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Cytogenetic and Genome Research

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Cytogenetic and Genome Research

A1

Risk Stratification in Children's Oncology Group (COG) B-Cell Precursor Acute Lymphoblastic Leukemia (B-ALL): Interactions of Cytogenetic and Molecular Approaches

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The COG uses a newly adapted 'risk stratification' for children with B-ALL to determine treatment. Initially, stratification is based on the NCI-Rome risk criteria of age and white blood cell count (WBC). Children >1 and <10 years with WBC <50,000 × 10^9/l are standard risk (SR); the others are infants or high risk. Following completion of four weeks of induction chemotherapy, genetic/cytogenetic criteria and measures of early response to therapy are used to refine risk stratification. SR pts with either *ETV6/RUNX1 (TEL/AML1)* fusion or 'triple trisomy' (TT, +4,+10,+17) are eligible for a SR-Low treatment arm; while all Ph+ or hypodiploid (hypo; mn <43 or DNA index (DI) <0.81) cases are eligible for a very high risk study. The genetic criteria are determined in two molecular and two FISH reference (ref) laboratories (lab), and are augmented by cytogenetics (cyto) from COG approved institutional (inst) cyto labs. Institutional results are required for a child to remain on study if ref labs get a poor sample or inconclusive results. Discrepancies between ref and inst labs are resolved individually. Both *ETV6/RUNX1* RT-PCR (all RT-PCR in ref labs) and inst FISH were considered for stratification in pts where data were inconclusive: six were RT-PCR-negative and inst FISH+; one was inst lab cyto Ph+ and *ETV6/RUNX1*+ via ref lab RT-PCR and FISH; one *ETV6/RUNX1* RT-PCR was inconclusive with negative ref lab FISH; poor sample quality prevented RT-PCR in an inst lab FISH *ETV6/RUNX1*+ case; and one case was RT-PCR+ and FISH negative. TT is determined by ref lab FISH on all SR pts with DI >1.06. One pt with DI = 1 was TT by inst FISH; one ref lab TT was inst FISH *MLL*+; one was ref lab TT and inst cyto was +4,+12,+17; a case with ref lab FISH +4,+10,?+17 was inst lab +4,+10 only; and one ref lab +4,?+10, +17 did not have +10 in the inst lab. One inst Ph+ pt was ref lab RT-PCR *BCR/ABL*– and *ETV6/RUNX1*+; three cases were RT-PCR *BCR/ABL* weakly positive, all were *BCR/ABL* negative by ref and/or inst lab FISH.

A2

A Case of Concurrent Relapsed ALL and Secondary AML

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Treatment-related or secondary AML has been frequently reported in individuals who have undergone therapy for ALL. Here we describe an unusual case with concurrent relapsed ALL and secondary AML. The patient was diagnosed with pre-B ALL in September 1998 at nearly 15 years of age. A bone marrow aspirate done at diagnosis showed trisomy 8 and a deletion of the short arm of a chromosome #9 [del(9)(p21)] in 12 of 24 metaphases examined. The patient achieved complete remission after four weeks of treatment and completed therapy in April 2001, when her therapy was electively discontinued. She remained in unmaintained remission until August 2002 when she relapsed. She was reinduced into a second complete remission and was then treated with a rotating pairs regimen that included etoposide. The patient completed this second course of therapy in April 2005 at which time her therapy was again electively discontinued. In August 2005 the patient presented with decreased platelets and 25% blasts. No flow cytometry was performed. The bone marrow aspirate showed trilineage dysplasia and was described as predominantly myeloid by morphology. There was some disagreement as to whether this represented relapsed ALL or secondary AML. Bone marrow cytogenetics showed a t(11;19)(q23;p13.1) in all 20 metaphases analyzed, consistent with secondary AML. A repeat bone marrow performed in September 2005 showed identical chromosomal results. Flow cytometry identified abnormal myeloid blasts with partial monocytic differentiation consistent with AML. A subsequent bone marrow in October 2005 contained mostly blasts and showed persistence of the t(11;19) translocation. However, two blast populations were identified by flow cytometry: a majority of abnormal precursor B-lymphoblasts (73%) consistent with relapsed ALL, and a minority of abnormal myeloid blasts (20%) consistent with persistent AML. FISH using probes specific for chromosome #8 (blue) and *MLL* separation (red and green) revealed two independent cell populations, one with 46% of cells with +8 and another with 42.5% of cells with *MLL* separation. Trisomy 8 and *MLL* separation were not observed in the same cells. These results confirmed the presence of two unrelated clonal processes. A bone marrow done in November 2005 showed the t(11;19) by cytogenetics, and similar flow cytometry results with 56% precursor-B lymphoblasts and 15% abnormal myeloblasts. Three subsequent studies had uninformative histopathological results due to hypocellularity of the samples. In two of these studies chromosomal analysis was performed and both showed the t(11;19). *MLL* and +8 FISH was positive in January 2006, with two separate clones present. The patient is currently awaiting bone marrow transplantation.

A3

The Effect of IL-2 on Increasing the Detection Rate of Abnormal Clones in Cancer Cytogenetic Studies: A Simple Strategy

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The detection of cytogenetic abnormalities in hematological disorders is becoming a powerful tool in providing an accurate diagnosis, in predicting the clinical course of patients and the choice of therapy. The general rate of abnormal cell detection for myeloid disorders is 80% versus 40% for lymphoproliferative disorders. In order to increase the detection rate of abnormal cells, different stimulants were added into different 72-hour cultures. Besides the 24-hour unstimulated culture, PMA/LPS, Interleukin-6, and Interleukin-2 (IL-2) stimulants were added into three different 72-hour cultures. This approach was applied on all types of hematological disorders including acute non-lymphocytic leukemia (ANLL), myelodysplastic syndromes (MDS), lymphoproliferative disorders (LPD), lymphoma (LYMP), myeloproliferative (MPD) disorders and acute lymphocytic leukemia (ALL). Overall, clonal chromosome abnormality was found in a number of cases: 23 AML, 56 MDS, 50 LPD, 24 LYMP, 21 MPD, and 34 ALL. IL-2 enhanced the detection rate by 25% in AML, 0.5% in MDS, 50% in LPD, 37% in LYMP, 0% in MPD, and 8% in ALL. IL-6 and PMA/LPS showed no significant enhancement in the detection rate of abnormal cells. We recommend IL-2 as the most efficient and the least expensive stimulant to use in cytogenetics studies of hematological disorders.

A4

ABL1 Amplification in T-PLL

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Although gene amplification is thought to be less common in leukemia than in solid tumors, there have been reports of *AML1* amplification in pediatric ALL and even adult AML and more recently *ABL1* amplification in T-ALL. We report two cases of *ABL1* amplification in T-cell prolymphocytic leukemia. T-PLL is a rare, aggressive disorder usually associated with rearrangement of the T-cell receptor genes on chromosome 14, abnormalities of chromosome 8, or deletions of 12p13 or the *ATM* locus at 11q23. Our first patient is a 56-year-old female who presented with flu like symptoms, cardiomegaly and pleural effusions. Evaluation of the pericardial fluid was consistent with a mature T-cell lymphoproliferative disorder most likely T-prolymphocytic leukemia. A pseudotetraploid karyotype with a deletion of 1q25 and double minutes was reported in 45% of the cells. FISH on interphase cells showed 71.5% to have high-level amplification (greater than 100 copies per cell) of the *ABL1* gene. The second patient is a 73-year-old female, initially evaluated for T-cell lymphoma and ultimately diagnosed with T-PLL. Routine cytogenetic analysis yielded very few hypodiploid cells. Interphase FISH revealed 3% of the cells to have low level amplification of *AML1* and the majority of cells to have only one copy of the *BCR* gene. The significance of *ABL1* amplification in T-PLL will be discussed.

A5

Direct-to-Consumer Genetic Laboratory Testing

Bradley Popovich

Chief Operating Officer, Sirius Genomics, Inc. Vancouver, BC, Canada

Dr. Popovich will discuss direct-to-consumer testing, the newest method for individuals to seek genetic testing without going through the traditional channels. The discussion will include testing currently available, future testing and how such testing may impact clinical laboratories.

A6

Identification of a Telomere-Like Sequence at 22q11.2 Using Double-Strand PRINS

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The presence of telomere or telomere-like repeats at intrachromosomal sites, known as interstitial telomeric sequences (ITSs), was described in many eukaryotic species as well as in humans. It has been reported that the ITSs might originate from ancestral intrachromosomal rearrangements (inversions and fusions), or result from differential crossing-over. Some ITSs were found to enhance chromosome breakages, induce high rates of chromosome rearrangements or closely correlate to the fragile sites or hot spots for recombination. Whereas the relationship between the sequence organization for telomeres and their function is clear, the presence of ITSs inside chromosomes is far from being understood. Using double-strand primed in situ labeling (PRINS) technique with telomere sequences as primers, we discovered a specific telomere signal on human chromosome 22q11.2 region (average detection frequency is 24.7% from 166 metaphases of 16 individuals), indicating a higher detection frequency than that defined by Azzalin et al. as 'very frequent' ITS category observed at 1q21 and 2q13 for 21 and 31 times, respectively, from a total of 232 metaphases. Having scanned in the genome database, we found a telomere-like sequence containing a 101 tandem repetitive 9-base unit, TTAGGGAGG and TTATGGAGG, located at 22q11.2 and amounting to 909 bp (http://www.genome.ucsc.edu/) in length. We designed new primers based on the sequence of the ITS in the genome database to perform PRINS. It resulted in a high signal frequency and specificity at 22q11.2. The average frequency of metaphases containing the signals at 22q11.2 reached 51%. Furthermore, the PCR using primers that flank the ITS revealed different patterns in some normal individuals and a case with DiGeorge syndrome, implying the presence of polymorphism in the ITS. Notably, the position of this ITS is in the same area of, and proximal to the common rearrangement sites for multiple disorders. The 22q11.2 region is highly unstable and frequently involved in chromosomal breakage and rearrangements, most of which appear to be distal to the ITS at 22q11.2. It is interesting to further investigate the underlying biological significance of whether the ITS at 22q11.2 plays any important role for its nearby genes or if any repair mechanism, when DNA damages occurred in this area, is involved in the ITS.

A7

Molecular Cytogenetic Characterization of a Unique and Complex de novo 8p Rearrangement

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Human chromosome 8p is prone to recurrent rearrangements with the inv dup del(8p) being most common. Such recurrent rearrangements are postulated to derive from non-homologous recombination mediated by repetitive sequences such as the olfactory receptor gene clusters. However, recent reports have shown that some of the rearrangements are unique and complex and are mediated by other repetitive elements within 8p. Each of these rearrangements is associated with different clinical manifestations. Here, we report a unique and complex 8p rearrangement with seizures as the major presenting feature.

A 4-year-old Hispanic girl, born at 37 weeks gestation to a G1P0 mother presented with failure to thrive. Her birth weight and height were below the 3rd percentile. No major organ malformations or complications were noted. Her height, weight and OFC were at the 50th, 45th and 35th percentiles, respectively. She had seizures and global developmental delay with the skills of an 18-month-old. She displayed poor auditory attention, impulsiveness and a decreased attention span. Physical examination showed upward slanting palpebral fissures, epicanthal folds, high arched palate and bilateral clinodactyly. Head CT showed a mild degree of atrophy and evidence of a mild Dandy-Walker variant in posterior fossa. Chromosome analysis showed a rearranged chromosome 8p, which on whole chromosome painting was determined to be a duplication. Subtelomeric FISH studies showed an 8p deletion. Since the proband's clinical phenotype differed from the common inv dup del(8p) phenotype, we have further characterized the rearrangement using a series of BAC clones from 8p. BAC-FISH analyses showed that our patient has a unique and complex rearrangement with a proximal deletion of 6.3 Mb spanning the 8p23.1→pter region, an intervening normal segment of 6 Mb spanning the 8p23.1 region, followed by duplication of 12 Mb spanning the 8p23.1→p21 region. These results were confirmed by array analysis using an 8p tiling path BAC array. In contrast to the more common inv dup, FISH analysis showed that the duplication in our case was tandem and thus making this the first case of dup del(8p) with seizures being the most prominent feature. BAC-FISH and array analyses mapped the distal breakpoint of the duplication between 23.4 and 23.6 Mb, while the proximal duplication/normal breakpoint is mapped at 12.62 Mb. The third breakpoint between the normal/deletion segment is mapped between 6.82 and 7.99 Mb with the most proximal deletion breakpoint mapping to 36 kb from the telomere. Two single copy genes, *LONRF1* (LON peptidase N-terminal domain and ring finger 1), a DNA and metal ion binding protein, is within the breakpoint region at 12 Mb; while the gene *SLC25A37* (Solute carrier family 25, member 37), a mitochondrial integral membrane protein, is at 23.4 Mb. Further studies are in progress with a fosmid tiling path 8p array to narrow the breakpoints so as to understand the mechanism of this complex rearrangement and also to elucidate the pathophysiology of our patient's presentation.

A8

Significance of a High Frequency of Chromosome Polymorphic Variations in Infertility

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Polymorphic variations of the heterochromatic regions of chromosomes such as 1, 9, 16 and Y are generally considered as normal and are usually ignored during diagnostic karyotyping. However, there is also the opinion that large 'de-novo' variations could have some clinical implications, compared to inherited ones. Usually a variation is seen in only one of the two homologues and the elongation of heterochromatin may be more than double the size of its counterpart on the other homologue. This should serve as an internal control and rule out most cultural artifacts. It had long been assumed that heterochromatin has no coding potential and is inert. It is now known that heterochromatin is not inert and is essential for viability of cells and organisms. Heterochromatin plays an essential role in spindle attachment, chromosome movement, meiotic pairing and sister-chromatid cohesion.

With the availability of refined molecular techniques, transcriptional activation of constitutive heterochromatic domains of the human genome including the pericentromeric heterochromatin of chromosome 9 has been demonstrated, by the assembly of nuclear stress bodies in response to environmental stress such as heat shock (Rizzi et. al., Mol. Biol. Cell, 2004, 15: 543–551). These are characterised by an epigenetic status typical of euchromatic regions. Recently, heat shock transcription factor on the Y chromosome has been mapped to the euchromatic AZFb region as multicopies. Deletion of this region results in severe male infertility. The heat shock factor family has been shown to be implicated in spermatogenesis both in animals and humans. The characterization of its genomic structure, the number of copies on the Y chromosome and the expression of the gene were recently reported (Tessari et. al., Mol. Hum. Reprod. 2004, 10:253–258).

In view of these findings and our observation of a high frequency of polymorphic variations in couples attending our infertility clinic, which will be discussed, it is suggested that chromosome variations should not be ignored and existing data could be re-evaluated. Karyotyping all prospective gamete donors for IVF and screening out those with variants may take us a step further in enhancing the success rate of IVF. Infertile individuals with large de-novo variations may stand a better chance with screened donor gametes (Madon et. al., RBMOnline 2005, 11:6,726–732). Confirmation from other laboratories and collaboration of cell biologists with cytogeneticists may help to determine the subtle clinical effects of heterochromatic variations, probably even in pediatric cases with attention deficit hyperactivity disorder and autism or in patients with psychiatric disorders. The mysteries of heterochromatin are now slowly being unraveled by scientists. Variants should be mentioned in karyotype reports without causing alarm, so that karyotyping need not be repeated at a later date if required. We do not however report 'normal' variants observed on prenatal diagnosis.

A9

Frequency of Telomere Rearrangements in Individuals Who Are Phenotypically Normal and Those with Developmental Delay/Mental Retardation

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Mental retardation (MR) affects 2–3% of the general population. Less than half of affected individuals have a known etiology for their disorder. Studies have shown that 5–7% of individuals with an unknown etiology of MR and normal G-banded karyotypes have a microdeletion and/or duplication of subtelomeric regions. Half of these individuals have inherited the deletion and/or duplication from a parent with a balanced cryptic translocation. While the incidence of visible cytogenetic reciprocal translocations is 1/600, the prevalence of balanced cryptic telomere rearrangements in the general population is unknown. As part of a large genetic dental study involving 500 families in Appalachia, unrelated individuals are being evaluated for the incidence of cryptic rearrangements using subtelomeric fluorescent in situ hybridization (FISH) DNA probes from Vysis. To date, blood samples from 328 unrelated individuals have been evaluated with subtelomeric FISH probes. We have not found any rearrangements within this population. However, two individuals have been identified who are mosaic for the X chromosome. Additional unrelated individuals are currently being evaluated to better determine the incidence of cryptic rearrangements in the general population. Supported by NIH/NIDCR grant # R01-DE0114899.

A10

Prenatal Diagnosis of 10 Cases with Smith-Magenis Syndrome

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Smith-Magenis Syndrome (SMS) is a clinically recognizable chromosome microdeletion syndrome due to its distinct physical, developmental and behavioral characteristics. It is caused by a deletion or mutation of the *RAI1* gene at chromosome 17p11.2. The incidence is about 1/15,000 livebirths. The clinical manifestations of this syndrome have been very well defined. Because its dysmorphic features are age dependent, the majority of SMS patients cannot be diagnosed until clinical symptoms become more obvious at their childhood. Therefore, prenatal diagnosis of SMS is very important. To date, only two cases of Smith-Magenis syndrome diagnosed prenatally have been described. The paucity of prenatally reported cases with a deletion of 17p11.2 limits the conclusion regarding prenatal indication and maternal age at the pregnancies with the disorder. We report ten prenatally diagnosed SMS cases from a total of 455,121 consecutive prenatal cytogenetic studies. Our study, the largest and most comprehensive study of the syndrome to date, would contribute more accurate recognition of Smith-Magenis Syndrome at prenatal diagnosis.

A11

When Interphase FISH and Cytogenetic Studies Show (Apparently) Discordant Results

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Interphase fluorescence in situ hybridization (FISH) analysis for aneuploidy screening of prenatal cases provides a rapid result for aneuploidies involving chromosomes 13, 18, 21, X, and Y. In the majority of cases, the rapid FISH analysis gives accurate results that correlate with the subsequent cytogenetic studies; however, a small subset of cases may show discordant results. We report on a group of prenatal cases in which the results of the interphase FISH analysis differ from the cytogenetic findings. Various explanations for these results will be discussed. Some explanations for these discordant results include mosaicism undetected in the interphase study, cross hybridization of centromere probes, small repetitive sequence regions, and overwhelming maternal cell contamination. Occasionally, an apparent discrepancy between the FISH and chromosome results can lead to the detection or clarification of an abnormality that might have been missed in the chromosome analysis. One case that will be presented involves a prenatal sample showing results consistent with trisomy 21 on the interphase FISH study while the chromosome analysis appeared to be consistent with a normal male. Upon further examination and additional FISH testing, an unbalanced translocation between the short arm of chromosome 4 and the long arm of chromosome 21 was identified. Since this was a subtle rearrangement involving two light staining regions, in the absence of the interphase FISH analysis it is likely that this translocation would not have been identified. These exceptional cases highlight the complementary nature of the information obtained from interphase FISH and traditional cytogenetic analysis.

A12

Structural Variations in the Human Genome

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Recent discoveries are demonstrating that the genomes of two 'normal' individuals can vary by hundreds of structural rearrangements including insertions, deletions, inversions, and copy number variations. Some of these structural genomic variations include genes known to be involved in adaptability, rather than viability. Consequently, investigations of these components of the human genome may soon yield valuable information on why people react differently to various drugs and environmental stimuli.

A13

Low Level Somatic Mosaicism Detected by CGH with a Targeted Chromosomal Microarray

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The current targeted chromosomal microarray (Version 5) from Baylor College of Medicine includes 860 FISH-verified BAC clones that span genomic regions implicated in approximately 75 known human genomic disorders. The array has multi-clone replication for each disease, with an average of 3–5 clones representing each deletion or duplication syndrome, 10 or more clones for the 41 subtelomeric regions and 4 to 6 clones for the 43 pericentromeric regions allowing for information integration and enhancing the specificity and sensitivity of our findings. To accomplish the data integration, we developed custom analysis software in R open source statistical computing language. Our system is also integrated with a relational database so that historical results can be mined to identify polymorphic clones and their genomic contexts.

To date, we have completed over 1000 analyses with the Version 5.0 microarray in which chromosome tests were previously reported normal or performed simultaneously. We identified seven new cases with low-level mosaicism and confirmed two cases with somatic mosaicism identified previously by another method. The seven cases include: two cases with mosaic trisomy 9, two cases with mosaic trisomy 14, one case with mosaic trisomy 22, one case with mosaic monosomy 7 and one case with mosaic Klinefelter syndrome. These results were confirmed by either chromosome analysis, FISH studies with T-cell stimulated and/or B-cell stimulated cultures or whole blood smears. We conclude that PHA stimulated T cells routinely used for chromosome and FISH analysis may not be the best source for detection of somatic mosaicism.

A14

High Resolution Oligo Array Comparative Genomic Hybridization (aCGH) in the Investigation of Autism and Neurodevelopmental Disorders

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Autism is a developmental disorder of brain function that appears early in life, generally diagnosed between 18 and 30 months of age. Children with autism have problems with behavior, social interaction, imagination and communications. Autistic traits persist into adulthood, but vary in severity. While the cause of autism is not known, many believe it to be a germline, inherited disorder. The need to identify affected individuals as early as possible is great in order for them to receive the necessary help and services as soon as possible to minimize the damage. The financial burden on the educational system and society easily reaches the billion dollar mark. The disorder spans all races and socio-economic boundaries.

Genomic copy number aberrations, such as chromosomal amplifications and deletions, have been associated with autistic children and their parents. Current microscopic-based diagnostics techniques such as karyotype analysis are inadequate for the resolution or the high-throughput required of a widespread diagnostic test. Here we present the development of a custom 60mer oligonucleotide microarray designed for two-color CGH. This array was designed to interrogate chromosomal aberrations at high resolution associated with autism and IDIC (Isodicentric) on chromosome 15. Peripheral blood lymphocytes (PBLs) from autistic patients, immortalized with Epstein Barr Virus (EBV) were assayed for chromosomal changes at chromosome 15 cytobands q11–13. Results show amplification of this region with well defined breakpoints and precise copy number changes unique to individual patients. Correlation of CGH to gene expression data will also be presented. By screening many patients in this manner and identifying common genetic aberrations, one can define the genes involved. If simple genetic copy number amplification is responsible for dysregulated gene expression of these DNA segments, then pharmaceutical intervention may be possible.

A15

Novel Cytogenetic Alterations Detected by Array CGH in Patients with Developmental Delay, Dysmorphology, and Mental Retardation

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Completion of the Human Genome Project and availability of high-density DNA clones have facilitated the development of rapid and highly sensitive assays to detect quantitative changes in genetic material. We have used microarray-based comparative genomic hybridization to study a cohort of 20 patients with developmental delay, dysmorphology, and mental retardation. Conventional cytogenetic analysis had yielded normal results for these patients. Virtually all patients carried large-scale deletion and duplication polymorphisms, some of which were new. Five of the 20 patients demonstrated unique deletion mutations of various sizes between 3 and 6 Mb. All of these mutations had occurred de novo. Three of the five mutations had not been previously reported and represented novel genetic syndromes. Of the five mutations, the first was a 15q11q12 deletion associated with Prader-Willi syndrome (PWS), although the patient did not exhibit a classic PWS phenotype. The second mutation was an interstitial deletion in 1p36, but the patient's phenotype did not resemble that of other patients with 1p36 deletions. The third mutation was an 8q22 deletion that resulted in a phenotype characterized by short palpebral fissures, hypertelorism, sparse eyebrows, everted upper lip, dysplastic ear helices, shiny and tight facial skin, and abnormal hair patterning. The fourth mutation was an Xq26q27 deletion overlapping the *F9* gene. The patient suffered from a bleeding disorder because of factor IX deficiency, as expected, but also exhibited macrocephaly and developmental delay. The fifth mutation was a deletion in 4q31, resulting in cleft lip and palate, heart defects, vertebral anomalies, and cryptorchidism. The cases described here add to an increasing number of microdeletion syndromes. As genes in the respective deletion intervals are further studied, these novel syndromes will provide valuable insight into mechanisms that regulate prenatal and neonatal development.

A16

Loci of Shared Segmental Aneuploidy in the Genomes of Healthy and Mentally Retarded Subjects Detected by Array-CGH

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By genome-wide segmental aneuploidy profiling with a 3,782 BAC array we detected in a panel of 131 unrelated affected children and 21 of their unaffected parents on average 35 autosomal loci of segmental aneuploidy (SA) per subject. In total, we identified 696 distinct loci of SA that were shared among patients and parents. These loci occur as deletion, duplication, or both,

in frequencies up to 45% within our study population. Subjects of Turkish and Arab/Berber origin showed significant overlap with Caucasians (87% and 92%, respectively). Plots of the cumulative number of shared loci of SA indicate that their total number may be finite. Loci of SA were distributed in the euchromatin with equal densities among Giemsa-light and Giemsa-dark bands. Out of the 68 most frequently occurring loci of SA, 21 contained sites of segmental duplication on the same chromosome, while 31 and 63 BAC clones were flanked by segmental duplications within a distance of 1 and 2 Mb, respectively. Comparison with a set of 39 randomly chosen BAC clones showed highly significant association of loci of SA with segmental duplications (P values of 0.0001, 0.0012 and <0.0001, respectively). Our data are consistent with the hypothesis that most loci of SA have been generated through non-allelic homologous recombination mediated by intrachromosomal sites of homology. Since loci of SA occur frequently in the general population, they should be taken into account before clinical conclusions are to be drawn upon detection of segmental aneuploidies in patients with congenital abnormalities or mental retardation.

A17

Applications of Array CGH Testing to Clinical Cytogenetics – What Should We Be Doing?

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There has recently been a large increase in interest from the cytogenetics community in using array CGH (arr cgh) technology for diagnostics. The medical value of using this technique as a supplement to conventional cytogenetics and potential replacement for FISH is becoming apparent. Different laboratories in both academic and commercial settings have offered some version of this testing, primarily for the detection of constitutional imbalances. An informal survey of our colleagues indicates that most acknowledge the need, potential and value of this technology. There is no current consensus, however, regarding specific aspects of implementing arr cgh into the general health care system. At the laboratory level, protocols for validation, confirmation, source of control DNAs, data interpretation and identification/reporting of copy number variants need to be considered. Reimbursement issues including source of the chip used and whether confirmatory studies should be billed also must be addressed. As a new technology, third party payers will also be reviewing published data to make a determination as to whether arr cgh is not experimental/investigational, and is medically necessary; the new test must meet both criteria to be payable. The American College of Medical Genetics is in the process of generating Standards and Guidelines for arr cgh testing, and these will likely serve as a good place to start, yet additional questions and the need for community consensus are likely to exist. The intent of this presentation will be to outline several of the issues noted and describe how these have been addressed by two academic testing centers (The Universities of Utah and Nebraska), including examples from approximately 500 arr cgh studies. The creation of specific cpt codes in 2006 for array CGH billing, inclusion of 'arr cgh' nomenclature in the ISCN 2005, and the general acceptance of this valuable technique by the cytogenetics community are all indicators that this will develop into a significant aspect of our testing repertoire in the future.

A18

Comparison of a Targeted BAC Microarray Analysis and Routine Chromosome and FISH Analyses in Chronic Lymphocytic Leukemia (CLL)

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B-cell chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western hemisphere; it accounts for about 25 percent of all leukemias in the United States. The clinical course of CLL is heterogeneous and difficult to predict. Cytogenetic studies using fluorescence in situ hybridization (FISH) have demonstrated that most cases of CLL contain non-random cytogenetic abnormalities with genomic imbalances such as deletions and gains are more frequently seen than translocations. These genomic aberrations in CLL are important independent predictors of disease progression and survival and are increasingly being used to contribute to treatment decisions and follow-up of CLL patients. Trisomy 12, 13q14 deletion, and 11q deletion are the most common clonal abnormality seen in CLL with prognostic significance. Deletion and mutations of p53 tumor suppressor gene at chromosome 17p13 has a strong implication for the clinical course of the disease. Some other chromosome aberrations identified in CLL include chromosome regions such as 6q, 8q, 3q, 18q, and 19. Although FISH has increased the sensitivity of detecting the above mentioned chromosome abnormalities it becomes time consuming and labor intensive to screen all possible implicated chromosome regions in CLL simultaneously. The current clinical CLL FISH probe panel consists of only the most common four chromosome aberrations seen in CLL. Therefore, a high throughput genomic analysis tool is required for a fast and a sensitive diagnostic analysis of CLL.

We have developed a targeted CLL microarray clinical diagnostic chip that will allow for a rapid and sensitive identification of genomic imbalances in CLL. The chip contains 232 FISH verified clones covering 17 chromosome regions implicated in CLL. Microarray analysis was performed with DNA purified from enriched B-cells from 31 CLL patients. In 27 out of 31 cases the microarray analysis was concordant with the CLL FISH panel analysis.

A19

Quality Watch: A New Program of the American College of Medical Genetics Laboratory Quality Assurance Committee to Enhance Quality Genetic Testing

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The Laboratory Quality Assurance Committee (Lab QA) of the American College of Medical Genetics (ACMG) is the authoritative body for developing standards and guidelines for assuring quality laboratory genetic services. The committee interfaces with other ACMG committees, related national groups, and agencies concerned with quality laboratory services. We recently formed the ACMG Quality Watch Subcommittee (QW) to address specific technical issues in genetic testing related to either product quality or genetic sequence variants that affect test accuracy. With regard to detecting and reporting adverse events resulting from lab product quality or errors with product use as labeled, the QW subcommittee is establishing a LIST SERV accessible through the ACMG website. This LIST SERV will be the initial communication venue for laboratory geneticists to discuss the putative problem that led to the adverse event, and assess whether it has occurred often enough to merit further investigation. Follow-up data will be collected electronically, via a targeted process moderated by a designated expert in biochemical, molecular, or cytogenetics. Formal reporting to manufacturers and FDA will ensue, if deemed appropriate, and timely information and instructions for all users will be posted on the QW LIST SERV. Concomitantly, the QW LIST SERV will also be used for discussing human gene sequence variants (polymorphisms) that alter test performance and accuracy. Again, submitted problems will be reviewed by an expert moderator and if determined to be a valid issue, posted on the website. A database listing these variant sequence changes is being discussed by the QW subcommittee, and will be accessible via the ACMG website. This database may be expanded to incorporate similar issues related to probe sets on microarrays. We will compare the two QW reporting platforms, outline the parameters for submitting a problem, discuss ways to encourage participation, and provide information that can be used to improve genetic testing quality.

A20

Case Load Survey, Workload Equation and How to Address Staffing Shortages in Cytogenetics

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Due to increasing caseload volumes in cytogenetic laboratories, there is a need for trained technologists and benchmarks for appropriate staffing per case workload. A workload survey of cytogenetics laboratories (in the US and Canada) was conducted. Participants were asked to provide information regarding numbers and experience levels of employees and indicate case volumes by specimen type. Employees were assigned a numeric value based on experience, while specimen types were weighted with a numeric value based on effort. Based on survey data, conclusions were drawn about potential efficiency values based on the number of full time employees versus case load.

In an independent effort, a cytogenetic workload equation was developed for Canadian cytogenetics laboratories. The equation is based on annual case volumes that are weighted by specimen type and procedure. The algorithm calculates the number of technologists required to complete the given cytogenetic studies. The survey and equation appear to be comparable with regard to estimating caseload per technologist per year. Such studies are useful in structuring cytogenetics laboratories; however, the issue of recruiting experienced cytogenetic technologists remains. We are developing a 'training position' with a career ladder in an effort to recruit recent college graduates into the cytogenetics laboratory. Our objective is to train and retain cytogenetic technologists to accommodate the growing caseload in cytogenetic laboratories.

A21

Analysis of a Repetitive DNA Sequence that Hybridizes by FISH to the Pericentromeric Regions of *Bufo* Chromosomes

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When genomic DNA from the toad *Bufo terrestris* is digested with *Hin*fI, a 23-kb band of apparently repetitive DNA is produced. Fluorescent in situ hybridization (FISH) using this band as a probe to *B. terrestris* metaphase chromosomes gives a hybridization pattern which is similar to that obtained by C-banding. This pattern can also be obtained on *B. fowleri* or *B. quercicus* chromosomes, but no hybridization occurs when using the band as a FISH probe to *Hyla chrysoscelis* or *Xenopus laevis* chromosomes. Southern blot analysis using the band as a probe to *B. terrestris* DNA digested with *Stu*I or *Bgl*II reveals ladders of bands, indicating that the bands are probably repetitive elements within the 23-kb band. Some of the fragments were cloned and Southern blot analysis confirmed their origin in the 23-kb band. These cloned fragments also hybridized to *Bufo* centromeres in FISH assays. One clone, designated pECT179, was sequenced, and its sequence was not found to have close homology to any other known centromeric sequences. Several of the other clones sequenced did not differ significantly from pECT179. This suggests that the 23-kb band is enriched with fragments having this repetitive DNA sequence and that it makes up a substantial portion of the *Bufo* genome.

A22

Paracentric Inversion Found in the Mother of a Child with Cri-du-Chat Syndrome: Evidence of Dicentric Chromosome Formation with Prezygotic Breakage Leading to a Viable Terminal Deletion

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We report the case of a child with a terminal deletion in the distal short arm of chromosome 5, 46,XX,del(5)(p14.3), with the clinical diagnosis of Cri-du-Chat syndrome. The father had a normal karyotype whereas the mother was a carrier of a paracentric inversion, 46,XX,inv(5)(p13.3p15.3). This finding in the mother was unexpected as the risk for a carrier of a paracentric inversion having a liveborn child with a recombinant chromosome leading to a genetic imbalance is thought to be extremely low. A recombination event between a chromosome with a paracentric inversion and its normal homolog will produce a dicentric and an acentric chromosome. Both recombinants are considered unstable and likely lethal for the developing embryo. Paracentric inversions are often diagnosed by conventional karyotype analysis using either G- or R-banding techniques. Using these techniques, a within-arm intrachromosomal insertion can be mistakenly interpreted as a paracentric inversion. The need to correctly distinguish these two types of chromosome rearrangements is emphasized by their different reproductive risks. For carriers of a within-arm intrachromosomal insertion, the general risk of having a liveborn child with a recombinant chromosome leading to a genetic imbalance is approximately 15%, and may be as high as 30%–50% for some rearrangements. It is therefore recommended that if a paracentric inversion carrier has a liveborn child with a chromosome abnormality thought to arise from a recombination event involving the inverted chromosome, the original interpretation of the parent's chromosomes should be questioned and molecular techniques employed to determine if the rearrangement is really an intrachromosomal insertion. With this in mind, fluorescence in situ hybridization (FISH) and microsatellite analyses were performed. These studies confirmed the paracentric inversion in the mother and that the deletion in the proband was maternal in origin. Therefore, this represents a case where a confirmed paracentric inversion likely resulted in a viable terminal deletion. We propose a mechanism involving dicentric chromosome formation with subsequent breakage and telomere healing during meiosis. This may illustrate a new mechanism of chromosome rearrangement leading to Cri-du-Chat syndrome. These results should also be considered in the medical management of patients with other terminal deletion syndromes.

A23

Genotype-Phenotype Correlation for Down Syndrome: A Study of Mosaicism for Trisomy 21

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Despite the prevalence of Down syndrome, little is known about the pathogenesis of the phenotype. The observed clinical variation in cases having mosaic Down syndrome (MDS) is hypothesized to be attributable to: (1) a 'threshold' effect of the trisomic cells; and (2) differences in the mechanism(s) of chromosomal malsegregation. To test the first hypothesis, lymphocyte and buccal mucosa nuclei from individuals having MDS were scored using FISH. To date, 70 probands have been ascertained. Overall, the proportion of trisomy 21 cells in the buccal mucosa smears was significantly higher than that observed in the cultured lymphocytes ($P = 0.0028$). This difference could not

be attributed solely to in vitro culturing since the percentage of trisomic cells in cultured and uncultured lymphocytes was not significantly different. The proportion of trisomic cells in the diagnostic (birth) chromosomal analysis was also significantly higher ($P = 0.05$) than that observed in the lymphocyte nuclei scored in this FISH study (patient ages ranged from 1 to 19 years) and may reflect tissue-specific changes in trisomic cell populations over time. A comparison of the FISH data to clinical findings showed that cases having higher buccal mucosa trisomic levels exhibited more phenotypic findings of ectodermal origin, while those having congenital heart disease (mesodermal origin) presented with higher lymphocyte (also mesodermal) trisomy levels. To test the second hypothesis, parental origin studies were performed using microsatellite markers, with the results being compared to clinical outcomes. Of the 38 families studied, to date, 27 cases resulted from a meiotic followed by a second mitotic error and 11 from a single mitotic error. No significant correlation was detected between clinical outcome and the mechanism(s) of chromosomal malsegregation in these MDS individuals. However, an older mean maternal age (31.8 years) was observed for the cases originating from both meiotic/mitotic errors as compared to those with only mitotic errors (27.4 years). Lastly, using expression microarray studies (in progress) of normal and trisomic lymphoblasts/fibroblasts clonally-derived from mosaic individuals, we plan to quantify: (1) the proportion of genes on chromosome 21 that show an imbalance in the trisomic cells; and (2) possible epistatic interactions with genes on other chromosomes. In conclusion, an analysis of both lymphocytes and buccal mucosa cells should be considered when evaluating individuals with MDS. Also, since a maternal age effect was seen in families having meiotic and mitotic malsegregation errors, the recurrence risk for these kindreds may be comparable to that of families having a child with non-mosaic trisomy 21. It is hoped that these continued studies will allow for a clearer understanding of the phenotypic outcome in people with mosaicism and trisomy for chromosome 21.

A24

Determination of the Origin of Female Cells in a Peripheral Blood Sample from an Unambiguously Male Newborn

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Our patient is a male infant with severe hydrocephalus born at 35 4/7 weeks gestation to a 37 y.o. previously G4 P0030 female. Maternal history was remarkable for three previous pregnancy losses, one of which was a second trimester loss complicated by severe hydrocephalus. Chromosome analysis of peripheral blood lymphocytes from the newborn showed 27 male cells and 2 female cells. FISH analysis of the same sample showed approximately 15% female cells in all three independent cultures initiated (one harvested at 48 hours and two harvested at 72 hours). Comparison of patient and parental acrocentric chromosome heteromorphisms ruled out fetal mosaicism. To clarify the origin of the female cell line, genotype analysis of 12 microsatellite markers from patient and parental peripheral blood samples was performed. All alleles present in the infant sample were present in the parental samples. Analysis was complicated by the fact that both parents (of Chinese descent) shared at least one allele at each marker. Consanguinity was denied. A small, unique peak representing the less abundant female cell line was observed for seven of the markers analyzed. For four of these markers, the small peak represented an allele shared by both parents; therefore parent of origin could not be determined. For the remaining three markers, the small peak represented a unique maternal allele. No unique paternal alleles were detected for the five markers for which it was theoretically possible. These results strongly suggest that the female cell line observed in this patient's peripheral blood is maternal in origin. Maternal cell microchimerism (the presence of maternal cells in fetal circulation) is typically limited to <1 nucleated cell in every 100–100,000 fetal cells. Additional tissues (buccal tissue and archival material from a colon biopsy) assayed by FISH for the presence of female cells were negative. At 4 months of age, FISH analysis of a peripheral blood sample showed all male cells. While the persistence of maternal cells in patients with immune disorders is well documented, this case showed an abnormally high percentage of maternal cells at birth, which subsequently decreased. The clinical significance of this phenomenon is unclear at this time, but it is of significant interest to cytogeneticists dealing with issues of potential contamination. Of note, sequence analysis of the maternal *L1CAM* gene showed a variant of unknown significance in a non-coding region of the gene. Her affected male child showed the same sequence variation.

A25

Inherited Derivative X;Y Translocation

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A 22-month-old boy with neurological delays and rhizomelic shortening of his limbs has an abnormal karyotype; 46,Y,der(X;Y)(p22.3;q11.2)mat. The mother has a mesomelic phenotype and height within normal limits. Her sister's son has learning disabilities. FISH studies using telomere probes on the patient's cells indicated absence of Xp and duplication of Xq or Yq onto the X chromosome. The X;Y translocation was confirmed using FISH paints for X and Y chromosomes. To help localize the breakpoint on the X chromosome, a FISH probe for the steroid sulfatase locus was present, indicating that the breakpoint on the X chromosome was distal to this site. X inactivation studies were performed on the mother's lymphocytes, showing inactivation of the derivative X in 73% of cells. X inactivation studies have been reported for seven other females with derivative X chromosomes; two had skewed X inactivation of the derivative X (90% or more), two had skewed inactivation of the normal X (89% or more), while three cases had random X inactivation, similar to our case. The X inactivation pattern can influence the phenotype in females. About 50 patients with a der(X;Y) have been reported in the literature, some of which have been inherited. The breakpoints on the X and Y chromosomes are the result of aberrant meiotic recombination that can occur due to sequence homology between the Xp22 and Yq11 regions. The breakpoints can differ among these familial cases, resulting in variable phenotype in males.

A26

Animal Cytogenetics: Thriving in an Era of Genome Sequencing

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Dr. Chowdhary and his colleagues have made tremendous contributions in equine cytogenetics including gene mapping and genomic characterization. Other areas of interest include mammalian chromosome evolution.

A27

Impact of Cytogenetic Evaluation on Prognosis of Patients with AML

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Non-random chromosome abnormalities are important, independent prognostic factors in acute myeloid leukemia (AML). Here we report a study of 27 adults with newly diagnosed AML. All patients received similar induction therapy, consisting of an anthracycline and continuous infusion cytarabine. Median follow-up for surviving patients was 7.7 months with a range from approximately 0.4 months to 18.9 months. The cytogenetic aberrations were correlated with the median overall survival (OS) of all patients. Clinical features and outcome were examined for each group. The patients with two or more abnormalities appear in more than one group. The significance of recurring chromosome abnormalities in our patients was compared to published data. The cytogenetic analyses of bone marrow (BM) or unstimulated

blood were performed using G-banding and/or FISH. All samples were unstimulated and processed using short-term (24-, 48-, and 72-hour) cultures. Twenty metaphase spreads and/or 200 nuclei for FISH analysis were analyzed in all patients.

Among 27 patients, five (19%) had a normal karyotype and 22 (81%) had one or more clonal abnormalities. The patients were grouped according to the presence of a recurrent abnormality noted in at least two patients and were categorized into Favorable, Intermediate and Adverse Risk Groups based on Cooperative Group Study data.

The Favorable Group had six patients with a median age of 43 years. Four of six had a t(8;21)(q22;q22) and two of six had a t(15;17)(q22;q22). The median OS in this group was 10.6 months (range 3.4 to 15.3 months).

The Intermediate Group had 16 patients (59%), with a median age of 60 years. Five had normal karyotypes (18.5%), nine had trisomy 8 (33%). Overall survival was 7.8 months (range 1 to 19 months). Nine patients of 27 had trisomy 8; four as the sole anomaly with a median age of 49 years and OS of 8.3 (1 to 14.7 months). Five patients had trisomy 8 and additional abnormalities; median age of 65 years and OS 4.9 months (1 to 10.6 months). OS was significantly less for the later group. Trisomy 8 was also analyzed as a separate risk group (i.e. nine patients). Four patients, median age of 49 years, had only +8 (15%) with median OS of 8.3 months (range 1 to 14.7 months) and five patients, median age of 65 years, who in addition to +8, had other complex aberrations. The median OS in the last subgroup was only 4.9 months (range 1 to 10.6 months). The difference in the outcomes between two subgroups was significant. This variability could be associated with differences in the age of the patients. This finding also correlates with reports that patients with +8 and additional abnormalities had a significantly worse OS than other patients with trisomy 8 as a sole cytogenetic abnormality.

Nineteen patients (70%) represented the Adverse Risk Group. Ten patients had complex chromosome abnormalities. The adverse risk group had a median age of 63 years and OS 6.2 months (range 1 to 15.2 months).

A28

A Novel t(4;17)(q12;q21) with Rearrangement of the 17q21 *RARA* Locus in a Case with Juvenile Myelomonocytic Leukemia

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Translocations involving the *RARA* locus on 17q21 have been identified in acute promyelocytic leukemia (APL). The majority of APL harbors the t(15;17)(q22;q21), resulting in a *PML-RARA* fusion transcript. Variant rearrangements involving *RARA* in APL are t(11;17)(q23;q21)(PLZF-RARA), t(5; 17)(q35;q21)(NPM-RARA), t(11; 17)(q13;q21)(NUMA-RARA), a der(17)(STAT5b-RARA) and t(3;17)(p25;q21). Here we report a case of juvenile myelomonocytic leukemia (JMML) carrying a t(4;17)(q12;q21) with a rearrangement of the *RARA* locus at 17q21 demonstrated by FISH (probe LSI RARA DCBA, Abbott-Vysis). FISH analysis using BAC probes derived from the 4q12 region indicated that the breakpoint is located proximal of the *CHIC* and *PDGFRA* loci. The more proximally located *FIP1L1* gene at 4q12 remains an attractive candidate gene. We will present ongoing FISH studies using probes spanning the 4q12 *FIP1L1* locus and experiments to test whether the translocation results in *FIP1L1-RARA* fusion gene. This is the first report on a rearrangement of the *RARA* locus at 17q21 in JMML until now exclusively demonstrated in APL.

A29

Recent Advances in Diagnosis and Management of Cancers – A Genetic Approach

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Introduction: The diagnosis, assessment of prognosis and minimal residual disease (MRD) of cancers depends on understanding of chromosomal and gene alterations using highly sensitive molecular techniques. **Objectives:** The aim of the study was to evaluate cytogenetic and molecular anomalies at diagnosis and sequential follow-ups in leukemias and cancers. **Methods:** Conventional cytogenetic and Fluorescence In Situ Hybridization (FISH) analysis were done in 420 cases of leukemias of which 195 cases were follow-ups. Ten samples of urinary bladder cancer and four of breast cancer were analyzed using FISH to assess aneusomies and *HER-2/NEU* amplification status respectively. Status of other genes like p53 and N-myc were also evaluated in various cancers. Seventy cases of Retinoblastoma were analyzed using conventional cytogenetics and mutational analysis was conducted in 14 cases using denaturation high-performance liquid chromatography (DHPLC). **Results**: Sequential cytogenetic and FISH analysis in leukemias revealed appearance of additional anomalies that were later correlated with hematological and clinical findings. Most of these cases revealed disease progression and relapse in subsequent follow-ups that was preceded by cytogenetic/molecular relapse. In some cases of leukemias and other cancers, FISH analysis revealed molecular changes and MRD that were not evident using conventional cytogenetics. FISH analysis in urinary bladder and breast cancers reveals prognostic significance of gene alterations and recurrence up to 6 months sooner than cytoscopy and cytology. Novel mutations were identified in some Retinoblastoma cases using DHPLC. **Conclusion:** Importance of analysis of chromosomal and gene alterations using highly sensitive molecular techniques in management of cancers is highlighted.

A30

Yin and Yang of False Positive and False Negative Results: Interpretation Complexities for MLL Fluorescence in situ Hybridization (FISH) Studies

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Structural rearrangements disrupting the *MLL* gene locus are prognostic indicators for aggressive treatment regimens in leukemia. Interpretation of FISH analyses to detect *MLL* rearrangements can be problematic due to the qualitative assessment of distance between orange and green signals in interphase nuclei which can lead to a False Positive result or chromosome condensation in metaphase cells leading to a False Negative result. Using the Vysis LSI® MLL BAP probe, in cells lacking an *MLL* rearrangement, two orange/green (yellow) fusion signals are observed. In cells with an *MLL* rearrangement, the expected pattern is: one fusion signal, one orange signal, and one green signal. A physical distance of greater than two signal diameters between orange and green signals of equal size and intensity determines whether the cell is positive for an *MLL* split.

MLL FISH studies were requested on a newly diagnosed pediatric leukemia case with abnormal cytogenetics: inv(1)(p36.1q23), add(8)(p23) or +8, del(10)(p12),add(11)(q25). Metaphase FISH results were reported as normal; however, low level positivity for split *MLL* signals was detected by interphase analysis. A technologist comment noting most cells with split signals did not have much distance between orange and green signals was considered consistent with chromatin decondensation or a False Positive. Upon review of the abnormal karyotypes, additional FISH analyses using a whole chromosome 10 paint probe (wcp10; Vysis, Inc.) and a chromosome 11 enumeration probe (D11Z1; Vysis, Inc.) revealed an insertion of chromosome 10 sequences into the long arm of chromosome 11. The small insertion resulted in a small split *MLL* signal in interphase, but chromosome condensation in metaphase cells resulted in an apparently normal *MLL* fusion signal. The interphase FISH results were initially suspected to represent a False Positive because of negative metaphase FISH results. In actuality, the metaphase FISH study was a False Negative and the interphase FISH very positive for an *MLL* rearrangement.

MLL FISH analyses were performed on three pediatric leukemia specimens, with low level positivity for split signals above established background limits. One specimen had hyperdiploidy by FISH in the vast majority of cells scored, with an *MLL* split detected in a small proportion of cells. This positivity was suspected to represent clonal evolution of disease resulting in a small emerging subclone; however, concurrent high hyperdiploidy and 11q23 *MLL* rearrangements are rare in pediatric leukemias. Upon review, in the small subset of nuclei with separate red and green signals, by critically changing the focal plane of the microscope, a thin thread of chromatin could be discerned connecting the two signals. The appearance of a split signal in these cells was thus determined to represent a False Positive for an *MLL* rearrangement.

Correlation with standard cytogenetics and clinical data are necessary to prevent errors in diagnosis or monitoring for minimum residual disease in leukemias with *MLL* rearrangements.

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A31

Correlations of Cytogenetic and Histological Studies in Renal Cell Carcinomas

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Renal cell carcinoma (RCC) is divided into several major histological subtypes, including benign adenoma, oncocytoma, chromophobe, papillary and clear cell. Most of the common subtypes can be distinguished by their specific pattern of chromosome abnormalities. In 2005, we karyotyped 42 cases of RCC. Of these, 34 (81%) had abnormal karyotypes. We conducted a study to determine the correlation of the abnormal karyotypes with the histopathological subtype of the tumors. Twenty seven tumors could be classified into one of the major subtypes. Of these, 16 were classified as clear cell, five papillary, four chromophobe and two low grade by histopathology. Eleven of the 16 (69%) clear cell, all five papillary, one of four (25%) chromophobe and both low grade tumors had karyotypes appropriate for their subtype. Only five clear cell and three chromophobe tumors were discordant.

We collected additional clinical information and re-examined the histological slides and karyotypes of the eight discordant tumors. One of the clear cell and all three chromophobes had simple karyotypes suggestive of low grade RCC, and were most likely derived from growth in culture of normal or low grade component of the tumors. Of note are the three chromophobes with karyotypes consistent with low grade tumors. Chromophobe tumors are hard to grow in culture, and the resulting karyotypes should be interpreted with caution. Of the remaining three RCC tumors signed as clear cell, none had loss of 3p or gain of 5q. One hyperdiploid tumor had a papillary component upon re-examination of the slides. Only the last two cases, one hyperdiploid and one hypodiploid could not be reconciled since the histology showed only the clear cell component of RCC.

Thus the concordance of karyotypes and histological subtypes of RCC in our laboratory were quite good. The most common reason for differences was growth, in culture, of the low grade or normal component of the tumor. This was especially common in chromophobe tumors. In cases of discordance, it is important to re-examine the slides and re-evaluate the karyotypes. This study further underscores the importance of clinical and pathologic correlation with cytogenetic analysis of solid tumors.

A32

Potential for False Positive Results Using Fluorescence in situ Hybridization Studies for Bladder Cancer

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Fluorescence in situ hybridization (FISH) tests designed to detect and monitor urothelial carcinoma are more sensitive than the standard-of-care cytological assessments; however, false positive results may arise due to both technical artifact and biological factors associated with patient condition. Benign conditions, such as presence of superficial (umbrella) cells that are often detected in instrumented urine (bladder or other washes) and occasionally in voided urine, may show abnormalities in DNA ploidy. Approximately 10% of our FISH analyses had a significant percentage of likely umbrella cells with tetraploidy (4, 4, 4, 4 pattern) or aneuploidy (3–4 signals for multiple probes). Concurrent cytological and clinical follow-up data revealed that patients with 3–4 signals in <10 cells typically did not have urothelial cancer. Thus, we have altered our positive result criteria to include:

1. a cut-off of >10 cells with polyploidy with only 3–4 signals for several probes

2. a cut-off of at least 4 cells with highly abnormal results (>5 signals for at least one probe and polyploidy for at least one additional probe).

In addition to umbrella cells, prostatic, seminal vesicle or colon (in the case of fistula) cells may be present in the urine of patients being evaluated for urothelial cancer. Aneuploidy is common in prostate cancer and ulcerative colitis and has been detected in benign seminal vesicle tissue by flow cytometric DNA ploidy studies. To assess the potential for false positive results associated with cells from the male reproductive tract, we studied samples from five prostates and three seminal vesicles using the UroVysion FISH protocol (Vysis). Prostatic and seminal vesicle tissue was disaggregated and resuspended in voided urine from a healthy donor. Test samples and clinical (urine) samples were masked and scored. Two of the seminal vesicle samples met criteria for positivity, while the remaining seminal vesicle and each of the prostate samples yielded normal results. These findings suggest that the presence of seminal vesicle cells in urine, may lead to false positive results. Laboratories performing FISH studies for bladder cancer must consider the potential for false positive results due to the presence of non-urothelial cells. FISH results should be correlated with clinical and other laboratory tests to determine the significance of the findings.

A33

Phelan-McDermid Syndrome in Several Family Members due to Malsegregation of a t(19;22)

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Cryptic terminal rearrangements are a significant cause of idiopathic mental retardation with or without dysmorphic features. The use of FISH has enhanced our ability to detect subtelomeric rearrangements and various studies have shown that 25–60% of unbalanced cryptic translocations are inherited from a parent who is a balanced translocation carrier. We describe the use of chromosome analysis and FISH to trace the transmission of a t(19;22) through several generations of an extended family. The proband was previously reported by Praphanphoj et al. (J Med Genet 37:58–61, 2000). Cytogenetic and FISH studies on the proband revealed a der(22)t(19;22)(q13.4;q13.3)mat resulting in loss of 22q13.3 and partial trisomy of 19q13.4. A careful family history and further cytogenetic testing has led to the diagnosis of the der(22) in two of the proband's cousins. All three individuals showed features consistent with Phelan-McDermid syndrome including neonatal hypotonia, absent speech, moderate to profound developmental delay, and minor dysmorphic features. Several individuals in this five generation pedigree were available for cytogenetic and FISH analysis leading to the identification of additional translocation carriers. Other family members were determined to be obligate carriers based on the birth of offspring who had the balanced or unbalanced translocation. Family and medical history led to the identification of additional individuals with phenotypic features of Phelan-McDermid syndrome. Although the familial occurrence of chromosome translocations is not unusual, this is the first report of multi-generational transmission of a deletion of 22q13 leading to Phelan-McDermid syndrome in multiple individuals.

A34

Chromosome-Specific Differences in the Frequency of Age-Related Genomic Changes: A Study of Micronuclei (Using SKY), Telomeres, and Uncultured Nuclei

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Although micronuclei formation is a well known consequence of age-related chromosomal loss, little is known about the chromosomal contents of micronuclei or their frequencies as compared to other measures of age-related, somatic cell aneuploidy. Thus, the primary goals of this study were to:

(1) assess the chromosomal composition of age-related micronuclei; and (2) identify possible correlations among chromosome-specific micronuclei frequencies, chromosome-specific telomere lengths assessed by FISH-based technology, and acquired aneuploidy frequencies determined by FISH. Individual-specific differences ($P < 0.005$) in micronuclei frequencies, ranging from 0.4% to 4.7%, were observed in the ten healthy females (ages 49–80) studied, to date. To identify the chromosomal contents of the micronuclei in these females we used SKY and sequential FISH with pantelomeric/pancentromeric probes, and found that the majority of micronuclei (81.6%) contained a single chromosome. A non-random pattern of exclusion of chromosomes into micronuclei ($P < 0.005$) was detected, with an over-representation of chromosomes X (37%), 1 (6.2%), 2 (6.5%), 3 (5.9%), 4 (9.0%), and 13 (5.6%) and an under-representation of chromosomes 14 (0.8%), 17 (0.3%), 19 (1.1%), 20 (0.8%), 21 (0.3%), and 22 (0.3%). Although individuals with overall or chromosome-specific decreased telomere lengths tended to have an increased frequency of micronuclei, this trend was not evident in every individual and there was no significant correlation over the data pooled from all females. The chromosome-specific frequencies of acquired aneuploidy in uncultured cells (completed for a subset of ten autosomes and the X chromosome) also showed a non-random pattern of chromosomal involvement ($P < 0.0001$). Interestingly, the frequency of X-chromosomal acquired aneuploidy in the uncultured lymphocytes was 2.2%, yet it was present in more than one third (37%) of all micronuclei. Also, chromosomes 16 (4.8%), 17 (4.68%), and 21 (3.10%) had significantly high frequencies of loss in the uncultured lymphocytes but had low frequencies of exclusion into micronuclei. Possible explanations for these apparent discrepancies include, but are not limited to: (1) different mechanisms leading to chromosome losses in interphase nuclei compared to micronuclei; (2) an epigenetic mechanism that alters the frequency of exclusion of a specific chromosome(s) into a micronucleus (i.e. the inactive X in females); and (3) a culture selection mechanism that prohibits the mitotic progression of cells lacking a specific chromosome (i.e. chromosome 17, which contains several tumor suppressor genes). In summary, based on the frequencies of acquired chromosomal aneuploidy as assessed through multiple independent assays, one can conclude that there are chromosome-specific and individual-specific differences in the frequency of micronuclei, and that these frequencies are not strongly correlated with chromosome-specific telomere length or aneuploidy frequencies in uncultured interphase nuclei. Furthermore, acquired aneuploidy appears to be a complex trait that is influenced by both genetic and epigenetic factors.

A35

Chromosome 15q Telomere Deletions: Association of *IGF1R* with Pre- and Postnatal Growth Retardation

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Patients with cytogenetically visible deletions of the distal part of chromosome 15, usually have intrauterine growth retardation (IUGR), global postnatal developmental delay and variable mental retardation. It has been assumed that hemizygosity for insulin like growth factor 1 receptor *(IGF1R)* gene, which maps distal to 15q26 and codes for a kinase receptor containing transmembrane protein, inhibits normal growth. Using genome-wide telomere FISH analysis, we identified two patients with de novo deletions of the 15q telomere region: one patient had a terminal deletion while the other was an unbalanced translocation with X/Yq. Both patients had intrauterine growth retardation, postnatal growth retardation and significant developmental delay. To delineate the size of the deleted material on chromosome 15q and determine if the *IGF1R* gene was included in these deletions, a combination of array comparative genomic hybridization (aCGH) and FISH analysis were utilized. For aCGH, a homebrew chromosome 15 BAC array, with variable clone spacing along the length of the entire chromosome, was used. The breakpoint in the patient with an unbalanced translocation was determined to be between 4.6 Mb and 5.5 Mb from the 15q telomere, while the patient with the terminal deletion had a breakpoint between 7.5 Mb and 8.0 Mb. The deleted portions in both patients include the *IGF1R* gene, indicating that they are hemizygous for this gene, which is believed to be responsible for their growth delays. A recent publication also reported a cryptic deletion of ~5.7 Mb, which included *IGF1R,* in a patient with IUGR, postnatal growth delay and other abnormal phenotypic features. Our data and the published data on 15q deletions suggest that this cytogenetic rearrangement should be considered in individuals with IUGR and postnatal growth retardation. In addition, since our two cases and the one in the literature were only identified using telomere FISH analysis, follow-up molecular cytogenetic analysis is warranted if routine G-banding is normal.

A36

Cryptic Duplication of 12q24.33→qter in a Child with Angelman Syndrome – Simultaneous Occurrence of Two Chromosomal Events

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We present a case of simultaneous occurrence of AS and 12q duplication in a 1-year 10-month old child with a history of cerebral palsy and seizures. The12q24.3 duplication was inherited from the mother with a balanced translocation between chromosomes 12 and 13 at bands 12q24.3 and 13p11.2. Her sample was submitted because of recurrent pregnancy loss. FISH and array-CGH were performed on this family to further delineate the breakpoints and the extent of 12q duplication. FISH using 12qtel probe revealed an extra signal on 13p11.2. Array-CGH (Signature Genomics Laboratories, Spokane, WA) revealed copy number gain of three BAC clones (RP11-46H11, RP11-386I8 and RP11-309H3) covering 423 kb of sequence on the terminal end of chromosome 12. FISH using more proximal clones (RP11-897M7, RP11-669N7 and RP11-117L5) revealed only two signals. In addition to the cryptic duplication resulting from the unbalanced translocation the child also has a deletion of the AS critical region at 15q12. An attempt was made to segregate the clinical features manifested due to AS deletion and those features that are probably due to a submicroscopic 12q duplication to address genetic counseling for future pregnancies. The literature review of all the 12q duplication cases involved a larger segment of 12q that was cytogenetically visible. Fortunately a subsequent pregnancy revealed a balanced translocation t(12;13) karyotype. The genes involved in the duplicated region are being investigated. The use of array-CGH offers an opportunity for genotype–phenotype correlations in such unique cases. This information can be used in counseling the mother as to whether she is at a greater risk of an abnormal outcome resulting from 12q deletion or duplication in future pregnancies.

A37

Re-examination of Carriers of Balanced Reciprocal Translocations: A Strategy to Detect Candidate Loci for Common and Complex Disease

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Reciprocal translocations have facilitated the identification of genes for especially early-onset monogenic disorders, e.g. by truncating specific genes. In contrast, limited data exist on their potential role in common and complex disorders. We have investigated the full spectrum of diseases in >700 carriers of balanced reciprocal translocations without known early-onset disorders in a nation-wide questionnaire-based study. The observed concordance with known linkage data, instances of familial co-segregation and number of unrelated carriers with similar breakpoints and traits support that some translocation breakpoints may reveal novel loci and genes for common and complex human diseases and traits.

A38

Beckwith-Wiedemann Syndrome in a Female with a de novo der(21)t(11;21)(p15.1;p11.2) Detected by Conventional Cytogenetic Analysis and FISH

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Beckwith-Wiedemann syndrome (BWS) is a disorder of growth characterized by macroglossia, omphalocele, macrosomia, ear creases and embryonal tumors. Cytogenetically detectable abnormalities involving 11p15 are found in 1% or less of cases. Chromosome abnormalities resulting in paternal 11p15 duplication produce the BWS phenotype. Chromosomal inversions or translocations involving the maternal 11p15 BWS locus will also produce the phenotype. Here we present an interesting case of a de novo chromosome abnormality resulting in the BWS phenotype. A peripheral blood specimen was received from a 12-day-old female with macrosomia, macroglossia, neonatal hypoglycemia and ear creases. Conventional cytogenetic analysis of the peripheral blood specimen from this patient showed an abnormal karyotype of 46,XX,der(21)t(11;21)(p15.1;p11.2). The GTG-banding results were confirmed by FISH using probes homologous to DNA sequences specific to the short (p) arm telomeric region of chromosome 11 (Vysis) as well as whole chromosome probes for chromosome 11 (CytoTrend). Cytogenetic evaluations of the peripheral blood specimens from both parents were normal, confirming the de novo nature of this rearrangement, although non-paternity could not be absolutely excluded. Thus, in this unique case the patient's phenotype is due to the presence of two copies of the paternal BWS locus at 11p15 not in typical tandem duplication. Although chromosome abnormalities are relatively rare in patients with BWS, cytogenetic analysis is important for these patients and should be performed simultaneously along with methylation studies. In patients with BWS as well as mental retardation, cytogenetic analysis is even more pertinent and should be performed first.

A39

Centric Fission as a Common Mechanism of Marker Chromosome Formation

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Supernumerary marker chromosomes occur in 0.03–0.04% of newborns, but are 10 times more prevalent in mentally retarded individuals. The mechanism of marker chromosome formation in humans has yet to be fully understood. Through high-resolution mapping of marker chromosomes, we are currently testing a hypothesis that invokes centric fission, the lateral breakage of a centromere into two distinct, functional entities. A panel of clones covering the pericentromeric regions of each human chromosome has been developed to ascertain the euchromatic makeup of marker chromosomes. Due to the extensive levels of segmental duplication in these regions on some chromosome arms, careful clone selection has been critical such that cross-hybridization between chromosome arms occurs at a minimum. Clones were chosen starting adjacent to the centromere gap and spanning 5 Mb into the p- or q-arm of each chromosome. Using this panel of clones, six marker chromosome cases have been mapped within ~1 Mb of the breakpoint. The markers are derived from chromosomes 1, 8, 15, 16, 18, and 20. Only one of these marker chromosomes contains euchromatin from both the p- and q-arms, whereas the remaining markers contain material only from one chromosomal arm. These five cases support centric fission as a common mechanism in marker chromosome formation. Further support for this hypothesis will be tested by analyzing the alpha-satellite arrays of the marker chromosomes. We predict that chromosomes containing larger alpha-satellite arrays are more prone to develop marker chromosomes.

A40

A Familial Duplication of 22q11.2 Including the *TUPLE1* locus

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A two-day-old girl was referred for chromosomal analysis because of multiple congenital anomalies including: a V-shaped cleft palate; hypertelorism; posteriorly rotated ears; wide-spaced, inverted nipples; and a bifid right great toe. Initial cytogenetic analysis at 550 G-bands revealed a normal female karyotype. Subsequent fluorescence in situ hybridization (FISH) analysis, using a probe (TUPLE1, Vysis) specific for 22q11.2, revealed a duplication of the *TUPLE1* region. Re-examination of the karyotypes failed to distinguish the duplicated chromosome 22 from its normal homolog. To delineate the duplicated segment, additional FISH assays were performed, using a series of RP11 BAC probes. Results from the BAC analysis indicate the proximal duplication breakpoint is between RP11-1053O2 (+) and RP11-711J20 (++) while the distal breakpoint is between RP11-165F18 (++) and RP11-317J15 (+). Based on data from the Human Genome Browser, the length of the duplication is estimated to be 2.2–3.1 Mb.

Though the child's birth parameters were appropriate for her gestational age, by five months of age, she was at the 5–10th percentile for length and weight and <5th percentile for head circumference. She was delayed in her milestones with poor head control. She has recently had several hospital visits for difficult breathing and upper respiratory infections.

FISH studies of parents revealed that the child's father also carries the same 2.2–3.1-Mb duplication of 22q11.2. The father reports he has a history of education-related issues, though he has no cleft lip/palate nor heart defect. *TUPLE1* FISH studies of the proband's paternal grandparents were normal with no evidence of the duplication.

Only a very few microduplication 22q11.2 families have been reported. This study shows: 1. the need to test parents when a microduplication is identified in a child, 2. the variability in phenotype between father and child though both carry the same duplication, 3. the limitation of standard karyotyping in identifying a 22q11.2 microduplication, even in patients known through FISH studies to carry the rearrangement.