

*Center for Liver Diseases
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LIVER UPDATE

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Inside this Issue

- Translational Research at the Center for Liver Diseases
- Publications
- Current Clinical Protocols

Upcoming Programs

- Hepatitis C Support Groups
Inova Fairfax Hospital
3300 Gallows Road, Falls Church, VA
Conference Rooms D, E, & F (located above the cafeteria)
Third Tuesday of every month at 7:30 p.m.

Inova Health System is a not-for-profit health care system based in Northern Virginia that consists of hospitals and other health services including emergency and urgent care centers, home care, nursing homes, mental health and blood donor services, and wellness classes. Governed by a voluntary board of community members, Inova's mission is to provide quality care and improve the health of the diverse communities we serve.

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Translational Research at the Center for Liver Diseases

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Since its inception in 2000, the Center for Liver Diseases has been involved in a spectrum of “patient-oriented research” protocols investigating a variety of liver diseases. The overarching goal of this research is to convert basic research findings into actionable improvements in patient care.

Translational research protocols are developed internally and carried out in collaboration with other institutions such as George Mason University, Celera Diagnostics, and the Department of Pathology of the Armed Forces Institutes of Pathology. These investigator-initiated projects include genomic and proteomic protocols related to obesity, non-alcoholic fatty liver disease (NAFLD), hepatic fibrosis, and hepatitis C (HCV). Integrative databases relate clinical and pathologic data to genomic and proteomic analyses of biologic specimens (e.g., serum, whole

blood, liver tissue, fat tissue, isolated stellate cells, etc.). The following is a summary of five projects related to NAFLD, hepatic fibrosis, and HCV, and their potential scientific and clinical impact.

Genomics of Non-alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD) is a common form of liver disease in the Western world. NAFLD represents a spectrum of clinico-pathologic entities with differential potential to progress to cirrhosis. Of the NAFLD subtypes, only non-alcoholic steatohepatitis (NASH) has been shown to progress to cirrhosis. Differential progression to cirrhosis among subtypes is not well understood, but may be related to individual genetic differences. At present there are no proven effective therapies for NAFLD or NASH.

Since 1997, our team has pioneered clinical and epidemiologic research in this area of clinical hepatology and NAFLD has remained a major focus since the establishment of the Center. Over the past three years, we have initiated genomic studies involving patients with NAFLD.

Patients (N=120) have been enrolled with each patient generating significant amounts of clinical and liver biopsy data along with a large number of serum and liver specimens.

Snap-frozen liver biopsy specimens are used for RNA extraction. Total RNA is extracted, amplified, and reverse transcribed into cDNA probes. The cDNA probes are hybridized with customized microarray gene chips and scanned. Gene expression profiles of patients with NASH are compared to those with steatosis alone. Statistically significant changes in gene regulation (up- or down-regulation) greater than twofold are considered important. Preliminary data suggest that some genes related to apoptosis are differentially expressed in patients with the progressive form of NAFLD. These findings will enhance our understanding of the pathogenesis of NAFLD and provide guidance in developing therapeutic approaches. The findings from these ongoing analyses have resulted in over a dozen abstracts for major international meetings.

see *RESEARCH*, page 2 ▶



► *RESEARCH, from page 1*

Proteomics of Non-alcoholic Fatty Liver Disease

NAFLD diagnosis is presently based on liver biopsy findings. Liver biopsy is the “gold standard” for establishing NASH and for staging liver disease, however the procedure is invasive and associated with potential risks and high costs. Proteomic technology may provide a non-invasive diagnostic marker for NAFLD. Surface Enhanced Laser Desorption/Ionization – Time of Flight Mass Spectrometry (SELDI-TOF MS) is a powerful method for determining differences in protein expression between patients with differing clinical conditions. In NAFLD, such differences may represent subtypes of NAFLD or stages of hepatic fibrosis. We are using SELDI-TOF MS for a proteomics study of NAFLD to determine differences in serum protein expression profiles between patients with NASH and other subtypes of NAFLD. Our ongoing prospective study includes 102 patients with frozen fasting serum from the time of liver biopsy. Patients’ protein expression profiles are determined with SELDI-TOF MS, and differential expression patterns are analyzed. Preliminary data indicates significant differences in multiple protein peaks among different groups. Specifically, increased protein expression

is apparent in NASH patients compared to other groups. Further identification of these proteins is underway. This data has been presented at two international liver meetings.

Genomics of Obesity

Obesity has reached epidemic proportions in the United States. The phenotypic expression of obesity is influenced by both environmental and genetic factors. In an ongoing prospective study of the genomics and proteomics of obesity, we are assessing differential gene expression in the adipose tissue of morbidly obese patients. Currently, 157 patients have been enrolled. Gene expression profiles in the intra-abdominal adipose tissue is determined in a similar fashion to the NAFLD Genomics project.

Preliminary data reveal that the visceral fat of morbidly obese patients contains differentially expressed genes related to energy expenditure. The contribution of these genes to the pathogenesis of obesity is the focus of future investigation. This preliminary data has been presented at three major international scientific meetings.

Genomics of Fibrosis

Quiescent hepatic stellate cells (HSC) may become activated and promote the

development of hepatic fibrosis after liver injury from any cause.

Continuous fibrogenesis related to chronic liver disease (e.g., viral hepatitis, NASH, metabolic diseases, etc.) can lead to cirrhosis. The steps in this fibrogenic process and the associated molecular pathways are unclear. One of our ongoing studies examines the transcriptional profile of “inactive” and “active” HSC from patients with end stage cirrhosis. Liver specimens from patients undergoing orthotopic liver transplantation are collected. Hepatic stellate cells are isolated, and RNA is extracted. Active and inactive cell types are examined with a custom cDNA microarray, and by semi-quantitative RT-PCR. The initial data suggests a number of genes with differential gene expression in active versus inactive stellate cells. Of particular interest is up-regulation of genes involved in the apoptosis signaling pathway. Preliminary data from this study is being presented in a major scientific meeting.

Genomics of Hepatitis C

Hepatitis C (HCV) is another common cause of liver disease in the United States. It is the most common indication for liver transplantation and cause of liver cancer in the United

Current Clinical Trial Protocols

- The Use of Growth Factors for the Management of Pegylated Interferon+Ribavirin Related Cytopenia in Patients with Chronic Hepatitis C , Non-responders to Previous Treatment.
- Use of Growth Factors for Treatment of Ribavirin-induced Anemia in Chronic Hepatitis C Genotype 1, Previously Untreated.
- A Number of Fully-Funded, Novel Treatment Protocols for Hepatitis C, All Genotypes, Previously Untreated.
- Clinical Research for Patients with Nonalcoholic Fatty Liver Disease.

States. Although the majority of HCV patients develop chronic infection, only 20 percent progress to cirrhosis. Despite major advances in antiviral treatment, only 50 to 55 percent can be cured. This differential rate in the progression of HCV-related liver disease and variable response to HCV treatment is the result of a complex interplay of host and viral factors. Viral factors such as HCV genotype and viral load interact with certain host factors to influence responsiveness to antiviral therapy. Our ongoing study examines this by profiling mRNA levels in peripheral mononuclear blood cells obtained prior to and during antiviral therapy. To date, 70 patients have been enrolled. Real-time RT-PCR is used to profile the mRNA levels of important genes. Initial data indicates that early differential gene expression related to apoptosis and interferon pathways may affect the response to therapy. This data is being presented at a major scientific meeting.

These studies represent examples of our translational research projects currently being carried out at the Center for Liver Diseases. Our hope is to extend clinical understanding of common chronic diseases such as obesity, Hepatitis C, and NAFLD, and to help develop new diagnostic markers (through proteomics technology) and therapeutic targets (through genomics technology). This “patient-oriented research” is expected to improve the health and health-related quality of life of our patients. For more information about the Center’s research, please visit our Web site at www.inova.org/inovapublic.srt/liver or gunston.gmu.edu/liverdisease or call us at 703-698-3182. ■

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