The Great Lakes Chromosome Conference
Proceedings of GLCC 2001

Session I: Cancer Cytogenetics, Chair, AnneMarie Block

I.1 Genetic alterations provide evidence of continuity between benign breast proliferations and overt malignancy.
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We have evaluated tissue sections from 12 cases with concurrent breast cancer and lesions representing various forms of benign breast disease (BBD). Using fluorescence in situ hybridization amplification with a HER-2/neu (ERBB2) probe(H2). Areas representative of malignant and BBD lesions from the same tissue sections were scored for average number of signals. Because of truncation artifact, normal centromeric signals are lost from approximately 35-40% of cells; therefore, values of 1.2-1.3+/- 10% were expected in diploid cells. The H2 and alpha 17 signals were examined concurrently to obtain an H2:17 ratio. The normally small fraction of S,G2 and M cells should result in slightly higher average values for normal H2 signal frequency, giving a ratio between the two that is close to but above 1. Because the centromeric signals remain intact and chromatid signals are duplicated during S phase, an increase in this ratio is indicative of proliferation. Although individual cases illustrated heterogeneity of some aberrations, several examples were identified in which genetic alterations provided evidence of continuity between the BBD lesions and overt malignancy.

I.2 The role of Alu repeat clusters as mediators of recurrent chromosomal aberrations in tumors.
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Cytogenetic analysis of tumor cells has revealed that recurring chromosome abnormalities are present in many tumors. There have been few attempts to understand the molecular mechanism of non-random genomic rearrangements. Mechanism involving repetitive sequences as hot spot for homologous recombination has been implicated as a proposed mechanism for chromosomal translocation. Alu elements, the most abundant interspersed repeats in the human genome, have repeatedly been found involved in gene rearrangements in humans. Ubiquitous presence of Alu repeats and their specific properties have lead to a number of possible functions for the Alu elements. There is now growing evidence that homologous recombination as both a pathway for DNA repair and a cause for genomic rearrangements is fairly frequent in mammalian cells. Key reaction in homologous recombination is performed by remarkably evolutionary conserved mechanism. Large number of Alu elements within primate genomes provides abundant opportunities for homologous recombination events. However unequal crossing-over between Alu repeats may happen and if it occurs interchromosomally it may result in deletion or duplication of sequences. We have demonstrated that some leukemia associated chromosomal rearrangements are frequently accompanied by deletions adjacent to the chromosomal rearrangement breakpoints. Analysis of sequence data from the each of the breakpoint regions suggested that large submicroscopic deletions occur in regions with a high overall density of Alu sequence repeats. This observation led us to propose that repetitive DNA sequences are intimately associated with the translocation process in general. Our findings are the first to show that the process of deletion formation is not disease specific per se but more likely determined by the factors such as the local density of repetitive DNA that could influence the somatic process of chromosomal rearrangement itself.

I.3 A review of balanced chromosome rearrangements: Workshop at the University of Chicago with Janet Rowley.
AnneMarie Block

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I.4 A novel chromosomal anomaly involving rearrangement of the MLL gene in a case of acute myelogenous leukemia.
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G-banding analysis of the peripheral blood from a 50 year old AML patient with monocytic features yielded a karyotype of 47,XY,+8,del(11)(q21q23) in all metaphases examined. Since the presence of a chromosome 11q23 breakpoint involving the MLL gene can have prognostic implications, molecular cytogenetic studies were undertaken in order to characterize the abnormality in detail. Metaphase FISH analysis using the MLL probe revealed that the 5' portion of MLL gene was inserted into chromosome 9 at band p22, while the 3' region remained on chromosome 11. WCP and M-FISH experiments confirmed that part of chromosome 11 had been inserted into 9p and demonstrated that the rest of the deleted segment of 11q (encompassing q21 to q23) had not been transferred elsewhere in the genome. With this information the karyotype was assigned as 47,XY,+8,der(9)ins(9;11)(p22;q23q23),del(11)(q21q23). FISH analysis revealed a cryptic rearrangement involving the MLL gene at 11q23. The t(9;11)(p22;q23) is a recurrent aberration associated with AML M5, which results in the fusion of the MLL gene and the AF9 gene (9p22) on the der(11) chromosome. MLL rearrangements are generally predictive of poor clinical outcome. The prognosis for children and adults with AML and the t(9;11), especially with the presence of trisomy 8, is associated with a more favorable outcome. RT-PCR was used to show that the cryptic rearrangement in this patient leads to the fusion of the MLL and AF9 transcripts on the der(9). The presence of the MLL-AF9 transcript is consistent with the clinical findings in this patient.

I.5 Hyperdiploidy and near tetraploidy in acute leukemia.
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I.6 Our experience with Her-2 Neu fluorescence in situ hybridization
James Higgins

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<abstract not available>

I.7 Variant Cryptic t(12;21) in childhood acute lymphoblastic leukemia detected in der(21) by FISH.
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The fusion gene formed by the cryptic translocation 12;21 is likely the most common cytogenetic abnormaliity in childhood ALL. It appears in 15=35% of pediatric B lineage ALL. It is associated with a distinct subgroup of patients between 1 and 10 years with an early B immunophenotype and a good prognosis. FISH analysis with TEL/AML1 translocation probe is usually a single fusion signal at the 12p13 TEL locus, one native TEL, one native AML1 and a residual AML1 signal. Our patient has two fusion signals in 60% of the cells, one of which is identified as a der(21). Chromosome studies were normal.

Special Presentation
A New Multiprobe Assay for DMD Hotspots.
Lisa G. Shaffer, Ph.D., Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA
*This presentation was given on behalf of Rainbow Scientific, USA, distriubutor of the Chromoprobe Multiprobe DMD System and Cytocell, Ltd, UK, maker of the Chromoprobe Multiprobe DMD System

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are X-linked recessive neuromuscular diseases caused by dystrophin gene mutations. Deletions are found in about 65% of patients. Mothers of affected males have a two-thirds chance of carrying a dystrophin mutation while the rest of affected males have de novo mutations. Current methods for detecting deletions include Southern blot analysis and multiplex PCR directed against exons mapping to deletion hot spots. In females, these methods are dependent upon dosage assessment to accurately identify carriers because of the normal X chromosome is also present. To eliminate potential biases in the interpretation of females, we developed exon-specific probes from the dystrophin gene and screened for potential carrier females using fluorescence in situ hybridization (FISH). Fifteen cosmid clones, representing 21 exons, were identified and used in FISH analysis of DMD/BMD families. We have shown that FISH is a reliable, alternate approach for establishing the carrier status in females. The ability to analyze these exons in an arrayed-fashion allows for the screening of clinically affected males as well. The panel of probes that we have developed is predicted to be able to detect about 98% of deletions found in DMD.

Session II: Clinical and Prenatal Problems, Part I, Chair, Marsha Speevak

II.1 When is a normal variant no longer normal?
Elizabeth Winsor, Martha Wood, Jiann Ng-Kcomt and Jana Muravska, Cytogenetics Section, Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto [ewinsor@mtsinai.on.ca]

Because there is variability in the size of the short arms of the acrocentric chromosomes it is sometimes difficult to distinguish between abnormalities and familial variants. Two recent publications illustrate translocation of material from other chromosomes distal to an NOR positive region on an acrocentric. (Engelen JJM et al.2001) De Novo Pure Partial Trisomy (6)(p22.1->pter) in a chromosome 15 with an enlarged satellite identified by microdissection. Amer J Med Genet 99:48-53. Benzacken B et al.(2001) Acrocentric chromosome polymorphisms: beware of cryptic translocations. Prenat Diagn 21:96-98. Case report (MSH): adult male tested because of a previous child with congenital abnormalities. Duplication of chromosome 15 short arm demonstrated by probes (Vysis) for Acro p-arm and D15Z1. Previous NOR staining did not clearly identify the duplication of 15p12. Interpretation: duplication of 15p not found in previous child and probably of no clinical significance.

II.2 The use of sequential FISH to interpret mosaicism level in an amniotic fluid.
E. Mak-Tam, M.J. Bajdo, E. Capua, G. Johnson, B. London, P. Wyatt. North York General Hospital, Toronto, Ontario, Canada [emaktam@nygh.on.ca]

The use of sequential FISH enabled the lab to distinguish between a level II (pseudomosaic) and a level III (true) mosaic in an amniotic fluid. In addition to one pure 45,X colony, a second cell from an independent culture dish was also suspicious for 45,X,-X or-10. Because of poor morphology and no other usable metaphases in this in situ colony, sequential FISH was performed using the VYSIS AneuVysion CEP X,Y and 18 probes. FISH results confirmed that the metaphase and all the interphase cells in the colony had one X. In total, 2 of 18 in situ colonies were 45,X. Eleven cells subcultured from another dish were all 46,XX. Insummary, the use of sequential interphase FISH can be a useful tool to resolve mosaicism levels particularly when the sample has a low mitotic index or poor
morphology.

II.3 Identification of the origin of a supernumerary marker chromosome following amniocentesis for advanced maternal age.
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A supernumerary marker chromosome was detected during routine prenatal testing for advance maternal age. C-banding was suggestive of a ring structure, as was the double ring observed in several cells. In order to identify the origin of the marker, amniocytes were subcultured and grown on a Chromoprobe-I Multiprobe slide (Cytocell). Following hybridization, three signals were observed in the chromosome 20 area of the slide. In order confirm the chromosome 20 origin of the marker, cells were hybridized with a chromosome 20 centromere probe (Oncor) where signals were seen on the two normal copies of chromosome 20 and on the marker chromosome. Both DAPI and PI counterstains were present on the marker suggesting that there was euchromatin adjacent to the centromeric signal. A review of the literature indicated that 5 cases of supernumerary marker chromosome

20 have been detected postnatally. Four of these had phenotypic abnormalities and MR while one had mild dysmorphology and normal intellect (Crolla 1998 Am J Med Genet 75:367-381). Further, two cases have been detected prenatally and resulted in normal offspring as assessed at 20 months of age (Viersbach et al. 1997 Am J Med Genet 70:278-283). In both instances, the marker was present in 80% of amniocytes examined. However, in one of the cases, the level of mosaicism was 9% in cord blood.

II.4 UPD Testing: Experience over 4 years in a clinical laboratory.
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Session III: Clinical and Prenatal Problems, Part 2, Chair, Marsha Speevak

III.1 A male infant with ambiguous genitalia and a 45,X/46,X,der(Y) karyotype.
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We report a case of an infant referred for chromosome studies due to ambiguous genitalia at birth. The infant was designated male due to the presence of testes detected by ultrasound, and male-like external genitalia. For rapid TAT, molecular studies were performed using PCR for AMELX and AMELY loci. The AMELY amplification was weak, so a second Y chromosome marker, SRY was used. The DNA was found to be SRY +, therefore the preliminary results were indicative of maleness. Followup cytogenetic studies revealed a 45,X karyotype in the first 10 cells examined. Further analysis (G-banding and FISH) revealed a marker chromosome in 7 % of cells. The marker was wcpY+ and SRY++. The implications of performing rapid preliminary molecular results that may appear to be discrepant with G-banding will be discussed.

III.2 Unusual X inactivation: an active Ring (X).
M. Shago, D. Antinucci, K. Mellick, N. Persaud, M. Sgro, T. Barozzino, D. Chitayat, and I. Teshima. Cytogenetics Laboratory1, Department of Pediatric Laboratory Medicine and Clinical Genetics3, Hospital for Sick Children, 555 University Avenue,
Toronto, and Division of Pediatrics2, Mount Sinai Hospital, University Avenue, Toronto, Ontario. [mshago@mtsinai.on.ca]

A newborn female of birth weight 1745 grams, delivered at full term, presented with facial dysmorphism including flattened mid face, a prominent forehead, a small chin, and small low set posteriorly rotated ears. Physical assessment revealed a short neck, short limbs, and very small hands and feet. Cardiovascular examination detected mild pulmonary stenosis and an eye examination indicated Riegers anomaly. Cytogenetic analysis of cultured lymphocytes revealed a karyotype of 47,X,r(X)(p?q?),der(Y)rea(X;Y)[49]/46,X,der(Y)rea(X;Y)[3]

by GTG banding, FISH, C-banding and spectal karyotyping. The mitotic instability of the ring chromosome was evident by its absence in 3/52 cells and the presence of a double ring in 2/52 metaphases examined. The derivative Y appears to be a complex rearrangement of chromosomes X and Y. By FISH, the SRY gene determining region was not present on the derivative Y while the XIST gene (within the X inactivation locus) was present on both the normal X and the r(X) but not on the der(Y). Replication studies suggest that the normal X is late replicating (inactive) and the r(X) is early replicating (active) in 50/50 cells examined. In normal females, X inactivation is a random event occurring early in embryogenesis. Abnormal Xs lacking XIST cannot undergo inactivation. Most abnormal X chromosomes that contain XIST, although they are randomly inactivated, are late replicating and inactive presumably as a result of secondary cell selection. The unusual skew of activation towards the r(X) in this case presumably results in the least amount of functional disomy of X-linked genes in the cells of this patient. Additional studies, including parental molecular cytogenetic investigation, are being pursued to provide an explanation for the derivation and origin of this unusual karyotype and its implication for prognosis and management of the patient.

III.3 Detection of a cryptic insertion by FISH.
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An infant was referred for chromosome studies due to unusual appearing feet. The initial G-banded chromosome analysis (500 band level) revealed adel(11)(p13p15). Since this deletion is usually associated with multiple congenital anomalies, particularly, the WAGR association, the cytogeneticist called the referring physician for additional details. The paediatrician insisted that the baby did not have aniridia and only had funny looking hands and feet. A concern arose that there may have been a sample mix up due to the lack of appropriate phenotypic features. A review of the samples received and harvested as well as procedures indicated that a sample mix up could not have occurred. As well, the senior technologist responsible for storage of abnormal cases for future teaching purposes queried the appearance of one chromosome 2. Because of these concerns, FISH using wcp11 was therefore performed to rule out the possibility of a cryptic insertion. An ins(2;11)(q31;p15.1p13) was detected by FISH and breakpoints determined by additional high resolution analysis. A procedural change has been instituted, so that all probable deletion cases be FISHed for the possibility of cryptic translocation or insertion.

III.4 Marker chromosome of variable morphology.
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Cytogenetic analysis was performed on a dysmorphic infant with developmental delay. A small supernumerary marker chromosome of variable morphology was found to be present in decreasing frequency with increase in age. Spectral karyotyping indicated the marker to be derived from chromosome 17, which was confirmed by FISH. A literature survey for other cases of duplication of the pericentromeric region of chromosome 17 was conducted. In some metaphases C-banding of the der(17) was very faint but indicative of two centromeres. Two smaller signals were also observed after FISH with a 17 centromeric probe. Centromere splitting is suggested to explain these findings and the variable morphology observed.

Session IV: Research Programs and Molecular Cytogenetics, Chair, Jeanette Holden

IV.1 FISH using Cytocell Multiprobe-T sytem and spectral karyotyping.
Yao-Shan Fan, Yang Zhang, Marsha Speevak, Sandra Farrell, Ikuko Teshima, Jack H. Jung, and Victoria M. Siu
London Health Sciences Centre, The Credit Valley Hospital, and The Hospital for Sick Children [mspeevak@cvh.on.ca]

Purpose: Genomic alterations occurring in the subtelomeric regions are considered to be an important cause of idiopathic mental retardation and developmental delay. This study was undertaken to further assess the frequency of subtelomeric aberrations in a selected population and to examine the feasibility of a clinical testing. Methods: Patients were selected based on the the following criteria: 1) mental retardation (IQ<70) or developmental delay with dysmorphic features; 2) a normal karyotype at the level of resolution of 450-500 bands; 3) exclusion of other possible etiologies by a full genetic assessment and relevant tests. Fluorescence in situ hybridization (FISH) was performed using multiple subtelomeric probes (Cytocell Multiprobe-T system). Abnormal findings were confirmed by spectral karyotyping (SKY) and Vysis probes, and family studies were carried out to determine inheritance. Results: A total of 134 patients were studied. Alterations were detected in 14 patients (10.4%), including four patients with a cryptic rearrangement:

der(1)t(1;3)(qtel;qtel),der(4)t(4;12)(ptel;qtel),der(5)t(5;20)(ptel;ptel),der(18)t(7;18)(ptel;qtel) respectively; two with del(1)(ptel), and eight with a common deletion del(2)(qtel)(D2S2986-). All four cryptic rearrangements were also visualized by SKY. Conclusion: The frequency of clinically significant subtelomeric aberrations was 4.5% (6/134) while the deletion of 2qtel detected by probe D2S2986 appeared to be a common variant (6.0%) in the study population. Both FISH with the multiprobe-T system and SKY are valuable clinical tests for establishing a definitive diagnosis for patients with unexplained mental retardation/developmental disorders.

IV.2 Unraveling the mystery of autism: From phenotyping and genotyping to prospective identification and prevention.
Jeanette J.A. Holden and Autism Spectrum Disorders Research Team, Cytogenetics and DNA Research Laboratory, Ongwanada Resource Centre, 191 Portsmouth Ave, Kingston, Ont, K7M 8A6 Canada [holdenj@post.queensu.ca]

Autism spectrum disorders (ASDs) are characterized by impairments in reciprocal social interaction and communication, and stereotypic activities, and affect at least 1/500 children. Sibling, twin, and family studies have shown that genetic changes play a major role in ASD. We have identified potential subgroups based on our genetic studies. We have formed an Autism Spectrum Disorders Research Team (ASD-RT), with a mandate to undertake research that will result in a better understanding of the etiology, pathogenesis, and effects of treatment of persons with ASD. The ultimate aims are to develop methods for the very early identification of infants ar risk and to intervene by appropriate means to prevent overt symptoms of autism. Our program of research will be presented, and includes: 1) Epidemiolog of ASDs in Canada; 2) Genetics of ASDs; 3) Phenotyping of ASDs; 4) a Prospective/Intervention Study on ASDs.

Invited Speaker Presentation
Probing the genome using scFISH.
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Chromosomal rearrangements are often delineated by in situ hybridization using large recombinant DNA probes. The sequences of these probes are generally not precisely determined, and their size precludes detection of small or cryptic abnormalities within corresponding or closely related genomic sequences. Nevertheless, these probes have been useful in demonstrating heterogeneous breakpoints and minimal regions of overlap in disease states. We have developed custom single copy (sc) probes from the draft genome sequence for fluorescence in situ hybridization (scFISH; Genome Res. 11:1086, 2001) and used this approach to precisely delineate chromosome abnormalities at a resolution equivalent to genomic Southern analysis. Probes, 1.5 to 10 kb in length, have been computationally designed, produced by long PCR, isolated, visualized and validated rapidly as this approach does not require cloning. We have validated more than 70 probes from 23 different chromosomal regions. We have utilized this methodology for defining deletion breakpoints (eg. Smith-Magenis syndrome), detecting submicroscopic (eg. Williams Syndrome, DiGeorge Syndrome) and microdeletions (eg. imprinting center mutations in Angelman and Prader-Willi Syndromes), and delineating translocation breakpoints in constitutional and acquired (ie. t[9;22]) chromosomal abnormalities. By combining FISH with sequence-based probe design, almost any euchromatic region can be readily characterized. This approach will refine and facilitate cytogenetic studies of common and rare abnormalities and provide insight into chromatin organization.