Other posters in each topic are scheduled for 4-day (W/T/F/S) or 2-day (F/S) display. See p. A-1 for complete listings.

894

Design of modified oligonucleotide probes to detect telomere repeat sequences in FISH assays. *J.G. Hacia[1], E.A. Novotny[1], R.A. Mayer[1], S.A. Woski[2], M.A. Ashlock[1], F.S. Collins[1].* 1) GMMB, NHGI/NIH, Bethesda, MD; 2) Dept. of Chemistry, U of Alabama, Tuscaloosa, AL.

Much current interest surrounds the structure of mammalian telomeres, and consequently, a robust and inexpensive fluorescent in situ (FISH) assay for telomere detection would be of considerable utility in both the research and clinical area. FISH probes based on unmodified oligonucleotides of peptide nucleic acid (PNA) homologs, complementary to one strand or the other of the hexamer repeat have certain disadvantages that caused us to examine alternative substrates for telomere hybridization. A series of dye-labeled oligonucleotide probes containing base and sugar modifications were tested for the ability to detect telomeric repeat sequences in FISH assays. These modified oligonucleotides, all 18-nt in length, were complementary to either the cytidine-rich $(C_3TA_2)_n$ or guanosine-rich $(T_2AG_3)_n$ telomere target sequences. Oligonucleotides were modified to either increase target affinity by enhancing duplex stability (2'-OMe ribose sugars and 5-(1-propynyl)-pyrimidine residues) or inhibit the formation of inter- or intramolecular structures (7-deazaguanosine and 6-thioguanosine residues) which might interfere with binding to target. Several dye-labeled oligonucleotide probes were found that could effectively stain the telomeric repeat sequences of either cytidine- or guanosine-rich strands in a specific manner. Such probes could be used as an attractive alternative to PNAs for investigating the dynamics of telomere length and maintenance. In principle, these relatively inexpensive and readily synthesized modified oligonucleotides could be used for other FISH-related assays such as triplet repeat expansions.

895

Interstitial deletions of chromosome 5 and 16 without phenotypic abnormalities: further confirmation. *J.L. Hand[2], V.V. Michels[2], R.P. Ketterling[1], M.J. Marinello[3], S.M. Jalal[1].* 1) Cytogenetics Laboratory, Department of Laboratory Medicine and Pathology; 2) Department of Medical Genetics, Mayo Clinic, Rochester, MN; 3) Genetic Diagnostic Laboratories, Inc., Buffalo, NY.

We describe two cases of euchromatic interstitial deletions without detectable phenotypic abnormalities. Patient one was a 19-year-old male who presented with progressive encephalopathy, progressive blindness, deafness, and spastic quadriparesis and was subsequently diagnosed with a peroxisomal disorder. Despite extensive evaluation, the specific peroxisomal disorder was not identified. We hypothesize that the son's phenotype may represent unmasking of a new autosomal recessive peroxisomal disorder in the deleted region. High-resolution chromosome analysis was interpreted as 46,XY,del(5)(p14.1p14.3). The asymptomatic mother had the same interstitial deletion. Use of wcp5 and SKY analysis confirmed that the abnormal chromosome 5 was not involved in a translocation. Chromosome 5p14 deletion has been reported previously in a family for three generations without phenotypic anomaly that included female to male and female to female transmissions. In the second case, an amniocentesis for advanced maternal age revealed an interstitial deletion in the long arm of chromosome 16. The karyotype of the fetus was 46,XX,del(16)(q13q22). The wcp16 painted the abnormal and the normal 16 entirely and there was no evidence of the presence of chromosome 16 DNA on any other chromosome. The pregnancy was carried to term and resulted in the birth of a normal daughter. The same deletion was observed in the phenotypically normal mother and a normal first child who is a 2-year-old son. Interstitial deletion of 16q21 has been reported previously to have a normal phenotype in a two generational family. These deletions do not seem to be associated with an abnormal phenotype due to imprinting. Interstitial euchromatic deletions without an apparent phenotypic anomaly include: Xq26,5p14,11p12, 13q21 and 16q21, a list that is growing.

896

Y-Autosome Translocations characterized by classical banding techniques and molecular cytogenetic studies. *D. Hansmann[1], Z. Storm[1], R. Raff[2], G. Schwanitz[2].* 1) Institute Prenatal Diagnosis, Meckenheim, Germany; 2) Institute of Human Genetics, University of Bonn, Bonn, Germany.

Y-Autosome translocations are rare events of chromosome rearrangements. In our own investigation group of about 30000 chromosome analyses from lymphocyte culture we diagnosed ten cases. 5 of them had breakpoints in euchromatic regions and showed a phenotype in agreement with the unbalanced karyotype. The other 5 cases were rearrangements with chromosome 15 and breakpoints in the constitutive heterochromatin. In chromosome 15 the breakpoints were located between 15p11.2 and p12/13, in the Y-chromosome between Yq11.23 and Yq12. In these 5 cases, where we investigated the probands and their relatives, each person was carrying only one of the translocation chromosomes, this means that the rearrangement had occurred in earlier generations. The translocations could be delineated by a combination of different banding techniques and FiSH. The way of genetic counselling is given, 2 of the pedigrees are demonstrated in detail.

897

Generation of repetitive sequence-depleted microdissected chromosome arm painting probe. *H. He[1], W. Huang[1], XY. Guan[2].* 1) American Lab Technologies, Inc, Rockville, MD; 2) Department of Clinical Oncology, University of Hong Kong, Hong Kong, China.

The application of fluorescence in situ hybridization (FISH) with whole chromosome painting probes, chromosome arm painting probes, and chromosome band-specific painting probes has greatly facilitated to detect chromosome rearrangements in both hereditary diseases and cancers. To meet the increasing demand for the high quality FISH painting probes, a subtraction strategy has been applied in our company to remove repetitive sequences from microdissected DNA probes before hybridization. Chromosome arm 5p has been chosen to test our new method. Briefly, 10 copies of 5p were microdissected and amplified using a degenerate oligo primer (UN1) by PCR. UN1 primer was then replaced by a unique sequence primer (R1) by PCR. These PCR products were then hybridized with biotin-labeled human repetitive sequences derived from a 120 kb BAC containing various human repetitive sequences. After hybridization, avidin was added into the reaction and phenol/chloroform subtraction was performed to remove proteins in the reaction solution including all avidin-bound biotin-labeled repetitive sequences and their specifically hybridized repetitive sequences in the microdissected DNA. The remaining unique DNA fragments were recovered by PCR with R1 primer. The intensity and specificity of the repeat-depleted 5p arm painting probe have been characterized by FISH without adding block DNA Cot-1. The intensity and hybridization specificity of the fluorescence signal was similar between a regular 5p arm painting probe with Cot-1 block and our repeat-deplete 5p painting probe. This repeat-depleted painting probe which no longer require adding block DNA will be used for FISH and will provide cheaper and quicker resource for the increasing demand of the high quality FISH painting probes.

898

Comparison of secondary cytogenetic abnormalities in pediatric T-cell versus B-cell lymphomas. *C.M. Higgins, M.M. Hess, B. Gordon, W.G. Sanger.* Human Genetics Laboratory, Univ Nebraska Med Ctr, Omaha, NE.

Cytogenetics of non-Hodgkin's lymphoma (NHL) have not been as intensively studied in children compared to adults. In childhood NHL, the majority of cases are neoplasms of B-lymphocyte origin (70%). Fewer lymphomas are of T-cell origin and most of these fall into a group of large cell lymphomas (LCL) known as anaplastic large cell lymphomas (ALCL). We present the cytogenetic and histologic results of eight t(2;5)-positive T-cell ALCL and compare the secondary clonal abnormalities with those of eleven pediatric B-cell neoplasms. Recurrent chromosome abnormalities in ALCL include t(2;5)(p23;q35) which leads to fusion of the nucleophosmin (NPM) gene on 5q to the anaplastic protein kinase (ALK) gene on 2p. This and other cryptic rearrangements may be important in the deregulation of ALK causing the pathogenesis of ALCL. The most frequent secondary cytogenetic features of ALCL from this analysis include the following disruptions, listed in decreasing frequency: 1q21, 10q24, 1q10, 3p23, 11q13, and 10q22. Loss of chromosome 4 and gain of the X chromosome were the most frequent numeric aberrations. Numerous breakpoints were noted on the long arms of chromosomes 1, 10, 11, and the entire length of chromosome 17. Most karyotypes were hyperdiploid followed by near-tetraploidy. Secondary to disruptions of 14q32 and 8q24, the B-cell lymphomas had a much more diverse pattern of additional abnormalities than the ALCLs. In decreasing frequency, repeated disruptions were at band 1q21, 1q42, and 15q26. The loss of Y and chromosomes 3, 4, and 15, in addition to extra copies of 8 and 18 were recurrent numeric abnormalities. Only chromosome 1 revealed a pattern of breakpoints along its entire length. Most of the pediatric B-cell cases were diploid and hyperdiploid. Similarities between the two groups of lymphomas were restricted to breakpoints clustered at 1q21 and the repeated loss of chromosome 4. The ALCL group had a much more consistent pattern of secondary abnormalities than did the B-cell tumors. Continuing studies may help elucidate the pathogenesis and progression of these pediatric lymphomas. Supported in part by the National Childhood Cancer Foundation.

899

Aneuploidy for chromosomes 12, 13, 17, 18, X and Y and deletions on the Y chromosome in sperm from twenty oligoasthenoteratozoospermic (OAT) patients undergoing ICSI. *S.F. Hoegerman[1], M.G. Pang[2], M.K. Rudd[1], N.K. Dahiya[1], M. Stacey[1], L. Lunsford[1], G. Doncel[3], A.A. Acosta[3], W.G. Kearns[1,3,4].* 1) Cent for Ped Res, EVMS, Norfolk, VA; 2) Biomedical Res Cent, Korea Adv Inst of Science and Tech, Taejon, Korea; 3) Jones Inst, East VA Med Sch, Norfolk, VA; 4) Inst of Genet Med, Johns Hopkins Univ School of Med, Baltimore, MD.

Objective: To determine aneuploidy frequencies and Y chromosome deletions from twenty OAT patients and six proven fertile donors. **Study Design:**Multi-probe, multi-color fluorescence *in situ* hybridization (FISH) and STS-PCR was performed on sperm to determine aneuploidy and Y chromosome deletions. **Materials and Methods:**Aneuploidy frequencies were determined in sperm from twenty OAT patients and six controls. Three-probe, three-color FISH was performed using direct labeled DNA specific for chrom. 12,13 and 17 (probe set I) and chrom. 18,X and Y (probe set II). Over 104,000 sperm were scored in this study. STS-PCR was performed to determine genomic deletions within the Y chrom. **Results:** In OAT patients, the per chrom. disomy for chrom. 12 ranged from 0.3 to 4.3%, for chrom. 13 from 0.2 to 3.5%, and per chrom. disomy for chrom. 17 ranged between 0.09 and 2.4%. In controls, the mean per chrom. disomy frequency was 0.8% for chrom. 12, 0.2% for chrom. 13, and 0.2% for chrom. 17. The per chrom. disomy for the sex chrom. ranged between 1.8 and 5.3% and the per chrom. disomy for chrom. 18 ranged between 0 and 1.5%. In controls, the mean disomy frequency for the sex chrom. was 0.4% and the mean disomy frequency for chrom. 18 was 0.2%. Diploidy ranged between 0.1 and 2.6% in OAT patients, with a control mean of 0.3%. Total aneuploidy ranged from 31 to 70% for OAT patients. Total aneuploidy in controls ranged between 4.1 and 7%. STS-PCR studies from 20 OAT patients identified one Y chromosome deletion, from both sperm and somatic cells of one patient. From this same patient, a significant increase ($p<0.05$) in autosomal and sex chromosome aneuploidy was found in his gametes. **Conclusions:**These findings showed significant increases of genetic abnormalities in sperm of all OAT patients studied.