

## **Integration of next generation sequencing and arrays into the future cancer cytogenetics service lab**

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An overview of the basic concepts, general applications, and the potential influences of next-generation sequencing (NGS) technologies on future applications in cytogenetics will be considered. Automation and large reductions in costs with high-throughput efficiencies in NGS are creating huge challenges for data management and interpretation. There will be increasing needs for applied bioinformatics and for different academic approaches for the successful integration of NGS technology in the service genetics setting. However the novelty of some of the excitement around discoveries from NGS suggest that successful integration of sequencing into the clinical laboratories provide new insights into cancer cytogenetics and for many human genetic conditions. Examples from recent findings concerning gene position in the nucleus using three-dimensional FISH will be mentioned. It is clear that there will be many new opportunities to apply cytogenomic methods in the service laboratory as NGS is more broadly applied in clinical settings.

## **Optimization of enzymatic digestion for fluorescence in situ hybridization on formalin-fixed paraffin-embedded tissue sections**

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Enzymatic digestion is the most critical step for performing fluorescence in situ hybridization (FISH) on formalin-fixed paraffin-embedded (FFPE) tissue sections. It directly affects signal quality as it eases access of the FISH probes to the genomic target DNA and reduces autofluorescence generated by intact proteins. Enzymatic digestion time is tissue-specific and fixation-dependent. Sub-optimal digestion can be identified by monitoring autofluorescence level, DAPI staining pattern and signal distribution. However, those indicators are quite subjective. A more objective method is to look for the fernlike formations under light microscopy, after immersion of the slides in 2 x SSC for 1 minute to inactivate the enzyme followed by tissue drying in thermal plate at 45°C. We have adopted this methodology at our laboratory, and successfully performed FISH assays on FFPE-tissue sections for C-MYC, BCL2 and BCL6 rearrangements in lymphomas, as well as 1p and 19q deletions in brain tumours.

## **Copy number alterations in prostate cancer: an *in silico* meta-analysis of publically available genomic data**

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With one in six men diagnosed, prostate cancer remains a serious global and public health issue. *In silico* meta-analysis permits the comprehensive and quantitative investigation of publically available microarray datasets on a large scale by harnessing data from multiple

studies, thus increasing statistical power and robustness. This meta-analysis examined primary and advanced prostate tumours from 546 and 116 individual patients, respectively, from 11 published datasets comprising five different platforms.

The most common alteration observed in primary disease is loss 8p (*NKX3-1*) in 49.3% of primary tumours, followed by 40.3% and 37.4% harbouring deletions at 6q12-22 and 13q13-31 (*RBI*), respectively. Other common large-scale deletions included 16q, 5q14-23, 17p, 3p13 and 12p. Gains are also observed, albeit at a significantly lower frequency but included gain of 8q (*MYC*), chromosome 7 and in advanced disease, Xq12 (*AR*).

The recurrent and adverse interstitial deletions of *PTEN* (10q23.31, 22.7% in primary and 69.8% in advanced disease) and formation of the *TMPRSS2-ERG* gene fusion (21q22.2-22.3, 24.2% in primary and 48.3% in advanced disease) were clearly identified in our combined analysis. Dividing the cohort based on *PTEN* loss demonstrated a significant difference in the proportion of the genome altered, no significant difference is observed when splitting with respect to the fusion gene. This is suggestive that loss of *PTEN* leads to heightened genomic instability.

Information from large-scale studies such as this can continue to enhance our understanding of the genomic changes observed in disease, providing biomarkers and targets for improved diagnostics and novel or personalized therapeutics.

### **Utility of cytogenetic and molecular genetic studies in bone and soft tissue tumours that required external consultation: the Kingston General Hospital experience**

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Diagnostics in bone and soft tissue (BST) pathology is often difficult due to a large number of existing histologic subtypes with overlaps in clinical, radiographic, histologic and immunohistochemical features. Genetic abnormalities have demonstrated an increasingly important role in BST tumour diagnostics. The present study evaluated the role of cytogenetic/molecular genetics in the diagnosis of BST tumours that required external consultation from the Department of Pathology and Molecular Medicine, Kingston General Hospital. Primary BST tumours in our laboratory information system, from 2008 to first quarter of 2013, were retrospectively reviewed. Twenty-nine specimens met our search criteria; nine bone and twenty soft tissue tumours. Specimens included tumour excisions as well as needle and excisional biopsies. One specimen was unsuitable for morphologic/genetic evaluation. Of the remaining twenty-eight, cytogenetic and/or molecular genetic testing was performed in 11 (39%) cases. Genetic testing was useful in narrowing the differential diagnoses in five cases, rendering a definite diagnosis in one case, providing prognostic and predictive information in one case each, confirming a clonal neoplastic process but not diagnostic in two cases, and uninformative in one case. Of interest was a spindle cell tumour for which molecular studies were pertinent in rendering a diagnosis of low-grade myxofibrosarcoma. It had a relatively simple karyotype with rearrangement of 2p23, but no demonstrable involvement of *ALK* by FISH or immunohistochemistry, suggesting that 2p23 rearrangement without *ALK* involvement may be a recurrent finding in a subset of low-grade myxofibrosarcomas. The present findings underscore

the importance of ancillary genetic testing, including classical cytogenetics in BST tumour diagnostics.

### **ALK gene rearrangement FISH testing for lung cancers: a Canadian multi-institution FISH and immunohistochemistry correlation**

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ALK gene rearrangement (ALK+) has been found in 3-5% of advanced non-small cell lung cancer patients. CALK was initiated to assess the feasibility of implementing ALK IHC and/or FISH assays across Canadian hospitals. Methods: FISH-confirmed 22 ALK+ and 6 ALK- tumors were used as study samples. Unstained sections and scanned images of HE-stained slides from each block, as well as from 20 normal lung tissues, were distributed to participating centres. IHC protocols with best signal to noise ratio using the 5A4 (Novocastra) or ALK-1 (Dako) antibodies were developed for various auto-stainers and implemented to suit the existing conditions of the participating centres. A common FISH protocol using the ALK break-apart probe (Abbott Molecular, Chicago, IL) was developed based on published reports. H-score was used to assess IHC and FISH signals were scored in 100 tumor cells/case by 2-3 pre-trained technicians or pathologists. Results: Independent IHC scores from 12 centres and FISH scores from 11 centres were collected and analysed. The intraclass correlation coefficients (ICC) between centres for IHC (Hscore) and FISH (% abnormal nuclei) were 0.84 and 0.68 respectively. The sensitivity and specificity of FISH results across centres using consensus FISH diagnosis (>80% consensus FISH diagnosis was reached in all cases) and a % abnormal red signal cutoff of 15% of cells, was 95.1% (sensitivity) and 95.8% (specificity) overall. One of 23 tumors revealed IHC-/FISH+ discrepancy, with the FISH revealing unusual signal configurations that suggested an atypical rearrangement occurring within the green (5' ALK) probed region. Potential reasons for occasional aberrant FISH results across labs were determined by a group review of each case. Reasons for FISH discrepant results included not using the circled H&E image to guide scoring, poor signal quality, polysomy resulting in increased artifactual separation of signals, loss of tumour in deeper sections, high level of intermixed non-tumour cells, and possibly "cherry picking" style of scoring, or not adhering to 2 signal-widths for defining a breakapart.

### **Influence of disease load and deletion size on CLL outcome in patients with an isolated deletion 13q**

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**Background:** Cytogenetic abnormalities are one of the factors influencing the variable clinical course of chronic lymphocytic leukemia (CLL). Although it is well described that an isolated deletion 13q represents a factor of good prognosis when detected by fluorescence in situ hybridization, there is still outcome heterogeneity in this subgroup. We sought to investigate whether the load of the malignant clone with isolated deletion 13q and the size of the deletion influence patient outcome, as previous studies have reported conflicting results.

**Methods:** We reviewed the BC CLL database to identify patients with isolated deletion 13q and to record the percentage of abnormal cells. We performed fluorescence in situ hybridization with an RB1 probe on 46 patients to investigate for the presence or absence of this locus. Kaplan-Meier analyses and log-rank tests were performed to estimate and compare treatment free and overall survivals of the different groups.

**Results:** 456/815 (56%) patients had a deletion 13q identified with a D13S319-D13S25 probe. 292 (36%) samples had an isolated deletion, 217 monoallelic, 51 mixed mono- and biallelic and 24 biallelic only. A longer treatment free survival (TFS) for patients with less than 60% of nuclei with a deletion 13q was observed compared to patients with 60% or more (median TFS not reached vs 13 years,  $p=0.031$ ). There was no difference in overall survival. 20/46 (43%) patients showed a deletion extending to the *RB1* locus, but the presence or absence of a *RB1* deletion in the small sample did not influence the outcome.

### **Incorporation of flanking probes reduces truncation losses for fluorescence in situ hybridization analysis of recurrent genomic deletions in tumor sections**

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Fluorescence in situ hybridization (FISH) on archival formalin fixed paraffin embedded (FFPE) samples is technically demanding; currently there are no comprehensive guidelines for the analysis of genomic deletions using FFPE tissue sections for clinical application in cancer. In this study we report a generalizable four-color deletion FISH approach to assist interpretative dilemmas associated with overlapping and truncated nuclei in FFPE tissue sections. The four-color FISH approach was developed using the *PTEN* deletion model in prostate cancer. We use a "chromosome enumeration" probe, a locus specific *PTEN* probe, and adjacent flanking probes. The sensitivity and specificity parameters of the four-color probe set were further characterized using a large number of well-characterized FFPE tumors and stringent scoring criteria to minimize truncation artifacts. Overall the approach reduced the frequency of misinterpretation and provided highly reproducible results that minimized inter- and intra-assay variability. In addition, since deletions may be of variable size it is possible to use each adjacent flanking probe to distinguish between microdeletions that are restricted to the *PTEN* region and larger deletions that extend into the regions detected by the flanking probes. The findings of this study allowed us to develop guidelines that will aid the interpretation of FISH signals in overlapping and truncated nuclei in FFPE tissue sections. Moreover this study will facilitate a more robust approach for FISH biomarker deletion assays as more tumor suppressor genes of clinical importance are discovered by next generation sequencing methods.

### **Autosomal structural mosaicism – a review of cases from thirteen years of constitutional karyotyping experience**

Ann M. Joseph-George, Daniel Antinucci, Gloria Nie, Ikuko Teshima, D. James Stavropoulos and Mary Shago.

Chromosomal mosaicism is the presence of more than one, karyotypically distinct population of cells, within a single organism. It arises post-zygotically, is variable in its extent between different tissues and is not always straightforward to ascertain in the laboratory when present at low levels. From a clinical perspective, phenotypic presentation related to the mosaicism may be variable and could be interpreted as normal variation in the population.

Autosomal structural mosaicism is rare and can involve intrachromosomal, interchromosomal or complex rearrangements with alteration resulting in balanced or unbalanced states. A retrospective review of approximately 30,350 cases from this laboratory was undertaken to establish the general prevalence of mosaicism in our patient population and to then examine further, a subset of these cases, specifically those with structural mosaicism for an autosome. These patients, the majority pediatric, were referred for varied indications to this diagnostic centre for postnatal constitutional karyotyping of their peripheral blood by G-banding analysis.

A summary of all cases with mosaicism will be presented briefly and will include a breakdown of cases within different categories. The chromosome and region involved, breakpoints, type of rearrangement and extent of imbalance (if any) will be reviewed to identify trends or any preferential chromosomal involvement. Interesting cases, mosaic for a structural rearrangement will be presented.

## **Challenging situations in the Cytogenetics Laboratory**

Group presentation

Organized by Rosemary Mueller, PhD FCCMG

The object of this session is to present interesting or surprising findings observed during the course of conventional or molecular cytogenetic investigations, to highlight cytogenetic observations that could have been misinterpreted or overlooked, to discuss testing algorithms.

1. "Constitutional translocation and low level trisomy 21 in lymphocytes of an MDS patient"  
Rosemary Mueller, Victoria General Hospital Cytogenetics Victoria, BC.
2. "Constitutional mosaic or acquired balanced reciprocal translocation?"  
Helene Bruyere, Vancouver General Hospital Cytogenetics, Vancouver, BC.
3. "Differential diagnosis of lipoma-like lipoblastoma"  
Maisa Yoshimoto, Children's Hospital of Eastern Ontario Cytogenetics, Ottawa, ON.
4. "Mosaic inv(14) in a woman referred for multiple miscarriages"  
Natacha Mosler, Hospital for Sick Children Cytogenetics, Toronto, ON.
5. "From GTG to PGD"  
Entela Ketri, Mount Sinai Hospital Cytogenetics, Toronto, ON.
6. "Prenatal diagnosis of trisomy 5 and mosaic del 13q with follow-up at 2 yrs. "  
Judy Chernos, Alberta Children's Hospital Cytogenetics, Calgary, AB.

Please join us next year and share your valuable story!

### **Cytogenetic investigation on an unusual prenatal case with discordant chromosome and microarray results**

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**Objectives:** To present an unusual prenatal case with discordant cytogenetics and microarray results.

**Methods:** A 41-year-old woman was referred (at 16w3d) for prenatal testing for advanced maternal age. QF-PCR was performed on the amniotic fluid specimen to rule out the common aneuploidies (chromosomes 21, 18, 13, X and Y). Seven *in situ* cultures (in 35 mm dishes) were established for routine cytogenetic analysis. Since blood was noted in the sample, an additional three T-25 flasks were set up.

**Findings:** QF-PCR was uninformative due to the presence of maternal cell contamination. Chromosome analysis from 5 independent primary cultures all showed an abnormal chromosome 4. Subsequent FISH analysis identified extra material on 4q to be chromosome 17 in origin. Parental chromosome confirmed the rearrangement occurred *de novo* in the fetus. The final karyotype reported was 46,XY,add(4)(q31.1)dn.ish der(4)t(4;17)(q31.1;q23)(wcp17+,362K14+). Meanwhile, cultured amniocytes from one primary culture, were referred to a commercial microarray laboratory for clarification of the cytogenetic rearrangement. The microarray result was unexpectedly reported to be normal. Subsequent chromosome analysis of the same culture used for microarray analysis showed a normal male karyotype, consistent with the microarray result. Analysis of all remaining cultures showed only the der(4) abnormality, except for one culture, which was mosaic. The couple was counselled about the possibility of a mosaic fetus or a vanishing twin. No obvious abnormalities were detected by level II ultrasound (18w0d). A second amniocentesis was performed. We identified a mosaic karyotype 46,XY,add(4)(q31.1)dn[22]/46,XY[8]. Microarray analysis by the same commercial laboratory on both uncultured and cultured cells of the repeat amniocentesis showed a mosaic result. The patient was admitted for termination and consented to a fetal autopsy.

**Conclusions:** More than one primary culture should be used for microarray analysis to minimize the chance of analyzing only one clone when mosaicism is present. Microarray analysis of uncultured amniocytes is preferred to eliminate culture bias.

**Learning Objectives:** The participant shall be able to potentially improve the detection of mosaicism for microarray analysis by using a minimum of two primary cultures. The use of uncultured amniocytes will eliminate culture bias.

### **Back to the future: Update on implementation of SOGC Guidelines for amniotic fluids in the Champlain LHIN**

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QF-PCR has been gradually introduced in diagnostic laboratories as a first-tier test for screening of aneuploidies involving chromosomes 13, 18, 21, X and Y in prenatal samples. The Society of Obstetricians and Gynaecologists of Canada (SOGC) and the Canadian College of Medical Geneticists (CCMG) published joint clinical practice guidelines in September 2011 regarding the use of QF-PCR for diagnosis of fetal aneuploidies. According to these guidelines, QF-PCR can replace conventional cytogenetic analysis whenever prenatal testing is performed solely because of an increased risk of aneuploidy for chromosomes 13, 18, 21, X or Y. Practically, it may be desirable to maintain a source of DNA for all pregnancies with a normal QF-PCR result to allow for other testing should abnormalities later be detected by ultrasound. We have been using QF-PCR to test for fetal aneuploidies in amniotic fluids from the Champlain LHIN since March 2009 and as of July 2012, we have implemented it as a stand-alone test for pregnancies at increased risk of aneuploidy after establishment of clear criteria for categorization of cases. As per our algorithm, pregnancies falling in the "trisomy-risk only" category are offered QF-PCR only, whereas pregnancies falling in the "other-risk" category are offered QF-PCR followed by standard karyotype. All abnormal QF-PCR results, regardless of risk category, are confirmed by standard karyotype; also amniocytes from all pregnancies with a normal QF-PCR result are frozen in pellets suitable for DNA extraction. By categorizing cases in such manner, we have reduced the need for chromosome analysis of cultured amniocytes by roughly 75%. We present here an update on our experience from testing approximately 500 amniotic fluid samples using this algorithm and discuss the challenges and future directions of QF-PCR as a first-tier test for all pregnancies and stand-alone test for specific clinical indications in the diagnosis of fetal aneuploidies.

### **Automation in the Cyto-Molecular Laboratory: DNA Extractions Made Easy**

Mark Adams

Genetics Technologist, Mount Sinai Hospital

The introduction of automated instrumentation can have a significant impact on a laboratory's ability to carry out testing on a large volume of samples in a short turn-around-time manner. Automated technology provides robust, reliable, consistent, and contained methods of DNA extraction from a variety of clinical samples. In our laboratory, we have devised several protocols for the automated extraction of perinatal tissue specimen (products of conception including umbilical cords, skin, cartilage and villi) as well as prenatal samples (direct amniotic fluid, direct chorionic villi sampling cell suspensions, and cultured cells). DNA yields, though dependent on sample type, amount of starting material, and specimen condition, are of high enough quality for each specimen type to be run on downstream applications such as rapid aneuploid screening using QF-PCR, and microarray. These automated extraction technologies can be implemented into the clinical laboratory, replacing the current manual techniques.

### **The Cytogenomics Array Group database (CAGdb) is a collaborative tool for individual laboratory microarray data management and data sharing**

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The Cytogenomics Array Group database (CAGdb) is a platform-independent, web-based tool developed as both an intra- and inter-laboratory repository for microarray case data. De-identified patient data can be entered with rich clinical detail (clinical features, parental and other follow-up data, interpretive category, comments, references, report text) and shared with other registered users. The intent of this tool is to allow individual laboratories to store and manipulate their own case data and to facilitate case interpretation and sharing amongst clinical colleagues. In addition to manual CNV entry, automated upload of CNV calls exported from array software in .txt file format is supported. The entire database or any subset of search returns can be exported in BED file format or viewed immediately in the UCSC genome browser. The CAGdb can also serve as an interface for submission to larger research database efforts (e.g. ISCA/ICCG at NCBI's ClinVar). Efforts to improve database functionality through continuous user input are ongoing. This database is operated as a non-profit resource, freely available to all participants. The group views professional education as a secondary mission and is partnering with the Cancer Cytogenomics Microarray Consortium (CCMC) for its second annual workshop this summer in Chicago.

### **Visual inspection findings in microarray data analysis**

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Various software algorithms and settings are used to detect copy number changes in genomic microarray testing. These analysis settings affect the number of CNVs being inferred per genome and stringency of these calls. The defined analysis settings will fail to detect CNVs below the set threshold of log 2 ratio and minimum number of probes to call CNVs. Therefore, the visual inspection of microarray data may identify such CNVs.

In this study, we reviewed CNV data from 4918 cases processed at the Hospital for Sick Children during 2010-2012. We found 16 CNVs that were not detected by the user defined software settings and were manually added, as well as 50 CNVs which needed manual corrections upon visual inspection by technologists before being reported.

The manually detected CNVs fall into three categories: deletions with low mean log 2 ratio, deletions with less than five probes, and mosaic regions less than five Mb in size. Some of these manually detected CNVs represent clinically significant CNVs such as 1p36 deletion syndrome, and 22q13.33 SHANK3 deletion. The manually corrected CNVs were merged, trimmed, extended, or separated after visual inspection. Thus, our study highlights the importance of visual inspection in microarray data analysis.

### **Clinical and molecular characterization of the recurrent 2q13 microdeletion and microduplication syndromes**

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While chromosome microarrays have allowed for characterization of an unprecedented number of novel syndromes, there are numerous recurrent copy number variants (CNV) for which the clinical significance remains poorly understood. Factors such as low allele frequency, reduced penetrance, and variable expressivity often limit our ability to accurately predict the clinical significance of these CNV. For example, a recurrent microdeletion/duplication at 2q13 has recently been reported to increase the risk for developmental delay and congenital anomalies; however, as only 12 cases have been described the spectrum of associated phenotypes remains unclear. Inheritance of 2q13 CNV from "phenotypically normal" parents further suggests that this variant can be benign in some individuals. We report herein 54 individuals with 2q13 deletions or duplications, thus considerably expanding the number of reported cases. Although preliminary analysis shows considerable phenotypic variability within this population it is hoped that further chart review will reveal similarities between patients. Comparison of case and normal control populations (n=51023 and 22246 respectively) shows that CNVs in this region are more common among cases than controls; the penetrance of the 2q13 deletion was further estimated to be 0.185 in this group. These findings suggest that while 2q13 deletions/duplications likely contribute to clinical findings in at least a proportion of cases it may be insufficient to cause a phenotype in others. This represents the largest cohort of patients with deletions/duplications of the 2q13 chromosome region reported to date and allows for further characterization of the phenotype and genetic features of CNV in this region.

### **Teaching an old assay new tricks: using genomic technologies to design a tool to measure BRCA1 function**

Harriet Feilotter, Director, Molecular Genetics and Service Chief, Laboratory Genetics, Kingston General Hospital, Kingston, Ontario

Testing for mutations in BRCA1 and 2 by exon sequencing is standard of care for Ontarians with a pre-specified risk of hereditary breast/ovarian cancer. The ongoing issue of patent protection of these genes, along with the problems that accompany identification of unclassified variants, prompted us to explore development of a functional assay for haploinsufficiency of these genes. Using whole transcriptome profiling in BRCA1 mutation carrier and BRCA1 wild type cell lines, we demonstrated that genes involved in cellular differentiation are differentially regulated in BRCA1 haploinsufficient cell lines compared to wild type controls. A brief overview of the current state of preclinical validation of the assay will be discussed.

### **Cancer genomics: Less is more**

John McPherson

Director, Genome Technologies, Ontario Institute for Cancer Research, Toronto, Ontario

Next-generation sequencing (NGS) enables deep sequencing of tumour samples and biopsies to reveal the landscape of somatic mutations. The ability to detect these variants is greatly affected by tumour cellularity and heterogeneity. Methods for enriching tumor samples are enhancing the sensitivity and specificity of mutant allele detection. In addition, reductions in the RNA/DNA input needs for analyses is greatly increasing sample availability.

## **Novel gene discovery for autism spectrum disorders by copy number variation detection**

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Utilizing a large cohort of Canadian autism spectrum disorder (ASD) cases (n=1158) and high-resolution microarray analysis, we have detected rare copy number variants (CNVs) that are enriched in ASD cases compared to control individuals. Although rare, we have detected recurrent CNVs, with variable breakpoints, in several subjects that overlap the brain-expressed genes, NRXN3, GPHN, ASTN1 and ASTN2. These apparently dosage-sensitive genes have known or projected roles in neurological development and function. Corroboration of our findings in the Canadian ASD research cohort has been aided by the ascertainment of individuals from various other national and international clinical and research laboratories, allowing for better detailing of the spectrum of associated clinical phenotypes. Collation of the genetic and clinical characteristics of these copy number variable patients will aid future interpretation of variants of uncertain clinical significance (VUS) and guide future basic research aimed at determining the pathogenic mechanism underlying altered dosage of these critical neurological genes in the development of autism.

## **QF-PCR rapid aneuploidy screen of cell free fetal (cff) DNA in supernatant of amniotic fluid (AF) from "bloody tap" amniocenteses**

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### **Background and objectives:**

Interpretation of prenatal analyses of blood stained AF samples has many ramifications mostly due to maternal cells contamination (MCC). We aimed to determine whether cff-DNA in residual AF supernatant obtained from bloody samples can be used for prenatal rapid aneuploidy screen.

### **Methods:**

Total of 20 blood contaminated AFs were analyzed in this case control, double blinded study. Cff-DNA was extracted from 1ml of residual AF supernatant (fresh or frozen) using QIAgen-DSP Virus Kit. The samples were processed using 3 algorithms: I) interphase FISH (n=20) (AneuVysion, Abbott/Vysis) and karyotype, II) QF-PCR analysis of direct AF (n=16) (ANEUFAST kit, Genomed AG Switzerland) and III) cffDNA (n=20). Results from the 3 algorithms were compared. Whole genome aCGH on 180K chip (BlueGnome) was performed on cff-DNA.

### **Results:**

QF PCR analysis of cff-DNA detected pure fetal profiles in all 20 samples. Results were in 100% concordance with karyotyping. Analysis of direct AF gave inconclusive results due to MCC (ranging 50-90%) in 4/20 samples by FISH and 5/16 samples by QF-PCR analyses. The remaining 11/16 samples processed by QF-PCR showed that the contaminating blood was of fetal

origin. An adequate amount of sufficient quality cff-DNA was obtained for successful aCGH 180K testing.

#### Conclusions:

AF supernatant is a valuable and underutilized source. Here we have shown that it is feasible to isolate cff-DNA from routinely discarded AF supernatant of sufficient quality and quantity to perform QF-PCR studies and microarray analysis providing timely and informative results even for problematic grossly bloody AF samples or culture failure.

### **The identification, importance and implementation of medulloblastoma molecular subgroups**

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Medulloblastoma is a malignant pediatric tumor of the cerebellum and the most common solid pediatric malignancy of the central nervous system (CNS). Recently, transcriptional profiling has highlighted the existence of four, core molecular subgroups of medulloblastoma with disparate molecular and clinical features. While comprehensively profiled at the level of the genome ( $n > 1000$ ) and nucleotide ( $n > 400$ ) each molecular subgroup harbors few highly recurrent somatic genetic events, which only infrequently converge on well-characterized signaling pathways. Improved understanding of the mechanisms of pathogenesis is required for the development of targeted therapies. We performed integrative molecular analyses of putative drivers of pathogenesis. Examination of CpG promoter hypermethylation, somatic single gene copy number aberrations (CNAs) single nucleotide variants (SNVs) reveals frequent involvement of genes involved in the formation and function of the synapse, converging on glutamate receptor and ion channel activity. We functionally validated the importance of ion channel activity across both normal cerebellar granule and medulloblastoma cells using optogenetically-engineered mouse model. Membrane depolarization of medulloblastoma cells through optogenetics resulted in cell cycle exit, neurite extension, and dramatically decreased malignant potential *in vivo*. Our results suggest cerebellar progenitor differentiation is mediated by membrane depolarization; and that this tumor suppressive mechanism is targeted by recurrent, highly convergent somatic genetic and epigenetic events in medulloblastoma. We conclude that membrane depolarization is a tumor suppressive event in the pathogenesis of medulloblastoma.

## **CMA Hodgepodge: amplified VUS, broken repair and other surprises**

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It has become increasingly clear over the recent past that the results of Cytogenomic Microarrays (CMAs) can be both clarifying and mystifying. Abnormal results often reflect textbook examples of chromosomal imbalances arising in the classical cytogenetic setting of balanced parental translocations, insertions and inversions. As well, *de novo* copy number abnormalities are frequently explained by the genomic context in which they reside such as regions susceptible to abnormal recombination such as segmental duplications and various forms of interspersed repeats. These types of abnormalities might be called well-described and easy to explain in counselling. Such cases can lead to satisfactory recurrent risk predictions. However, on occasion we are confronted with 'hard to explain' cases that are so rare and poorly understood that recurrence risks may be unknown. In these situations, we can only speculate regarding the mechanisms of abnormal chromosome behaviour that led to the patient's chromosomal disorder. Surprisingly, mechanisms that we normally see in somatic genetic disease seem to fit in some cases. In this talk, I will demonstrate that theories developed from the observation of the replication and repair behaviour of cancer cell types are relevant in some cases of pathogenic copy number variation in the germline.

## **Aneuploidy in humans: what we know and what we wish we knew**

Terry Hassold

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With the advent of the human genome project in the 1990s, DNA markers became available, allowing us to determine the parent and meiotic stage of origin of human aneuploid conditions. This approach has been extensively used to study the origin of trisomies, with one over-arching conclusion: the vast majority of clinically recognized trisomies derive from errors in the development of the egg and, in particular, from nondisjunction occurring in the first maternal meiotic division (MI). However, against this general background, it has also become apparent that there is considerable chromosome-to-chromosome variation, indicating that individual chromosomes behave differently with respect to mechanisms of meiotic nondisjunction. In this presentation, we will summarize results of these parental origin studies of aneuploidy and discuss the involvement of the first identified molecular correlate of nondisjunction - aberrant meiotic recombination - in the genesis of these abnormalities. We will also look forward, discussing the development of mouse models of human aneuploidy and recent advances in molecular cytogenetics that make it possible to directly analyze human meiosis "as it happens" in fetal oocytes and in spermatocytes. These latter studies have identified remarkable differences between human males and females in the way in which chromosomes find and synapse with one another, in the packaging of chromatin, and in the control of the meiotic recombination pathway. Further, they suggest that errors in fetal oogenesis are relatively common and may contribute to male:female differences in meiotic nondisjunction, as well as to the maternal age-related increase in aneuploidy levels in our species.