affected female was 41, X. No evidence of mosaicism was observed. This would explain the manifestation of this X linked recessive disorder in female littermates of affected males without invoking hypotheses of mutation, lyonization, or mosaicism. The karyotype of the common rat (Ratus norvegicus) has been well described with G and R bands. The utility of cytogenetic analysis of tissue culture cell lines is well accepted. The application of cytogenetic analysis to breeding colonies of experimental animals (i.e. rats, mice) may often be overlooked. Application of routine cytogenetic analysis may simplify explanation of unusual phenotypic events in such colonies.

SEX CHROMOSOME ANEUPLOIDY AND INFERTILITY IN DOMESTIC GOATS N.P. Healy1, A.M. Willey1–2, T. Casimo1, W. Caler1, 3, ½adsworth Center for Laboratories and Research, Albany, NY, Miner Institute for Agriculture, Chazy, NY

The alpine goat herd maintained at Miner Institute has provided extensive opportunities to investigate herd management practices. Clearly fertility is critical to milk and meat production. The selection for pedigree and body type are typical criteria in dairy goat management. A 1988 doe kid was retained in the Miner herd and bred to kid as a yearling. She and her mother failed to produce in April 1989, although a full, sib produced twin kids and a full brother was known to be fertile. On further consideration the yearling doe was described as buck-like in behavior, having a masculine head, and very small labial-vaginal orifice.

Utilizing routine (human) clinical peripheral blood chromosome analysis protocol, karyotype evaluation of the yearling was undertaken. Three cell lines were identified, normal female, X chromosome monosomy, and X with marker presumably Y derived. Little appears in the literature on sex chromosome aneuploidy in goats. Culling of infertile animals is routine. It is unlikely that cytogenetic analysis would be of financial benefit except in unusual circumstances. However, this may not be an unusual cause of infertility.

A NOVEL ROBERTSONIAN TRANSLOCATION IN A FAMILY OF DOGS. P.B. Jacky (1), D.M. Stone (2), W.D. Mickelsen (3), and D.J. Prieur (2). (1) Department of Pathology/Cytogenet Genome Res, Kaiser Permanente Northwest, Portland, OR, (2) Department of Veterinary Microbiology and Pathology and (3) Department of Veterinary Clinical Medicine and Surgery, Washington State University, Pullman, WA.

Cytogenetic evaluations are rarely part of the diagnostic protocol of dogs. Recently, we were requested to conduct a chromosome evaluation of a 5-year-old female Walker Hound with reproductive problems. Analysis of unbanded, homogeneously-stained chromosomes from peripheral blood lymphocytes disclosed 77 instead of the normal canine diploid 78 chromosomes, and 3 metacentric instead of the expected 2 submetacentric X chromosomes of a normal female dog. G-banding disclosed that the submetacentric chromosomes consisted of the two × chromosomes and a Robertsonian centric fusion of two acrocentric autosomes, chromosomes 21 and 33. Subsequent cytogenetic analysis of two full-sister siblings disclosed
one littermate with the same translocation and one with a normal female chromosome constitution. The propositus was artificially inseminated with sperm from a karyotypically normal male Walker Hound and gave birth to 9 live grossly normal pups, 6 females and 3 males. Another female pup was born dead but was grossly normal. Cytogenetic analysis of the pups disclosed that 4 (3 males and 1 female) of the 9 had the same translocation. The remaining 5 pups (5 females) had normal female chromosome constitutions. This is a previously unreported Robertsonian translocation in dogs. The distribution of normal and affected offspring from the mating between the propositus and a normal male appeared to be Mendelian. The translocation is not incompatible with life, nor was there a reduction in the expected litter size for this breed. This translocation, when used in conjunction with other chromosomal markers, should be valuable in the mapping of canine genes. (Supported by NIH grant RR00515).

KARYOTYPIC CHANGES IN TRANSFORMED CELL LINES OF Nyctereutes procyonoides (Japanese and Chinese Raccoon Dogs). J.P. Park* and D.H. Wurster-Hill, Dartmouth Medical School, and Dartmouth College, Hanover, N.H.

Fibroblasts from a male Chinese raccoon dog (Nyctereutes procyonoides #A202, 2n=54 + B chromosomes) were retrieved from cryogenic preservation, cultured in McCoy’s 5A medium with 20% fetal bovine serum (FBS) and left without further manipulation for 10 weeks. Spontaneous transformation was manifested by development of foci, karyotypic changes, and the ability to survive past 40 subsequent passages. Transformation was induced in fibroblasts from a male Japanese raccoon dog (Nyctereutes viverrinus #A179, 2n=38 + B chromosomes). Lipofectin™ (BRL) was used as an alternative to conventional calcium phosphate or DEAE-dextran transfection. The cells were co-transfected with the plasmids pSV2-neo and dIA2441. dIA2441 is a construction incorporating an SV40 deletion mutant (Virology 162, 76–89, 1988). pSV2-neo contains the dominant selectable marker neo from Tet which confers resistance to G418 (Geneticin®, Gibco). The co-transfection was performed at a 10:1 plasmid ratio (20µg dIA2441:2µg pSV2-neo) for each culture dish. The cultures were incubated in 3ml Opti-MEM® (GIBCO). At 24 hrs., 3ml McCoy’s 5A medium supplemented with 20% FBS was added to each culture and the cultures were incubated undisturbed for an additional ten days. The cultures were then passed and allowed to grow to confluence (six days) at which time Geneticin® (300µg/ml) was added to each experimental culture and to 2 cultures which were not transfected but otherwise handled in parallel. Severe mortality was observed after 3 days Geneticin® exposure in both control flasks as well as in the majority of experimental flasks. After one week of undisturbed Geneticin® exposure, three independently derived experimental flasks (A179CL1,4,&6 respectively) showed limited patches of growth. These cultures were given fresh supplemented medium without Geneticin® and allowed to grow undisturbed for one week. Each culture was then re-exposed to Geneticin® (300µg/ml) for two weeks. The cells have since been cultured routinely and are passed approximately every four-five days. At subculture (SQ S, A202 consisted mostly of paratriploid cells, but a smaller population of paratetraploid or hypertetraploid cells was also observed. Dicentric fusions of acrocentrics were seen in early passages with fusion involving a B chromosome first seen in SC13. Over a period of 4 months (SC 5–21) the incidence of dicentric fusions increased and the modal number dropped slightly from 81–82 to 73. One ring chromosome and a fusion involving 2 B chromosomes were seen at SC21. By passage 41, dicentric fusions, including (B:B) fusions, were common, and a hypertetraploid population had become dominant. All 3 A179 cell lines were characterized by paratetraploid modal numbers, although CL4 with 2n=70 had fewer
chromosomes than the other two lines with $2n=77–81$. Two of the lines display some pulverization, ring formation and double minutes. The evolution of these cell lines is being monitored with particular interest in the behavior of the $B$ chromosomes.

NEW EVIDENCE FOR TANDEM CHROMOSOME FUSIONS IN THE KARYOTYPIC EVOLUTION OF ASIAN MUNTJACS. C. C. Lin, R. Sasi, Y-S. Fan and Z-Q. Chen. University of Alberta and University of Alberta Hospitals, Edmonton, Alberta, Canada. A clone of highly repetitive DNA designated C5', was isolated from cellular DNA of female Chinese muntjac cells. The base pair sequence of this clone is 80 – 85% homologous to that of the satellite A clone and other highly repetitive DNA clones previously obtained from the Indian muntjac. Using C5 as a probe for in situ hybridizations to chromosome preparations of cells of both the Chinese and Indian muntjacs, we were able to show that these repeated sequences occur in centromeric heterochromatin of the chromosomes of both Chinese and Indian muntjac species. More significantly, non-random clusters of hybridization signals were detected on the arms of chromosomes of the Indian muntjac. These latter hybridization sites are postulated to be regions of interstitial heterochromatin and could be the remnants of centromeric heterochromatin from ancestral Chinese muntjac chromosomes. Our observations provide new supportive evidence for the tandem chromosome fusion theory that has been proposed for the evolution of the Indian muntjac karyotype.

SYNAPTONEMAL COMPLEX ANALYSIS OF MOLE RATS (SPALAX EHRENBergeI) : UNUSUAL POLYMORPHISMS OF CHROMOSOME 1. Ira F. Greenbaum, David W. Hale, Philip D. Sudman and Eviatar Nevo. Department of Biology, Texas A&M University, College Station, TX; The Jackson Laboratory, Bar Harbor, ME; Museum of Natural Science, Louisiana State University, Baton Rouge, LA; Institute of Evolution, University of Haifa, Mount Carmel, Haifa 31 999, Israel

Two unusual structural polymorphisms in the largest chromosomal pair of the Israeli mole rat, Spalax ehrenbergi, were analyzed from surface-spread and silver-stained preparations of synaptonemal complexes. A C-band negative polymorphism for the length of the lp arm was visible as axial length differences during late zygonema and early pachynema. This region underwent synaptic adjustment resulting in a fully paired, mid-pachytene synaptonemal complex with equalized axial lengths. The somatically variable and nonargentophilic secondary constriction in the lq arm was evident as a distinct silver-stained thickening along the synaptonemal complex. Presence of this structure on the synaptonemal complex varied both among individuals and among cells within individuals. The intraindividual variation of this region is hypothesized to represent differential biochemical activity with its cellular visualization being regulated in a manner similar to that of nucleolus organizer regions.

ENHANCED DETECTION OF PRENATALLY DIAGNOSED SEX CHROMOSOME MOSAICISM UTILIZING IN SITU HYBRIDIZATION. B.E. Ward, D.J. Shook, and K.W. Klinger. Integrated Genetics, Framingham, MA.
In an effort to evaluate the utility of in situ DNA hybridization for prenatal diagnosis, we have compared cytogenetics to in situ hybridization of interphase nuclei in a case of low level sex chromosome mosaicism. The results demonstrate that in situ hybridization is an appropriate adjunct to cytogenetics in elucidating mosaicism. Cytogenetic analysis following amniocentesis identified two colonies with a 47, XXY chromosome constitution and 23 colonies which were 46, XY. The patient desired further evaluation of the potential mosaicism and a PUBS was performed. Analysis of fetal lymphocytes revealed a 47, XXY chromosome constitution in 15% of metaphases (5/33). Over 250 interphase nuclei from the PUBS were examined by in situ hybridization. 96% of those nuclei hybridized with Y specific probes demonstrated a single hybridization signal. Nuclei hybridized with an X chromosome probe revealed two patterns: 74% of these nuclei had a single hybridization signal while 22% had two hybridization signals indicating two X chromosomes. This pattern is consistent with a 47, XXY constitution in 22% of nuclei examined. The level of mosaicism detected by in situ hybridization (22%) is equivalent to that detected by cytogenetics (15%). The significant number of nuclei available for examination by in situ hybridization suggests that it is a powerful technique for the detection and quantification of mosaicism. The utilization of in situ hybridization in conjunction with cytogenetics therefore offers an enhanced ability to diagnose and characterize mosaicism when compared to cytogenetics alone.

DETECTION OF DELETIONS AND CRYPTIC TRANSLOCATIONS IN MILLER-DIEKER SYNDROME BY FLUORESCENT IN SITU HYBRIDIZATION. A. Kuwano, S.A. Ledbetter and D.H. Ledbetter. Baylor College of Medicine, Houston, TX 77030.

Fluorescent in situ hybridization using two cosmid probes (41A and P13) from 17p13.3 was performed on five Miller-Dieker syndrome (MDS) patients with microscopic or submicroscopic deletions. Both cosmids were co-hybridized with the chromosome 17 alpha satellite probe (to identify the 17s) using the Oncor Chromosome In Situ Kit. Case 1 involved an MDS patient with a der(17)(p+). The mother had the same der(17), but G-banding analysis failed to reveal the reciprocal product (i.e., a “half-cryptic” translocation). In situ hybridization showed that the mother had two positive signals in 90% of her cells, one on the normal 17 and the other on 3qter.

Case 2 involved an MDS patient with apparently normal karyotype. Because a large DNA deletion was found, the hypothesis was entertained that the patient represented an unbalanced cryptic translocation involving two G-negative telomeres (i.e., a “full-cryptic” translocation). In situ studies of her father and normal brother showed an 8q;17p translocation. These results dramatically changed the recurrence risk for this family from a minimal risk for a de novo deletion to a high risk for translocation carriers, but prenatal diagnosis can be offered. Three additional MDS patients were studied as part of a blinded analysis of MDS vs. normal controls. Analysis of 20 cells from these patients showed one chromosome 17 labelled in 85–95% of cells analyzed. For four normal controls, both 17 homologs were labelled in 90% of cells. This high efficiency suggests in situ hybridization with cosmid probes is a rapid and reliable method of deletion analysis, and that this strategy may be extended to other microdeletion disorders.

CHARACTERIZATION OF ROBERTSONIAN TRANSLOCATIONS USING FLUORESCENCE IN SITU HYBRIDIZATION. DJ Wolff, AR Brothman, PN Mowrey, KN Rosenbaum, RL Ladda, and S Schwartz. Div Human Genetics, Univ of Maryland School of Medicine, Baltimore, Cytogenet Genome Res Div, Eastern VA Medical School,
Norfolk, Dept Ped/Genetics, Penn State Univ, Hershey, Div Clinical Genetics, Children’s Natl Med Ctr, Washington, DC.

Molecular studies utilizing probes for specific DNA sequences in the centromeric and short arm regions of acrocentric chromosomes are useful for studying Robertsonian translocations. Fluorescence in situ hybridization was used to characterize 40 Robertsonian translocations from 28 different families: two t(13;13), one t(15;15), four t(21;21), six t(13;14), three t(13;15), three t(13;21), one t(14;15), thirteen t(14;21), five t(14;22), and two t(21;22). Five probes were used for the analysis: (1) αXT(640) 22–94, specific for alphoid repeat sequences on both chromosomes 14 and 22, (2) LI.26, which hybridizes to alphoid sequences on chromosomes 13 and 21, (3) pTRA 20, chromosome 15 alphoid sequence specific, (4) Beta satellite probe, specific for beta satellite sequences in all acrocentrics, and (5) pA285, which hybridizes to ribosomal DNA sequences. The probes were labeled with biotin via nick-translocation with biotin-dUTP or by a PCR amplification technique, and subsequent hybridization in situ to metaphase spreads was visualized with fluorescence microscopy. Six of 7 de novo homologous translocations were monocentric, with one t(13;13) chromosome being dicentric. Of the 33 nonhomologous Robertsonian translocations, 29 were dicentric. The four monocentric included all three of the t(13;21) and one t(21;22). Preliminary results using the ribosomal gene probe (pA285) revealed that no rDNA sequences were detectable in seven translocations studied thus far. Recently a probe for beta satellite sequences in all acrocentrics, 29 were dicentric. The four monocentric included all three of the t(13;21) and one t(21;22). Preliminary results using the ribosomal gene probe (pA285) revealed that no rDNA sequences were detectable in seven translocations studied thus far. Recently a probe for beta satellite sequences in all acrocentrics was obtained. This probe will be used to determine if these sequences are involved in Robertsonian translocation formation. Our results indicate that Robertsonian translocations result from breakpoints in different locations in short arm and/or centromeric regions of acrocentrics. These molecular studies, combined with cytogenetics, are useful for precise elucidation of the structure of Robertsonian translocations.

IDENTIFICATION OF CHROMOSOMAL MARKERS AND REARRANGEMENTS UTILIZING FLUORESCENCE IN SITU HYBRIDIZATION. S Schwartz1, DJ Wolff1, and JL Zackowski2. •Division of Human Genetics, University of Maryland School of Medicine, Baltimore; ¾ivision of Pediatric Genetics, Univ. Florida, Gainesville.

It has always been a major difficulty in clinical cytogenetics to elucidate the origin of unidentifiable chromosomal material. In this continuing study, we have applied fluorescence in situ hybridization to 32 samples with marker chromosomes or structural rearrangements. To study these, we have primarily used DNA probes containing alphoid sequences from the centromeric regions from almost all 22 autosomes and the X and Y chromosomes. For one case, we utilized a chromosome 11 library. Thus far, we have identified the unknown material in 25 cases. These include: (a) Nine cases had an unidentified sex chromosome marker. Seven were derived from an X chromosome and two from a Y chromosome, both of which were iso(Yp); (b) Seven cases had a supernumary satellited marker chromosome. Four were inv dup (15), one was from either chromosome 14 or 22, and two were from either chromosome 13 or 21; (c) Three cases had an unknown supernumary non-satellited marker chromosome. One was derived from chromosome 11, one from chromosome 18 and one appeared to contain alphoid DNA common to both chromosomes 11 and 17; and (d) Six cases contained structural rearrangements. This group included an iso(18p), iso(llq), and a sat(Yq). The satellite material on this latter chromosome was derived from chromosome 15.

This study is of interest for several reasons: (1) It demonstrates that fluorescence in situ hybridization can be used effectively to identify unknown chromosomal material; (2) The findings from this study along with others reported in the literature demonstrate that the majority of sex chromosomal markers originate from the X-chromosome; (3) The majority of autosomal
satellited markers appear to be derived from chromosome 15. However, these markers may originate from all the acrocentric chromosomes; (4) Determination of the origin of autosomal non-satellited markers is possible, but, multiple alphoid probes must be used; and (5) These techniques can also be utilized to precisely delineate the nature of structural rearrangements.

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X-linked ichthyosis results from deficiency of the enzyme steroid sulfatase. As the name implies, patients with the disease have scaly skin. Ninety percent of affected patients have the entire 146 kb spanning the STS gene deleted on the distal X chromosome short arm (Xp22.3). In these families prenatal diagnosis and carrier testing can be completed in two days by hybridizing two different cosmid probes subsequently labeled with fluorescein or Texas red and counterstaining interphase nuclei blue with DAPI. STS gene probe labeled with Texas red hybridizes specifically to the steroid sulfatase gene on the X chromosome. A second flanking probe labeled with yellow-green with fluorescein hybridizes to both the X and Y chromosomes in normal and STS deleted X chromosomes. In this fashion normal males, affected males, normal females, and carrier females were distinguished unambiguously in interphase nuclei. Although normal males and carrier females each show two yellow fluorescein spots and one red STS spot, given the sex of the donor unambiguous diagnosis can be established. In a blind study of six cell lines, 83–100% (average 92%) of the interphase nuclei scored had the predicted genotype. This protocol has been used successfully on direct and cultured chorionic villus cells, direct and cultured amniocytes, lymphocytes, and fibroblasts to make it generally useful for all STS patient studies.

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We previously reported an “atypical” Down syndrome case identified by phenotypic features, chromosome banding analyses and superoxide dismutase (SOD1) gene dosage studies, which suggested a triplication of chromosome 21 material in a marker chromosome 12 described as 12q+ (Clin. Genet. 1983;24:97–102). We have analyzed this rearranged karyotype by in situ hybridization with probes for the beta-amyloid gene locus (21q21.2), for the SOD1 locus (21q22.1) and for the anonymous loci D21S16 (21q21.2), D21S15 (21q22.3) and D21S19 (21q22.3 → qter). Both tritium labeled and biotinylated probes showed that these loci were absent from the 12q+ marker chromosome. Chromosome “painting” with a chromosome 21 library, pBS 21, showed that the material translocated to chromosome 12 was not from chromosome 21.

We are currently extending this analysis to determine the nature of the translocation. The chromosomal origin of the additional material on the 12q+ chromosome is particularly interesting because it may be responsible for the Down syndrome-like features.
CHARACTERIZATION OF A MARKER CHROMOSOME WITH FLUORESCENT IN SITU HYBRIDIZATION. A. Yenamandra, H. Markalous, C. Lieber,* F. Desposito, Division Of Human Genetics, Department Of Pediatrics, NJ And Hackensack Medical Center,* Hackensack, NJ

A 39 year old female was referred for amnio-centesis because of advanced maternal age. Cytogenetic analysis of twenty GTG-banded metaphases revealed a marker chromosome in the fetal karyotype 45, X,+marker. The marker was small, with a CBG-positive band at the centromere, AgNOR negative, QFQ-band negative and no distinct GTG-bands. Parental chromosomes were normal including a normal appearing paternal Y chromosome which was highly fluorescent. While we presumed the marker chromosome represented either a deleted X or Y chromosome, the exact nature of this marker could not be identified. The pregnancy was continued and resulted in the birth of an apparently normal male infant with normal external genitalia and bilaterally descended testes. Peripheral blood lymphocytes of the infant showed the same 45, X,+marker chromosomal pattern. The marker was tested for Y chromosome origin by fluorescent in situ hybridization with a biotin labelled Y centromeric DNA probe (DYZ3). The marker demonstrated Y-specific hybridization confirming the presence of Y centromeric DNA sequences. In situ hybridization with labelled DNA probes is a useful tool in the identification both prenatally and postnatally of marker chromosomes particularly when a sex chromosomal variant is suspected.

LACK OF KINETOCHORE PROTEINS IN MOUSE-HUMAN HYBRID K. L. Sterne and B. K. Vig, Department of Biology, University of Nevada, Reno, NV 89557–0015 Most, but not all, chromosomes in a mouse x human somatic cell hybrid, HYG-1, exhibit the presence of kinetochore proteins. In early passages, the number of chromosomes not exhibiting kinetochore proteins varied between one and 24 (198/23389 or 0.85% chromosomes). After five months of continuous culturing the frequency of such chromosomes dropped (between 0 and 3 or 0.45%) as did the number of human chromosomes (0 to 8 per cell). In contrast, the parental human and mouse cells exhibited only a rare chromosome without kinetochore proteins. Hoescht stained acentric chromosomes showed intact pericentric and, by inference, the centromeric region. Based on their frequency, the acentric fragments could be ruled out as the cause of chromosomes lacking kinetochore proteins.

Immunoblots of the proteins showed that the antibodies in the serum used for kinetochore detection recognizes all major CEN proteins. The electron microscopic study of some metaphase chromosomes lying off the main cluster did not exhibit trilamellar kinetochore structure. Is a lack of formation of kinetochore, or incomplete assembly of the kinetochore protein complex, one factor responsible for loss of the segregant genome?

Another phenomenon of interest in the freshly generated hybrid was the apparent detachment of the kinetochore, or pericentric/centric region, from some chromosomes. These autonomous elements tended to aggregate into clusters enclosed in a nucleus. Such cells were observed only in the early passages of HYG-1.
PRESENCE OF CENTROMERIC DNA (MINOR SATELLITE) IN INACTIVE CENTROMERES OF MOUSE CHROMOSOMES. H. J. Yoo, B. Richards and B.K. Vig, Department of Biology, University of Nevada, Reno, NV 89557–0015 Several cell lines of mouse exhibit dicentric and multicentric chromosomes. Some of these chromosomes display regular segregation. The orderly separation of these chromosomes is attributed to the inactivity of the so-called accessory centromeres. Only one centromere in these chromosomes is functional and binds to the spindle microtubules. When these chromosomes are tagged with antikinetochore antibody, the functional centromeres show kinetochore proteins whereas the accessory centromeres are devoid of kinetochores.

Mouse centric and pericentric region is composed of two satellite DNA fraction – a major component and a minor one. The major fraction is located primarily in the pericentric region. The minor component, however, is reported to be specifically present in the centromere. In order to find out if the accessory centromeres in mouse lack centromeric DNA, we hybridized the chromosomes with biotinylated DNA from the minor satellite. This DNA hybridized with both the active and inactive centromeres. The data indicate that a lack of kinetochore proteins on the accessory centromeres is not due to the fact that the accessory centromeres shed off the minor satellite fraction from the centromere region. It is not known if this DNA has accumulated mutations which result in a lack of binding to the kinetochore proteins.

CHANGING MODALITIES FOR CLINICAL CYTOGENETIC LABORATORY QUALITY ASSURANCE: AN EIGHTEEN YEAR EXPERIENCE, A.M. Willey, N.P. Healy, Wadsworth Center for Laboratories and Research, Albany, NY 12201

Clinical cytogenetic laboratories serving physicians and residents of New York state have been included in a laboratory accreditation program since 1972. This program is legislatively mandated under the 1964 NYS Clinical Laboratory Improvement Act. Since its inception the quality assurance program for cytogenetics laboratories has included physical inspection of the laboratory, credential review of the cytogeneticist director, and successful participation in proficiency tests.

Director credential requirements have been constant, with minimum documentation of doctoral degree (M.D., Ph.D.) and four years postdoctoral clinical laboratory experience, two of which are in clinical cytogenetics.

Laboratory inspection checklists are based on record and equipment maintenance; case documentation, reports and follow-up; culture success and reporting times; and procedure manual review for testing offered. The addition of new procedures such as Fragile X analysis, prenatal diagnosis from CVS, and blood dyscrasia or tumor cytogenetics have been paralleled by addition of inspection requirements.

The principal of proficiency testing is to evaluate the laboratory’s ability to process clinical materials and produce a clinical report. The time consuming, labor intensive nature of clinical cytogenetics has limited the test modalities in this category. Test materials have included prepared stained slides, photographs, karyotypes, case descriptions from stated nomenclature and theoretical questions. Combinations of these modalities facilitate continuous laboratory evaluation with limited laboratory disruption, and flexibility in establishing performance criteria for a variety of laboratory types. The intended outcome of improving laboratory performance can be documented by observation of submitted test materials over time.
DETECTION OF MONOSOMY 7 IN INTERPHASE CELLS FROM PATIENTS WITH HEMATOLOGIC MALIGNANCIES. E. Bryant and C. Friedman. Fred Hutchinson Cancer Research Center, Seattle, WA

In situ hybridization (ISH) with alpha satellite DNA probes has multiple applications in clinical cytogenetics. We applied ISH with a biotin labelled alpha satellite DNA probe for locus D7Z/ (commercially obtained) to detect monosomy 7 in interphase cells of patients with hematologic malignancies. Bone marrow samples from seven test patients with cytogenetically documented monosomy 7 and from ten control patients with a normal karyotype or clonal abnormalities not involving chromosome 7 were evaluated. The results of interphase analysis correlated with conventional cytogenetic analysis. Monosomy 7 was correctly identified in each of the seven test cases. In four test cases, the percent of interphase nuclei with monosomy 7 was similar to the percent of abnormal metaphase cells. Interphase analysis detected cells without monosomy 7 in two cases with 100% of metaphases showing monosomy 7. In one case interphase analysis indicated a higher percentage of abnormal cells than metaphase analysis. Six patients with monosomy 7 were evaluated at various times following bone marrow transplant. In one case, interphase analysis showed 10% abnormal cells in a sample not adequate for chromosome analysis. Monosomy 7 was confirmed in a subsequent sample in both metaphase cells and interphase nuclei. In the ten control cases 1.3–4.75% of cells showed a single hybridization signal. The number of false-positive monosomic cells indicates a potential limitation of interphase analysis for detection of monosomy below 5%. Conventional cytogenetics provides an accurate representation of dividing cells, but interphase results give more accurate information on the entire cell population and can be applied to a small number of cells.

CLINICAL AND CYTOGENETIC CORRELATIONS IMPLICATING CHROMOSOME BAND 21q22 WITH A UNIQUE PHENOTYPE AND CLINICAL COURSE IN CML BLAST CRISIS. N. Rao, MJ. Pettenati. Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC

The specific role of major chromosomal changes associated with blast crisis is not clear, although they have important implications for prognosis. We recently detected 2 patients with Ph+ positive CML and in myeloid blast crisis with the additional clonal abnormalities of a t(6;21)(q21;q22.1) and a t(3;21)(q26.3;q22.1), and with the breakpoint 21q22.1 being common to both. A literature review of the cytogenetic abnormalities of Ph+ positive CML in blast crisis identified 30 cases involving aberrations of band 21q22. The review identified clinical/cyto genetic correlations suggesting strong cytogenetic evidence implicating region 21q22 region with a unique phenotype and a clinical course characterized by abrupt progression to myeloid blast crisis. The male to female ratio was 2:1. All but 1 patient showed signs of myeloid differentiation. 81% of the patients had an accelerated phase of 4 weeks or less in contrast to the median duration of 12 months normally seen. Molecular analysis of 21q22.1 in CML patients in blast crisis may elucidate the role of this region in the progression of the disease. It is interesting to note that the alpha- and beta-interferon receptor sites and gamma-interferon receptor-2 site have been localized to 21q22. It is possible then that patients with cytogenetic abnormalities involving this region may result in no or reduced sensitivity to interferon.

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COMPARATIVE CYTOGENETIC AND BIOCHEMICAL ANALYSIS BETWEEN CYCLOPHOSPHAMIDE SENSITIVE AND RESISTANT LINES OF ACUTE MYELOID LEUKEMIA IN THE LEWIS BROWN NORWAY HYBRID RAT. William Kearns, Todd Koelling, and Andrew Yeager. The Johns Hopkins University School of Medicine, Baltimore, MD.

An animal model of acute myeloid leukemia (AML) has been developed in the Brown Norway (BN) rat and has successfully been introduced into the Lewis x BN F1 hybrid (LBN) and designated LBN AML. The original LBN AML is sensitive to cyclophosphamide (CY). Recently, a CY-resistant cell line has been established. To better characterize this animal model of human leukemia, we analyzed and compared the chromosomal and biochemical makeup of both the CY-sensitive and CY-resistant LBN AML lines. The CY-sensitive LBN AML cultures contained two cell lines: Line I (88%): 41, XX,-1,-2,-9, del(12)(ql6), +der(l)(t(l;?)(pl3;q31), +der(2)(t(2;9)(pl1;ql1)); Line II (12%): 41, XX,-1,-2,-9, del(12)(ql6), del(20)(q13), +der(l)(t(l;?)(pl3;q31), +der(2)(t(2;9)(pl1;ql1)). The recently developed Cy-resistant AML cells contained two cell lines: Line I (88%): 41, XX,-1,-2,-9, del(3)(q36q42.1), del(4Xq42.2), +t(5;?) (q35;?), +t(8;?) (q24;?), del(12)(ql6), +H(ler(l)(t(l;?)(pl3;q31), +der(2)(t(2;9)(pl1;ql1)); Line II (12%): 42, XX, probably represents host contamination. The new chromosomal aberrations in the CY-resistant line I [del(3)(q36q42.1), del(4)(q42.2), +t(5;?) (q35;?), and +t(8;?) (q24;?)] suggest a possible interrelationship between these secondary karyotypic abnormalities and acquisition of resistance to the chemotherapeutic agent CY. Furthermore, resistance to CY in LBN AML cells may be due to elevated aldehyde dehydrogenase (ALDH) activity; specifically, ALDH-1.

MICRODISSECTION OF DNA SEQUENCES FROM 8Q INVOLVED IN NEOPLASIA: Linda A. Cannizzaro, Ph.D., Leslie Boghosian-Sell; Fels Institute for Cancer Research & Molecular Biology, Temple University School of Medicine, Philadelphia, Pa. 19140:

Chromosome microdissection is a powerful mechanism used to dissect out sequences from a defined chromosome region that may be involved in the initiation or progression of malignancy. One chromosome region, the long (q) arm of chromosome 8, is now under intense investigation in our laboratory due to its significant involvement in alterations manifested in different types and stages of malignancy. At least four different malignancies, including benign salivary gland tumors, acute non-lymphocytic leukemia (ANLL), Burkitt lymphoma, and renal cell carcinoma, all consistently demonstrate alterations of 8q-specific sequences usually in the form of a translocation event. Our investigations are currently focused on isolating sequences by microdissection from 8q as well as from a cell line established from a patient with renal cell carcinoma who carries a t(3;8)(p21;q24) chromosome translocation. Sequences isolated in this manner can be studied in greater detail with a variety of molecular analytic methods and the results of such analyses can be compared to homologous regions from individuals who do not manifest any malignant disease. The microdissection method offers an excellent alternative to other conventional methods for isolating disease associated loci. We will describe the microdissection method, some of the technical difficulties as well as its potential value especially when used in conjunction with other cytogenetic and molecular genetic analyses. Since the microdissection method is so new, it is essential that we confirm the regional chromosome location of DNA sequences isolated by this method with either radioactive or fluorescent in situ hybridization to ensure that possible contamination from nearby chromosomes within the metaphase has not taken place. Sequences isolated from either normal 8q or the renal cell
carcinoma breakpoint will be mapped in relation to each other and the breakpoint region itself to generate a physical map of the 8q region. Our ultimate goal will be to define the sequences along this chromosome region which are responsible for the manifestation of renal cell carcinoma.

CHROMOSOME ABNORMALITIES IN FeLV-INFECTED AND FeLV-FREE FELINE TUMOR CELL LINES. S. Gulino, K. Walen†, D. Wurster-Hill*, University of California, and †State of California Public Health Service, Berkeley, CA 94720. *Dartmouth Medical School, Hanover, N.H. 03756. Chromosome abnormalities are found in feline leukemia virus (FeLV) infected tumor cells as well as in tumor cells free of the virus. Here we ask whether chromosome abnormalities and viruses can each cause, or play a role in the causation of tumors.

Cytogenetic analysis on 3 cell lines derived from tumors in domestic cat (Felis catus) two of thymic origin and one of multicentric lymphoma origin, has been carried out in an attempt to determine whether the FeLV was associated with chromosomal abnormalities in these tumors. One thymic tumor and the multicentric lymphoma cell lines were FeLV-infected. The other thymic tumor cell line was FeLV-free.

The normal diploid chromosome number in domestic cat is 38. All three cell lines had structural and numerical abnormalities and modal numbers of 37, 38 and 39, respectively. Marker chromosomes were consistent within cell lines. One marker was common to the virus-free cell line and one of the virus-positive cell lines. Since both the virus-infected and virus-free cell lines have chromosomal abnormalities, it can be concluded that, although the FeLV virus may cause chromosome abnormalities, it could not be considered to be the only causative agent of the abnormalities observed. These abnormalities may, however, be a triggering factor in oncogenesis.

CHARACTERIZATION OF MARKER CHROMOSOMES IN THE PPC-1 PROSTATIC CARCINOMA CELL LINE USING IN-SITU HYBRIDIZATION WITH NON-RADIOACTIVE CHROMOSOME-SPECIFIC PROBES. Arthur R. Brothman and Ankita M. Patel, Eastern Virginia Medical School, Norfolk, VA 23507.

Consistent chromosomal loci involved in rearrangements in human prostate cancer have yet to be identified. Although there are few primary prostatic cell lines available, these are useful in the examination of late stage chromosomal changes associated with the disease. The analysis of a primary prostatic cell line, PPC-1, has previously shown that chromosomes 1, 2, 3, 4, 5, 9, 10, 11, and 12 are most frequently involved in rearrangements; a modal chromosome number of 84 was observed with double minute chromosomes also detected. A weakness in studies using routine banding techniques is the inability to determine the etiology of certain “unidentifiable” marker chromosomes. The availability of chromosomal specific DNA probes has greatly helped in our determination of the origin of chromosomal rearrangements. We have used alpha satellite repeats for the centromeres of chromosomes 1, 7, 10 and 12 and whole chromosome paint probes for chromosomes 1–4, 6–8 and 12 for determination of the origin of nine marker chromosomes to date. We have determined that chromosomes 4, 6, 7 and 8 are involved in rearrangements in addition to the other chromosomes noted above; chromosomes 1 and 8 appear to be most frequently rearranged. Our current evaluation of the PPC-1 cell line has therefore yielded approximately 50% more information over our initial study. Whether these chromosomal loci are involved in rearrangements in other patients with the disease remains to be determined.
CYTOGENETIC STUDY ON 56 CASES OF UTERINE LEIOMYOMAS. J. Hu and U. Surti. Magee-Womens Hospital, University of Pittsburgh, Pittsburgh, PA.

Fifty-two cases of typical leiomyomas and four cases of unusual leiomyomas (one bizarre leiomyoma, one lipoleiomyoma, one epitheloid leiomyoma and one cellular leiomyoma) were studied. Consistent chromosomal abnormalities were found in twenty cases (36%). Three out of four unusual leiomyomas and seventeen out of fifty-two typical leiomyomas showed clonal chromosomal changes. Abnormalities involving chromosomes 12 and 14 with or without additional changes were identified in seven cases. Complex translocations involving chromosome X, 3 and 14 as well as X, 5, and 14 were detected in one case of each. A non-reciprocal translocation of 14 and 15, and a monosomy 22 with a derived 14 were also found in one case each. Deletion of chromosome 7 as only change was found in three cases. Reciprocal translocations of chromosome 7 and 13, 7 and 17 were observed in one case each. Trisomy 7 was found in one case. Other clonal structural changes were found in three cases. According to our study, chromosomes 14, 12 and 7 are more frequently affected in uterine leiomyomas. The most common breakpoints observed are 14q22–24, 12ql3–15 and 7q22–32. Genes critical in tumor initiation or progression may exist in these regions. Since three out of four cases of leiomyomas with unusual histology showed abnormal karyotypes, it seems likely that the frequency of chromosomal abnormalities in these cases may be higher than those with typical histology.

COMPLEMENTARITY OF INTERPHASE CHROMOSOME STUDIES AND CYTOGENETIC ANALYSIS IN HUMAN RENAL TUMORS. Wolman, SR, Balazs M, Waldman FM. Michigan Cancer Foundation, Detroit, MI and Dept. of Laboratory Medicine, UCSF, San Francisco, CA.

Fluorescent in situ hybridization (FISH) was used to complement cytogenetic studies of human renal tumors. Chromosome-specific centromeric probes were applied to samples disaggregated from tissue blocks of tumors from which fresh samples had yielded cytogenetic results after short-term cultures. Cells were dissociated from paraffin-embedded formalin-fixed tissues for FISH by methods routine in flow cytometry, and were dried on slides after Carnoy’s fixation. Biotin-labeled chromosome probes were: pUC1.77 for chromosome #1, p7atet for #7, pl7H8 for #17, and pY for the Y chromosome. After hybridization, slides were counterstained with propidium iodide. Probes for chromosomes 1, 7, and 17 yielded clean signals with disomic control frequencies near 90%; 97% of controls showed a single bright spot for the Y. Eight cases were compared: In seven, aneusomies of # 1 and 17, not identified in culture, were observed. In 2/3 cases, trisomy 7 in culture was substantiated by FISH and in 4/4 cases loss of the Y was reconfirmed. Disaggregation of whole nuclei from thick sections permits a more sensitive approach to assessment of chromosome number per cell because the cellular substrate is more uniformly representative of the tumor than in cells of traditional microscopic sections that may not encompass the whole nucleus. Our results include limited validation of cytogenetic culture results, evidence of selection in culture of tumor sub-populations, and demonstration that the FISH method is applicable to archival tissue blocks after prolonged periods. Conditions of culture prior to harvest and inherent heterogeneity within tumors permit selection for nonrepresentative subgroups within tumors and emphasize the need for alternate approaches to evaluation.
CYTOGENETIC STUDIES OF LOW GRADE GLIOMAS

A. Al Saadi, F. Latimer, and T. Robbins, Anatomic Pathology, William Beaumont Hospital, Royal Oak, Michigan

Glioblastoma multiforme (GBM) is the most malignant glioma which may develop through progression of lower grade gliomas (LGG) or spontaneously. Several cytogenetic studies have been reported on GBM, but very few such studies are available on LGG. In order to determine whether GBM evolve from mutant cells with certain chromosomal abnormalities in LGG, cytogenetic studies of such tumors are needed. We report here cytogenetic studies of three types of LGG namely low grade astrocytoma (AA), ependymoma (EA) and oligodendroglioma (OA). Short term cultures (3–6 days) of tumor samples were used. Twenty five G-banded chromosome spreads were analyzed from each tumor whenever possible. No chromosome abnormalities were observed in 3 of 8 low AA three of which were juvenile. In each of the remaining 5 AA an aneuploid cell line was present. In two, loss of chromosome 22 and in one loss of a sex chromosome were the only abnormalities. One AA had two hyperdiploid clones: 48, XX,+18,+18 and 47, XX,+8, and one had a 45, X and a 47, XX,+9 clones. Four of the AA with abnormal clones recurred as GBM. One of two EAs had a clone with a 45, XY,-17,-21,+ring karyotype and the other had no abnormalities. Two of five OA had no abnormalities, one had a near triploid clone with 70, XXX,+4,+12,-3 karyotype and the other two each had multiple structural abnormalities. Although there is some resemblance between reported chromosomal abnormalities in GBM and those we found in LGGs, the tumors with abnormal clones irrespective of the type of abnormality appear to progress to more aggressive tumors such as GBM.

IDENTICAL KARYOTYPE IN TWO CHOROID PLEXUS TUMORS

F. Latimer, A. Al Saadi, T. Robbins. Anatomic Pathology, William Beaumont Hospital, Royal Oak, Michigan

Choroid plexus papilloma (CPP) is a rare neoplasm with a wide spectrum of cytologic and behavioral features that ends in anaplastic, invasive and metastasizing neoplasm referred to as choroid plexus carcinoma (CPC). These neoplasms are primarily found in children and young adults. Cytogenetic studies of such tumors are virtually non-existent. We report here cytogenetic results on a CPP in an 18-year-old male and a CPC in a 23-year-old female. Short term cultures (3–5 days) of tumor samples were used in this study. Each of 35 analyzed chromosome spreads from the CPP was hyperdiploid. All but seven had a 54, XXY,+5,+7,+9,+12,+14,+19,+20 karyotype. Each of the remaining seven cells had 53 chromosomes with essentially identical karyotype to those with 54 chromosomes, but each missing a different chromosome. Of the 25 chromosome spreads from the CPC, 10 had 46, XX karyotypes and 15 had 54, XX,+5,+7,+9,+12,+4mar. The four markers could not be identified precisely, but are reminiscent in morphology to the extra autosomes in the CPP. It is not clear from these results whether the cells with 54 chromosomes found in the less aggressive CPP, were already malignant or became malignant with further, cytogenetically undetectable, mutations. The close similarity of the karyotypes of both choroid plexus tumors suggest that at least some of the chromosomes are primary and are characteristic of choroid plexus tumors. Additional studies of similar tumors are needed to confirm these results.

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Our preliminary studies showed a bimodal distribution of sister chromatid exchange (SCE) induction by diepoxybutane (DEB) in lymphocytes from 58 normal blood donors. Approximately 24% of individuals were twice as sensitive to DEB induction of SCEs; also, cells from sensitive individuals contained a 4.4-fold increase in DEB-induced chromatid aberrations. Analysis of variance indicated that persons sensitive to induction of SCEs had higher baseline SCE frequencies. We have now completed analyses of baseline and DEB-induced SCE frequencies in an additional 53 normal subjects. The distribution of SCE scores in lymphocytes treated with 6 μM DEB appeared bimodal; 44 subjects had SCE frequencies ranging from 52 to 82 SCEs/cell and 9 subjects had induced frequencies of 106 to 129 SCEs/cell. The mean baseline SCE frequency for persons relatively resistant to DEB induction of SCEs was 8.0 ± 0.9 whereas sensitive persons had a mean of 10.4 ± 0.7 (p < 0.001; Students t-test). These results confirm our earlier observation that sensitivity to induction of SCEs by DEB is correlated with high baseline SCE frequencies. The mechanisms responsible for DEB sensitivity or its association with individual variations in baseline SCEs are unknown, however, preliminary studies of identical and fraternal twins indicate that DEB sensitivity is influenced by familial factors. DEB induction of SCEs may be a marker of individuals in the population who are at increased risk for genotoxic damage by certain classes of mutagens.

A COMPARISON OF CHROMOSOMAL ABNORMALITIES IN SPERM AND LYMPHOCYTES IN A PATIENT TAKING LOW DOSE METHOTREXATE. A.M. Estop, F. Levinson, K. Cieply, and V. Van Kirk. West Penn Hospital, Pittsburgh, PA, USA

The possibility of differential sensitivity of germ and somatic cells to chromosome damaging agents has been suggested. We have performed sperm and lymphocyte chromosome studies in a 34 year old male patient affected by Rheumatoid Arthritis who had been taking low dose methotrexate (2.5 mg/week) for 2.5 years.

At the time of analysis sperm count, motility and morphology were within normal ranges. Sperm chromosome analysis was performed by fusing human sperm with golden hamster eggs. A total of 174 cells were analyzed. Four percent were hypohaploid and 1.2 percent were hyper-haploid. These values are within the normal range for our 7 control donors. The frequency of cells with structural aberrations (mostly chromosome breaks and gaps, chromatid gaps and fragments) was 14% which is outside of the distribution of structural aberrations in our control donors where the highest frequency is 8.3%. Twenty one breakpoints were accurately identified; 15 of these (71.5%) are in locations where common fragile sites have been described. Lymphocytes were grown in RPMI minus folic acid supplemented with 10% fetal bovine serum and in RPMI supplemented with 20% of fetal bovine serum plus the addition of FUdR. One hundred twenty five cells were studied; 5.6% showed some type of structural aberration and 86% of these aberrations had breakpoints in common fragile sites. These results indicate that chromosome lesions in the sperm of this patient occur non randomly and could be a consequence of his exposure to methotrexate. Moreover, sperm cells show a
higher frequency of chromosome aberrations than lymphocytes, supporting other reports suggesting a higher sensitivity of sperm cells to chromosome breakage.

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DISCORDANT CYT0GENETIC RESULTS AT CVS: ANALYSIS OF 15 CASES. Z. Gibas and L. Gibas. Division of Medical Genetics, Thomas Jefferson University, Philadelphia, PA. 19107

Discordant findings in chorionic villus direct preparations and long term cultures are a major source of diagnostic problems in Chorion Villus Sampling (CVS). We present a detailed analysis of 15 cases which discordant results were initially detected at CVS.

A chromosomal abnormality was present in the direct preparation in 4 cases and in long term culture in 11 cases. The abnormality was mosaic in 7 cases and non-mosaic in the remaining 8 cases. Sex chromosome aneuploidy was present in 4 cases, autosomal trisomy in 5, structural rearrangement in 5 and polyploidy in 1 case. There were 3 cases of trisomy 2, all in long term culture (2 mosaic, 1 pure). Follow up amniocentesis was performed in two cases and showed a normal karyotype. Structural abnormalities were observed in culture in 3 cases and in the direct preparation in 2 cases. Mosaicism was present in one case and non-mosaic abnormality was observed in 4 cases. Follow up studies confirmed the presence of an abnormality in only one case involving an unbalanced Robert-sonian translocation resulting in a Down syndrome. Out of two cases of a viable autosomal trisomy detected in culture, only one was confirmed in the fetus. Sex chromosome aneuploidy present in culture was confirmed in 2 out of 3 cases.

We conclude that trisomy 2 present in long term cultures of chorionic villi is probably of placental origin. Structural abnormalities (except for Robertsonian translocations) present either in the direct preparation or in long term culture only, are likewise usually of placental origin and do not reflect the true karyotype of the fetus. Follow up studies are probably not indicated in such cases.

DISTURBANCES OF MITOTIC CENTROMERE FUNCTION AND PRENATAL DETECTION OF PSEUDOMOSAIC ANEUPLOIDY.


Premature centromere division (PCD) and C-anaphase are manifestations of disturbed centromere function induced by hypersensitivity to phytohemagglutinin and/or colchicine resistance. A distinct form of PCD is associated with Roberts syndrome, but PCD and C-anaphases have been observed as variant, dominantly inherited traits unassociated with phenotypic abnormalities. An association with aneuploidies is rarely reported.

We wish to report the prenatal detection of trisomy 21 pseudomosaicism in a 20 year old woman referred for amniocentesis at 27.6 weeks gestation because of intra-uterine growth retardation and oligohydramnios. A total of 81 cells were examined from 5 different culture flasks: Three of 15 cells from one flask showed trisomy 21 and one of 25 cells from another revealed trisomy 20. The remaining 77 cells had a normal male karyotype. At least 25% of 196 cells screened retrospectively showed C-anaphases and tetrasomy.

The infant was delivered by C-section at 35 weeks because of fetal distress and weighed only 1430 grams. He had problems associated with prematurity but showed no signs of Down syndrome. A detailed neurodevelopmental assessment at 6 months was normal.

A cord blood sample revealed 5 of 15 cells with differing trisomies; 13, 17, 18, 22 and X respectively. PCD, C-anaphases and tetrasomy were noted in 80% of 100 cells screened. A
peripheral blood sample 2 weeks later revealed one of 50 cells with trisomy 21 and 50% of 100 cells screened showed PCD and C-anaphases.

The role of the above centromeric disturbances in the etiology of nondisjunction and relevant investigations to elucidate centromeric function will be discussed.

Abstracts – 29th Annual American Cytogenet Genome Res Conference

THE DILEMMA OF CHROMOSOME MOSAICISM IN PRENATAL DIAGNOSIS
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Two cases with true chromosome mosaicism diagnosed prenatally were mosaic for sex chromosomes. Case 1 was a 46, XY/47, XXY mosaic. Case 2 was a 45, X/46, XY mosaic. The 45, X/46, XY mosaic is not the most common chromosome mosaicism encountered in prenatal diagnosis. This kind of mosaicism has drawn considerable attention in the recent years because of the difference in the phenotypic outcome between postnatal and prenatal diagnosed cases. This kind of mosaicism presents a diagnostic dilemma to the cytogeneticist. The clinical and cytogenetic profiles on these cases will be presented. The implications of chromosome mosaicism in Genetic amniocentesis will be discussed. An attempt will be made to review current literature on this subject. The importance of cytogenetic confirmation using in-situ culture techniques will be emphasised. The need for ultrasound examination and follow up will be pointed out and the implications for genetic counselling will be discussed.

PROBABLE SMITH-MAGENIS SYNDROME RESULTING FROM MATERNAL MOSAICISM FOR AN INTERSTITIAL DELETION OF 17p. J.L. Zackowski, D.J. Driscoll, B.A. Gray, and R. Zori. Division of Genetics, Department of Pediatrics, University of Florida College of Medicine, Gainesville, FL.

A two month-old female was referred for genetic evaluation because of slanted palpebral fissures, tongue protrusion, abundant neck skin and a small ASD. Cytogenetic analysis of peripheral blood lymphocytes revealed the presence of a short arm deletion of one chromosome 17 in all 20 metaphase cells examined. The karyotype was tentatively interpreted as 46, XX, del(17)(pter→pl3.1::cen→qter). Parental chromosomes were studied to rule out the possibility of a parental rearrangement involving 17p. Paternal chromosomes were normal; however, 11 of 20 maternal metaphases contained the same deletion seen in the proband.

As deletions of 17pl3.3 are known to cause Miller-Dieker syndrome, a brain MRI was ordered for the proband. Moderate hydrocephalus, but no lissencephaly, was noted. The absence of lissencephaly rules out Miller-Dieker syndrome. Thus, it is probable that the chromosome deletion is interstitial, rather than terminal. Smith-Magenis syndrome is associated with interstitial deletions of 17pl3.2. The broad, high forehead, telecanthus, and apparently mild mental retardation seen in the mother, as well as additional features noted in the proband during an examination at age 5 months (broad forehead, low-set cupped right ear, telecanthus, and mild hypotonia), are features seen in Smith-Magenis syndrome. Thus, revised karyotypes for the daughter and mother may be 46, XX, del(17)(pter→pl3.1:cen→qter), and mos46, XX/46, XX, del(17)(pter→pl3.1:cen→qter), respectively.
This familial deletion with subtle physical findings is interesting as: (1) it may be within the spectrum of Smith-Magenis syndrome, thought to be an under-diagnosed chromosome deletion syndrome; and (2) the non-mosaic deletion present in the proband was present in mosaic form in the mildly affected mother.

CONSTITUTIONAL MOSAICISM FOR DIFFERENT 5;8 TRANSLOCATIONS IN SUSPECTED CRI-DU-CHAT SYNDROME. Susanne Gollin, Sofia Shekhter-Levin, and Daniel Graff. Departments of Human Genetics, Environmental and Occupational Health, and Pediatrics, University of Pittsburgh and the Pittsburgh Cancer Institute, Pittsburgh, PA.

A 26-year-old male was referred for cytogenetic analysis because of possible cri-du-chat syndrome. Clinical features include profound mental retardation, microcephaly, absence of speech, spastic quadriplegia with contractures, kyphoscoliosis, and moderate to severe mitral valve prolapse. Parents are unavailable for study and family history is incomplete, apart from the mother’s comments that cousins have dysmorphic features and mental retardation. Cytogenetic analysis of peripheral lymphocytes revealed an apparently mosaic abnormal male karyotype: 46, XY,-5,-8,+der(8)t(5;8)(q11;p23), +mar (67% of 34 cells)/46, XY,-5,-8,+der(8)t(5;8)(q11;q24),+mar (33% of cells). Although the marker chromosomes in the two cell lines appear similar, their origins are unclear. We speculate that the marker chromosome is a ring chromosome 5, r(5)(pl4q11) resulting in del(5)(pl5) and clinical features of cri-du-chat syndrome.

The finding of two translocations in the same patient involving the same breakpoint on one chromosome and two different breakpoints on another chromosome is unusual. The mechanism of origin of these two cell lines is unclear and parental chromosome studies are not possible at this time. Since the breakpoints are near the telomeres of chromosome 8, one might invoke an intermediate step involving a ring chromosome 8, r(8)(p23q24) and two breaks in chromosome 5, at 5pl4 and 5q11 leading to the marker chromosome that we speculate to be a r(5)(pl4q11) and alternative splicing of the broken ring 8 with the 5q segment resulting in the two derivative chromosomes 8.

PRENATAL DIAGNOSIS OF A RECIPROCAL TRANSLOCATION USING DNA LIBRARIES. C. ROSENBERG, G. STETTEN, K.J. BLAKEMORE, V.V. KEARNS, AND P.L. PEARSON. THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE, BALTIMORE, MD.

We used DNA libraries to characterize a reciprocal translocation involving chromosomes 13 and 14. The case was ascertained through an affected child who lived for several months with a partial trisomy of chromosome 14 (46,XY,-13,+der(14), t(13;14)(q22;q22) mat). The mother was found to carry the balanced reciprocal translocation 46, XX, t(13;14)(q22;q22) and was referred for prenatal diagnosis with her second pregnancy. Direct chromosome analysis of a chorionic villus biopsy at 12 weeks revealed the same unbalanced karyotype as had been reported in the previous child. This represents an unusual adjacent type 2 segregation of chromosomes in meiosis. This pregnancy was terminated and samples were obtained for confirmation and further studies.