

## **DRAFT**

### **Working Group 11. Gallbladder and Biliary Disease**

#### **Introduction & Background**

Diseases of the gallbladder and biliary tree include gallstones, acute cholecystitis, acalculous cholecystitis, sclerosing cholangitis, biliary atresia, choledochal cysts, gallbladder cancer and cholangiocarcinoma. These serious diseases can cause considerable morbidity and mortality. Gallstones are by far the most common cause of gallbladder disease.

Gallstones affect at least 26 million Americans (12% of adults) and the prevalence of stones appears to be rising. Many gallstones are “silent,” but approximately a third eventually lead to symptoms, complications and need for surgery. Gallstone disease necessitates an estimated 750,000 cholecystectomies per year and medical expenses in excess of \$6 billion in the year 2000, making it one of the most costly of digestive disorders. At present, 3,000 deaths (0.2% of all deaths) are attributed to complications of cholelithiasis and gallbladder disease yearly.

The majority of gallstones are composed of cholesterol, but may also contain calcium and bilirubin. Gallstones form when the concentration of cholesterol or calcium bilirubinate in bile exceeds the limited solubility level, and these compounds precipitate out of solution. Gallstones increase in prevalence with age. Risk factors for developing cholesterol gallstones include obesity, major weight loss, medications, genetic predisposition, ethnic background, and, among women, multiparity. Sudden development of symptomatic gallstones can complicate major weight loss, particularly after obesity surgery (gastric bypass). While gallstones rarely result in death, they can complicate management of other diseases, and gallstone pancreatitis can result in prolonged disability and death. Gallstones and other biliary diseases are important risk factors for the development of gallbladder cancer and cholangiocarcinoma, both of which are rare in the general population but increasing in frequency. Gallbladder cancer is common in selected populations, such as Native-Americans and Hispanic Americans of Mexican descent.

A smaller proportion of gallstones are pigment stones. Risk factors for “black” pigment stones include hemolysis and increased enterohepatic cycling of bilirubin as may occur with ileal disease or intestinal resection. Brown pigment stones are associated with biliary stasis, particularly if there is chronic anaerobic bacterial infection.

Other non-gallbladder related biliary diseases include sclerosing cholangitis, biliary atresia, cystic fibrosis, polycystic liver disease and malignancies such as cholangiocarcinoma. Many of these conditions are discussed in other parts of this Action Plan. Sclerosing cholangitis is the most common of these biliary conditions and is believed to be an autoimmune disease. The diagnosis of sclerosing cholangitis is difficult and there are no therapies that are of proven benefit. Sclerosing cholangitis is a frequent reason for liver transplantation and is often associated with development of cholangiocarcinoma, a highly malignant neoplasm.

### **Recent Research Advances**

The proximal cause of cholesterol gallstones was not well-characterized until the early 1970's when NIH-funded investigators demonstrated the importance of the physical chemical nature of bile in maintaining cholesterol in solution. Gallstones were believed to form when the concentration of cholesterol exceeded its solubility in the bile salt and phospholipid-rich bile of humans. Alterations in bile salt, phospholipid or cholesterol synthesis and secretion appeared to underlie development of gallstones. Animal models were developed for cholesterol gallstone disease which demonstrated the importance of proteins in bile as well as the presence of precipitating (nucleation) factors and motility abnormalities of the gallbladder. Recently, inbred strains of mice have been used to demonstrate the importance of genetic factors in the development of gallstones. Studies in the inbred strains of mice have also shown the importance of newly identified bacterial flora colonizing both distal intestine and gallbladder bile in leading to precipitation of cholesterol and formation of stones. Recent advances in genetics, genomics, and proteomics offer unprecedented opportunities for understanding the molecular basis of the crucial defects leading to gallstone disease. These advances may well lead to practical and effective means of prevention and treatment of gallstone and biliary disease.

The bedrock of management of gallstone disease for more than 100 years has been cholecystectomy. The introduction of laparoscopic techniques in the late 1980s transformed the field and improved medical care of patients with gallstones considerably. At present, over 90% of gallbladder surgery is done laparoscopically, which minimizes hospital stay and time out from work and normal activities. At the same time as the advances in laparoscopic surgery were occurring, increasingly sophisticated imaging and endoscopic techniques helped improve the ability to localize and identify biliary tract diseases (including neoplasms and strictures in addition to stones). Endoscopic techniques, such as endoscopic retrograde cholangio-pancreatography (ERCP), now allow relatively noninvasive approaches to diagnosis and management of complicated biliary disease and cancers. Nevertheless, imaging of the gallbladder and biliary tree is problematic, and both diagnosis and monitoring of biliary disease are hampered by the relative inaccessibility of the biliary system and lack of specific means for its visualization.

## Research Goals

Major goals for research are the prevention and treatment of gallstones and other diseases of the biliary tract. The following specific research goals are highlighted as important.

**Gallstones:** While fundamental elements of gallstone composition and formation are known, questions of diagnostic and therapeutic importance to gallstone formation and pathogenesis remain. For example, the molecular basis of cholesterol gallstone formation is beginning to be defined with the identification of several “*Lith*” genes associated with cholesterol gallstone susceptibility in murine models on a lithogenic diet. The complete characterization of the role of these *Lith* genes and their products represents a research goal that may be achievable in the near future. A more challenging goal is to apply knowledge of murine *Lith* genes to the identification of homologous genes in humans associated with susceptibility to form cholesterol gallstones. While many of the identified *Lith* genes relate to cholesterol and lipid metabolism, important genetic factors related to mucin formation, nucleation factors and gallbladder motility should also be sought. Epidemiological and family studies of carriers of these genes would be a necessary complement in this effort to identify genetic contributors to cholesterol gallstone formation in patients (Matrix cells A1 and C2).

In addition to exploring the importance of genetic factors in gallstone pathogenesis, another more challenging goal is to characterize the role of the enterohepatic bacteria, specifically of the genus *Helicobacter*, in cholesterol gallstone formation in both animal models and human patients. Evidence of gallbladder infection with bacteria including *Helicobacter* species has been noted in studies of human cholesterol gallstones, but the role that these bacteria might play in gallstone formation remains unknown. Conducting studies in humans in addition to murine models is important in this area because of possible species differences in enterohepatic flora. Future research should also consider the contributions to gallstone formation of microbial interactions with the gallbladder epithelium (Matrix Cell B2). If bacterial infection appears to be important in causing gallstones in humans, clinical approaches to treatment and prevention of gallstones using antibiotics to treat and eradicate the bacterial infection should be considered.

In the case of pigment stones, advances should be encouraged to further understanding of the solubility of calcium bilirubinates, including their enzymatic and non-enzymatic hydrolysis, nucleation, precipitation and polymerization in bile. Better understanding of how to inhibit bacterial beta-glucuronidase and regulate enterohepatic recycling of bilirubin may lead to means of preventing pigment stones.

Of equal interest to delineating the pathogenesis of gallstones is an improved understanding of the origins of pain and inflammation in the gallbladder both in the presence and the absence of detectable gallstones or biliary tract abnormalities (such as in acalculous cholecystitis, sphincter of Oddi dysfunction, biliary dyskinesia and post-cholecystectomy biliary pain syndrome). The mechanistic basis for biliary pain is still not well understood nor its seasonal or circadian periodicity. The cause and appropriate diagnostic evaluation and therapy of the non-gallstone related biliary dysfunction are particularly challenging. An important way to advance knowledge in this area is through the development of more effective and safer means

of management of retained biliary stones. At present, ERCP is commonly used for bile duct stone extraction, but continues to carry some risk of complications. An intermediate-term research goal is the development of a cross-sectional and longitudinal epidemiological study of biliary pain to allow for analysis of potential risk factors such as genetics, microlithiasis, nucleation factors, gallbladder motility, and sphincter of Oddi function, and pilot studies of treatment and prevention (Matrix Cell B1).

Translation of knowledge gleaned from basic and clinical research into clinically usable diagnostic tools will include the research goal of identifying biomarkers of lithogenic bile through proteomic or metabolomic assays of plasma or urine. These biomarker assays would permit early identification of individuals at high risk of developing gallstones (Matrix Cell B3). With these diagnostic tools available, practical and effective strategies to prevent cholesterol gallstones in high-risk populations may be possible (Matrix cell C3).

**Primary Sclerosing Cholangitis:** Recent estimates of the prevalence of cholangiopathies, diseases that affect the biliary epithelium such as primary sclerosing cholangitis (PSC), have shown that these conditions are more common in the U.S. population than previously estimated. Whereas the etiology of PSC is unknown, its co-incidence with inflammatory bowel disease suggests that autoimmunity is responsible for inflammatory and fibrotic damage to the bile ducts in this condition. However, the role of autoimmunity in the etiology of PSC is still unsettled and other possible mechanisms, such as infection, environmental exposures and heightened immune responses to enteric bacteria deserve careful analysis. To better understand the effects of chronic inflammation on biliary epithelium, a research goal for the next few years is to develop reliable small animal models for cholangiopathies to allow for research on pathogenesis, prevention and treatment (Matrix Cell A2).

**Gallbladder Cancer and Cholangiocarcinoma:** Cholangiocarcinoma and gallbladder cancers are usually detected at a late stage, and adequate therapies are lacking. Cholangiocarcinoma refers to adenocarcinomas affecting the biliary tree which can be either intra- or extra-hepatic and are often related to chronic biliary diseases such as PSC. Gallbladder cancer refers to adenocarcinoma arising in the gallbladder and typically occurs in patients with gallstones and chronic cholecystitis. In certain high-risk populations, the incidence of gallbladder cancer has been rising. An important research goal is to improve early diagnosis and management of these cancers, so that necessary steps such as liver transplantation for PSC or cholecystectomy for gallstones can be taken to prevent these fatal cancers. The identification of genetic or biological markers for gallbladder cancer and cholangiocarcinoma in high-risk populations would facilitate diagnosis and early detection (Matrix Cell C2). This research goal would benefit from the creation of a database to collect information on a cohort of patients at high risk of gallbladder cancer, such as Native Americans, for large-scale studies of novel diagnostic and therapeutic approaches (Matrix Cell C1).

**Biliary Tract and Gallbladder Imaging:** Effective diagnosis and monitoring of disease in the biliary tract is hindered by the structure's relative inaccessibility to biopsy and the limits of available imaging technology. Current methods to visualize the biliary tract are often invasive or not sufficiently informative for diagnosis and staging of disease. An important research goal, which would improve the diagnosis and management of several diseases of the

biliary tract and gallbladder, is the development of innovative and effective molecular imaging techniques to visualize these tissues, as well as cellular processes at the molecular, organelle or cellular level. Molecular imaging might provide improved diagnostic assessment, early detection and staging of neoplasia, and noninvasive evaluation of gallbladder motility, degree of inflammation, sphincter of Oddi function, and physical composition of gallstones or sludge within the biliary tract. In addition, these improved imaging techniques might provide a means to monitor the effectiveness of a therapeutic regimen by directly assessing changes in disease activity (Matrix Cell A3).

### **Steps to Achieve Research Goals**

Progress in gallbladder disease research would be strengthened with the availability of more advanced molecular tools, including molecular libraries of liver and gallbladder expressed genes, proteomic and metabolomic resources, and molecular probes and antibodies, such as to measure murine *Lith* & human *LITH* genes and their products.

The development of molecular markers for lithogenic bile and innovative molecular imaging technology to visualize the biliary tract and gallbladder requires the formation of interdisciplinary teams of investigators, including clinicians, endoscopists, radiologists, biomedical engineers, physical chemists, molecular biologists, and geneticists.

Large-scale clinical studies of populations at high risk of developing gallbladder disease require collaborative networks of clinical centers, either existing or newly established. Existing networks and clinical trials include: (1) the Longitudinal Assessment of Bariatric Surgery (LABS) Consortium, which could provide patient materials to evaluate biomarkers for lithogenic bile and the effects of obesity and rapid weight loss on formation of gallstones, and (2) the High-Dose Ursodiol Trial in PSC, which could be used to help identify biomarkers for disease progression or cholangiocarcinoma.

The ability to pursue the promising research opportunities now available in the area of gallbladder and biliary disease requires a pool of new, highly trained investigators to conduct and continue this research. Encouraging the training and career development of more young investigators is important to the future success of these research efforts.

## Matrix of Research Goals in Gallbladder and Biliary Disease

	Short-term Goals (A) (0-3 years)	Intermediate-term Goals (B) (4-6 years)	Long-term Goals (C) (7-10 years)
<b>High Risk (3)</b>	<b>A3.</b> Develop molecular imaging techniques for visualization of biliary tree that would provide accurate assessment of size, shape, position, motility & inflammation and means of early detection and staging of neoplasia.	<b>B3.</b> Identify plasma or urine markers for lithogenicity of bile (proteomics, metabolomics).	<b>C3.</b> Develop practical & effective approach to or means of prevention of cholesterol gallstones in high risk populations.
<b>Intermediate Risk (2)</b>	<b>A2.</b> Establish small animal model for cholangiopathies that would allow analysis of effects of chronic necroinflammation on biliary epithelium.	<b>B2.</b> Characterize role of enterohepatic species of <i>Helicobacter</i> and other candidate bacteria in development of cholesterol gallstones in both mice and humans.	<b>C2.</b> Identify (at least 5) human <i>LITH</i> genes associated with increased risk of gallstones, based upon homology with murine genes & family studies. Develop noninvasive biomarker for cholangiocarcinoma.
<b>Low Risk (1)</b>	<b>A1.</b> Fully characterize (at least 10) murine <i>Lith</i> genes related to cholesterol gallstones.	<b>B1.</b> Develop cohort study of calculous and acalculous biliary pain to allow for analysis of risk factors & roles of genetic factors, microlithiasis, gallbladder motility, sphincter of Oddi dysfunction and nucleation factors.	<b>C1.</b> Establish prospective database on cohort of patients with high risk of gallbladder cancer (e.g., Native Americans) to allow development and assessment of means of early diagnosis and management.