Meeting Report

1999 Association for Molecular Pathology Annual Meeting

Genetics Session

The Genetics Plenary Session opened the meeting with Dr. Nick Schork of Case Western Reserve University, who presented an overview of the theoretical basis of quantitative trait loci analysis, genetic epidemiology, and linkage analysis which were illustrated with examples from his work in hypertension and heart disease. Increasingly, these complex but clinically very important traits are becoming better understood using a multigroup approach to generate large data sets and increasingly sophisticated statistical methods to dissect out the important genetic components. Dr. Margo Heath-Chosey of Abbott Labs presented a seminar entitled "Moving Pharmacogenomics from the Laboratory to the Clinic." She discussed how tests can be used to improve drug responses, illustrating her talk with data on single nucleotide polymorphisms (SNP) in the promoter region of 5 lipoxygenase responsible for Zileuton hepatotoxicity. She speculated on the likely development of DNA chips to tailor drug therapy to the individual patient.

The first Genetics workshop featured Dr. Thomas Prior of Ohio State University, who presented an overview of spinal muscular atrophy (SMA) illustrated with actual cases from his laboratory practice. Deletions in exon 7 of the telomeric *SMN* gene are responsible for 95% of cases. He also presented some of his atypical and unusual cases. Dr. Domnita Crisan of William Beaumont Hospital provided an extensive overview of angiotensin-converting enzyme (ACE) polymorphisms and their relevance to vascular disease, particularly as related to coronary artery restenosis after angioplasty and stenting, including her experience to date at William Beaumont Hospital.

The second workshop, entitled "Challenging Cases and their Solutions," provided a forum for laboratory directors to share selected challenging or difficult cases from a variety of common genetic disease assays to point out the pitfalls involved and how these problems were resolved. Themes included the importance of continuing to perform cytogenetics on patients referred for Fragile X syndrome testing, the need to maintain good clerical and labeling practices in the laboratory, and the need to consult with colleagues when a case is atypical, utility of using Southern blot analysis in the molecular diagnostics

of Duchenne Muscular Dystrophy, the potential problems associated with the largely ignored problem of nonpaternity. Dr. Elaine Lyons of Salt Lake City presented an interesting case in which an abnormal melting curve from a Lightcycler-based analysis lead to the discovery of a mutation (T281K) in *cis* with the common C282Y mutation at the *HFE* gene in a patient referred for hereditary hemochromatosis testing.

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Hematopathology Session

The first speaker was Timothy McDonnell, M.D., Ph.D., from the Department of Molecular Pathology of the University of Texas M. D. Anderson Cancer Center, who spoke on cell death regulation and the pathogenesis of hematolymphoid malignancies. Dr. McDonnell began his talk with a historical perspective, referring to the initial discovery of the t(14;18) in follicular lymphoma and the efforts (including his own) to clone and characterize the *bcl-2* gene on chromosome locus 18q21. He then discussed how the characterization of the *bcl-2* oncogene product led to the recognition that *bcl-2*, unlike other oncogenes, is involved in inhibiting apoptosis (programmed cell death). The remainder of his talk focused on current research, including the recent discoveries of a family of genes that either facilitate or inhibit apoptosis, the interaction of these proteins with each other, and their possible roles in the pathogenesis of malignant lymphomas.

The second speaker was Dr. Janet Rowley, M.D., D.Sc. (Hon.) from the Section of Hematology/Oncology of the University of Chicago, whose topic was "Chromosomal Translocations in Acute Leukemias." Dr. Rowley began her talk by briefly discussing the more common chromosomal translocations that occur in acute leukemias. She then focused on treatment-related acute myeloid leuke-

mia and translocations involving the *MLL* gene on chromosome locus 11q23, including the efforts of her team to understand the epidemiological aspects and map breakpoints. Dr Rowley finished her talk by discussing spectral karyotyping (SKY) and its value in identifying abnormalities not readily apparent by conventional cytogenetic analysis.

The first workshop, "Molecular Genetic Techniques for the Quantitative Determination of Bone Marrow Engraftment," was organized by Steven Schichman, M.D., Ph.D., from the University of Arkansas for the Medical Sciences and Timothy Stenzel, M.D., Ph.D., from Duke University Medical Center. Other speakers included David Olson, B.S., from Fairview-University Medical Center and Richard Hays, B.S., from St. Jude Children's Research Hospital. The presenters discussed the relative merits of current methods and commercially available kits to quantify bone marrow engraftment, with selected illustrative cases.

The second workshop discussed the results of the ongoing Hematopathology sample exchange program, and was organized by Rita Braziel, M.D., from Oregon Health Sciences University, Adam Bagg, M.D., from the Hospital of the University of Pennsylvania, and Daniel Arber, M.D. from the City of Hope National Medical Center. The results of four molecular assays performed on unknown specimens in a number of participating laboratories were compared. The assays included Southern blot analysis for the immunoglobulin (Ig) heavy chain and T cell receptor β chain genes, and PCR analysis of the Ig heavy chain gene, the T cell receptor γ chain gene, and JH/bcl-2. The results were interesting and provocative. Southern blot analysis appeared to be the more reliable method, compared with PCR, to assess the Ig heavy chain gene. A false positive rate of 4 to 5% was observed with PCR assays to assess the T cell receptor γ chain gene, and a low frequency of bcl-2 minor breakpoint cluster region breakpoints was observed in JH/bcl-2-positive cases of follicular lymphoma.

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Infectious Diseases Session

In the Infectious Disease Plenary Session at the 1999 AMP meeting, we were treated to two excellent presentations. The first, by Dr. Michael Pfaller, Professor of Pathology at the University of Iowa College of Medicine, addressed the use of molecular methods to study various microorganisms. A variety of approaches can be used not only to identify but also to discriminate subtly differing types within a single species. Plasmid typing, arbitrarily primed PCR, ribotyping, pulsed field gel electrophoresis, and other approaches have proven useful to both public health and hospital epidemiologists monitoring the spread of certain microorganisms. Dr. Pfaller closed his talk with pertinent real-life examples where such molec-

ular studies were instrumental in investigation of the spread of nosocomial infections in the hospital setting.

The second speaker, Dr. Fred Tenover from the Center for Disease Control and Prevention in Atlanta, Georgia, described the detection of resistance to antimicrobial agents by molecular means. Resistance to treatment is becoming an increasingly serious problem worldwide. Using methicillin-resistant *Staphylococcus aureus* as a prototype, he described several molecular approaches (PCR, bDNA, Taqman, Invader) for rapid detection of the *mec* gene sequences responsible for resistance. Other significant clinical problems discussed included vancomycin-resistant enterococcus, multidrug-resistant *Streptococcus pneumoniae*, multidrug resistant *Mycobacterium tuberculosis*, and resistance among a variety of viruses. Dr. Tenover closed with a discussion of some of the issues complicating these studies, such as the difficulties in study and analysis of multiple genes and mutations, sorting out silent carriage of genetic information versus expression of the phenotype, and whether results reported should indicate the presence of a genetic marker (MecA positive) or indicate the phenotype (methicillin-resistant).

In the workshops, Dr. Stephen Day of Visible Genetics, Inc. presented an inclusive overview of methods used to isolate DNA and RNA from a variety of organisms and sample types. In a second workshop oriented toward molecular infectious disease testing, Drs. Angela Caliendo of Emory University in Atlanta and Karen Kaul of Evanston Hospital and Northwestern University Medical School conducted a very practical and interactive session on the various practices and approaches used in molecular diagnostics laboratories, addressing such topics as controls, standards, quantitation, assessment of amplification inhibition, prevention of contamination, reimbursement, and regulatory issues.

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Solid Tumors Session

The Solid Tumor Section Plenary Sessions at the 1999 AMP Annual Meeting were presented by Dr. Gary J. Miller from the University of Colorado Health Sciences Center and Dr. Tom Frank from Myriad Genetic Laboratories and the University of Utah School of Medicine. Dr. Miller highlighted the molecular heterogeneity of prostate cancer. Starting from traditional histological grading and pathological staging of prostate carcinoma, the presentation then focused on problems and pitfalls in the application of biological markers of prognosis in this tumor. His initial studies of DNA ploidy in prostate cancer established that this was largely a surrogate marker for tumor volume, most larger tumors being nondiploid. Subsequent studies of the geographic heterogeneity of *p53* mutations, LOH patterns, and E-cadherin expression have suggested that the observed heterogeneity in these potential prognostic markers may represent a serious limitation for the

use of prostate biopsy material to define biological prognostic markers in individual patients. In summary, this presentation illustrated the unique advantages of the integration of clinical data, surgical pathology, and molecular analysis in furthering our understanding of prostate carcinoma.

The second plenary session was presented by Dr. Frank and dealt with laboratory determination of hereditary susceptibility to breast and ovarian cancer. The main focus of the presentation were mutations in *BRCA1* and *BRCA2*, which are associated with a significantly increased risk of breast cancer and ovarian cancer. Dr. Frank drew on his substantial experience as Medical Director of Myriad Genetic Laboratories to present an overview of the biology of *BRCA1* and *BRCA2*, as well as of the complex issues involved in cancer risk assessment in the setting of different family histories and different ethnic populations. He also presented the contrasting molecular diagnostic approaches using the detection of founder mutations compared to nonrecurrent mutations. Counseling issues and clinical management options were also presented. Finally, Dr. Frank commented on the perceived risks of insurance discrimination, which according to recent studies may have been overstated previously. His plenary session presentation is summarized in a recent review (Arch Pathol Lab Med 1999, 123:1023–1026).

There were also two Solid Tumor Section Workshops. The first focused on *RET* gene alterations in familial and sporadic cancers of endocrine glands. Dr. Barbara Zehnbauer from Washington University School of Medicine and Dr. Walter Noll from Dartmouth-Hitchcock Medical Center spoke on activating *RET* mutations in *MEN2A*, *MEN2B*, and familial and sporadic medullary thyroid carcinoma. Dr. Noll presented the background and general concepts in the diagnosis and management of patients with these forms of familial cancer, and Dr. Zehnbauer focused on details of mutation testing and genotype-phenotype correlations in these disorders. Dr. Giovanni Tallini from Yale University School of Medicine then spoke on the other major form of *RET* alteration in cancer, namely *RET* gene fusions in sporadic papillary thyroid carcinomas. Many different types of *RET* fusions have been described, but the common features of the fusion partners include the presence of active promoters, as well as dimerization domains which lead to dimerization of these chimeric *RET* proteins, resulting in autophosphorylation and constitutive activation.

The second workshop was presented by Dr. Carlos Cordon-Cardo from Memorial Sloan-Kettering Cancer Center, and the topic was the molecular analysis of cell

cycle alterations. The presentation focused on the biological significance, detection methods, and prognostic utilization of *p53* alterations using, as an example, studies of bladder cancer and soft tissue sarcomas from his laboratory. Dr. Cordon-Cardo and Dr. William Bennett from City of Hope Medical Center are now busy planning the Solid Tumor sessions for next year's AMP meeting in Denver, and we look forward to enjoying the fruits of their efforts.

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Special Topics Session

The Special Topics Plenary Session highlighted technological innovations and featured two speakers, Dr. Rolfe Anderson and Dr. Donald Hunt. Dr. Anderson is the Manager of Integrated Device Development at Affymetrix (Santa Clara, CA). His presentation focused on innovations in microfluidics and efforts to develop a prototype "lab on a chip." Using videotape, he demonstrated to the audience a prototype device that can perform a variety of molecular biology reactions such as polymerase chain reaction (PCR) and restriction enzyme digestions in miniature compartments. Once a reaction is performed, the products can be transported to other chambers using pressure differentials. The presentation provided the audience with an opportunity to see the intersection of molecular biology and miniaturized chemical engineering processes.

Dr. Hunt is a Professor of Chemistry and Pathology at the University of Virginia. Dr. Hunt's presentation focused on advances in mass spectrometry that are facilitating the ability to identify individual proteins in complex mixtures, even when present in very minute quantities. His presentation raised the critical issue that when there is a perturbation in a gene, such as a mutation, one is ultimately interested in how that affects protein expression and function. Through advances such as those occurring in mass spectrometry, one can envision a horizon in which molecular diagnosticians will be assessing gene structure and expression through a combination of technologies.

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