Chapter 15
Gallbladder and Biliary Disease

A1. Fully characterize at least 10 murine *Lith* genes related to cholesterol gallstones. A total of 23 murine *Lith* genes have been identified using quantitative trait locus analyses of inbred strains of mice susceptible to diet-induced cholesterol gallstones. Several candidate *Lith* genes involved in lipid uptake and secretion have been identified, but none definitely linked to these genetic sites. Recently, genes that regulate inflammation as well as innate and adaptive immune responses have been found in the same regions as several of the *Lith* genes (Lyons MA. Biochim Biophys Acta 2006;1761:1133). (2006 0%; Total 20%)

A2. Develop small animal model for cholangiopathies that would allow analysis of effects of chronic necroinflammation on biliary epithelium. Further use of the *Mdr2* knockout (-/-) mouse suggests that the side-chain modified bile acid, nor-ursodeoxycholate, is more effective than standard ursodeoxycholate in decreasing biliary inflammation and the chronic cholestasis of this animal model of sclerosing cholangitis. Thus, this animal model may provide means of screening for therapies for chronic cholestatic liver disease (Fickert P. Gastroenterology 2006;130:465). (2006 10%; Total 30%)

A3. Develop molecular imaging techniques for visualization of the biliary tract that would provide accurate assessment of size, shape, position, motility, and inflammation, as well as a means of early detection and staging of neoplasia. Studies of positron emission tomography have provided further support for the reliability of this means of detection of cholangiocarcinoma (Prytz H. Hepatology 2006;44:1572). (2006 0%; Total 10%)

B1, Develop a cohort study of calculous and acalculous biliary pain to allow for analysis of risk factors and roles of genetic factors, microlithiasis, gallbladder motility, sphincter of Oddi dysfunction, and nucleation factors. In a cohort of normal adult volunteers, gallbladder dysmotility was found to correlate with insulin resistance (Nakeeb A. J Gastrointest Surg 2006;10:940), providing a potential link between obesity, diabetes, and gallstone disease, as well as affording new approaches to prevention. This cohort is also being evaluated for genetic factors linked to gallstones and is being used to assess plasma markers of lithogenicity of bile. (2006 10%; Total 20%)

B2. Characterize the role of enterohepatic species of *Helicobacter* and other candidate bacteria in development of cholesterol gallstones in both mice and humans. Several *Helicobacter* species have been linked to formation of gallstones in both murine models and humans. *Helicobacter* species associated with gallstone susceptibility produce urease, an enzyme that breaks down urea into ammonia and bicarbonate, thus creating an alkaline pH locally, which might promote calcium precipitation, a factor known to play a role in gallstone formation (Belzer C. Gut 2006;55:1678). *Helicobacter pylori*, the well-known

human pathogen that is linked to duodenal ulcer disease, does not promote gallstones in murine models (Maurer KJ. Am J Physiol Gastrointest Liver Physiol 2006; 290:175). (2006 10%; Total 20%)

B3. Identify plasma or urine markers for lithogenicity of bile using proteomics or metabolomics. Cohorts are being developed for assessment of plasma and urine markers that might correlate with lithogenicity of bile using proteomic approaches. Grant applications in this area are encouraged through program announcement (PA 07-016), "Proteomics: Diabetes, Obesity, and Endocrine, Digestive, Kidney, Urologic, and Hematologic Diseases." (2006 0%; Total 0%)

C1. Establish prospective database on cohort of patients with high risk of gallbladder cancer to allow development and assessment of means of early diagnosis and management. No prospective studies have yet been initiated. (2006 0%; Total 0%)

C2a. Identify at least 5 human *LITH* genes associated with increased risk of gallstones, based upon homology with murine genes and family studies. While several rare human gene variants have been linked to familial gallstone disease, no specific sites have been linked to typical cholesterol gallstone disease in humans. Investigation of human homologues to several candidate murine *Lith* genes (*ABCB11* and *LXRA*) found that they were not associated with typical gallstone disease (Schafmayer C. Hepatology 2006;44:650; Puppala S. Am J Hum Genet 2006; 78:377). (2006 0%; Total 10%)

C2b. Develop noninvasive biomarkers for cholangiocarcinoma. Proteomic profiling of serum and tissue from patients with cholangiocarcinoma have identified several patterns of protein peaks that correlate with the presence of cholangiocarcinoma, but no specific serum protein(s) has been identified that reliably predicts the presence of this cancer (Scarlett CJ. Hepatology 2006;44:658). Research grant applications on development of biomarkers are encouraged in program announcement (PA 07-052), "Development of Disease Biomarkers." (2006 10%; Total 10%)

C3. Develop practical and effective approach to or means of prevention of cholesterol gallstones in high-risk populations. Human trials on prevention of gallstones have not yet been initiated, but several studies in animal models have suggested potential novel approaches. Fibroblast growth factor (FGF)-15 has been identified as playing an important role in gallbladder filling (Choi M. Nat Med 2006;12:1253). FGF-15 is induced in the terminal ileum as a result of bile acid signaling through FXR and then leads to gallbladder filling by acting on cAMP-linked receptors on smooth muscle cells in the biliary tract. Lack of gallbladder filling may predispose to gallstones, which perhaps explains the link between diseases of the terminal ileum and gallstone formation. Furthermore, agonists of FGF-19 (the homologue in humans) might play a role in prevention of gallstones. In another study in mice, targeted deletion of *Gpbar1* (a gene

involved in regulation of cholesterol secretion) led to resistance to gallstone formation in response to a high-fat diet (Vassileva G. Biochem J 2006;398:423). Thus, inhibitors of this cell-surface receptor for bile acids may be a means of decreasing the likelihood of gallstones. (2006 0%; Total 10%)

Figure 17. Estimated Progress on Gallbladder and Biliary Disease Research Goals, 2006 (Year 2) [Cross-hatching indicates recent year's progress.]