The Great Lakes Chromosome Conference (GLCC) 2011 Collated Abstracts

Diagnostic assessment of AML

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As the efficacy and specificity of available options for management of AML rapidly improve, laboratories are under increasing pressure to analyze specimens with greater sensitivity, greater accuracy, and greater urgency. We need to be able to rule in or rule out an expanding list of specific aberrations in order to provide optimal support to clinicians. Further, there is a challenge to make sure that there is consistency across regions in their approach to testing so that equivalent access to the same test accuracy and comprehensiveness is reasonably available. In some ways, the diagnostic challenge of AML is a microcosm example of the same challenges we face with other test indications. How can we best work within budget constraints to use existing technologies while we take up the promise of new technologies like expression panels and arrays, and how much time will we have to make the transition?

Cytogenetic diagnostic and prognostic findings in lipomatous tumours

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The largest single group of mesenchymal tumours are adipocytic tumours due to the high prevalence of lipomas and angiolipomas; and liposarcomas represent the single most common type of soft tissue sarcoma. These tumours currently have the best studied cytogenetics of all soft tissue sarcomas. Most types of adipocytic tumours have distinctive genomic aberrations and as a result detection of these cytogenetic alterations can be of significant assistance in diagnosis. The World Health Organization classifies adipocytic tumours into eleven benign, one intermediate and five malignant subtypes. These classifications are largely based on clinical features and histologic patterns; however, some more aggressive adipocytic tumours are difficult to distinguish from benign lipomas; and dedifferentiated liposarcomas from other high-grade undifferentiated sarcomas. In such cases molecular cytogenetic testing can provide considerable help in the diagnosis and the alter prognosis. The spectrum of tumours seen at the British Columbia Cancer Agency, illustration of characteristic cytogenetic abnormalities and clinicopathologic features will be explored.

Cytogenetic analysis of chromosome instability syndromes

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Although molecular genetic testing is available for the rare, autosomal recessive chromosome instability syndromes Fanconi Anemia, Ataxia Telangiectasia, and Bloom syndrome, cytogenetic assays for the chromosomal abnormalities associated with these syndromes continues to be an integral part of patient assessment. A summary of volumes and results for a 10 year period will be presented, along with an overview of the clinical features of the patients. Complications of such testing, including the difficulty of dealing with low white blood cell counts, and of distinguishing chromosome abnormalities caused by chemotherapeutic/radiation treatment from those attributable to the syndromes will be discussed.

Variability in quality amongst labs analyzing the same pediatric ALL pellet

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The Quality Management Program - Laboratory Services (QMP-LS) functions under the Ontario Medical Association; as part of its mandate it functions to coordinate and provide external quality assurance (EQA) programs for many disciplines of laboratory medicine and pathology, including Cytogenetics. As part of this process, fixed cytogenetic pellets are regularly used as a uniform source of survey testing material. The results, including the laboratory report and karyotyped metaphase are submitted to QMP-LS for assessment. In Ontario, this EQA activity is mandatory under Ontario Laboratory Accreditation.

During this talk, an overview of the function and structure of QMPLS will be given, with a discussion regarding the EQA program for Cytogenetics. The results of one Cytogenetics survey will be reviewed, illustrating a surprising variation in the quality of karyotyped metaphase images derived from uniform fixed pellets and highlighting the importance of post-culture steps in the production of quality cytogenetic preparations.

FISH testing for HER2 in breast cancer: brief reviews of guidelines, controversy and advancement

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Human epidermal growth factor receptor 2 gene (HER2, ERBB2) is amplified in approximately 18%-20% of breast cancers. HER2 amplification is associated with poor prognosis in breast cancer and the patients with HER2 amplification can benefit from Herceptin treatment. FISH assessment of HER2 status is considered standard of care in management of breast cancer.

This presentation is to provide brief reviews of 1) guideline recommendations for HER2 testing of breast cancer; 2) controversies on cut-off HER2/CEP17 ratios for HER2 positivity and definition and reporting of HER2 genetic heterogeneity (GH); and 3) emerging techniques such as bright field in situ hybridization and microarrays. Current data support FISH as primary HER2 testing because of better test accuracy, reproducibility and predictive value. While the 2007 ASCO/CAP guidelines with 3 diagnostic ranges are commonly used, increasing evidence favours the initial US FDA criteria with for HER2 positivity. The definition of GH in the 2009 CAP guidelines may lead to misinterpretation and clinical significance of reporting of the GH remains unclear. Use of microarrays has contributed significantly in our understanding of cytogenomics of "polysomy" and "monosomy" 17 in breast cancer.

The Cytogenetic Lab at London Health Sciences Centre and University of Western Ontario has performed FISH testing for HER2 of FFPE breast cancer samples since 2006. Our annual caseload approximate 240 FISH tests; reflexes from ~1000 cases initially tested by IHC. Some of our interesting cases are also part of this discussion.

Interpretation of cytogenetic biomarkers on an individual basis: Beware, interindividual variation is present…

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Cytogenetic biomarkers such as chromosome aberrations (CAs), sister chromatid exchanges (SCEs) and micronuclei (MNs) are being increasingly used to monitor occupational and environmental exposure to many contaminants. Also, these biomarkers are useful in clinic during investigation of chromosomal instability syndromes. During my PhD's research project, we measured those cytogenetic biomarkers in healthy young adults before and after a genotoxic challenge caused by benzo-a-pyrene *in vitro* exposure, a carcinogenic chemical. We found that an important interindividual variation was present among our subjects, with and without exposure. Indeed, a spontaneous CA frequency (without gaps) ranging from 1 to 10% for women, and from 0 to 4% for men, with a mean CA frequency of 1.6 ± 0.3% for the group was present. Also, MN frequencies were highly variable between individuals, as we obtained spontaneous MN frequencies (per 1000 cells) ranging from 3.5 to 21‰ for women, and from 1 to 17‰ for men, with a mean MN frequency of 9.9 ± 1.2‰ for the group. Finally, individual mean SCEs ranging from 5.9 to 12.0 were measured in our cohort, representing a 7.6 ± 0.12 mean SCEs for the group. Use of high frequency SCE cells (HFC), identifying outlier individuals, points towards 3 subjects with a high percentage of HFC cells, two with «normal» SCEs (7.7 and 7.8), and the third having the highest SCEs (12.0) of the group. Together, these results suggest that individual interpretation of cytogenetic biomarkers should be realized using a combination of biomarkers and careful control of confounding factors.

"Double hit" diffuse aggressive B cell lymphomas with features of lymphoblastic leukemias: importance of FISH in the diagnosis

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We describe three patients with high grade B cell non-Hodgkin lymphoma with phenotypes overlapping between precursor-B lymphoblastic leukemia (B-ALL) and diffuse large B cell lymphoma, all with MYC and IGH-BCL2 translocations.

Three adult patients (ages 61, 63, and 83) all presented with lymph node masses. One of the patients had a history of follicular lymphoma. In two of the cases, the morphologic and immunophenotypic features were consistent with B-ALL, including strong expression of TdT and CD10, and lacking CD20. The third patient had a leukemic component with WBC of 112 x 10^9 / L, CD20 negative by flow, with a diagnosis of unclassifiable B-cell lymphoma with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. Overlapping or atypical phenotypes prompted cytogenetic investigations for MYC and BCL2 rearrangements, and FISH analysis on cytospin preparations and isolated nuclei from sections of paraffin-embedded tissue revealed MYC and IGH/BCL2 rearrangements in all three cases, with complex single patterns including extra signals for the intact and rearranged genes tested for.

The importance of recognizing a "double hit" lymphoma is due to its association with a very poor survival, which results in a more aggressive treatment regimen for these patients. These cases illustrate that a subset of dual translocation lymphomas may be diagnosed as B-ALL due to an immature B cell immunophenotype and systemic involvement. The typical karyotype as part of a B-ALL workup would be very time consuming and the presence of dual translocations may be missed due to the complex abnormalities. The presence of atypical features for B-ALL including pleomorphic nuclear morphology, and expression of restricted light chain immunoglobulins and bcl-2 protein, should prompt FISH analysis to provide an expedited "double hit" diagnosis.

Clinical importance of monitoring the emergence and decay of BCR-ABL resistant mutant clones in *Imatinib* and *Dasatinib* - -treated CML patients

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Chronic myeloid leukemia (CML) patients have been successfully treated with imatinib (Gleevec) and other potent tyrosine kinase inhibitors (dasatinib, nilotinib) which target the activated ABL kinase of *BCR-ABL* fusions. However more than 100 distinct ABL mutations have been characterized that are often responsible for treatment failure due to the progressive expansion of drug-resistant clones, an event invariably associated with poorer clinical outcomes. As the progress of the disease is conventionally monitored by evaluating BCR-ABL transcripts, the lack of or the loss of a molecular response calls for a mutational analysis, done by DNA sequencing. Any given CML patient is at best tested once during treatment, at relapse, and rarely so at diagnosis.

We developed a more sensitive method of mutant detection, with the objective of examining the fate of mutant clones that arise prior to or in the course of treatment, a more genuine assessment of drug sensitivity under selective pressures in vivo, with achievable doses. A panel of 19 mutations in 14 codons was selected. Fourteen mutations in 11 codons were detected by allele-specific (RT)-PCR, in the context of b2/b3a2 translocation subtypes and validated (by prior sequencing): E255K, M244V/I, E275K, D276G,T315I/N, M351T, L298V, E355G, F359V, H396P/R and V379I. Nine CML patients, all in chronic phase at diagnosis, were studied longitudinally. For 6 patients, 3 log reduction was never achieved with imatinib.

The mutational tests by sequencing were ordered either 2 to 5 yrs after initiation of imatinib treatment (n=5); or not ordered after 4 to 6 yrs (n=2); or only ordered 3 to 16mo after dasatinib treatment. The tests were ordered 2 to 4 yrs, and 10mo after the threshold 3 log reduction was not achieved under imatinib and dasatinib, respectively.

The mutation(s) identified by sequencing were detected on average 12 months, and up to 52 months earlier by PCR. With one exception, a single mutant was identified by sequencing per patient, but all tumors examined were found to be mutant polyclonal by PCR. Pre-existing mutations were found by PCR at diagnosis or first presentation in the majority of cases.

T315I mutants were identified in 8 patients but 4 resisted imatinib or dasatinib or both drug treatments, in agreement with their relatively high abundance. Similar clonal evolution towards resistance characterized E255K mutants in 5 patients. The following mutants appeared to persist under imatinib treatment but to decline after switching to dasatinib: F359V, M244V, M244I, E275K, L298V, H396R, D276G.

This study, together with other reports suggest that the early emergence of co-existing mutants, even at low level, is a significant factor in the progression of the disease in therapeutically treated CML patients. Laboratory tests could be implemented to multiplex the most frequent and most resistant mutations. (*supported in part by PALM Enrichment Funds, The Ottawa Hospital*)

Characterization of recurrent and rare 12p and 21q chromosomal abnormalities in childhood ALL and AML

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Transcription factors *ETV6* and *RUNX1,* localized in 12p13 and 21q22 respectively, play an important role in hematopoiesis and leukemogenesis. Translocation t(12;21), generating the fusion gene *ETV6-RUNX1,* is the most common rearrangement in B lineage childhood acute lymphoblastic leukemia (ALL). However, 12p and 21q abnormalities that doesn't involve those genes are less studied. The aim of our study is to establish the frequency and the characterization of 12p and 21q abnormalities in *de novo* ALL and acute myeloid leukemia (AML). Overall, 59 cases present 12p (44 ALL and 4 AML) and/or 21q (46 ALL and 2 AML) rearrangements. As expected, most 12p abnormalities involve *ETV6* (98 %) and most 21q involve *RUNX1* (93 %). Besides translocation t(12;21), the most common anomalies in ALL are deletion of the wild-type allele of *ETV6* (34 % of 12p abnormalities) and *RUNX1* amplification (16 % of 21q). In AML, rare translocations have been identified by FISH or by karyotype: t(7;12), t(12;19) and t(12;13). Moreover, a further analysis of t(12;13) revealed a novel *ETV6* partner, *FOXO1*, which is frequently rearranged in alveolar rhabdomyosarcoma, but has never been described in hematologic malignancies. In conclusion, our study point out the importance of using a strategy combining karyotype and FISH to identify secondary or rare structural abnormalities.

Cytogenetic investigation of sex chromosome abnormalities: unexpected results

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Sex chromosome aneuploidy is the most commonly observed chromosome specific abnormality in humans. Structural abnormalities of the X and Y chromosome have also been well described and in most instances, both numerical and structural abnormalities are identified using conventional G-banding, with further characterization possible using special stains and fluorescence in situ hybridization (FISH) analyses. Here we report on the cytogenetic findings of two cases in which structural abnormalities of the Y chromosome were identified by FISH. Our first case is that of a 42 year old male who presented clinically with mental retardation, physical anomalies and behaviour issues. G-banding analysis identified the expected XY sex chromosomes and a supernumerary marker chromosome in all cells analyzed. Additional FISH analysis identified the marker chromosome to be Y chromosome derived. However, the normal appearing Y chromosome had 2 copies of SRY at the terminal ends of both arms. His final karyotype was reported as: 47,XY,+mar.ish der(Y) (wcpY+,SRY++), +der(Y)?i(Y)(p10) (wcpY+,SRY++). Case 2 is of a 14

year old female referred for short stature. G-banding analysis revealed a mosaic karyotype; 45,X[19]/46,XY[16]. Additional FISH and C-banding analysis determined that the normal appearing Y identified by G-banding analysis was actually an isochromosome Yp. The final karyotype was reported as: 45,X[19]/46,X?Y[16].ish idic(Y)(q11.23) (wcpY+, SRY++, DYZ3++, DYZ1-). Interestingly, follow-up microarray analysis of case 2 detected loss of all probes that map to the Y chromosome only. Based on the identification of unexpected Y chromosome structural abnormalities in these two case studies, we suggest further cytogenetic analysis be undertaken in cases involving Y chromosome abnormalities.

Group presentation on challenging situations in the cytogenetic laboratory

Josée Lavoie, Montreal Children's Hospital

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

1- **Spot check!**
Amanda Fortier and Josée Lavoie, Montreal Children's Hospital

2- **Too many breakpoints to figure out the mechanisms: inversions, multiple deletions/duplications and intervening normal sequence all come together**
Jia-Chi (Jack) Wang, McMaster University Health Sciences Centre
We report on three cases with multiple chromosomal rearrangements at chromosome 2q, 13q and 19p/19q. These cases were characterized by G-banding, 4x180k oligo microarray and dual color BAC-FISH analyses. The mechanisms responsible for these complex rearrangements remain to be determined.

3- **Testing paradigms in multiple myeloma**
Adam C. Smith and Monica Hrynchak, BC Cancer Agency - Vancouver Centre
Testing for cytogenetic abnormalities in Multiple Myeloma is used to aid in the prognostic staging of patients in addition to other criteria. The NCCN recommends a barrage of cytogenetic and FISH testing to help in staging and treatment decisions. In an effort to provide the most relevant staging results while minimizing cost to the health care system a strategic and reflexive approach is used at the BCCA. However, karyotypic changes such as hypodiploidy may obscure a clear indication to perform a reflex test. Therefore, how much testing is really required to enable sound treatment decision making.

4- **New algorithm for testing POCs, validation at MSH**
Elena Kolomietz, Mount Sinai Hospital

5- **The "46,XY,? something" karyotype; when microarray studies do not aid in the interpretation of G-banding results**
Elizabeth McCready, Children's Hospital of Eastern Ontario
We describe an aberrant banding pattern on the long arm of chromosome 3 including an extra band within 3q21. Despite an apparently unbalanced karyotype by G-band studies, array CGH studies performed elsewhere were normal. Investigation of the aberrant banding pattern and implications for counseling are discussed.

6- **Follow-up on a pediatric patient with multiple abnormal clonal repopulation post-transplant for Ph-+ve ALL**
Ronald F Carter, McMaster University Health Sciences Centre

7- **Trisomy 4 and C-MYC amplification in an AML case.**
Yana Ahuzhen and Joan Halbgewachs, Lakeridge Health

Caught in the act! A cell line with a dicentric chromosome 9 gives rise to two stable cell lines with different rearrangements of chromosome 9

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A 29 year old woman underwent amniocentesis at 21 weeks gestation because of an elevated maternal serum screen risk for trisomy 21. At this time, detailed ultrasound revealed an oomphalocoele. This was her second pregnancy: she had one living child from her first pregnancy and there was no unusual or contributing clinical history in this pregnancy. Cytogenetic analysis of G banded metaphases from cultured amniocytes detected three related female cell lines. One cell line was characterized by a del(9)(p22) and was detected in cells distributed in six colonies over four independent slides. A second cell line showed an addition to 9p that was interpreted as der(9)(9qter->9p22::9p22->9p12:); this rearranged chromosome was seen in cells in seven colonies distributed on four slides. Vigilant searching identified a potential third cell line on one slide that was described as dic(9)(9qter->9p22::9p22->9qter). Additional FISH testing was used to verify the interpretation of G banding analysis.
The cell line with the large dicentric chromosome 9 is presumed to have given rise to both the del(9) and the der(9) as segregants after breakage in band 9p22. Surprisingly, both daughter cell lines as well as the original cell line bearing the dicentric 9 were captured as metaphases in this analysis.

Validation of BlueGnome 24sure array CGH for comprehensive chromosome analysis of embryos

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Genomic imbalances are a major cause of constitutional disorders. Therefore, aneuploidy screening has become the cornerstone of preimplantation, prenatal and postnatal genetic diagnosis. Array comparative genomic hybridization (array CGH) has been successfully used as a rapid and high-resolution method for the detection of genomic imbalances. However, up until now, array CGH has been performed using a significant quantity of DNA derived from a large pool of cells.
Aneuploidy screening of a single cell would allow addressing basic questions about the chromosomal constitution of gametes and early embryos. In the clinic, this screening could be used to select a single embryo with the highest chance to implant. The use of preimplantation genetic diagnosis to assist the identification and preferential transfer of euploid embryos should improve implantation rates, reduce miscarriages and ultimately lead

to an increase in live birth rates. The development of microarray technology combined with whole genome amplification (WGA) has recently emerged as a promising solution for comprehensive analysis of the full chromosome content of a single cell.
Our study aimed to evaluate the use of single-cell array-CGH for the whole genome aneuploidy screening of polar bodies and preimplantation embryos in a pre-clinical setting.

A prenatal case with trisomy 18 and mosaic variant Klinefelter syndrome due to isochromosome Xq

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Objective To present the first case of a trisomy 18 with mosaic variant Klinefelter syndrome due to isochromosome Xq diagnosed prenatally.

Method and Results Both the fluorescence in situ hybridization (FISH) with probes for chromosomes X, Y, 13, 18 and 21 on uncultured amniocytes and cytogenetic analysis of cultured amniotic fluid using G-banding at 450-475 band level resolution were performed in a 21-year-old woman at the gestation age of 19 5/7 weeks due to a screen positive risk for trisomy 18 of 1/5 (20%) and a screen positive risk for Smith-Lemli-Opitz syndrome (SLOS) of 1/30 (3.33%) as indicated by serum integrated prenatal screening (SIPS). The FISH result indicated positive for trisomy 18 with mosaic Klinefelter syndrome, since 10% of nuclei had two signals for the X chromosome and one signal for the Y chromosome, while fetal chromosome analysis revealed a karyotype of 47,XY,+18[[18]/48,XY,i(X)(q10),+18[3]. Both parental karyotypes were not available. Fetal ultrasound evaluation showed a single umbilical artery and bilateral choroid plexus cysts.

Conclusion The chromosomal complement of this fetus is unbalanced with trisomic for chromosome 18 in all of the cells and trisomic for the long arm of chromosome X in about 10% of cells. To our knowledge, this is the first report concerning a trisomy 18 with mosaic variant Klinefelter syndrome due to isochromosome Xq.

Discordant phenotypes in a mother and daughter with mosaic supernumerary ring chromosome 19 explained by a cryptic recombinant chromosome

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Reported here is a patient with severe intellectual disability, microcephaly, short stature and dysmorphic features possessing two cytogenetic abnormalities, an apparently balanced paracentric inversion in the long arm of chromosome 7, and a small supernumerary ring chromosome derived entirely of material from chromosome 19. Although the inversion 7 was observed in all cells, mosaicism was observed for the ring chromosome. Interestingly, the apparently identical cytogenetic abnormalities were detected in the patient's mother who with the exception of a few dysmorphic features had a normal phenotype. To investigate the phenotypic discordance, the level of mosaicism of the ring chromosome was assessed and a-CGH was performed. The level of mosaicism could not adequately explain the discordance; however, a-CGH led to the identification of a cytogenetically cryptic pericentric inversion in the mother, in addition to the paracentric inversion identified within the same chromosome 7.

Interstitial deletions 14q and CGH microarrays: When the clinical severity of the phenotype does not correlate with the size of the abnormality

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We present four cases of chromosome 14 interstitial deletions that were characterized by array CGH (aCGH). In the first two instances, a *de novo* 14q12 deletion of approximately 3 Mb was found in two young girls with central nervous system abnormalities. These deletions involved 3 genes each and had in common the loss of *FOXG1*, mutations of which are associated with a congenital variant of Rett syndrome. Further analyses by FISH confirmed the deletion in both patients. In the two remaining cases, fetal karyotype showed an interstitial deletion in 14q31. Amniocentesis was performed for increased risk of aneuploidy (presence of soft markers and increased nuchal translucency respectively). In both cases, a similar deletion was suspected on the parental karyotype, and later confirmed by FISH analyses. aCGH on both probands revealed that the deletions in 14q31.1q31.3 were approximately 7 Mb in size, involved respectively 10 and 7 genes, and had in common the loss of 6 genes: *TSHR*, *GTF2A1*, *SNORA79*, *STON2*, *SEL1L* and *FLRT2*. Two similar cases, with slightly larger deletions of the same region, have been reported in the literature. Mild cognitive anomalies and dysmorphic features seem to be associated with deletions of this region. In conclusion, these four cases increase our knowledge regarding the clinical significance of chromosome 14 interstitial deletions. They also illustrate the importance of parental analyses following the discovery of a chromosomal anomaly by aCGH since rather large deletions can be associated with mild clinical severity and either be inherited or *de novo*.

Identification of a supernumerary neocentromere marker and a supernumerary derivative chromosome in an autistic patient

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Array comparative genomic hybridization (aCGH) is a clinically valuable molecular cytogenetic method for analyzing DNA copy-number variations. Not only aCGH can detect submicroscopic anomalies, but when combined with fluorescence in situ hybridization (FISH), it allows comprehensive description of genetic alterations missed by conventional cytogenetic analysis. We have recently detected by G-banding karyotyping, a mosaic supernumerary marker chromosome (SMC) in an autistic patient (47,XY,+mar[25]/46,XY[5]). FISH analysis with alpha satellite probes showed that this SMC originated from either chromosome 1, 5 or 19. aCGH revealed a 5p11q11.2 (≈ 7,7 Mb) pericentromeric duplication corresponding to the SMC and a 10q11.21q11.22 interstitial duplication (≈ 4,2 Mb). We performed FISH analysis on the patient and his parents' chromosomes to confirm the location of these copy number duplications and to study their inheritance. We observed the coexistence of a de novo derivative chromosome 5 with a tiny de novo mosaic neocentromeric marker corresponding to the duplication 10q11.21q11.22 in this child. Neocentromeric markers are rare chromosomal aberrations where a new centromere has formed in a non-centromeric location. To our knowledge, this is the second case in the literature of a neocentromeric marker formed in region 10q11.21q11.22. This case illustrates the need to confirm the location of copy number deletions and duplications detected by aCGH with visual techniques such as FISH analysis to allow a comprehensive description of genetic alterations.

Microarray testing for genomic imbalances - challenges and unforeseeable clinical implications

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Background: Microarray testing offers a much higher resolution for the evaluation of genomic imbalances than can be achieved by conventional karyotyping. However, the ability to detect small copy number changes in the genome also comes with challenges. We present a case with a small gain in the dystrophin gene identified by array comparative genomic hybridization (CGH) testing to illustrate the challenges that Genetics Laboratories and Clinical Geneticists face with advancing technologies.
Case report: The proband was a 16 month-old girl with global developmental delay. She was born to healthy non-consanguineous parents of Lebanese descent. Clubfeet were noted at birth and she developed infantile spasms and seizures at 5 months of age. Decreased lateral eye movements with bilateral Duane anomaly were noted. Dysmorphic features were evident on physical examination. The muscle tone appeared increased peripherally with brisk reflexes. Magnetic resonance imaging of the brain showed absence of the posterior pituitary bright spot, prominent lateral and third ventricles, and features suggestive of

cerebral atrophy. Previous cytogenetic investigation yielded a normal female karyotype at 450 band resolution. Angelman and Rett syndrome molecular testing were negative. Other laboratory investigations were also unremarkable. Array CGH testing on an oligonucleotide platform revealed a 76.8 kb gain within the dystrophin gene. Molecular testing by multiplex ligation-dependent probe amplification (MLPA) has confirmed the array finding, with duplication of exons 34 to 42 of the dystrophin gene which was predicted to be in-frame. The array CGH finding however was believed not to account for the child's phenotype. Parental studies by molecular method showed that the mother was a carrier of the same duplication. The mother was asymptomatic with no history of weakness and her serum creatine kinase was normal. There was no family history of weakness in maternal male relatives. She was 22+ weeks pregnant at the time of investigation but declined prenatal testing. Prenatal ultrasound examination revealed normal fetal growth and anatomy and normal amniotic fluid volume. The fetal sex appeared to be female. Duplication of the dystrophin gene involving only exons 34 to 42 has not been reported in the literature and its implication in a male carrier is currently unknown; clinical features are predicted to range from asymptomatic to Becker/Duchenne Muscular Dystrophy with Becker phenotype being the most likely.

Conclusions: Microarray testing offers a powerful tool for detecting copy number changes across the genome. However, there are challenges in confirming and interpreting the results particularly when the aberrations are small. Array finding(s) thought not to be contributory to the presenting phenotype of the patient may still have profound implications in genetic counseling for the individual and his/her family.

Genomic microarray versus G-band analysis for the diagnosis of developmental delay and multiple congenital abnormalities

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The implementation of genomic microarray analysis revolutionized the diagnosis of patients with developmental abnormalities, often providing a diagnosis when G-band analysis is negative. While genomic microarray will not detect balanced chromosome rearrangements, the ability to efficiently interrogate the whole genome at an ever-increasing resolution has greatly improved diagnostic yield, and make this the most attractive option as a front-line test for the diagnosis of developmental delay and multiple congenital abnormalities. The opportunity to identify genomic copy number changes of smaller size and gene content, has led to challenges in clinical interpretation that did not commonly exist with karyotype analysis. Previous assumptions associated with G-banded chromosome studies have become less relevant in the face of smaller copy number changes identified by genomic microarray, which can be associated with reduced penetrance and variable expressivity. The advantages and limitations of data generated by microarray analysis as compared to G-banded chromosomes will be discussed.

Interpretation of copy number variants from microarray data using genomic databases

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The advent of microarray technology has made discovery of clinically relevant unbalanced structural variants feasible and cost effective in a diagnostic setting. However, increasing platform resolution and the accompanying increase in the number of variants detected coupled with a lack of applicable control has led to challenges in clinical interpretation. Public repositories cataloguing copy number variants (CNVs) from healthy control individuals can aid in this interpretation. The Database of Genomic Variants (DGV; http://projects.tcag.ca/variation/) was developed in 2004 to provide information about structural variation in the human genome for biomedical research. Currently, the database holds nearly >100,000 published variants representing approximately 16,000 independent chromosomal loci. The main user group of the database and its associated genome browser are clinical geneticists utilizing the data to facilitate the interpretation of structural variation data from patients. With this user group in mind specific aims of the DGV are: to provide information about genomic structural variants identified in control samples, to serve as a resource to both clinical and research labs; to show variation in genomic context in a simple genome browser; and to afford detailed data analysis using a query-analysis tool. The increase in the number of studies and data archived has also led to several challenges including the increasing complexity around data curation. Correct usage of the DGV in the context of these new challenges will be discussed. We will also describe the relationship and collaboration between DGV and two new databases recently launched, including DGVa (started by European Bioinformatics Institute) and dbVAR (started by the National Center for Biotechnology Information).

Quantification of microarray findings by trio analysis: the numbers tell the story

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Credit Valley Hospital

As microarray technology has progressed from BAC arrays to high resolution oligo and SNP arrays, the confirmation of Copy Number Changes (CNCs) and the subsequent determination of inheritance has become increasingly challenging. Although fluorescence *in situ* hybridization (FISH) is required for determination of structural changes, its utility is limited by the quality of the FISH probes available for the region of interest, the relative size of the probes to the CNCs, and the genomic position of the CNCs. Confirmation of inheritance of an unbalanced CNC from a carrier parent can be done by microarray; however, this is a very expensive option. Quantitative PCR (Q-PCR) is an alternative methodology that is used to measure the genomic DNA of the CNC region relative to a stable, reference diploid locus. The main drawback of Q-PCR is that each experiment is custom designed and requires PCR efficiencies to be equivalent between the reference and the target amplicons. Published methods are cumbersome, requiring dilution series of

genomic DNA to produce standard curves of the reference and target amplicons. As well, the dye SYBR Green is known to inhibit PCR and intercalate inconsistently, depending upon the amplicon. In our laboratory, we have found that careful selection and validation of primer pairs, the use of a saturating dye rather than SYBR Green, and inclusion of multiple controls in trio analyses negate the need for standard curves. This makes it possible to reliably determine the inheritance of CNCs in a single, simple experiment based upon the ΔΔCt and/or Pfaffl equations. Q-PCR is a cost-effective and efficient way to confirm small regions of duplication and deletion detected by array CGH that were too small to confirm by FISH.

Evaluation of aberration calls with aCGH by automated algorithm analysis

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Evaluation of cytogenetic aberrations have been greatly improved with the use of Array Comparative Genome Hybridization (aCGH). The resolution and power of this technology comes from the use of thousands of probes to span the entire genome to detect copy number changes. The challenge is to interpret the thousands of probes to determine whether a substantial change has occurred. Various software are available to mathematically determine the aberration and help determine pathogenicity. Our laboratory has evaluated and used three software packages in the last three years to determine aberration calls; Agilent's DNA Analytics, Bluegnome's Bluefuse Multi, and OGT's Cytosure Interpret. Although each package has its own features which set them apart, it is the aberration detection component that is the critical part for clinical labs. We will have a overview of our lab's experience dealing with automated algorithm analysis and discuss how we analyze our data. In addition, we will discuss the issue of manual calls that may be "missed" by the algorithm, as well as the benefits and pitfalls of these additions.

Array CGH analysis of a complex chromosome rearrangement: lessons for interpretation of copy number changes

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Introduction of a new technology, such as array CGH, in the diagnostic laboratory setting can be both satisfying for the ability to provide answers to previously undiagnosed patients as well as frustrating due to the ambiguity of some findings and uncertainty of clinical significance. We report here a long-standing patient of the Alberta Children's Hospital genetics clinic with a complex chromosome rearrangement who was finally given a clinical diagnosis following analysis by array CGH. This case demonstrates both the satisfaction and frustrations of this new diagnostic modality. Iterative learning from analysis of array CGH analyses in our laboratory over the past two years has led to the development of an evaluation tool for interpretation of data and for a standardized classification system of results.

Patient-derived induced pluripotent stem cells to assess the function of copy number variations associated with autism spectrum disorders

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Copy number variation (CNV) analysis of Autism Spectrum Disorder (ASD) patients and family members has revealed the presence of inherited and *de novo* genomic DNA gains and losses. CNVs in several genomic regions have been correlated with ASD, but the biological effect of each CNV remains to be elucidated. As neuronal tissues are in limited supply for functional studies, the development of patient-specific induced pluripotent stem cell lines will allow for a robust source of cells carrying ASD-related CNVs for future studies. Numerous cell lines have been developed from patients and family members carrying genomic DNA losses at cytobands 11q13.3, 16p11.2 and Xp22.11. Future work to differentiate iPS cells to neuronal lineages of functional importance to ASD will allow the elucidation of the effects of various CNVs on the development of ASD phenotypes.

Personalized medicine. Your future is in your genes

Peter Ray PhD, FCCMG, FACMG
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Current medicine is based on a limited knowledge of the molecular basis of disease and how different people respond to different treatments. The result is a "one size fits all" approach to medicine. However our growing understanding of genetics and the development of technology to efficiently analyze the genome is allowing us to provide better diagnoses, safer drug prescribing, and more effective treatment of the diseases and conditions that have affected us throughout history. Personalized medicine is moving us closer to more precise, predictable and powerful medicine—customized for the individual patient.

This is providing a significant challenge to diagnostic laboratories to keep up to date with the explosion of knowledge and technology. Laboratories need to adapt, retool and retrain at a pace unheard of in the past.

The H. Allen Gardner Memorial Lecture

A personal journey from stem cells to clinical cytogenetics to cloning of the Duchenne Muscular Dystrophy gene to the molecular nature of chromosomal rearrangement and back to stem cells.

Ronald Worton, CM, PhD, Scientific Director Emeritus
The Ottawa Hospital Research Institute

To honour Dr. Gardner and everything that he stood for, I am grateful to have this opportunity to speak to you today. I shall say more about Allen and his achievements at the talk.

My goal in this talk is to convey to you some of the excitement of biomedical science over the last 50 years, the time span of my own scientific career, using examples of discovery from my own experience. I hope you will see how scientific progress is shaped by four things (i) new enabling technologies, (ii) innovative thinking applied to important problems, (iii) creative drive and hard work of students and research fellows and often (iv) a large dose of serendipity.

Fifty years ago this spring, right here in Toronto, Drs. Jim Till and Bun McCulloch described stem cells in bone marrow responsible for maintaining the blood-forming system, and 3 years later I had the great good fortune to join their group as a graduate student. I will talk about that discovery and the enabling technology that allowed stem cells to be quantified and studied.

In 1971 I moved to the new Genetics department at the Hospital for Sick Children and for 11 years I was privileged to run the cytogenetic service laboratory and later the prenatal diagnostic laboratory at SickKids. During this time a cytogenetics fellow, Dr. Christine Verellen, made me aware of a female with muscular dystrophy and a t(X;21) translocation that she later showed to be the cause of her disease. This led to a 5 year project, beginning in 1982 to isolate the Duchenne muscular dystrophy (DMD) gene by molecular cloning of the translocation junction, use the cloned gene to identify the protein product, *dystrophin*, and demonstrate its absence from the muscle of boys with DMD. It also kick-started a 5-year effort to understand the nature of mutations causing the disease, particularly the deletions, duplications and translocations that disrupt the 2.5 megabase dystrophin gene. This work, virtually all carried out by students and fellows, will be described in some detail.

In 1996, with the DMD story behind us, I had the opportunity to move to the General Campus of The Ottawa Hospital (then the Ottawa General Hospital), to develop a new Research Institute. One of the research themes I chose to develop was neuromuscular disease, with the intent of recruiting people who might take such research to the next level toward a cure, or at least a beneficial treatment. In 1999 one issue of Science magazine focused on new developments in stem cell research – particularly the newfound ability to grow human embryonic stem cells in culture. This led to much speculation about the use of human stem cells to repair or regenerate tissue destroyed by degenerative disease. It led me to think about the possibility of muscle stem cells (not yet identified at that time) to regenerate muscle in boys with DMD. I will end my talk with a discussion about work at the Ottawa Hospital Research Institute and across Canada in the Stem Cell Network devoted to the pursuit of stem cell therapy for degenerative disease.