The Great Lakes Chromosome Conference (GLCC) 2010
Collated Abstracts

CIZ gene rearrangement in pediatric CD10-negative acute lymphoblastic leukemia

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The *CIZ (ZNF384)* gene, located distal to the *TEL (ETV6)* gene at 12p13.31, is a putative zinc finger transcription factor which is recurrently rearranged in acute leukemia. To date, 23 patients with *CIZ* gene rearrangement have been reported. Most of these patients are children or young adults with B-precursor acute lymphoblastic leukemia (ALL). Rearrangements of the *CIZ* gene result in attachment of various 5′ partner gene sequences to form *CIZ* fusion genes. The *CIZ* gene has three known partners: *TAF15* at 17q12 (16 cases), *EWSR1* at 22q12 (4 cases), and *E2A* at 19p13 (3 cases). We present seven new pediatric ALL patients with *CIZ* gene rearrangement. The patients, five females and two males, ranged in age at diagnosis from 2 to 15 years. All of our patients had lymphoblasts with a CD10-negative or CD10-low immunophenotype, similar to the antigenic profile seen in *MLL* gene-rearranged ALLs. Follow up on the patients ranges from 10 to 40 months, and none of the patients have relapsed. These patients were diagnosed at our institution over the last 3.5 years. The t(12:19)(p13;p13) and t(12;22)(p13;q12) mediating the *E2A-CIZ* and *EWSR1-CIZ* translocations are difficult to identify by G-band analysis because the *CIZ*, *E2A*, and *EWSR1* genes are near the distal ends of their respective chromosome arms. Identification of the rearrangements was facilitated using dual colour breakapart probes for the *E2A*, *CIZ*, and *EWSR1* loci. Four of the patients had *E2A-CIZ* gene rearrangement and one had *EWSR1-CIZ* gene rearrangement. The remaining two patients had *CIZ* gene rearrangement involving novel regions on chromosomes 6 and 22, suggesting the presence of two aditional *CIZ* partner genes. During the time period of the study, approximately 240 pediatric ALLs were analyzed, of which 40 were CD10-negative/low. Our data suggests that *CIZ* gene rearrangement may have an incidence of ~3% in pediatric ALL, with an incidence of at least 18% in CD10-negative pediatric pre-B ALL. Since *CIZ* gene rearrangement may be associated with a more favorable prognosis than *MLL* gene rearrangement, FISH analysis with probes to detect *CIZ* gene rearrangement is recommended in patients with CD10-low/negative ALL.

Identification of two novel *RUNX1* translocations in acute leukemia

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The *RUNX1* gene is a key regulator of hematopoiesis and is frequently targeted by chromosomal translocations in *de novo* and therapy-related leukemias. Abnormal RUNX1 proteins, resulting from translocations or mutations, are important contributing factors to leukemogenesis. We will describe the molecular characterization of two translocations involving *RUNX1* in leukemia.
The recurrent t(1;21)(p22;q22) translocation was detected in a case with therapy-related acute myeloid leukemia. Fluorescence *in situ* hybridization (FISH) with *RUNX1-RUNX1T1* and bacterial artificial chromosome (BAC) probes, confirmed the rearrangement of *RUNX1* in this case. Using

FISH, RT-PCR and sequencing, we cloned a new fusion partner of *RUNX1* on chromosomal band 1p22.3. This novel fusion gene generates several alternative out-of-frame fusion transcripts, producing truncated RUNX1 isoforms.
We have also identified a novel cryptic translocation, t(15;21)(q26;q22). This cytogenetic abnormality was detected in cells of an adult patient with a t(9;22) positive biphenotypic acute leukemia. FISH with BAC probes confirmed a breakpoint within *RUNX1* intron 1. To our knowledge, this breakpoint region was only reported in t(12;21) B-lineage acute lymphoblastic leukemia. No fusion transcripts were detected, but a large deletion of ~700kb was identified near the breakpoint on chromosomal band 15q26.1.
Molecular characterization of novel *RUNX1* translocations is essential to better define the clinical diversity of *RUNX1*-related leukemias and to improve the understanding of oncogenic mechanisms associated with these rearrangements.

Chronic Lymphocytic Leukemia: Reeling in FISH and clinical outcomes

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults >60 years in the Western world. The clinical course is extremely variable with overall survival ranging from months to decades. Some patients present with a stable form of the disease while many progress to an advanced stage, requiring treatment. Over 80% of CLL patients have prognostically significant recurrent cytogenetic and/or FISH abnormalities including trisomy 12 and 13q, 11q and 17p deletions. FISH for these chromosome abnormalities is part of the routine standard of care for CLL patients in most centres. However, there are CLL patients with these FISH abnormalities who do not respond as expected suggesting that additional genetic factors may be involved in disease initiation and progression. Therefore, refinement of these FISH prognostic subgroups may be helpful in order to better risk-stratify patients and tailor treatment.
Cytogenetic abnormalities including deletions and translocations involving the IGH gene locus located on chromosome 14q32 are common in many hematological malignancies. However, they are thought to be rare events in CLL and their clinical significance is unknown. Review of CLL FISH data from the VGH Cytogenetics Laboratory between 2003-2009 revealed a statistically significant greater number of CLL patients with IGH translocations (26%) and/or IGH deletions (20%) as compared to the reports in the literature (~10%). Clinical and FISH data from the 184 patients tested at the VGH Cytogenetics Laboratory has been collected and compiled. Statistical analysis is being perfomed to determine the treatment-free interval and overall survival for each FISH abnormality, including the subgroups with IGH translocations and deletions. These findings will be presented. Results of this study will provide novel information into the clinical significance of IGH abnormalities in CLL and may lead to a revised FISH classification system that includes assessment of the IGH locus.

Genome-wide survey for DNA copy number alterations of prognostic and predictive significance in Non-small-cell lung carcinoma

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Lung cancer remains the leading cause of cancer death in Canada with an overall 5-yr survival rate of 16%. Up to 40% of lung cancer patients are potentially curable by surgery, yet their risk of dying from the disease remains high at 50%. Post-surgery chemotherapy is a toxic therapy but may improve cure rate. New methods of classifying lung cancers are needed for making more informed decisions on chemotherapy, based on specific molecular markers present in each cancer. Using a CGH microarray, we have identified small regions of chromosomes that when gained or lost in lung cancers, impact patient outcome. After testing individual genes within these regions by quantitative polymerase chain reaction, DNA copy number gains located on 1p, 8q, 11q, 12q, and 14q are showing a significant association with a worse prognosis in the absence of chemotherapy, and/or an improved response to chemotherapy.

Identification of novel fusion genes in the blast phase of chronic myeloid leukemia

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Chronic myeloid leukemia (CML), characterized by the t(9;22) chromosomal translocation, is one of the best example of a cancer treated by targeted molecular therapy. Imatinib mesylate, the tyrosine kinase inhibitor of BCR-ABL1, induces complete molecular response in the majority of CML patients. However, imatinib is less effective for patients with more advanced disease. Blast phase CML is characterized by hematopoietic cell differentiation arrest and uncontrolled proliferation of the blast cells which rapidly become resistant to imatinib. The acquisition of secondary chromosomal abnormalities and other molecular aberrations in addition to the Philadelphia chromosome, reflects the clonal evolution of this disease. However, the molecular mechanisms involved in the transition from chronic to blast phase CML (CML-BP) are not completely understood. We now report four additional chromosomal translocations in patients with CML-BP refractory to imatinib therapy: t(1;21)(p36;q22), t(7;17)(p15;?q22), t(8;17)(q?11;?q22) and t(2;12)(q31;p13). By fluorescence in situ hybridization (FISH), RT-PCR and sequencing analyses, we have characterized four novel fusion genes involving *RUNX1*, *PRDM16*, *MSI2*, *HOXA9* and *ETV6* genes. Interestingly, several of these are bona fide regulators of hematopoietic stem cell self-renewal and differentiation. In light of the functional studies performed in mice, these novel fusion genes likely contribute to the blastic transformation of CML.

Clinical utility of fluorescence *in situ* hybridization assay in detecting *PTEN* deletions in formalin-fixed paraffin-embedded sections of prostate cancer

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We have developed a four-colour FISH tumour suppressor gene deletion assay that comprises a centromeric “chromosome counting” probe, a target gene probe, and control flanking probes either side of the target probe. We have evaluated the reliability of this assay using *PTEN* deletion analysis of formalin-fixed paraffin-embedded tissue sections derived from prostate cancer. We initially determined the breakpoint regions associated with *PTEN* deletions in prostate cancer tissue microarrays by FISH (n= 330) and available online SNP databases (n= 117). Four-color FISH analyses showed that the most frequent deletion at 10q23 was a recurrent interstitial genomic loss, restricted to several hundred kb in size and always included the *PTEN* gene. The second most frequent class of deletion was more heterogeneous and involved *PTEN* and the neighbouring loci. *In silico* copy number analysis of the 10q23 region in the publicly available dataset identified a partial *PTEN* deletion as the smallest overlapping region of deletion, which mapped specifically to our *PTEN* probe. The clinical value of this assay is that presence of *PTEN* deletion by FISH is associated with earlier disease recurrence (based on PSA levels), and homozygous deletion is strongly associated with hormone refractory prostate cancer and metastatic disease. Therefore, this assay will be helpful in planning more appropriate treatment for men with a new diagnosis of prostate cancer.

How cytogenetic tools can be used to investigate the aneugenic and clastogenic activities of a known human carcinogen.

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Benzo-a-pyrene (BaP) is a polyaromatic hydrocarbon compound used as a model for its carcinogenic properties. Cytogenetic tests, such as chromosomal aberrations (CAs) and micronuclei (MNs) can be used to provide a mechanistic comprehension of the genotoxic effects in human cells, and to discriminate between clastogenic and aneugenic activities. In our study, human cultured lymphocytes were exposed to different concentrations of BaP dissolved in DMSO (0 – 0.1 – 1 – 5 and 10µg/ml) for 24 hours. Cells obtained from 20 different subjects were harvested 24 hours after the end of exposure and examined for CA and MN frequencies. FISH with a pancentromeric probe was also done on MNs. Globally, following BaP exposure, a significant increase in the CA and MN frequencies is observed in our cohort. Further analysis of the CAs showed that men had significantly more chromosomal breaks and complex aberrations following BaP exposure (20% more than the control), compared to women. FISH analysis of the MNs showed that BaP exposure causes the formation of MNs containing one or more centromere (10% more C+ MNs than control), and they preferentially contain more than one chromosome (78% of all C+ MNs). The increased CA frequency observed after BaP exposure confirms the presence of a clastogenic effect of this product, already demonstrated in the literature using CHO cells. Yet, our study shows for the first time, the aneugenic properties of BaP revealed by the presence of more than one chromosome in most induced MNs, which certainly contributes to the carcinogenicity of this compound.

Interesting cytogenetic cases with unusual or challenging observations

J Lavoie, Thomas M-A, Carter RF, Fortier A, Hazourli S and Winsor E

The goals of this group presentation are to share with colleagues interesting findings observed during the course of routine cytogenetic investigation and to highlight some cytogenetic observations that could be misinterpreted or overlooked.

1- Multiple clonal trisomies are seen in a blood specimen from a man with multiple primary cancers. The end of the story!
   Josée Lavoie, Montreal Children's Hospital

2- Interesting findings in the course of postnatal array CGH investigations.
   Mary Ann Thomas, Alberta Children's Hospital

3- A case of extreme cytogenetic structural instability in pediatric acute leukemia post transplant failure.
   Ronald F Carter, McMaster University Health Sciences Centre

4- Interesting karyotype results after normal rapid FISH testing.
   Amanda Fortier, Montreal Children's Hospital

5- Simultaneous presentation of two recurrent reciprocal translocations in a case of acute myeloid leukemia.
   Sawcene Hazourli, Leukemia Cell Bank of Quebec, Maisonneuve-Rosemont Hospital, Montreal

6- Unusual case of level III mosaicism in an amniotic fluid.
   Elizabeth Winsor, Mount Sinai Hospital

We would like to invite participants to present challenging cases in future meetings. Good examples are: 1) Cases where the observations, despite being likely important clinically, could be sufficiently inconsistent with the clinical indications for testing that it is considered important to investigate in detail before drawing any conclusions about their phenotypic effect, 2) Cases where an anomaly could have been overlooked, leaving the underlying genetic condition undiagnosed or 3) Near misses that makes you revisit your quality control measures.

My Gene_ Arraytion: The Sudbury Experience with the CGH Microarray

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Array CGH has become a powerful tool for the detection and analysis of genetic imbalances. Patients which had previously been shown to be normal on a G-band karyotype can now be re-analyzed with CGH to detect very small deletions or duplications. Some of these will be benign copy number variants (CNVs) but others will have clinical implications . This powerful technique may provide answers to the cause of a child's developmental delay or dysmorphism.
In Sudbury, we have been performing aCGH and reporting results for more that a year on both constitutional and haematological samples. To date, the total number of these cases exceeds 200.

We will present examples of constitutional cases including a newborn with a ring chromosome and a child with an apparently balanced inversion of chromosome 7. These cases will help illustrate our adventures in array CGH.

The role of molecular microsatellite identity testing to detect sampling errors in prenatal diagnosis.

Winsor EJT, Akoury H, Chitayat D, Steele L, Stockley TL.

Objective: The objective of this study was to determine the risk of sampling error in amniocentesis and chorionic villus sampling (CVS) in singleton and multiple pregnancies. Data from this and other published studies was used to discuss current practice guidelines for molecular identity testing.

Method: Clinical and laboratory records of all patients undergoing molecular-based identity testing in our clinical laboratory from July 2002 until March 2008 were reviewed. DNA microsatellite testing was performed to determine zygosity in multiple pregnancies and maternal cell contamination (MCC) in both singleton and multiple pregnancies.

Results: MCC was detected in 6/148 (4%) CVS and 1/87 (1%) amniotic fluids from singleton pregnancies. In two of the CVS, only maternal cells were found. In 2/24 (8%) twin pregnancies, the same fetus was tested twice. In a total of 285 pregnancies (235 singleton, 24 twin, 26 with ≥ 3 fetuses), without molecular identity testing, four women would have received erroneous results.

Conclusion: Current guidelines recommend molecular identity testing for MCC in conjunction with molecular diagnostic testing, but not for cytogenetic testing. No published guidelines were found for zygosity testing in multiple pregnancies. We suggest that identity testing be considered for all prenatal testing of multiple pregnancies, especially if CVS is performed.

A project to expand the capacity of genetic testing laboratories in Ontario

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Ontario has a network of genetic testing laboratories that includes 8 molecular genetic, 11 cytogenetic and 4 metabolic genetic laboratories. All genetics laboratories are licensed by the Ontario Ministry of Health and Long-Term Care (MOHLTC) and receive designated operating funds from MOHLTC. The combination of increased demand for genetic testing and funding reductions in recent years has resulted in the laboratories losing ground such that <15% of clinically available genetic tests are currently performed in the province. Consequently, there has been a sharp increase in the number and cost of genetic testing referrals to laboratories outside of the country. As part of an ongoing Genetic Services Strategy by the MOHLTC, a decision was made to repatriate 5 genetic tests currently sent out of the country including genomic microarray testing

for multiple congenital anomalies/developmental delay and 4 cardiac genetic tests: arrhythmogenic right ventricular cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy and long QT syndrome. The goals of this repatriation are to increase the capacity of genetic testing laboratories in the province and maintain the high quality of testing while reducing the cost of sending tests out of country. In order to ensure that high quality testing is maintained for repatriated tests, an Expert Panel (EP) composed of cytogeneticists, molecular geneticists and clinical geneticists from across Ontario and Canada was established to provide research and advice to the MOHLTC regarding the quality criteria for the identified tests. Over a 6 month period, the EP researched current practices within and outside the province, national and international accreditation standards and professional society recommendations and external quality assurance (EQA) programs for the tests slated for repatriation. The work of the EP culminated in March 2010 with a report to the MOHLTC containing a series of recommendations for establishing the quality management program for these tests. Recommendations covered minimum criteria for testing methodology, the development of clinical criteria for testing, external quality assurance, and changes to accreditation requirements. The EP also identified stakeholders that should be included in clinical and laboratory discussions and potential barriers and limitations to the development of a quality testing program for repatriated tests. Laboratories selected by the MOHLTC to perform the testing will undergo scope extension reviews under the Quality Management Program – Laboratory Services, Ontario Laboratory Accreditation (OLA) program. An ongoing evaluation of the quality of repatriated testing will be established to ensure that the quality of testing provided within the province meets or exceeds that of out-of-country testing. It is expected that the combination of expert input and ongoing evaluation will ensure a smooth transition to repatriated testing and set the stage for future repatriation efforts or will have transferable principles to other molecular genetic tests.

Case reports - three postnatal *de novo* chromosomal anomalies

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Three postnatal *de novo* terminal chromosome deletions were identified through cytogenetic analysis of cultured peripheral blood lymphocytes. The first case was ascertained in a one-month-old boy with swallowing disorder, undescended testes, mild hypotonia, and mild micrognathia. Chromosomal analysis for case 1 demonstrated a *de novo* terminal deletion at 10q26.1. The second case was ascertained in a 6-month-old boy with progressive microcephaly, developmental delay, seizure disorder, and excessive involuntary movements. Chromosomal analysis for case 2 demonstrated a *de novo* terminal deletion at 17p13.3, which is diagnostic of Miller-Dieker syndrome. The third case was ascertained in a 21-month-old boy with short stature, failure to thrive, an atypical Russell Silver phenotype and a negative result for UPD. Chromosomal analysis for case 3 demonstrated a *de novo* idic(Y)(p11.3). Here, we report three *de novo* chromosomal anomalies in three boys, with literature reviews on the related cases. Our data indicates that there is still a place for conventional karyotyping as the first line of testing in patients with multiple congenital anomalies, developmental delay and mental retardation.

Evaluation of human spermatozoa nuclear organization by Fluorescence in Situ Hybridization (FISH)

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Objective: Human spermatozoa have a unique, well-organized nuclear architecture different from somatic cells. Telomeres play a fundamental role in organization of the sperm nucleus and looped chromosome configuration by forming telomere dimers i.e. contact between two ends of one chromosome. Improperly packaged sperm chromatin will have a high probability of disrupting the extremely structured sequence of fertilization. The evaluation of nuclear organization in spermatozoa is difficult due to the compactness of chromatin and the absolute necessity for the chromatin decondensation, which itself alters native sperm nuclear architecture. The purpose of our study was to identify sperm nuclear decondensation (ND) protocol that results in minimal disturbance of sperm architecture as well as to compare the telomere-telomere interaction of chromosome 1 in men with proven fertility and infertile patients.

Methods: Three widely used ND protocols for FISH on human spermatozoa were evaluated in this study: 0.5N NaOH; 10mM dithiothreitol (DTT) and lithium diiodosalicylate (LIS); 2.5mM DTT and heparin followed by direct labeling with sub-telolomeric (ST) arm specific Aquarius 1q (green) -1p (red) probes. Images were acquired using unbiased sampling and were analyzed employing Visiopharm Integrator System. The distance between ST probes was calculated after taking into account the effect of decondensation and was referred as normalized ST distance.
The results are expressed as mean ± SD.

Results: Only 40% of cells in fertile and infertile groups had expected normalized ST distance of <0.6μm between two arms of chromosome 1, confirming looped chromosome configuration. Spontaneous Comparison of ND protocols is provided in the table:

Protocol	Head area (μm²)	Fold increase	ND (%)	Mild ND (%)
Neat sample	12.8 ± 2.7*	n/a	n/a	n/a
1. NaOH	30.2.± 7.6	2.3 ± 0.6**	94	83**
2. DTT-LIS	50.2 ± 25.7	3.9 ± 2.0	95	28
3. DTT-heparin	48.1 ± 13.3	3.8 ± 1.0	95	15

* P<.001 between neat and all protocols; ** P<.001 between NaOH vs. DTT-LIS and DTT-heparin protocols. Mild decondensation was defined as 1.5-3 fold increase in nuclear area.

Conclusions: Combined denaturation and decondensation with 0.5N NaOH demonstrated superior results providing uniform mild ND with over 80% of nucleus suitable for FISH sperm architecture assessment. The similar observation of telomere-telomere interactions of chromosome 1 in men with fertility and infertility might be related to the nature of analyzed chromosome. Chromosome 1 has the largest amount of chromatin mass, and as a result would be most affected by any pathological process disrupting normal chromosome architecture including sperm chromatin damage.

aCGH reveals that G-banding pattern of a chromosome segment, when translocated, may change; diagnostic implications

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We present a case with prenatally detected abnormal chromosome 11 from amniocentesis. Initial G-banding showed the presence of additional material of unknown origin at distal 11q. Telomere FISH identified that the abnormal 11 had the distal 11q replaced by a distal segment of 11p. aCGH by Signature Genomics showed a 16.9 Mb duplication of 11p15.1pter (195,983-17,138,226) and a 5.5 Mb deletion of 11q24.3qter (128,928,236-134,434,130). Metaphase FISH using BACs confirmed the presence of the dup at 11q24.3. This is a de novo aberration since both parents had a normal karyotype by G-banding and telomere FISH. The fetal karyotype is interpreted as 46,XX,der(11)(pter->q24.3::p15.1->pter).
Retrospective review of the der(11) at 500-800 band levels showed that in most (13/19) cells the translocated 11p segment looked different from the native 11p; shorter and with atypical G-banding pattern. In the remaining 6 cells the translocated 11p looked similar to the native 11p.
This case illustrates that G-banding pattern of a chromosome segment, when translocated, may change or appear to be atypical. This could be one of the possible reasons why the chromosome bands have not always correlated with array CGH results in the past. We hypothesize that cytogenomic rearrangement may affect the dynamics and degree of chromatin condensation (at metaphase) and decondensation (at interphase) in the cell cycle and consequently G-banding staining property and pattern in the chromosome segments involved. A change in banding pattern can make it difficult or impossible to accurately diagnose a chromosome abnormality. This can be more problematic for 1) those specimens (e.g. prenatal or cancer) with limited number of analyzable metaphase cells and/or suboptimal quality; and 2) chromosome regions of small size and/or with fewer distinctive G-bands. Use of a combination of G-banding, FISH and aCGH capable of defining molecular breakpoints may enable not only more accurate diagnosis but also better understanding of the effects of cytogenomic rearrangements on G-banding patterns.

Sequential GTG banding and FISH identify cryptic chromosomal rearrangements and the origin of marker chromosomes in mosaic situations

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Three cancer cytogenetic cases are used as examples to demonstrate the usefulness of sequential GTG banding and FISH to uncover mosaic chromosomal anomalies. The first case is an 80-year-old female with high-grade lymphoma, having trisomy 8 in 10% and IGH rearrangement in 25% of cells. Sequential GTG banding and FISH confirmed the rearrangement involving IGH/BCL6, consistent with a diagnosis of follicular lymphoma. The second case is a 55-year-old male with pancytopenia, having monosomy 9 and multiple marker chromosomes. Sequential GTG banding and FISH excluded the Philadelphia chromosome and confirmed that the marker chromosomes identified were derived from chromosome 21, consistent with a diagnosis of MDS. The third case is a 67-year-old male with high-grade lymphoma, having gains of chromosomes 2, 8, 14, 20 and 21, i(6)(p10), del(7)(q22), derivative chromosomes 3, 4 and 5, and addition of unknown genetic material to chromosomes 11 and 16. Sequential GTG banding and FISH confirmed a rearrangement involving BCL6, consistent with a diagnosis of diffuse large cell lymphoma. Using these cases, we demonstrate the power of

sequential GTG banding and FISH in identifying cryptic chromosomal rearrangements and the origin of marker chromosomes in mosaic situations. This technique provides significant diagnostic value in patient care.

Unexpected rearrangement complexity revealed by oligonucleotide array complete genomic hybridization: When an apparently balanced translocation isn't balanced

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Balanced chromosomal rearrangements are frequently observed during the course of cytogenetic investigations. Balanced reciprocal translocations are detected in approximately 1 in 500 individuals. Most rearrangements are two break rearrangements of no phenotypic consequence. Occasionally, a de novo translocation may cause a deleterious phenotype, which are often explained by appealing to the following four mechanisms: the translocation causes a change in gene dosage by disruption of a gene, cryptic deletions or duplications around the breakpoint alter gene expression, a position effect can alter gene expression over large distances or imprinted gene expression is disrupted secondary to missegregation. However, when a familial translocation of no apparent phenotypic consequence is inherited and causes a deleterious phenotype it is often difficult to find a causal link. We present two pedigrees with the same apparently balanced translocation [t(9;12)(q22;q13)] detected by conventional cytogenetics that when inherited resulted in a deleterious phenotype. Oligonucleotide array complete genomic hybridization revealed a more complex rearrangement including an insertional translocation and probable meitotic recombination required to explain the cytogenetic findings observed by microarray. These cases illustrate the clinical utility of microarray for the elucidation of complex, unbalanced rearrangements that cannot be explained using conventional cytogenetics.

New Technologies in the Molecular Cytogenomic Era

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Since the initial discovery that chromosomes are the cellular structures upon which hereditary information is carried, the field of Cytogenetics has evolved at an incredible pace. The development of molecular cytogenetic techniques, including FISH and microarray technologies, has allowed us to examine the genome at increasingly finer resolutions and is quickly becoming the standard of care for a variety of medical genetic referrals. Advancements to these techniques continues and we are now witnessing the emergence of new tests that have the potential to significantly impact the clinical investigation of genomic disease. For example, the incorporation of SNP data into genomic microarrays promises the identification of disease-associated long contiguous stretches of homozygosity and uniparental disomy that may have otherwise gone undetected by more traditional cytogenetic techniques. The emergence of techniques such as molecular combing and optical mapping further promises to enhance our ability to investigate even the smallest chromosomal rearrangements. Finally, technical advancements and cost-reductions has made whole genome sequencing increasingly accessible; the ability to query a patient's whole genome for both chromosomal and Mendelian mutations in a single assay may indeed become routine in the

foreseeable future. Despite their diagnostic potential, many of these new techniques remain in their infancy. The discovery and utility of clinically relevant information obtained from these new technologies remains to be determined and will require considerable effort and collaboration between cytogeneticists, clinicians, bioinformaticians and the research community.

Microarray CGH in hematological disorders

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Microarray CGH is now cheaper and faster than multiple F.I.S.H. testing. It also reveals much more information. In cancer, obtaining all the relevant information while avoiding the collection of unwanted details is achieved by using custom built arrays that target cancer genes. The 8x60K Sudbury Cancer Array has been developed with Agilent and OGT oligonucleotides. Its use and advantages will be illustrated in various hematological disorders.

BACS-ON-BEADS ™ (BoBs ™) Workshop PerkinElmer

John Duck, Viola Freeman and Jack Wang
Hamilton Regional Laboratory Medicine Program
Hamilton Health Sciences

PerkinElmer was invited to present the BACS-ON-BEADS (BoBs) technology to the Genetics staff at the Hamilton Regional Laboratory Medicine Program. BoBs technology is used to rapidly detect gains and losses of the 5 common aneuploidies (13, 21, 18, X and Y) and 9 microdeletion syndrome regions. BoBs technology works by attaching specific DNA probes, that have been constructed from PCR amplified BACs, to fluorescently coded Luminex® beads. Both the labeled sample and reference DNA are hybridized to BACS-ON-BEADS probes under controlled environment. By using the BoBsoft™ analysis software, which takes the output file generated by the Luminex® 100/200™ instrument, the signal intensities from the sample and reference DNA are compared. This will permit any copy number changes to be seen in the targeted regions. During the two-day workshop the instruments required to perform the technique were set up. The complete procedure and data analysis were demonstrated using our in house patient samples. The presentation will show the technical procedure and data analysis of the result. This technology allows a quick turn around time (24 hr) on a large volume of patients (96 reactions per run) with very little DNA (100 ng) is required.

Rapid aneuploidy detection for low risk pregnancies: A suitable replacement for G-banding?

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Rapid aneuploidy detection (RAD) is a molecular technique that uses microsatellite markers on chromosomes 13, 18, 21 and the sex chromosomes to evaluate DNA obtained from amniotic fluid or

chorionic villi for the presence of aneuploidies. It is used extensively in Europe, but has not yet gained popularity in North America due to the reduced sensitivity in detecting all chromosome anomalies in comparison to G- banded analysis. However, it is not currently clear whether the limitations associated with RAD outweigh the disadvantages of G-banded analysis which is costly, has a long turnaround time and may lead to results with low predictive value, such as supernumerary markers, de novo balanced rearrangements and mosaicism. To examine the feasibility of using RAD as the primary test for prenatal diagnosis of chromosome abnormalities, we decided to establish simplified risk criteria that can be used by our counseling staff to select patients with the highest likelihood of any kind of chromosome abnormality versus patients at low risk. Patients received both RAD, with results available within 24-48 hours of the amniocentesis, plus G-banded analysis which had a 12-21 day turnaround. We categorized our patients into risk groups and evaluated the detection rate of RAD only versus RAD plus G-banded analysis. Through this prospective study, we tested in theory the use of QF-PCR as the primary method of prenatal chromosome abnormality detection, with G-banded analysis reserved for a subset of cases deemed at higher risk. Data will be presented that supports the use of RAD only in low risk pregnancies.

Impact of miRNA in Osteosarcoma: an integrated analysis of genomic, expression and miRNA profiles

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Osteosarcoma (OS) is an aggressive sarcoma of the bone that is characterized by a complex and composite karyotype. The level of genomic complexity and heterogeneity of this tumor prevented the identification of recurrent simple chromosomal abnormalities or gene rearrangements, like in many other sarcomas. We have shown that some regions are more prone to harbor genomic imbalances and chromosomal breaks: *1p35-p36*; *6p12-21*; *8q24*; *17p11-p12* and *19p13*. However, due to this genomic instability, no prognosis biomarker has been identified in OS yet. In addition to the previously published genomic and expression profiles, we integrated a new layer of gene expression regulator: MicroRNA (miRNA). It is thought that miRNA, a class of small non coding RNA, are involved in many biological processes, including oncogenesis. The expression profile of 723 human miRNA was established for a series of 7 OS. It showed that less than 5% of the miRNA (38) were differentially expressed compared to osteoblasts. Most of them were underexpressed (28): only 10 miRNA exhibited overexpression. The gene copy profiles for the matching samples were integrated to the new miRNA profiles: only 9 miRNA showed a positive correlation between expression and locus copy number. In OS, the gene copy number seems to play little role in the regulation of miRNA expression. The in-silico analysis also included the identification of miRNA target genes (Miranda, TargetScan, PicTar). Co-expression of miRNA and the target gene pairs was established by integrating the available data sets for OS expression profiles with the miRNA profiles. The gene copy number status for these target genes was also compared with their expected up or down-regulation (by the differentially expressed identified miRNA). By this means we identified a list of genes regulated either by miRNA (GADD45A, WIF1…) or by other means such as gene copy number (FOS, PLK2…). This integrated in-silico approach of genomic/ expression/ miRNA profiling allowed us to identify: i) the miRNA OS signature; ii) the mechanism of miRNA regulation in OS; iii) sets of genes regulated by miRNA; and iv) sets of genes regulated by gene copy number. This study

represents a new step into the comprehension of OS oncogenesis, the identification of new therapy targets, and potential prognostic biomarkers.

Evidence-based Assessment of Genomic Microarray Testing in Pediatric and Prenatal Populations

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Objectives: Genomic microarray testing is now routinely used for the evaluation of chromosomal imbalances in patients suspected of having a genetic syndrome, most commonly in patients who have already had a normal karyotype analysis but increasingly as a first-line test. Less commonly but increasingly, genomic microarray testing is used for prenatal diagnosis following amniocentesis or chorionic villus sampling when a chromosomal syndrome is strongly suspected but a normal karyotype is found (in the presence of abnormal ultrasound findings, for example). Genomic microarray may be performed using a targeted array that assays loci known to be associated with specific conditions or phenotypes, or may be performed with a genome-wide array, with probes at a specified resolution. To determine the analytical and clinical validity and clinical utility of genomic microarray testing in both pediatric and prenatal populations, an evidence-based health technology assessment was performed. **Methods**: The ACCE model, which was developed by the National Office of Public Health Genomics (NOPHG) at the Centers for Disease Control and Prevention (CDC), was used as the basis for the assessment. Utilizing this model, data regarding the sensitivity, specificity, risks, benefits, and quality control issues associated with genomic microarray testing were evaluated. Conclusions were based solely on published data from retrospective and prospective case control studies and meta-analyses. **Results**: Sufficient data exist that support the analytical and clinical validity and clinical utility of performing genomic microarray testing in patients with developmental delays, learning disabilities, mental retardation, dysmorphic features, and/or congenital anomalies. Studies that examine the use of genomic microarrays in prenatal populations, however, are limited. The benefits of genomic microarray testing include increased resolution, the ability to detect copy number changes at multiple loci throughout the genome in a single assay, and the ability to characterize unclear abnormalities identified by conventional cytogenetics, such as supernumerary marker chromosomes. In any setting, the main limitation of genomic microarray testing is the identification of variants of unclear clinical significance, an issue that is currently being addressed with the development of several copy number variant databases. In addition, genomic microarray testing will not detect certain chromosomal abnormalities, such as balanced rearrangements or polyploidy or low level mosaicism.
Conclusions: The published data support the use of genomic microarray testing, either as an adjunct to standard karyotype analysis or as a first-line test, in the diagnostic work-up for individuals suspected of having a genetic syndrome. In addition, there is a clear clinical benefit to performing genomic microarray testing in miscarried fetuses and stillborn infants where specimens may be limited or compromised. There is sufficient evidence to suggest that the use of targeted microarrays as an adjunct to conventional karyotype analysis is beneficial. Evidence is currently insufficient, however, to adequately weigh the risks and benefits of using genome-wide arrays in the prenatal population.

Array CGH Elucidation Of A Complex Chromosome 21 Rearrangement In A Child With Developmental Anomalies And Thrombocytopenia.

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A 2 ½ year-old male patient presented for genetic assessment due to intermittent thrombocytopenia, with several episodes of petechiae with acute infectious illnesses. Platelet levels were found to be significantly decreased with no evidence of other hematologic disease. Other medical concerns included bilateral vesico-ureteral reflux, hypospadias, laryngomalacia and short stature. He had poor feeding in the first few months of life. He demonstrated global developmental delays with behaviour concerns. All growth parameters were below the 3rd percentile. Dysmorphic features included coarse hair, short up-slanting palpebral fissures, epicanthal folds, hypertelorism, depressed nasal root, bulbous nasal tip, thin vermillion border with a tented upper lip. His ears were low-set and posteriorly angulated. He had short fifth fingers with clinodactyly of the 5th digit on the left hand. Family history was unremarkable.

Investigations showed a de novo chromosome abnormality with additional satellites on the distal long arm of one chromosome 21. FISH studies demonstrated a deletion of the 21q subtelomere region, with a diminished signal for the AML1/RUNX1 locus. Due to the clinical significance of haploinsufficiency for the RUNX1 gene, further investigations were undertaken to determine if the gene was completely deleted. Genotyping of microsatellite markers predicted a single copy of the RUNX1 gene. A panel of BAC FISH probes in the vicinity of RUNX1 showed deleted or diminished signals but not in the predicted pattern according to the human genome assembly. Array CGH using a hematology design chip suggested an interstitial deletion of 9 BACs in the 21q22,12 RUNX1 region. Subsequent array CGH analysis using a whole genome 105K oligo array revealed a complex chromosomal rearrangement of chromosome 21 with interspersed duplications and deletions and elucidating the perceived discrepancy of previous genetic results.

Incorporating findings from array CGH studies into clinical cytogenetics and molecular diagnostics

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The advent of robust array comparative genomic hybridization (CGH) and SNP/CNV platforms, combined with some recent refinements of FISH technologies provides new challenges for cytogenetic and molecular genetics service laboratories. Clinical applications arising from the discoveries of "next gen" sequencing platforms will also create new demands that will impact on the hospital genetics laboratory services. This presentation will review how new approaches will be required to address innovations in constitutional genetic analyses, and will contrast these to the somewhat different approaches that will be needed for future cancer testing. In the context of personalized medicine there is increasing awareness that many tumours, previously considered to have a distinct histologic and clinical subtype, can be further subdivided into unique molecular subtypes. Many of the genetic changes within these subtypes can be used to make treatment decision or they might inform oncologists on the best drug to use. For solid tumours close interaction with pathologists in the implementation of novel FISH and molecular approaches will also

be required. Since many tumours are characterized by chromosomal instability and unexpectedly high levels of genetic mutation, there will be significant challenges in deciding when and how an assay should be implemented as a new hospital service test. In constitutional molecular analyses the role of bioinformatics will be crucial to assigning appropriate clinical value to genomic findings and for providing timely and comprehensive information for the clinical geneticists and counselors. The complementary nature of molecular, genomic and cytogenetic information that will be available in future test algorithms will require more integrated approaches to reporting and to training at all levels.