The Great Lakes Chromosome Conference 2005

Sess on I: Cancer Cytogenet cs

Amp f cat on 12q12-q15 n non-Hodg n ymphoma: A mo ecu ar cytogenet c study of chromosoma structure-dosage changes and corre at on w th gene express on data.

Va a S Lestou[1,2], Laur e Sehn[3], O ga Lud ovs [2], Mu esh Chhanabha [2], R chard J K asa[3], Joseph M Connors[3], Randy D Gascoyne[2], Wan L. Lam[4], Doug as E Horsman[2]

1. Department of Paed atr cs and Laboratory Med c ne, Hosp ta for S c K ds, Toronto, Canada
2. Department of Patho ogy and Laboratory Med c ne, BC Cancer Agency, Vancouver, Canada
3. Department of Med ca Onco ogy, BC Cancer Agency, Vancouver, Canada
4. Department of Cancer Genet cs and Deve opmenta B o ogy, BC Cancer Research Centre, Vancouver, Canada

Chromosome reg on 12q12-q15 s one of the most frequent y amp f ed reg ons n fo cu ar and d ffuse arge B ce ymphomas (FL and DLBCL). We have underta en a comprehens ve ana ys s of the chromosoma structure of 12q12-q15 n FL and DLBCL, us ng mo ecu ar cytogenet c techn ques to def ne the boundar es of the amp con, de neate the m n ma core doma n, and determ ne the genes conta ned w th n th s segment for corre at on w th ex st ng express on prof e data and c n ca outcome. Forty-three NHL cases demonstrat ng 12q+ abnorma t es by G-band ng were further ver f ed by MFISH ana ys s. Mu t co our band ana ys s for chromosome 12 (MBAND12) was used to ref ne the amp f ed reg on to 12q13.1-q14.3, wh ch revea ed a var ety of conf gurat ons (tandem dup cat ons, r ng chromosomes) and var ab e copy numbers (2-8 t mes). Locus-spec f c FISH was performed us ng 17 BAC probes for 12q12-q15 prepared from an RPCI-11 BAC cont g. F ve d fferent patterns of dup cat on or amp f cat on of th s reg on were dent f ed, rang ng from who e chromosome tr somy, who e arm dup cat on, to reg ona dup cat on of var ab e s ze. The borders of th s amp con extended across the q-arm from centromer c bp 53889578 to te omer c bp 71883142, correspond ng to chromosoma bands 12q13.1-q14.3. Th s reg on of amp f cat on spans ~17.3 Kb, wh ch conta ns the genes *T* , *R* *R* *S* *PP* *P* *T* *R* and *P* (3-8 cop es). The core amp con, however, was on y 11.6 Kb ong w th amp f cat on of SAS, CDK4, RAP1b and MDM2 and was nvo ved n a ana yzed cases. These resu ts corre ate c ose y w th up-regu ated express on, as demonstrated n a re ated LYMPHOCHIP study. These resu ts suggest that cand date genes w th n 12q13-q14.3, such as SAS and MDM2, are preferent a y dup cated or amp f ed ead ng to up-regu ated express on, contr bute to tumour progress on, and are assoc ated w th adverse outcome n FL and DLBCL.

A Summary of Cytogenet c Resu ts n New D agnoses of Ped atr c Acute Lymphob ast c Leu em as

Audrey O'Ne , Lynne Fa st, Rosemary Mue er Un vers ty of A berta Hosp ta Cytogenet cs Laboratory, Edmonton, A berta. (rosemarymue er@cha.ab.ca)

Acute ymphob ast c eu em a accounts for ~1/3 of ch dhood cancer. Surv va rates have mproved dramat ca y from near 20% to 80% n the past 25 years because of ref nements n mu t-drug treatments and because of r s ad usted chemotherapy ntens ty. Stud es suggest that 85-90% of ch dren w th ALL have a v s b e cytogenet c abnorma ty and that more than ha f of these are current y prognost ca y mportant.

Cytogenet c aberrat ons are a very mportant too for strat f cat on of pat ents at d agnos s nto def ned r s groups. Unfortunate y, bone marrow spec mens for ped atr c ALL can have a poor y e d of metaphases and can have poor chromosome morpho ogy.

Our exper ence w th ten consecut ve bone marrow spec mens rece ved over s x months w th the nd cat on of new d agnos s of acute eu em a n a ped atr c pat ent w be d scussed. A cases had cytogenet c abnorma t es and abnorma resu ts were apparent by G-band ng n 9/10 cases. N ne of 10 cases were u t mate y d agnoses of pre B ALL and 1/10, a d agnos s of AML. By mp ement ng the recommendat ons of the Ch drens' Onco ogy Group n Cytogenet cs, our ab has made a mar ed mprovement n obta n ng usefu resu ts n these spec mens.

Mo ecu ar genet cs study of mucosa-assoc ated ympho d t ssue (MALT) ymphomas w th f uorescence n s tu hybr d zat on techn que from arch va b opsy spec mens

Ingr d La and Dr. Kathy Chun, Cancer Cytogenet cs, Bant ng Inst tute, Un vers ty of Toronto, 100 Co ege Street, Toronto, Ontar o, M5C 1G5 ngr d. a @utoronto.ca

Mucosa-assoc ated ympho d t ssue (MALT) ymphomas ar se from the cont nuous growth of B-ce ymphocytes often w th contr but on from genet c aberrat ons. A common trans ocat on assoc ated w th MALT ymphoma s t(11;18)(q21;q21), resu t ng n the fus on of the MALT1 gene on chromosome 18 w th API2 on chromosome 11. MALT ymphomas preferent a y deve op n the stomach and ungs, a though they aff ct var ous s tes nc ud ng organs ac ng nat ve ympho d t ssue. Ex st ng terature nd cates two patterns of deve opment and progress on. In the presence of t(11;18)(q21;q21), accumu at on of secondary genet c aberrat ons s nsuff c ent to transform marg na zone B-ce ymphomas (MZBCL) to d ffuse arge B-ce ymphomas (DLBCL). In the absence of t(11;18)(q21;q21), a tendency for ncreased accumu at on of genet c aberrat ons may advance the ymphoma nto h gh-grade DLBCL.

S xty-two b opsy samp es from MALT ymphoma pat ents, arch ved at the Un vers ty Hea th Networ , were exam ned for the occurrence of MALT1 gene rearrangements. We w shed to corre ate the f nd ngs to c n ca features nc ud ng tumour s te, recurrence, and progress on to h gh-grade ymphoma. Based on th s nformat on and the pat ent's c n ca man festat ons, the se ect on of opt ma treatment opt ons can be made accord ng y. To dent fy MALT1 gene rearrangements, Vys s MALT1 (18q21) Dua co our, Brea Apart Rearrangement probe was ut zed n f uorescence hybr d zat on (FISH). FISH was performed on paraff n t ssue samp es from MALT ymphoma pat ents d agnosed between 1992-2003. Samp es negat ve for MALT1 rearrangements were subsequent y FISHed w th centromer c probes CEP3 and/or CEP18 to dent fy aneup o d es. The data from th s study are concordant w th the now edge n ex st ng terature regard ng MALT ymphomas.

Recurrent secondary cytogenet c abnorma t es n ch dhood t(12;21)-pos t ve acute ymphob ast c eu em a

Shanth n Kangesan[1], Oussama Ab a[3], Rona d Grant[3], Johann H tz er[3], G or a N e[1], Char es Ye[2], Mohamed Abde ha eem[2], I u o Tesh ma[1], and Mary Shago[1].
Department of Paed atr c Laboratory Med c ne, D v s ons of Mo ecu ar Genet cs[1] and Haematopatho ogy[2] and Paed atr cs, D v s on of Haemato ogy/Onco ogy[3], The Hosp ta for S c Ch dren and the Un vers ty of Toronto.

The t(12;21)(p13;q22) trans ocat on, wh ch resu ts n the fus on of the ETV6 (TEL) and RUNX (AML1) genes, s present n 25% of ped atr c B-precusor acute ymphob ast c eu em a (ALL) pat ents. A though secondary cytogenet c abnorma t es are often present, there s very tt e nformat on ava ab e n the terature address ng the s gn f cance of these add t ona cytogenet c changes. In add t on, there are conf ct ng reports n the terature regard ng the mpact on prognos s of two of the more frequent y detected secondary abnorma t es: de et on of the non-trans ocated ETV6 a e e, and the presence of add t ona cop es of the trans ocated 21 chromosome. The a m of th s study was to exam ne the nature and frequency of secondary cytogenet c abnorma tes n t(12;21)-pos t ve ALL pat ents from the Hosp ta for S c Ch dren. From 2000 to 2005, 61 ALL pat ents tested pos t ve for the t(12;21) by FISH. The mean age of the pat ents at d agnos s was 4.9 years, and the age range was <1 to 12 years. The ma e to fema e rat o was 1.3 to 1. Invest gat on of pat ents was by a comb nat on of FISH, G-band ng and SKY. The most frequent secondary a terat on was a de et on of the non-trans ocated ETV6 a e e, seen n 39% of the pat ents. An extra copy of the ETV6-RUNX fus on s gna was present n 11% of the pat ents. Add t ona numer ca abnorma t es (ga n or oss) were seen n approx mate y one-th rd of the cases, w th the most common ga n be ng +21 (15%). Recurrent de et ons occured n chromosome reg ons 6q and 11q. A fract on of cases demonstrated cytogenet c comp ex t es nc ud ng two abnorma ce nes (21%) or comp ex rearrangements nvo v ng the ETV6-RUNX fus on (10%). The second phase of th s study a ms to exam ne whether these secondary cytogenet c changes have mpact on pat ent outcome, and n part cu ar to determ ne whether add t ona cytogenet c changes are assoc ated w th an ncreased r s of re apse.

A pat ent w th fam a test cu ar cancer and mu t p e chromosome anoma es n the per phera b ood.

Stephen Paut er[1], Er c W ndqu st[2], Scott Ernst[2], D ana Munav sh[3] and J e Xu[3]
[1]Uro ogy and Surg ca Onco ogy, St. Joseph Hea th Care; [2]Med ca Onco ogy, [3]Cytogenet cs, London Hea th Sc ences Center and Un vers ty of Western Ontar o, London, ON

A 32 year-o d ma e (pat ent 1) proband presented w th a 8.5 cm r ght test cu ar mass. A past h story of eft test cu ar sem noma 14 years ear er was treated w th ad uvant externa beam rad otherapy to the retroper toneum. Fam y h story was pos t ve for test cu ar cancer (TC) n h s father. Rad ca orch dectomy was performed. Patho og ca ana ys s demonstrated a stage T3N0M0 c ass c sem noma. Metastat c wor up was negat ve and two cyc es of ad uvant carbop at n was adm n stered. Twenty months ater, h s 22 year-o d brother (pat ent 2) presented w th a 2.3 cm eft test cu ar mass. Metastat c wor up was negat ve and he underwent a eft rad ca orch dectomy. Patho ogy revea ed a T1N0M0 m xed germ ce tumor (embryona and sem noma). Th s pat ent underwent three cyc es of b eomyc n, ectopos de, and c sp at num chemotherapy. Because of the fam y h story, rout ne cytogenet cs of 72 h PHA st mu ated cu ture of per phera b ood of both pat ents was done to ru e out fam a chromosome rearrangement that may pred spose to TC.

Pat ent 1: Four of 50 G-banded ce s had an abnorma ma e aryotype:
46,XY,t(1;21)(q23;q22),t(5;21)(p13;q22)[1],
46,XY,t(17;18)(q11.2;q21.1), nv(11)(p11.2q13)[1],
46,XY,t(3;6)(p21;q23),der(5)t(5;?)(p15.1;?)[1] and
46,XY,t(4;15)(p14;q11.1),t(7;9)(p15;q22)[1]. Cytogenet cs of repeat b ood from th s pat ent showed that 8 of 50 ce s exam ned were abnorma : 46,XY,t(14;16)(q21;q23)[1],

46,XY,t(3;14)(q32;q13)[1], 46,XY,t(4;10)(q12;p11.2)[1], 46,XY,t(1;3)(p22;p13)[1], 46,XY,t(5;16)(q35;q12.1)[1], 45,XY,-6,der(14)t(6;14)(p11.2;p11.2),der(15)t(6;15)(q21;q22)[1], and 46,XY,t(2;22)(q13;q11.2)[2].

These aberrat ons are not nd cat ve of recogn zed chromosome nstab ty syndromes. Notab y the f nd ngs, most y apparent y ba anced trans ocat ons, are d fferent from 1) somat c aberrat ons seen n TC (most y aneup o d); and 2) therapy-re ated secondary anoma es n eu em a. Pat ent 2: No chromosome anoma es were seen n 30 ce s exam ned. Parenta aryotyp ng was not performed. The somat c aberrat ons seen n the proband are postu ated to be treatment effect from chemotherapy. Fo ow-up cytogenet cs w determ ne how ong the somat c aberrat ons rema n n the post-therapy b ood. Further stud es nc ud ng sporad c TC pat ents pre- and post treatment w he p determ ne f there are patterns of somat c rearrangements w th re at onsh p to effect of type, dosage and durat on of TC therapy and f there s genet c pred spos t on to suscept b ty to the genotox c treatment ead ng to somat c anoma es.

De et on of 1p36/19q13 n a bra n tumor detected by FISH but m ssed by PCR

J e Xu[1], Brenda Ham ton[1], D ana Munav sh[1], Joseph Megyes[2], V m a Harry[3], Subrata Cha rabart[3], and Robert Hammond[3]
[1]Cytogenet cs, [2]C n ca Neuro og ca Sc ences, [3]Patho ogy, London Hea th Sc ences Center and Un vers ty of Western Ontar o, London, ON

De et on of chromosome reg ons 1p36 and 19q13 s reported y a common f nd ng n bra n tumor and s cons dered a favorab e prognost c mar er, espec a y n o godendrog oma. To assess prognost c va ue of the de et ons n our sett ngs, we app y para e nterphase FISH of paraff n s des us ng probe sets (Vys s) for 1p36/1q25 and 19q13/19p13 and PCR assays for oss of heterogene ty for D1S508 and D1S199 at 1p and D19S596 and D19S112 at 19q. We corre ate the mo ecu ar cytogenet cs f nd ngs w th c n copatho og c resu ts. Here we present a rare case w th d screpancy between the two tests. Th s 37 year-o d r ght handed fema e presented w th a 1 year h story of se zures. Imag ng stud es dent f ed a eft fronta obe mass w th foca enhancement. She was ta en to the operat ng room where the es on was part a y resected v a a r ght fronta cran otomy. The tumor showed typ ca features of an o godendrog oma. The PCR assay of DNA extracted from the forma n-f xed paraff n-embedded t ssue showed no ev dence of de et on of the se ected mar ers. Interphase FISH of 200 nuc e dent f ed 70.5% of the ce s w th 1 s gna and 29.5% of the ce s w th 2 s gna s for 1p36 and 85% of the ce s w th 1 s gna and 15% w th 2 s gna s for 19q13, respect ve y. Th s FISH f nd ng nd cates de et on of 1p/19q n a s gn f cant proport on of the ce s and s cons stent w th c n copathog c d agnos s and suggests a more favorab e response to ad uvant therapy. Th s case prov des a warn ng that caut on be exc sed n nterpretat on of negat ve f nd ng by PCR assay a one and emphas zes use of both FISH and PCR to enhance a chance of detect on.

Session II: Clinical Problems

Atypical molecular/cytogenetic aberrations in microdeletion syndrome patients

Shago, M. The Hospital for Sick Children & University of Toronto, Toronto, Ontario.

The majority of microdeletion syndrome patients have common deletions mediated by flanking low copy repeats. These patients can be diagnosed using commercial FISH probes directed at the deleted region. However, atypical deletions, rearrangements, or point mutations, undetectable by FISH, cause the genomic disorder in some patients. Alternate genomic changes leading to DiGeorge, Williams, and Smith-Magenis syndromes will be reviewed.

Two cases of *ir* supernumerary marker chromosomes of autosomal origin in newborns

Rosemary Mueller, Lynne Faist, Shirley Lammers University of Alberta Hospital Cytogenetics Laboratory, Edmonton, Alberta.rosemarymueller@cha.ab.ca

Case 1: Cytogenetic analysis of the amniotic fluid cells from the first pregnancy of a woman with the indication of late maternal age revealed two cell lines: 47,XY,+mar[12] /46,XY[3]. FISH studies were unable to identify the origin of the marker chromosome using probes for the centromeres of chromosomes 13,14,15,18, 21, 22, X and Y; a probe for acrocentric p arms was also negative. Parental karyotypes were normal. No abnormalities were noted by ultrasound examination of the fetus.
At birth, the baby was found to have a heart abnormality and renal dysplasia. Chromosome studies in a peripheral blood specimen from this baby showed 47, XY,+mar[15]/46,XY[15]. Multi-color FISH studies demonstrated chromosome 20 to be the origin of the marker.
Case 2: An eight day old baby was referred for cytogenetic analysis because of poor growth, poor feeding and features of Down syndrome. Cytogenetic analysis of this child's peripheral blood cells revealed the presence of a marker chromosome in all cells examined. The marker chromosome was about the size of a G group chromosome, had a G-banding pattern and was C-band negative. Parental karyotypes were normal. Multi-color FISH studies of the marker chromosome demonstrated chromosome 10 as the origin. The marker chromosome showed two signals by FISH analysis with a 10q subtelomeric probe. The child's karyotype was reported as 47,XX,+mar. ish inv dup(10)(qter->q25.2::q25.2->qter).

The Case That Never Ends

Judith Reid and Christine Asquith-Smith, Cytogenetics Laboratory, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada

An amniotic fluid from a fetus at 16 weeks gestation was received for a routine "AMA" study (prenatal diagnosis). Chromosomal analysis revealed a 45,X karyotype in the 10 colonies analysed.
The couple elected to terminate the pregnancy and "delivered" what appeared to be a normal male fetus.

Th s d scordant resu t tr ggered a ong (? end ess), mu t faceted nvest gat on wh ch cu m nated n a f na determ nat on that the fetus was n fact mosa c w th 2 d fferent ce nes: 45,X/46,X, d c(Y)(q11.2).

Outcome of 8 cases of sod centr c Yp chromosome w th prenata nformat on

H. Bruyere[1], M.D. Speeva [2], B. de Frem nv e[3], S. Farre [2], E. W nsor[4], B. McG vray[1], D. McFadden[1], V. Adouard[3], D. Terespo s y[2], F. Pr eur[3], T. Pantzar[1], M. Hryncha [1]

1. Un vers ty of Br t sh Co umb a, Vancouver, BC
2. Department of Laboratory Med c ne, Cred t Va ey Hosp ta , M ss ssauga, ON
3. Centre Hosp ta er Un vers ta re de Sa nt-Et enne, France
4. Mount S na Hosp ta , Toronto, ON

Ob ect ves To present the postnata outcome of prenata y detected cases of sod centr c chromosomes for the short arm of the Y chromosome.
Methods Prenata and postnata cytogenet c data and c n ca f nd ngs n e ght cases of sod centr c Yp ascerta ned n 5 nst tut ons were rev ewed.
Resu ts Seven of the e ght cases reported were ascerta ned on the bas s of rout ne prenata cytogenet c d agnos s. One term nated case showed a ma e foetus w th norma externa gen ta a. S x cases resu ted n the b rth of a norma ma e nfant w th subsequent norma growth and psychomotor deve opment, w th fo ow-up rang ng from 3 months to 7 years. One case was ascerta ned because of ncreased nucha trans ucency and a cytogenet c d agnos s of 45,X was made. Rev ew of the amn ot c f u d s des and b ood cytogenet c ana ys s revea ed the presence of an sod centr c Yp after the b rth of an nfant w th amb guous gen ta a.
Conc us on Prenata d agnos s of sod centr c Yp appears to be compat b e w th norma ma e deve opment n the ma or ty of cases.

D abetes Me tus n a Neonate w th dup (6)(q23.3q24.2)

Shu n Zhang(1), Jenn fer MacKenz e(2) and Karen Harr son (1)
D v s on of Genet c Serv ces, Department of Patho ogy and Mo ecu ar Med c ne (1), Department of Ped atr cs (2) K ngston Genera Hosp ta , Queen's Un vers ty. K ngston, ON, K7L 2V7

Neonata d abetes Me tus (NDM) s a very rare cond t on w th an est mated nc dence of 1 n 400,000 neonates. We report here a 10-day neonate w th s ght dysmorph c features and hyperg ycem a. Cytogenet c study dent f ed a dup cat on of chromosome 6 nvo v ng the q23.3q24.2 reg on. F uorescence n s tu hybr d zat on (FISH) us ng a chromosome 6 pa nt probe conf rmed the chromosoma or g n of the dup cat on. Both parents were found to possess norma aryotypes, nd cat ng that the dup cat on s *r s* . Abnorma genom c mpr nt ng of 6q24 reg on has been reported to be assoc ated w th trans ent neonata d abetes. Paterna dup cat ons nvo v ng 6q24, paterna un parenta sod somy (UPD) and methy at on defects at a CpG s and over app ng exon 1 of ZAC/HYMAI gene, have a been mp cated n the pathogenes s of neonata d abetes.

Prenata detect on of a de novo and apparent y ba anced comp ex chromosome rearrangements nvo v ng 6 brea po nts

D ana Munav sh[1], Brenda Ham ton[1], Sh r ey Nan[1], Jac Jung[2], He en C. Ett er[3], and J e Xu[1]
[1]Cytogenet cs; [2]Med ca Genet cs, and [3]Patho ogy, London Hea th Sc ences Centre and Un vers ty of Western Ontar o, London, ON

We present a rare case of prenata detect on of a comp ex chromosome rearrangement (CCR). A 32 year-o d fema e was referred to the Prenata D agnos s C n c at 20 wee s

gestat ona age because of materna serum screen ng (MSS) nd cat ng an ncreased r s of Down syndrome (1 n 34). There was no h story of any s gn f cant materna ness or exposure to teratogens or on z ng rad at on. The fam y h story was not s gn f cant for any add t ona r s factors. Ser a u trasound exam nat ons had demonstrated adequate nterva feta growth w th no ev dence of any ma or feta anoma es. The on y anoma y detected was a 2.7 mm eft s ded choro d p exus cyst. After a rev ew of the c n ca nformat on ava ab e, th s coup e dec ded to proceed w th an amn ocentes s.

Prenata nterphase FISH of uncu tured amn ocytes us ng a set of the 5 probes (for 21, 13, 18, X and Y) showed a norma resu t suggest ve of a fema e fetus. G-band ng ana ys s of 12 n s tu co on es and SKY revea ed an abnorma fema e aryotype w th an apparent y ba anced CCR character zed by the presence of a 4;9 trans ocat on and 3 der vat ve (der) chromosomes. der (1) resu ted from trans ocat on nvo v ng chromosomes 1, 2 and 13. der(13) resu ted from 1;13 trans ocat on. der(2) resu ted from 1;2 trans ocat on. The f na aryotype s 46,XX,der(1)t(1;2)(p13.3;q31)t(1;13)(q25;q32), der(2)t(1;2)(p13.3;q31),t(4;9)(q27;q22.3),der(13)t(1;13)(q25;q32). Th s s nterpreted a de novo CCR s nce the parents had a norma aryotype.

The parents were counse ed regard ng a s gn f cant y ncreased r s of feta anoma es because of the de novo CCR nvo v ng 6 brea po nts and ncreased e hood of the presence of subm croscop c rearrangements of c n ca s gn f cance. The parents chose to term nate the pregnancy w th perm ss on for deta ed patho ogy. Autopsy f nd ngs reported a fema e cons stent w th the c n ca gestat ona age of 22 wee s. Externa anoma es nc uded ong s ender d g ts, f at feet w th protuberant hee s w th a ow set anter or ha r ne. No nterna anoma es were detected. Cytogenet cs was performed on a post-mortem s n b opsy conf rm ng the amn ot c f u d resu t. Th s case prov des an add t ona examp e that SKY s very usefu for character zat on of CCR and prenata management of a h gh-r s pregnancy.

Large per centr c Invers ons and the r Recomb nants

Judy Chernos, Cytogenet cs Laboratory, A berta Ch dren's Hosp ta , Ca gary, A berta

Subte omere FISH s w de y used for dent fy ng crypt c rearrangements n affected nd v dua s w th norma GTG banded aryotypes. However, t can a so be a usefu too n character z ng abnorma chromosomes to eva uate the r brea po nts and rearrangements n more deta . Large per centr c nvers ons can be d ff cu t to dent fy by rout ne chromosome ana ys s. Due to the h gh r s for me ot c recomb nat on w th v ab e mba ance ead ng to s gn f cant c n ca prob ems n offspr ng, t s mportant to dent fy these nvers ons carr ers. Three cases of arge per centr c nvers ons (nv 5, nv 21 and nv 17) ascerta ned through offspr ng w th recomb nat on aneusomy are presented.

The f rst case of a ba anced trans ocat on p us recomb nant dup(5q) nv(5) demonstrates how an add t ona chromosome rearrangement may d rect attent on from other c n ca y s gn f cant cytogenet c anoma es. The second case nvo v ng an nvers on 21 w th brea po nts n the acrocentr c short arm shows how nvers ons can be m s nterpreted. The th rd case ustrates an unexpected subte omere FISH pattern for a per centr c nvers on due to the un que term na brea po nt on the ong arm of an nverted chromosome 17. These three cases demonstrate how subte omere FISH stud es can be used to dent fy per centr c nvers ons and to character ze the r brea po nts.

Se ect on cr ter a for prenata nterphase FISH: Is there an dea f t?

Marsha D. Speeva , PhD The Cred t Va ey Hosp ta Genet cs d v s on of Laboratory Med c ne, 2200 Eg nton Ave W M ss ssauga,ON L5M 2N1

In Canada, the cost of perform ng f uorescence hybr d zat on (FISH) s absorbed by the cytogenet cs aboratory. S nce FISH s both expens ve and abour ntens ve, t s a techno ogy that s used ud c ous y n se ected c n ca s tuat ons. For examp e, n prenata d agnos s (PND), on y a sma percentage of pat ents qua fy for rap d screen ng for the presence of common aneup o d es by FISH. To ncrease the detect on rate and decrease the number of cases that qua fy for FISH, we n t ated a prospect ve study to eva uate referra cr ter a for FISH and cytogenet c outcome.
Mater a s: A amn ot c f u d cases rece ved n the aboratory between Apr 1, 2003 and March 31, 2005 were prospect ve y categor zed by r s , ass gned to be e ther FISH e g b e or ne g b e and at the end of the accrua per od the resu ts were rev ewed.
Methods: Based on the resu ts of a sma retrospect ve study of 600 f u d cases, we estab shed a set of 8 c n ca r s categor es for FISH e g b ty and app ed them prospect ve y to our PND samp es. Of part cu ar nterest were the 'grey area' r s categor es of nucha th c en ng (NT) 3.0-3.5mm and u trasound soft s gns.
Resu ts: The tr somy detect on rates for each r s category var ed from <4% (est mated r s 5% or ess) to 58% (non- etha u trasound abnorma t es). Soft s gns, nucha th c en ng (NT) and est mated r s s of 6% or greater gave ntermed ate detect on rates of 13-25%.
Conc us ons: Soft s gns, nucha th c en ng (espec a y n the 3.0-3.5mm range) were surpr s ng y strong nd cators for the presence of a common aneup o dy. NT >3.5mm were a so at r s for anoma es other than the common tr som es. Est mated r s (based on ntegrated prenata screen, materna serum screen or materna age) of <6% s nsuff c ent to warrant rap d FISH ana ys s. L m t ng the FISH e b b e pat ents to those w th u trasound f nd ngs (both m nor and ma or) and/or est mated r s s based on b ochem ca screens or age of 6% or greater wou d max m ze the detect on rate of common aneup o d es n th s ser es.

CASE STUDY: Prenata Detect on of Two Fam a Structura Rearrangements

Kath een Br er ey, Ch dren's Hosp ta of Eastern Ontar o, 401 Smyth Rd, Ottawa, K1H8L1 br er ey@cheo.on.ca

An amn ot c f u d spec men from a 37 year o d pat ent was subm tted to the aboratory w th a referr ng d agnos s of IPS pos t ve (r s 1/170), as we as advanced materna age. Fam y h story was unremar ab e and the coup e had a phenotyp ca y norma 4 year o d ch d. Chromosome ana ys s revea ed two structura rearrangements, one materna n or g n the other paterna . Convent ona cytogenet cs test ng on per phera b oods from the parents revea ed an apparent y ba anced trans ocat on n the mother and homozygos ty for a structura y rearranged chromosome n the father.

Sess on III: New Techno og es

The H gh ghts Of Des gn ng And Imp ement ng A Cytogenet cs Informat on System.

Eva M. Cappa, Mount S na Hosp ta , Cytogenet cs Laboratory, Department of Patho ogy and Laboratory Med c ne, Toronto,ON.

A br ef overv ew of Mount S na Hosp ta 's exper ence n deve op ng, va dat ng and mp ement ng a cytogenet cs aboratory nformat on system. H gh ghts of the aboratory's bac ground (h story), dec s on to des gn "new" system, resources requ red, cha enges encountered and status of expectat ons to date.

A new age for mon tor ng Chron c Mye ogenous Leu em a: Mo ecu ar too s for detect ng m n ma res dua d sease.

Marsha D. Speeva , PhD The Cred t Va ey Hosp ta Genet cs, D v s on of Laboratory Med c ne, 2200 Eg nton Ave W M ss ssauga,ON L5M 2N1

Chron c Mye ogenous Leu em a (CML) s a parad gm for the ut ty of understand ng onco og ca d sease at the genet c and ce u ar eve s. W th now edge of the mo ecu ar bas s of CML and the consequences of abnorma prote n act v ty n the ce , t was poss b e to rat ona y des gn the drug G eevec to combat the d sease. Now that CML has become contro ab e, t s mportant to fo ow how we pat ents respond to new c asses of drugs such as G eevec. Th s nvo ves deve opment of methods of detect on we beyond that poss b e by FISH.

Quant tat ve reverse-transcr ptase PCR (Q-PCR) s the mo ecu ar method of cho ce now used to fo ow pat ents' responses to G eevec when the Ph+ mar er has dropped be ow FISH detectab e eve s. A case rev ew of pat ents fo owed at CVH suggests that FISH and Q-RT-PCR together prov de a broader perspect ve of how we pat ents respond to treatment.

Cytogenom c Resources for Research – an update from The Centre for App ed Genom cs

Ann George[1], Mart n L [1], Jenn fer S aug[1], Mar Schachows oy[1], Y ng Q [1], Ju e Seo[1], Jun- un Zhang[1], L nda Dan L [1], Jo-Anne Herbr c [1], Mary Shago[2] and Stephen W. Scherer[1].
(maryann@genet.s c ds.on.ca)
Cytogenom cs Fac ty, The Centre for App ed Genom cs, Room 9109 E m, Department of Ped atr cs and Laboratory Med c ne, The Hosp ta for S c Ch dren, 555 Un vers ty Avenue, Toronto ON Canada M5G 1X8

The Centre for App ed Genom cs (www.tcag.ca) s a Canad an nfrastructure estab shed to fac tate nnovat ve research and deve opment n genet cs and genom c b o ogy. The Genome Resources, Gene Iso at on and Cytogenom cs fac ty s one of f ve nfrastructures that operate w th the ob ect ve of prov d ng core resources and techno og es to support arge-sca e research pro ects, as we as for serv ce-re ated research. Th s fac ty offers a number of convent ona cytogenet c and mo ecu ar cytogenet c serv ces w th a focus on FISH app cat ons. Cytogenet c resources for research nc ude: chromosoma preparat ons and aryotyp ng us ng G-band ng from var ous human t ssues, mu t -spec es ce ne cu tures and mouse embryon c stem ce s. Latest app cat ons of FISH techno ogy fac tate prec se def n t on of crypt c chromosoma rearrangements, a terat ons n genome organ zat on and gene mapp ng wh ch, w thout th s eve of reso ut on wou d be mposs b e. Serv ces offered range from nterphase and metaphase FISH, transgen c mapp ng n m ce, array Comparat ve Genome Hybr d zat on, as we as preparat on of custom-made probes from the n-house brary resource.

Canad an Cytogenet c Emergency Networ (CEN) for b o og ca dos metry fo ow ng rad o og ca /nuc ear acc dents

Douglas R. Boreham[2,], Susan M. Miller[1], Catherine L. Ferrarotto[1], R. Wilkins[1] and JoAnna Dowling[2,3]
[1]Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa, Canada
[2]McMaster Institute of Applied Radiation Sciences, McMaster University, Hamilton, Canada
[3]Genetics Department, Credit Valley Hospital, Mississauga, Canada

We are developing a network of laboratories across Canada to provide the capacity for rapid biological dose estimates using the dicentric chromosome assay (DCA) in emergency situations. The DCA measures dicentric and ring chromosomes, which are induced by radiation, in circulating blood lymphocytes blocked in metaphase. This method has been used internationally for over 30 years and has been standardized by ISO (ISO 19238).

In cases where only a small number of dose estimates are required, up to 1000 metaphases per blood sample are analysed, allowing detection of exposures as low as 0.15 Gy. However, in dealing with a large number of samples from potentially exposed individuals, where turnaround time is critical, the detection threshold can be raised to 1 Gy, thus reducing the number of metaphases to be analysed.

In a major emergency situation, even with the combined capacity of four core

the most cha eng ng aspects of FISH test ng n c n ca pract ce, name y va dat on of new assays and use of contro s.

A va dat on method nvo ves fam ar zat on, p ot study and c n ca nvest gat on. Fam ar zat on stud es are performed on PHA st mu ated b ood metaphase ce s and nterphase nuc e from f ve norma ma es. The fam ar zat on stud es assess equ pment, s gna ntens ty and ntegr ty, potent a nterfer ng factors such as cross hybr d zat on, and estab sh ana yt ca sens t v ty and spec f c ty for metaphase ce s. The p ot study s done on f ve norma and f ve abnorma samp es us ng the FISH assay on the ntended t ssue type for c n ca pract ce. The p ot study tests the expected scor ng cr ter a, ana yt ca sens t v ty, and norma cutoff for the FISH strategy used. The c n ca nvest gat on nc udes 25 norma spec mens and a ser es of representat ve abnorma samp es nc ud ng var ant chromosome anoma es and mosa cs. The resu ts are used to def ne the reportab e abnorma reference range (the owest and h ghest percentage of ce s w th an abnorma pattern) and norma cutoff (percentage of ce s requ red to d scr m nate w th 95% conf dence between a fa se-pos t ve resu t and a true abnorma c one). The new test s mp emented n c n ca pract ce once va dat on s comp ete and the f na standard operat ng procedure s wr tten.

A regu atory agenc es requ re the use of standard contro spec mens for FISH test ng. Test ng contro s w th c n ca FISH assays he ps to 1) ensure that the procedure s wor ng appropr ate y, 2) estab sh that the correct probe(s) s app ed, 3) ver fy that scor ng cr ter a are cons stent y used, 4) he p to nterpret performance va ues of the assay, and 5) mon tor performance of the assay over t me. Contro strateg es d ffer between qua tat ve and quant tat ve FISH test ng. Resu ts of qua tat ve FISH tests are genera y norma or abnorma . Most m crode et on FISH assays are qua tat ve tests because mosa c sm se dom occurs n these cond t ons. In contrast, FISH assays that estab sh tumor burden or measure mosa c sm are quant tat ve FISH tests. For examp e, a c n c an w mon tor the percent of abnorma nuc e for a pat ent w th eu em a over the course of the r treatment. Contro s for FISH ana yses may be nterna or externa . Interna contro s use a s te other than the target ocus and are suff c ent for qua tat ve metaphase tests. Externa contro s nc ude norma and/or abnorma spec mens, and are usefu for quant tat ve nterphase FISH assays.