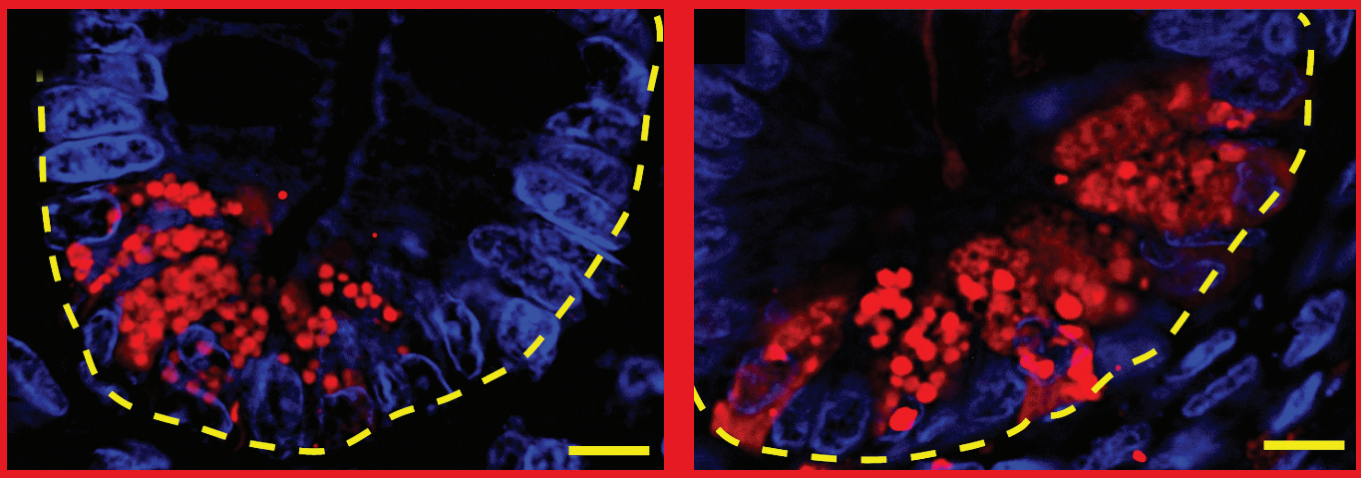

**Autophagy is an important process that the cell uses to degrade damaged cellular components and to help eliminate some pathogenic bacteria. These images show the effect of the Crohn's disease-associated mutation *ATG16L1* in Paneth cells from human participants who do (right panel) or do not (left panel) carry the mutated gene. The Paneth cell, a type of intestinal cell, is important in defending against the microbial flora. The protein Atg16 is responsible for proper placement of the autophagy machinery within the cell. When Atg16 is mutated, an increased proportion of Paneth cells contain disorganized or diminished granules (red dots) which serve as a storage depot for antimicrobial peptides and a protein called lysozyme. This new finding sheds light on previously unrecognized abnormalities in Paneth cell storage granules in the intestinal crypt in people with Crohn's disease who carry the mutated *ATG16L1* gene. Additional information on autophagy and its role in Crohn's disease can be found in this chapter. Also described in this chapter, NIDDK-supported research suggests that autophagy is triggered by an abnormal aggregation of mutant proteins in alpha-1-antitrypsin deficiency disease. Autophagy has also been implicated in anemia due to abnormal red blood cell maturation, as described in the chapter on Kidney, Urologic, and Hematologic Diseases.**

*Images provided by Drs. Thaddeus S. Stappenbeck and Herbert W. Virgin IV and reprinted by permission from Macmillan Publishers Ltd: Nature, 456: 259-263, copyright 2008.*

# Digestive Diseases and Nutrition

*Digestive diseases are among the leading causes of hospitalization, surgery, and disability in the U.S. These conditions include disorders of the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. Disorders of the digestive tract exact a significant toll on many Americans each year. For example, approximately 135 million people each year suffer from non-food-borne gastroenteritis, a typically infectious inflammation of the GI tract associated with such symptoms as diarrhea, nausea, and vomiting.[1] Additionally, liver and biliary diseases affect a large portion of the population and represent a huge burden, in terms of quality of life as well as health care costs, such as the estimated 6 billion dollars spent annually in the U.S. on gallbladder disease care.[1] NIDDK-supported scientists are vigorously pursuing research to understand how widespread these diseases are across the U.S., to identify the causes of these diseases and how they progress, and to test new interventions for treatment and prevention of these costly diseases, including drugs, surgery, and behavior modification.*

Digestive diseases that affect the GI tract include inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis. These diseases are marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. IBD often strikes early in life, with a peak age of onset in adolescence or young adulthood. To address this condition, surgery may be required, including removal of the affected region of the intestine. Scientists are dissecting the complex interactions among the genetic, environmental, and cellular factors that contribute to the development of IBD. Helping to catalyze the design of novel therapeutic strategies will be the continued discovery of predisposing genetic variations and their interactions, as well as other factors, such as potential autoimmune and microbial influences. Research on controlling intestinal inflammation has potential benefits not only for patients with inflammatory bowel diseases, but also for those at risk of developing colorectal cancer. Screening programs for colorectal cancer are aimed at reducing mortality through early detection, particularly in those individuals at higher risk.

Intestinal disorders also include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Gastroesophageal reflux disease, in which stomach acids rise up into the esophagus, is a common functional bowel disorder that can lead to a condition known as Barrett's esophagus. This condition, in which cells lining the esophagus transform into an intestinal-type cell, is associated with a heightened risk of esophageal cancer, the most rapidly rising cancer in the U.S. Gastroparesis is another functional bowel disorder that is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. A common cause of gastroparesis is diabetes, which is thought to damage nerves leading to the stomach and controlling movement of food. Fecal incontinence, or impaired bowel control, is another bowel disorder that poses a major public health burden, particularly in the elderly.

Some digestive diseases can be triggered by the body's reaction to certain foods. For example, in individuals with celiac disease, the small intestine is damaged when the immune system reacts to the protein gluten—a component of wheat, barley, and rye. This reaction interferes with the ability to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, growth failure. The only

[1] *Sandler RS, et al: Gastroenterology 122: 1500-1511, 2002.*

current treatment for celiac disease is maintenance of a gluten-free diet, which is difficult for many people. The greater challenge now facing patients and their health care providers is to improve methods capable of diagnosing celiac disease early, before damage occurs or other conditions develop. Recent and continued advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

The microorganisms that inhabit the gastrointestinal tract are increasingly appreciated as important factors in maintaining or tipping the balance between digestive health and disease. These microbes can affect intestinal health in some surprising ways, depending on their interactions with each other, with host cells, and with nutrients ingested by their host. Scientists are gaining insights into the ways these GI microorganisms influence the development and function of the digestive tract, as well as other systems throughout the body such as those with immune and metabolic functions.

The liver is an organ within the digestive system that performs many centralized functions in the body, including metabolism and distribution of nutrients such as fats. When the liver is functionally compromised by disease, this can have serious adverse impacts on health, and can sometimes lead to complete liver failure. Some liver diseases primarily affect children—such as biliary atresia, a progressive inflammatory liver disease—while others more commonly affect adults—such as a form of nonalcoholic fatty liver disease known as nonalcoholic steatohepatitis (NASH). Some are caused by viral infection, such as hepatitis B and C, or by genetic mutations, such as alpha-1-antitrypsin deficiency, while others arise from diverse factors such as autoimmune reactions, drug toxicity, and other, unknown triggers. Many of these forms of liver disease, such as chronic hepatitis C, place individuals at elevated risk for developing liver cancer. A healthy liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. The number of livers available from deceased donors is limited. Therefore, research is of critical importance to identify and treat liver disease, preserve liver function in people with liver disease, and explore treatment options beyond cadaveric liver transplants, including the use of tissues from living donors.

The number of overweight and obese Americans has risen dramatically in the past two decades and is now at epidemic levels. Obesity is associated with numerous serious diseases, including type 2 diabetes, heart disease, and cancer. Multiple factors contribute to obesity. As scientists elucidate the molecular factors that control appetite, metabolism, and energy storage, they are identifying potential targets for the development of new pharmacologic agents to promote safe, long-term weight loss. In addition to new pharmacologic interventions for obesity, existing bariatric surgical techniques are being evaluated for their long-term impacts on weight loss and well-being. Investigators are also continuing research to help people achieve healthy lifestyles that include increased physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the Obesity chapter.)

## GENETICS OF INFLAMMATORY BOWEL DISEASES

**The Number of Genes Associated with Crohn's Disease Soars to 30 and Beyond:** Using genome-wide association methodology and combined research data, three international research groups conducted a joint study that identified 21 new genes or gene regions associated with Crohn's disease (CD), bringing the total number to more than 30. Crohn's is a complex disease involving genes, the immune system, and microbes that interact to cause the intestinal inflammation. Although the precise number of CD-associated genes is not known, scientists estimate that 100 or more genes having varying levels of influence on disease susceptibility may be implicated in CD. Importantly, even genes with modest contributions to disease risk may have significant consequences. The three research groups had previously conducted individual genome-wide association studies that identified 11 genes associated with CD. In this joint study, data from the previous investigations were combined to create a large study cohort capable of detecting the more subtle CD variants. The new study's analysis of the combined data identified the 11 original CD genes and discovered 21 additional CD genes, bringing the total number of CD-associated genes to 32. The scientists confirmed the results of the new study by conducting a replication analysis with an independent group of

individuals with CD, and a mixture of family-based and population-based healthy (control) participants. The findings of this multi-national study demonstrate the advantage of very large study cohorts in detecting genes that may each have only modest effects in complex diseases, but that may interact with environmental factors and perhaps with each other to influence disease susceptibility. Although the functions of some of these CD variants are unknown, several are related to biochemical pathways that promote inflammation. All of the genes and genomic regions identified by this study provide important information for understanding the molecular architecture of CD and identifying potential targets for future therapies.

*Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhart AH, Targan SR, Xavier RJ; The NIDDK IBD Genetics Consortium, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot J-P, de Vos M, Vermeire S, Louis E; The Belgian-French IBD Consortium; The Wellcome Trust Case Control Consortium, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwilliam R, Tremelling M, Deloukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, and Daly MJ: Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet 40: 955-962, 2008.*

### Specialized Intestinal Cells and Crohn's Disease:

Scientists have recently discovered that a gene associated with Crohn's disease plays an important role in specialized intestinal cells, known as Paneth cells. Crohn's disease, a type of inflammatory bowel disease, is a chronic disorder that causes inflammation of the digestive tract resulting in abdominal pain and diarrhea. Previous research revealed that some patients with Crohn's disease harbor a mutation in a gene called *ATG16L1*. This gene is involved in an important biologic process called autophagy, which cells use to degrade damaged cellular components and to help eliminate some pathogenic bacteria. But it was not known how the ATG16L1 protein contributes to Crohn's disease onset and/or progression. Because the ATG16L1 protein is responsible for proper placement of the autophagy machinery within mammalian cells, researchers created mice having reduced levels of ATG16L1 to begin to determine the role of this protein in autophagy in the intestine. Reduced levels of ATG16L1 had no effect on the overall appearance of the intestine, but there were obvious changes in Paneth cells that line the intestinal wall. Paneth cells have granules filled with antimicrobial agents which are secreted in response to invasive bacteria. In contrast to mice with normal levels of ATG16L1, mice with reduced levels of this protein contained disorganized granules and marked changes in their granule-secreting pathways, as well as other defects. Examination of mice deficient in a different autophagy gene (*ATG5*) revealed similar Paneth cell abnormalities, verifying that the autophagy process is involved in the granule secretion pathway of Paneth cells. Further investigation revealed that intestinal Paneth cells from patients with Crohn's disease contained disorganized or diminished granules, which was similar to that seen in mice with reduced levels of ATG16L1. Thus, this study demonstrates previously unrecognized abnormalities with respect to Paneth cells and provides additional understanding of a compromised host defense in Crohn's disease.

*Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK, Kishi C, Kc W, Carrero JA, Hunt S, Stone CD, Brunt EM, Xavier RJ, Sleckman BP, Li E, Mizushima N, Stappenbeck TS, and Virgin HW IV: A key role for autophagy and the autophagy gene Atg16L1 in mouse and human intestinal Paneth cells. Nature 456: 259-263, 2008.*

## SCREENING FOR COLON CANCER

### Disparities Found in the Prevalence of Colon Polyps for African Americans and Caucasians:

New research suggests that more extensive screening for pre-cancerous polyps may help address disparities in rates of colon cancer. African Americans are at greater risk of developing colorectoral cancer and dying from this disease than are Caucasians. The reasons for these disparities are not clear. However, colon cancer is frequently preventable if pre-cancerous polyps are detected and removed before they can form tumors. Therefore, scientists designed a study to gain insight into whether there are differences between African Americans and Caucasians in the number of polyps and polyp location as detected by colonoscopy. They reasoned that any differences found could inform

recommendations for earlier or enhanced screening. Screening colonoscopy data were analyzed from subsets of African American and Caucasian men and women who had no prior symptoms of colorectal disease. The analysis revealed that the African Americans had greater numbers of pre-cancerous polyps (polyps 9 millimeters or larger) than the Caucasians. The number of pre-cancerous polyps detected in African American women younger than 50 years was found to be particularly high. Although all people over age 60 were more likely to have polyps located in the upper (proximal) colon than younger patients, African Americans over age 60 had a greater number of proximal polyps than Caucasians of the same age. This study provides important information showing that African Americans who do not yet show symptoms of colorectal disease are more likely than Caucasians to have pre-cancerous colon polyps. Evidence linking upper colon polyps to age suggests that people over age 60—both African Americans and Caucasians—would benefit more from colonoscopy, a procedure which examines the entire colon. This is significant because another technique for detecting polyps—the sigmoidoscopy—does not screen the proximal colon. Notably, this study suggests that increased early colonoscopy screening for African Americans has the potential to significantly reduce their disproportionate burden of colon cancer.

*Lieberman DA, Holub JL, Moravec MD, Eisen GM, Peters D, and Morris CD: Prevalence of colon polyps detected by colonoscopy screening in asymptomatic black and white patients. JAMA 300: 1417-1422, 2008.*

## BARRETT'S ESOPHAGUS RISK FACTORS

**Fruits and Veggies Help Protect Against Barrett's Esophagus:** A recent study shows that eating more fruits and vegetables, which are rich in antioxidants as well as other substances that may affect health, is associated with a reduced risk of developing Barrett's esophagus (BE). BE is a condition in which the cells lining the esophagus transform into a type of cell that is found in the intestine. Patients with BE are at high risk of developing a form of cancer called esophageal adenocarcinoma, a cancer that has risen substantially in the U.S. in recent decades. Previous studies revealed an association between eating more fruits and vegetables and having a decreased risk of developing esophageal cancer, as well as an association between dietary antioxidants (which are among the many substances found in fruits and vegetables) and reduced risk for esophageal adenocarcinoma. The current study was designed to determine if a higher intake of fruits and vegetables, and either dietary or supplemental antioxidants, was associated with lower risk of BE. The study began by interviewing three groups of volunteers: (1) patients recently diagnosed with BE and other study participants; (2) healthy individuals; and (3) individuals with a history of gastroesophageal reflux disease (GERD), a condition that is often a precursor to BE. All study participants completed a "food frequency" questionnaire to assess their dietary habits during the previous year. Participants' diets subsequently were analyzed according to the number of servings of fruits and vegetables they consumed and whether or not they took antioxidants in the form of dietary supplements. Comparison of the diets of BE patients with those of the "healthy" group revealed that eating more fruits and vegetables correlated with a reduced risk of BE, with a greater benefit achieved from more daily servings. In contrast, dietary supplements containing antioxidants did not lower the risk of BE, even for those who ate few servings of fruits and vegetables. Comparison of the diets in the "GERD" participants with the healthy group showed that fruit and vegetable consumption was also associated with reduced risk of GERD. The results of this study indicate that eating more fruits and vegetables is associated with a reduced risk of BE, which may in turn reduce the risk of developing esophageal cancer.

*Kubo A, Levin TR, Block G, Rumore GJ, Quesenberry CP, Buffler P, and Corley DA: Dietary antioxidants, fruits, and vegetables and the risk of Barrett's esophagus. Amer J of Gasteroenterol 103: 1614-1623, 2008.*

## IRRITABLE BOWEL SYNDROME CONTRIBUTORS

**New Insights into the Pain Associated with Irritable Bowel Syndrome:** Researchers have found that patients with irritable bowel syndrome (IBS) perceive visceral pain associated with IBS differently than healthy volunteers, and exhibit altered brain activity responses to both pain and anticipation of pain. In addition, pain responses are heightened in IBS patients who have experienced physical abuse.

IBS is a relatively common, painful, and distressing disorder characterized by cramping, abdominal pain, bloating, constipation, and diarrhea. IBS affects more women than men. It is a debilitating disorder that can prevent individuals from working, attending social events, or traveling even short distances. The coping mechanisms that are employed by individuals to cope with pain are determined by the degree of pain that is anticipated. Inhibition mechanisms are employed to minimize discomfort when the expected pain is tolerable, whereas amplification mechanisms are used to enhance arousal and vigilance when the expected pain is dangerous. Researchers thus designed clinical studies to evaluate the pain responses of IBS patients, explore whether IBS patients exhibit increased sensitivity to anticipated visceral pain, and determine if prior abuse plays a role in the intensity of pain experienced by some IBS patients.

In one study, functional magnetic resonance imaging (fMRI) was used to evaluate the brain activity of women with IBS and healthy women volunteers in response to anticipation and delivery of mild and moderate pain induced by rectal distention. When compared to healthy volunteers, IBS patients experienced a heightened perception of pain and altered brain activity both when anticipating the pain, which was cued, and during the actual painful rectal distention procedure. Brain activity patterns of the healthy volunteers corresponded to known inhibition responses that would help to minimize pain perception. When the IBS patients were anticipating the pain, the brain imaging showed less of this inhibitory response, which then correlated with increased brain responses during the actual pain. These results confirm a hypersensitivity to visceral pain in IBS patients and suggest a relationship among pain perception, altered brain activity in response to pain stimuli, and the anticipation of pain.

In a second study, researchers assessed whether a history of sexual or other physical abuse affected the degree of pain IBS patients reported and, using fMRI, whether prior abuse correlated with differences in brain responses to painful stimuli. The responses to induced pain of women with IBS who had been abused were compared to those of women with IBS who did not have abuse histories as well as to women who did not have IBS. Women with both IBS and a history of abuse reported significantly greater pain in response to rectal distention, and they exhibited brain activity patterns that demonstrated reductions in pain inhibition responses and other alterations in brain activity associated with pain. Analyses of the increased perceived pain and the decreased pain inhibition of patients with histories of abuse revealed that abuse has a synergistic effect that intensifies IBS-related pain. This finding is consistent with the poorer treatment outcomes observed in IBS patients who have been abused.

Visceral pain has a major impact on the lives of individuals with IBS. In these studies, scientists explored pain perception of IBS patients, gaining new insights into patient responses to anticipated and perceived pelvic pain and the effects of prior abuse on IBS-related pain. The findings of these studies provide researchers with greater understanding of IBS-related pain, which may lead to improved treatment strategies and outcomes.

*Ringel Y, Drossman DA, Leserman JL, Suyenobu BY, Wilber K, Lin W, Whitehead WE, Naliboff BD, Berman S, and Mayer EA: Effect of abuse history on pain reports and brain responses to aversive visceral stimulation: An fMRI study. Gastroenterology 134: 396-404, 2008.*

*Berman SM, Naliboff BD, Suyenobu B, Labus JS, Stains J, Ohning G, Kilpatrick L, Bueller JA, Ruby K, Jarcho J, and Mayer EA: Reduced brainstem inhibition during anticipated pelvic visceral pain correlates with enhanced brain response to the visceral stimulus in women with irritable bowel syndrome. J Neurosci 28: 349-359, 2008.*

## CELIAC DISEASE IN CHILDREN

**New Antibody Test To Detect Early Childhood Celiac Disease:** Scientists have developed a new strategy to facilitate early detection of celiac disease and to help patients and doctors monitor adherence to the gluten-free diet required by people with this disease. The strategy uses a new test to detect antibodies against small modified gluten proteins, called deamidated gliadin peptides (DGP); these antibodies are made aberrantly by the immune systems of patients with celiac disease. Celiac disease affects approximately 1 in 133 individuals in the U.S. When individuals with celiac disease ingest gluten, a component of wheat, rye, and barley, their

immune systems mount a reaction against it, which damages the small intestine. To avoid or repair this damage, individuals with celiac disease must adhere to a gluten-free diet. There is great individual variation in the intensity and spectrum of symptoms associated with celiac disease. Many of the symptoms, including weight loss, short stature, and failure of infants to thrive, are related to malabsorption of nutrients by the damaged small intestine. Therefore, it is especially important to diagnose this disease as early as possible in infants and children to prevent the growth problems associated with celiac disease.

In this study, scientists used a new test to compare levels of antibodies to DGP to levels of another type of antibody made in celiac patients, the transglutaminase autoantibody (TGAA). An autoantibody is an antibody made by the immune system against one of the body's own proteins; celiac is thus considered an autoimmune disease. The antibody levels were tracked over time using blood samples collected from 50 children (6 months to 17 years of age), beginning prior to their disease diagnosis. The children were participating in the NIDDK-sponsored Celiac Disease Autoimmunity Research (CEDAR) study. These children were at high risk for developing celiac disease due to their genetic predisposition to celiac disease and/or another autoimmune disease, type 1 diabetes. The CEDAR study is, in fact, a branch of a larger study, the Diabetes Autoimmunity Study in the Young (DAISY). All of the children studied were TGAA positive, and 39 of the children were also positive for DGP antibodies at the time TGAA was first detected. Notably, in 9 of these children, DGP antibodies were found even before TGAA was detected, demonstrating that the DGP antibody test was indicative of celiac disease in these children before they tested TGAA positive. After the initial evaluations, 30 children elected to follow a gluten-free diet while 20 continued on a normal diet. For the 30 children on the gluten-free diet, both TGAA and DGP antibody decreased over time. However, DGP antibody levels declined more quickly than TGAA levels after starting a gluten-free diet. These results suggest that measurement of DPG antibody may be more useful than measurement of TGAA in monitoring adherence to a gluten-free diet. By following the development of antibodies to DGP and TGAA in children at risk for celiac disease, scientists have shown that the new DGP antibody test will permit earlier detection of celiac disease in certain individuals and will provide a more sensitive tool for evaluating the success of a gluten-free diet intervention.

*Liu E, Li M, Emery L, Taki I, Barriga K, Tiberti C, Eisenbarth GS, Rewers MJ, and Hoffenberg EJ: Natural history of antibodies to deamidated gliadin peptides and transglutaminase in early childhood celiac disease. J Pediatr Gastroenterol Nutr 45: 293-300, 2007.*

## INHERITED DISEASES OF NUTRIENT METABOLISM

**Menkes Disease—A New Strategy for Therapeutic Design:** Scientists have developed a novel approach to screening for potential treatments for a fatal childhood disease, Menkes disease. Children with this disease suffer seizures, floppy muscle tone, neurodegeneration in the brain, and failure to thrive beginning a few weeks after birth, along with other symptoms. Menkes disease is caused by mutations in a gene that encodes a copper-transporting protein; copper is critical for many biological processes. Although treatment with copper injections can improve outcomes if begun shortly after birth, development may still not be normal, and the disease is often not detected early enough. The scientists first developed a model of this devastating disease in zebrafish, a small fish highly amenable to experimentation. Aptly named "calamity," this zebrafish model has similar symptoms to human Menkes disease. To explore potential new therapeutic approaches, the scientists then considered the nature of the gene and the disease-causing mutations. As is the case with many genes, the protein-coding information occurs in non-contiguous segments along a strand of DNA. Normally, the intervening segments are excised and the protein-coding information joined together in a process called splicing, which occurs in special copies of the gene called mRNA copies. A mutation in the zebrafish gene caused aberrant splicing, resulting in a lack of normal copper-transporting protein. To try to counteract this defect, the scientists next designed a series of molecules that would interact with specific stretches of mRNA around the mutant splice site. After injecting these molecules—called "antisense oligonucleotides"—into zebrafish embryos, they identified a few that reduced the aberrant splicing and restored normal neurologic development. Because

different types of mutations cause disease in different ways, this approach would not ameliorate the effects of all Menkes disease mutations. However, this strategy may eventually lead to new treatments for many patients with this disease and other genetic diseases. It also demonstrates the value of a zebrafish model of human genetic disease for rapid screening of potential therapeutics.

*Madsen EC, Morcos PA, Mendelsohn BA, and Gitlin JD: In vivo correction of a Menkes disease model using antisense oligonucleotides. Proc Natl Acad Sci USA 105: 3909-3914, 2008.*

## LIVER FAT METABOLISM AND CONTRIBUTORS TO NONALCOHOLIC FATTY LIVER DISEASE

**New Role for Molecular Factor in Regulating Liver Fat Metabolism:** Research sponsored in part by the NIDDK has uncovered a new function for a previously identified protein that acts within liver cells to regulate lipid (fat) metabolism. The liver serves as a major metabolic center within the body, receiving nutrients for processing soon after their digestion in the intestine. When carbohydrates are consumed in excess of the body's needs, the liver can incorporate them into lipids such as triglycerides for storage. A long-standing surplus of lipids produced by the liver can contribute to conditions such as the metabolic syndrome that are marked by abnormal lipid levels. Lipid synthesis in the liver is a process that is regulated by several transcription factors that control the production of enzymes involved in carbohydrate and lipid metabolism. The transcription factor XBP1 was originally known to have a key role in a completely different cellular process—turning on genes involved in protein secretion by pancreatic cells and plasma B immune cells. In these studies, however, scientists found that XBP1 plays an additional, functionally distinct role in regulating lipid metabolism in the liver. Scientists used a mouse model, in which XBP1 synthesis was controlled, to isolate the specific actions of XBP1 in liver lipid metabolism. The requirement of this factor for normal lipid synthesis in the liver was demonstrated by the low levels of lipids, such as triglycerides, observed in the liver and bloodstream when XBP1 synthesis was blocked. In contrast, when mice with functional XBP1 were fed a high-carbohydrate diet, they produced high levels of XBP1, which directly activated genes in the liver involved in lipid synthesis. With these studies, researchers have recognized the dual functions of the XBP1 transcription factor as a regulator of both lipid synthesis in the liver and protein secretion in other cells. The opportunity now exists to develop inhibitors of XBP1 action within the liver as a potential treatment for patients with abnormally high lipid levels.

*Lee A-H, Scapa EF, Cohen DE, and Glimcher LH: Regulation of hepatic lipogenesis by the transcription factor XBP1. Science 320: 1492-1496, 2008.*

**Gene Variant Linked to Disparity in Nonalcoholic Fatty Liver Disease Risk:** Genome-wide scanning has revealed gene alterations associated with variation in risk for nonalcoholic fatty liver disease in different ethnic groups. Nonalcoholic fatty liver disease refers to the accumulation of excess fat in the liver in the absence of heavy alcohol consumption, which can lead to liver inflammation, cirrhosis, and the need for transplant. This form of liver disease has become more common in the U.S. population as the numbers of obese and overweight individuals have increased, and is currently the leading cause of liver disease in this country. However, within the general population, some ethnic groups—such as Hispanics—appear to be disproportionately affected by this form of fatty liver disease, while others—such as African Americans—are more resistant to its development. Researchers sought to identify an inherited factor that might explain these risk disparities. To find this genetic factor, they utilized the same technique of genome-wide scanning that has proven so successful recently in identifying genes associated with other diseases through scanning the genomes of participants in a large population-based study called the Dallas Heart Study. Participants were of Hispanic, African American, and European American ancestry. After comparing the genomic data with a noninvasive, imaging-based measure of liver fat and a blood test for liver inflammation, they uncovered a variant in the gene *PNPLA3* that was strongly associated with increased levels of liver fat and inflammation. (The variant resulted in a change in the protein encoded by the gene.) This gene variant was found to be more common among Hispanics, and the variant was also associated with higher levels of liver fat and inflammation in this group. Conversely, a

different variant of the same gene was associated with lower liver fat in African Americans. While the precise effects of the *PNPLA3* gene variants on the function of the encoded proteins remain to be established, researchers are pursuing additional studies to define their proposed roles in liver fat metabolism. Based on their distinctive associations with nonalcoholic fatty liver disease risk amongst ethnic groups in the U.S., these gene variants could provide a basis for needed diagnostic tests to predict who is at greater risk for developing this form of fatty liver disease and whether their disease will progress.

*Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, and Hobbs HH: Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 40: 1461-1465, 2008.*

## HEPATITIS C TREATMENT AND EARLY DETECTION OF PROGRESSION TO LIVER CANCER

### Immune Molecule Gives Clue to Differential Treatment Responses in Chronic Hepatitis C:

The standard treatment for chronic hepatitis C—a combination of the antiviral drugs peginterferon and ribavirin—is known to be less effective in African Americans compared to Caucasians. The reason for this differential effect has been elusive, but scientists recently uncovered an important clue in the form of a molecule that sits on the surface of immune cells and affects their ability to fight infection by the hepatitis C virus (HCV). This research was part of a larger study funded by the NIDDK known as the Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C, or "Virahep-C," aimed at identifying reasons for differential responses to these antiviral drugs in some African Americans compared to Caucasian Americans and developing ways to improve treatment.

In this series of experiments, investigators measured levels of an immune cell molecule known as PD-1 in study participants, both before and after receiving antiviral treatment for chronic hepatitis C. PD-1 is short for "programmed death-1," reflecting in part the fact that its presence on cells indicates that they are functionally exhausted. Found on the surface of T cells of the immune system, PD-1 inhibits the ability of these cells to defend against HCV. Participants with persistent chronic HCV were found to have higher pre-treatment levels of PD-1 on their T cells than individuals without HCV. In addition, African American participants for whom this therapy was not effective had higher pre-treatment levels of PD-1 on their T cells than those with successful treatment results. No such differences were found in Caucasian participants who did or did not respond to therapy. Participants from both racial groups who responded to therapy showed a decrease in PD-1 levels over the course of therapy.

Based on these findings, the researchers concluded that high levels of the PD-1 molecule on the surface of immune cells prior to treatment plays an important role in determining whether African American patients, in particular, respond to antiviral therapy for chronic hepatitis C. This study provides some of the first evidence to explain why antiviral therapy against chronic hepatitis C has not been as effective in some racial groups. Through further understanding of how PD-1 and other immune system elements contribute to treatment responses in different racial groups, scientists hope to build the knowledge base necessary to improve future therapy for all patients with chronic hepatitis C.

*Golden-Mason L, Klarquist J, Wahed AS, and Rosen HR: Cutting edge: Programmed Death-1 expression is increased on immunocytes in chronic hepatitis C virus and predicts failure of response to antiviral therapy: Race-dependent differences. J Immunol 180: 3637-3641, 2008.*

**Unique Gene Expression Patterns Found in Early Liver Cancers:** In the U.S. population, hepatitis C virus infection is the leading cause of hepatocellular carcinoma, a form of liver cancer that is increasing in prevalence in this and other countries. Understanding the molecular events within liver cells that drive the development of hepatocellular carcinoma would help in identifying its early origin at a stage where it may respond to more effective, targeted therapies. An international research group with NIDDK support recently conducted a study using liver tissue biopsies from individuals with an early stage of hepatocellular carcinoma due to hepatitis C to identify unique molecular changes. Using technologies such as the gene microarray, they analyzed the genomes (collection of genes) and transcriptomes (collection of messages

made, or "expressed," from the genes, a sign of their activation) in these liver tumors during the early stages of development. The researchers detected changes in the expression of genes that are involved in specific pathways signaling for cellular processes relevant to cancer, such as proliferation or tumor suppression. Different tumors were found to have unique patterns of altered gene expression, which would allow them to be classified by subtype. For example, the gene encoding vascular endothelial growth factor A (VEGFA), which is essential for promoting the growth of new blood vessels to tumors, was found to be overexpressed in some tumors, while other tumors displayed changes in expression of molecules involved in different signaling pathways. Additionally, the researchers found extra copies of large chromosomal segments in some tumors, some of which correlated with increased expression of the VEGFA gene. The investigators were able to use the molecular changes they observed to predict the risk of cancer recurrence after surgical removal of the tumor. These findings provide a wealth of potential biomarkers for predicting treatment response based on characteristics of a particular patient's liver tumor, as well as new targets for developing more personalized liver cancer therapies.

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## LIVER TRANSPLANT OUTCOMES

**Evaluating Outcomes in Recipients and Donors of Living Donor Liver Transplants:** Investigators from the NIDDK-supported Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) have conducted two studies that quantify the impacts of living donor liver transplantation (LDLT) on patients receiving a transplant, as well as on the individuals who donate portions of their livers for this procedure, using data from a network of transplant centers. The A2ALL study is being conducted at nine transplant centers across the U.S. to provide information on the risks and benefits of LDLT for both recipients and donors.

Compared to liver transplantation using deceased donor organs, which are in limited supply relative to demand, LDLT reduces waiting time. However, it is also a more complex procedure in which only a partial liver can be transplanted, and poses some risks to the healthy donors, who derive no direct benefit from their donation. Thus, it is important to simultaneously assess: (1) recipient outcomes to determine whether there are patient survival benefits from proceeding with a LDLT instead of waiting for a liver from a deceased donor; and (2) donor outcomes to determine the risk of complications from undergoing this voluntary procedure.

In the study of recipient outcomes, records from the centers were analyzed to determine if LDLT increased survival of adult patients compared to those who received a deceased donor liver transplant or no transplant. Study investigators found that, over an average of 4 years of follow-up time, LDLT was associated with lower mortality than the alternative of waiting for a liver from a deceased donor. Patients receiving LDLT were 44 percent less likely to die during the follow-up period than individuals who did not receive LDLT. The study also demonstrated that recipient survival with LDLT increased as the transplantation team became more familiar with the procedure. Once the centers were more experienced in LDLT, defined as performing more than 20 procedures, the mortality risk was 65 percent lower for patients who received LDLT compared to those who did not.

Focusing on the outcomes among the donors of the livers, a second study evaluated reports of complications they experienced across the participating centers. In the period during and after transplantation, 38 percent of donors experienced one or more complications from the procedure. The most common complications, including bacterial infections and biliary leaks (leaking of bile from a defect in the wall of the bile duct), were of low severity, but some were potentially life-threatening and required hospitalization. In contrast to the study of recipient outcomes, greater surgical experience performing LDLT had no effect on minimizing donor complications. Factors that were associated with development of donor complications were high levels of the liver enzyme alkaline phosphatase prior to transplantation, and the need for blood transfusion during the procedure.

Additional studies are planned to assess impacts on donor pain, quality of life, and other predictors of donor complications.

This study provides evidence that, while LDLT can increase survival for patients with end-stage liver disease—especially with improving experience of transplantation centers—it continues to carry some health risks for donors that are not ameliorated by greater surgical experience. These new data will be useful to patients who may be contemplating LDLT relative to other treatment options; to potential healthy volunteer donors considering the risks of the procedure, both for themselves and the recipients, as well as the benefits; and to their physicians.

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# *National Commission on Digestive Diseases*

Diseases of the digestive system span a wide range of conditions—from functional gastrointestinal (GI) and motility disorders, inflammatory bowel diseases, and celiac disease, to liver and gallbladder diseases, to pancreatic diseases and GI cancers. Collectively, these diseases represent an enormous public health burden. A strong commitment to advancing research is required to combat digestive diseases.

Since its establishment by the former NIH Director, Dr. Elias Zerhouni, in 2005, the National Commission on Digestive Diseases made substantial progress toward its goal to improve the health of the Nation through advancing digestive diseases research. The Commission was responsive to the mutual interest in this research area shared by the Congress, the NIH, and the research community. Within the NIH, the NIDDK provided leadership and support for the Commission.

As part of its charge, the Commission assessed the state-of-the-science in digestive diseases and the related NIH research portfolio, in order to identify research challenges and opportunities for inclusion in its long-range research plan for digestive diseases. The Commission's efforts benefited from the diverse expertise of its members, who represented the academic and medical research and practice communities, the patient advocacy community, and the NIH and other Federal health agencies. The research plan was also informed by a separate, parallel, NIDDK report on the current burden of digestive diseases in the U.S.

In 2006, the Commission initiated a research planning process by convening two public meetings, defining the topic areas within digestive diseases research that comprise the research plan, assigning Commission members to chair topical Working Groups, conducting an open call for additional experts to serve as Working Group members, and laying the foundation for the Working Groups' deliberations through the identification of research goals and other recommendations. Dr. Zerhouni addressed the Commission members at their November 2006 meeting to express his support and appreciation for their efforts.

During a subsequent public meeting held by the Commission in June 2007, the Working Group chairs presented their Groups' research recommendations for specific digestive diseases, along with steps to achieve the proposed goals. At the following meeting in November 2007, the Commission considered the entire draft research plan and invited public comments. A formal public comment period held in early 2008 invited additional stakeholder input on the draft research plan, which was posted on the internet. Following incorporation of public input, the Commission convened in May 2008 to discuss the draft research plan, which was subsequently finalized and approved by all members of the Commission. The final research plan, entitled "Opportunities and Challenges in Digestive Diseases Research: Recommendations of the National Commission on Digestive Diseases," will be released in early 2009 in both electronic and print formats. The research plan was transmitted to the NIH Director and Congress, as specified in the Commission's charter, and will also be widely disseminated to the research and health care communities interested in digestive diseases research, including professional and patient advocacy organizations; leaders of NIH Institutes, Centers, and Offices, as well as other Federal agencies; private research organizations; and individual scientists and advocates who provided input through the Working Groups. The Commission's 10-year research plan is intended to guide the NIH—along with the investigative and lay communities—in pursuing important research avenues for combating digestive diseases.

The Commission's research plan will be available in electronic form through its website: *http://NCDD.niddk.nih.gov*. Hard copies of the publication will also be available through the National Digestive Diseases Information Clearinghouse, and ordering information will be provided on the website.

## STORY OF DISCOVERY

# *Hepatitis B Research Progress: A Series of Fortunate Events*

Over the span of a few decades in the U.S., hepatitis B has been transformed from a disease newly infecting 200,000-300,000 individuals annually to one infecting approximately 46,000 individuals in 2006, the most recent year surveyed.[1] This impressive public health achievement can be attributed largely to immunization programs using a safe and effective hepatitis B vaccine, and screening of the blood supply for the virus. Therapy for chronic hepatitis B has similarly improved from a point in time when no effective treatment was available, to the current armamentarium of seven FDA-approved treatment options. These gains in hepatitis B control and care are based on years of careful research, marked by a confluence of serendipity and concerted effort by U.S. and international scientists into understanding the cause and course of hepatitis B, effectively treating it, and preventing its spread. NIH-sponsored research has contributed greatly to advancing knowledge of hepatitis B over the years. This Story of Discovery highlights some of the landmark accomplishments to date in hepatitis B research and their far-reaching impact through translation into improved medical care and public health in this country and around the world.

**Silent Disease with a Global Reach**

Hepatitis B is an inflammation of the liver caused by infection with the hepatitis B virus (HBV), which results from exposure to an infected person or their blood or blood products. Infection with HBV can result in acute or chronic forms of hepatitis. Symptoms can include fatigue, nausea, fever, loss of appetite, stomach pain, diarrhea, dark urine, light stools, and jaundice (yellowing of the eyes and skin). However, hepatitis B often is a "silent disease," quietly inhabiting the body for several decades before provoking symptoms or progressing to cirrhosis (scarring of the liver that prevents normal function) and/or hepatocellular carcinoma (liver cancer). This delayed appearance of symptoms can hinder efforts to detect the disease at an early stage and to prevent further transmission. Common ways in which HBV is passed on include: from mother to baby at birth; sex without use of a condom; use of tainted needles or tools for injection drug use, tattoos, or body piercing; accidental needle-stick; or sharing a toothbrush or razor with an infected person. Receiving a blood transfusion in the U.S. used to be another common mode of transmission, during the 1980s and earlier, prior to effective screening of donor blood for the virus.

Chronic hepatitis B currently affects an estimated 1.25 million people in the U.S., resulting in approximately 5,000 deaths each year.[1] Recent estimates from the World Health Organization indicate that more than 350 million people worldwide have chronic hepatitis B, out of 2 billion infected with the virus.[2] In particular, individuals from parts of the world where hepatitis B is endemic, such as parts of Asia and sub-Saharan Africa, are at increased risk of developing chronic hepatitis B, which is the leading cause of cirrhosis and hepatocellular carcinoma worldwide. People infected with the human immunodeficiency virus (HIV) are also at high risk of being co-infected with HBV, due to common, bloodborne transmission routes.

**Discovery of a Bloodborne Threat to Liver Health**

Hepatitis epidemics, which likely included hepatitis B as one cause, have spanned the course of human history, dating back to antiquity and observations on an epidemic of jaundice by Hippocrates. Yet it wasn't until 1883 that a German scientist first described what was later thought to be this particular form of viral hepatitis in a group of people who had developed jaundice after receiving a smallpox vaccine

## STORY OF DISCOVERY

prepared from human blood. Similarly, an outbreak of hepatitis-related jaundice affecting approximately 50,000 U.S. Army personnel during World War II was later attributed to HBV infection transmitted through a contaminated yellow-fever vaccine, based on research performed in the late 1980s with support from the Veterans Affairs Medical Center, the NIDDK and the National Cancer Institute (NCI) within the NIH, and the National Research Council.

The infectious agent responsible for these hepatitis outbreaks, the hepatitis B virus, was identified by Dr. Baruch Blumberg while working at the NIH in the 1960s—a discovery that later earned him the Nobel Prize in Physiology or Medicine in 1976. Strangely enough, Dr. Blumberg and his laboratory did not set out to find the virus causing hepatitis B. As part of their research to identify forms of blood proteins that differ across populations or ethnic groups, they were testing blood from hemophilia patients who had received multiple blood transfusions. When proteins in a donor's blood are slightly different from those in a recipient's own blood, the body may mount an immune reaction, including the production of antibodies that stick to the foreign proteins. Thus, the scientists were looking for antibodies in the hemophilia patients as potential markers of differences between their blood proteins and the donors'. In this case, however, the scientists would soon learn that some of the antibodies reflected the presence of a bloodborne infectious agent.

In 1963, Dr. Blumberg and Dr. Harvey Alter identified an antibody in the blood of a patient in New York with hemophilia that reacted against a protein in blood collected from an Australian aborigine. This protein was named the "Australia antigen" or "Au." This finding piqued their curiosity as to why a patient in New York would produce an antibody against a protein found in the blood of an individual so geographically, ethnically, and culturally distinct as an aborigine living in Australia. They went on to test samples from individuals around the globe and found the antigen in some of these as well, more commonly in people who had received multiple blood transfusions or were from Asian or tropical regions.

A clue that the Australia antigen might be linked to liver disease came in early 1966 when Dr. Blumberg's group noted that one patient's blood first tested negative and then positive for the antigen—a shift associated with clinical signs of chronic hepatitis in the form of elevated liver enzymes. Also around this time, one of the technicians in Dr. Blumberg's laboratory developed a case of acute hepatitis, which was accompanied by a positive test for the antigen. Additional clinical studies during the late 1960s in the U.S. and Japan also found hepatitis associated with the Australia antigen. Soon after, blood banks in the U.S. and abroad started screening donors to ensure that this apparent bloodborne cause of hepatitis was not passed on to transfusion recipients.

The Australia antigen was confirmed to be part of a virus causing hepatitis B (now known as HBV) in 1970 by a research group in London that visualized viral particles in blood from patients with hepatitis who had tested positive for the antigen. Once the protein heretofore known as the Australia antigen was revealed to be HBV, scientists realized how the hemophilia patient in New York could harbor antibodies to a protein found in the blood of an Australian aborigine; presumably, both individuals were infected at some point with HBV. In the following years, research would continue to yield illuminating details about HBV. For example, further investigations of the Australia antigen identified it as a protein on the surface of HBV. NIH-supported studies also revealed the distinctive circular shape and other characteristics of the HBV genome. Valuable knowledge of the mechanisms HBV uses for infection and replication, and its overall life cycle, was gained from research in unique animal models

that could simulate human HBV infection, such as ducks, woodchucks, and ground squirrels, as well as from cells grown in the laboratory.

Basic research sponsored by the NIH into understanding HBV components, infection strategy, and resulting disease processes would later prove to be essential as a basis for additional prevention strategies, as well as effective diagnostic and treatment approaches.

Investigations of the disease resulting from HBV infection also showed that chronic hepatitis B could lead also to a form of liver cancer known as hepatocellular carcinoma. For example, in 1981, a study sponsored in part by the NCI of over 20,000 Chinese government workers showed that chronic hepatitis B was strongly associated with development of and death from hepatocellular carcinoma after 5 years. Later, in 2005, the National Institute of Environmental Health Sciences' National Toxicology Program would list the hepatitis B virus as a known human carcinogen in its annual Report on Carcinogens.

**Medical Success Story: Effective Prevention of Hepatitis B**

Soon after discovery of the Australia antigen, researchers developed tests to detect HBV in blood that could be applied to diagnosis and screening of populations for the infection. Screening of the donor blood supply for the virus had an important impact on reducing disease transmission in patients requiring transfusions. But for prevention in the general population, a vaccine was needed.

Basic research on the natural history of HBV infection led to the preparation in the 1970s and 1980s of the first hepatitis B vaccines based on heat-inactivated and blood plasma-derived viruses. Clinical research on the plasma-derived vaccine conducted with NIH support showed that it was effective at protecting against HBV infection.

Researchers later developed an improved, "recombinant" version of the vaccine by inserting the hepatitis B surface protein gene into yeast or mammalian cells, which facilitated its purification and preparation for the vaccine. These vaccines also protect against infection by the hepatitis D virus, which requires HBV in order to replicate.

Since the establishment in the U.S. in the 1980s of vaccination programs and donor blood screening for hepatitis B, new cases of acute hepatitis B have declined by more than 80 percent.[3] The immunization strategy initially recommended by the Centers for Disease Control and Prevention (CDC) in 1991 entailed universal vaccination of children. In 1992, Federal programs began routine hepatitis B vaccination of infants, and vaccination of adolescents was added in 1995. The vaccine is also currently recommended for individuals in high-risk groups, such as family members of patients with chronic hepatitis B and individuals who inhabit or emigrate from parts of the world with high rates of infection. Worldwide, beginning in the 1990s, the World Health Organization has called for all countries to add the hepatitis B vaccine to their national immunization programs, which presents a challenge in many parts of the developing world. Multi-national public-private partnerships, such as the Global Alliance for Vaccines and Immunization, are working to improve vaccination rates in these areas.

**Many Treatment Options for Hepatitis B**

Early trials of therapy for hepatitis B focused on the immune-cell chemical interferon. Studies conducted during the 1970s through the 1990s, in the U.S. with NIH support and also abroad, demonstrated the efficacy of treating hepatitis B with interferon, which decreases the stability of HBV genetic material, as well as viral assembly. However, interferon carries potential side effects, including fever, fatigue, headache and muscle aches, and depression. More recently, advances in understanding the viral life cycle and pathogenesis of hepatitis B have paved

the way for identifying new therapeutic agents known as nucleoside/nucleotide analogues, some of which were originally developed to treat HIV infection. These drugs protect against hepatitis B by directly inhibiting replication of HBV through targeting its polymerase enzyme. Currently, seven antiviral drugs are approved by the FDA to treat hepatitis B: interferon-alpha, peginterferon, lamivudine, adefovir, entecavir, telbivudine, and tenofovir. However, no definitive guidance yet exists on the most effective use of these drugs, either alone or in combination. Other issues that remain to be resolved concerning use of these drugs against hepatitis B include how to ensure a lasting response once treatment is stopped and how to avoid the development of viral resistance with long-term treatment, in which the virus mutates over time to escape suppression by the antiviral drug. The drugs also differ in terms of efficacy, safety, likelihood of viral resistance development, method and frequency of administration, and cost. The many HBV types (genotypes) in existence also affect response to therapy and disease progression. New antiviral therapies are currently being tested against hepatitis B in clinical trials.

In addition to pharmaceutical agents, liver transplantation is an effective treatment for individuals with hepatitis B who develop cirrhosis and end-stage liver disease. However, organs for transplant remain in short supply.

To resolve the many issues concerning optimal use of available therapies against hepatitis B, the NIDDK has sponsored several consensus-building conferences that bring together experts in the field to make recommendations based on available evidence. For example, in September 2000 and in April 2006, the NIH sponsored workshops to review current health care practices and develop recommendations for optimal management of hepatitis B. Proceedings from these meetings were published in scientific journals. In October 2008, the NIDDK convened an NIH Consensus Development Conference on Management of Hepatitis B together with the NIH Office of Medical Applications of Research, The Johns Hopkins University School of Medicine, and other entities within the NIH and the Department of Health and Human Services.

The purpose of this 3-day conference was to examine important issues in hepatitis B therapy, including which groups of patients benefit from treatment and at what point during treatment, as well as which groups do not show a benefit. The external experts serving on the conference panel addressed major questions regarding hepatitis B management related to current burden, natural history, benefits and risks of current treatment options, who should or should not be treated, appropriate measures to monitor treatment, and the greatest needs and opportunities for future research on hepatitis B. Additional information on this conference is provided in the accompanying feature on “NIH Consensus Development Conference on Management of Hepatitis B.”

**More To Discover Through Research**

Despite the impressive scientific gains made over the past decades toward preventing and treating hepatitis B, much remains to be learned about this disease, including details of the disease processes associated with HBV infection, as well as ways to optimize approaches to treatment and control. To further advance knowledge of hepatitis B, the NIDDK is funding the Hepatitis B Clinical Research Network. Established in fall 2008, the Network consists of 12 clinical centers, a data coordinating center, a virology center, and an immunology center. The Network is conducting translational research on chronic hepatitis B, focusing on understanding disease processes and applying this knowledge to more effective strategies to treat and control the disease. Its focus has been informed by the research recommendations of recent NIH-sponsored meetings and planning activities on this topic.

## STORY OF DISCOVERY

Through scientific endeavors such as the Hepatitis B Clinical Research Network and investigator-initiated research, conferences, and research planning efforts including the trans-NIH *Action Plan for Liver Disease Research* and the new National Commission on Digestive Diseases' research plan, the NIH is now building upon the extraordinary legacy of past research advances to make additional contributions toward alleviating the burden of hepatitis B, in the U.S. and throughout the world.

[1] *http://www.cdc.gov/hepatitis/PDFs/disease_burden.pdf*

[2] *http://www.who.int/mediacentre/factsheets/fs204/en*

[3] *http://www.cdc.gov/ncidod/diseases/hepatitis/resource/PDFs/disease_burden.pdf*

Additional information on hepatitis B and research progress on this disease is available through the following NIH resources:

- NIH Fact Sheet on Hepatitis B, available at: *http://www.nih.gov/about/researchresultsforthepublic/HepatitisB.pdf*
- Materials for the public provided by the National Digestive Diseases Information Clearinghouse (*http://digestive.niddk.nih.gov/index.htm*), including the brochure "What I Need to Know About Hepatitis B," available in electronic or printed form.

# *NIH Consensus Development Conference on Management of Hepatitis B*

In October 2008, the NIH convened an independent panel of experts for a Consensus Development Conference on the Management of Hepatitis B to weigh available evidence on managing hepatitis B and to offer recommendations concerning future research. This conference examined important issues in hepatitis B therapy, including which groups of patients benefit from currently available treatment and at what point during treatment, as well as which groups do not show a benefit. The NIDDK and NIH Office of Medical Applications of Research, and the Johns Hopkins University School of Medicine sponsored the conference, with additional support from the National Cancer Institute, National Institute of Allergy and Infectious Diseases, Centers for Disease Control and Prevention, and Food and Drug Administration.

The consensus panel included experts in fields such as hepatology and liver transplantation, gastroenterology, public health and epidemiology, infectious diseases, pathology, oncology, family practice, internal medicine, and biostatistics, as well as a public representative. The panel's deliberations were informed by presentations during the conference by experts in the field of hepatitis B management, a review of the published medical literature performed by the Agency for Healthcare Research and Quality, and input from conference participants.

Based on available evidence, the panel recommended several avenues for future research relating to hepatitis B management and control, including:

- prospective clinical studies to define the natural history;
- large randomized clinical trials, including those using placebo controls, to test therapies alone and in combination in terms of health outcomes such as liver failure, cancer, and death;
- identification of elevated hepatitis B virus DNA in blood and elevated liver enzymes as the most important indicators for progression to cirrhosis and liver cancer;
- prevention of disease transmission through routine hepatitis B screening for new immigrants who arrive from countries where hepatitis B prevalence is greater than two percent;
- identification of patients with hepatitis B who should or should not be treated based on whether active disease is present; and
- pursuit of translational research on hepatitis B through the new Hepatitis B Clinical Research Network, the research directions of which will be informed by the conference's recommendations.

As an independent report, not a statement of NIH policy, the panel's statement will be used to inform planning of future research on these important challenges and opportunities in hepatitis B management. The panel's full statement and additional information about this consensus conference are available at: *http://consensus.nih.gov/2008/2008HepatitisBCDC120main.htm*

# Liver Disease in Alpha-1-Antitrypsin Deficiency: Organ-specific Complications Arise from a Misfolded Protein

*Dr. David Perlmutter*

*Dr. David Perlmutter is a Vira I. Heinz Professor and Chairman of Pediatrics at the University of Pittsburgh. He is also the Physician-in-Chief and Scientific Director of Children's Hospital of Pittsburgh. Dr. Perlmutter has carried out basic research on alpha-1-antitrypsin deficiency for more than 20 years. His work has led to many new concepts about the underlying causes of liver disease in this genetic condition and has suggested several new concepts for approaches to prevent chronic liver injury, liver cancer, and lung disease that sometimes result from alpha-1-antitrypsin deficiency. Dr. Perlmutter spoke at the January 2008 meeting of the NIDDK Advisory Council to share some insights from his ongoing studies of alpha-1-antitrypsin deficiency. Following are highlights of that presentation.*

Alpha-1-antitrypsin deficiency (a condition also referred to as Alpha-1) is a genetic disorder caused by defective production of the protein alpha-1-antitrypsin (alpha-1AT). It affects about 1 in every 1,800 live births.[1] In normal individuals, alpha-1AT protein is produced in the liver and secreted into the bloodstream. Its main site of action is in the lungs, where it protects the delicate tissue from damage. People with Alpha-1 carry a mutation in the gene encoding alpha-1AT, which results in a protein that retains some of its biological function but is poorly secreted, and thus does not reach the lungs and may accumulate—sometimes forming large aggregates—within the liver.

Mutant alpha-1AT can cause two different medical problems: pulmonary complications such as emphysema may arise because the protein does not perform its function in the lungs; and liver complications such as inflammation and cancer may arise because the mutant protein can build up in the liver. While lung complications are hallmarks of Alpha-1, most patients do not develop serious liver disease; in fact, only 8 to 10 percent of the people with Alpha-1 will do so. This wide variation in the severity of liver symptoms among people with Alpha-1 strongly suggests that additional genetic and/or environmental variables contribute to the development of clinical liver disease. The identity of these factors is unknown. One hypothesis Dr. Perlmutter posed was whether "protected" individuals—those who carry the alpha-1AT mutation but do not develop liver disease—are somehow able to metabolize the mutant alpha-1AT, while patients who are susceptible to liver disease are not. The first questions Dr. Perlmutter addressed concerned the mechanisms by which this mutant protein was degraded in the liver, and whether these pathways were less effective in people whose livers have aggregates of mutant alpha-1AT.

**Alpha-1AT Processing in the Liver**

Using a series of experiments in cultured cells, Dr. Perlmutter and his colleagues found that a metabolic pathway known as the "autophagic pathway" was involved in the degradation of mutant alpha-1AT in the liver. Autophagy is the degradation of a cell's own components by its internal digestive pathways—literally, autophagy is a process by which a cell eats part of itself. It is a tightly-regulated process that plays a part in normal cell growth and metabolism and helps to maintain a balance between the synthesis, degradation, and recycling of cellular components. It is also a major mechanism by which a cell under stress—starvation, for example—reallocates scarce nutrients to essential processes. The autophagic

pathway seemed to be particularly important in the disposal of the very large aggregates of protein found when very high levels of the mutant protein were produced, as the aggregates of alpha-1AT were able to activate the autophagic response.

**Other Pathways for Disposing of Mutant, Misshapen, or "Misfolded" Proteins**

Dr. Perlmutter's research team next turned to two other well-characterized cellular pathways activated in response to the accumulation of misfolded proteins in general to see if they were involved in the metabolism of mutant alpha-1AT. The first was the "unfolded protein response" pathway, which is activated in response to the presence of misfolded or defective proteins. When the researchers looked at markers for activation of the unfolded protein response pathway, however, they were unable to detect increased activity in the presence of mutant alpha-1AT. A second pathway, the "ER overload response" pathway, is activated when the endoplasmic reticulum—a specialized area within the cells where proteins are prepared for secretion—becomes "backed up" with proteins that cannot get out of the cell. In contrast to the unfolded protein response, the ER overload response pathway did show increased activity in the presence of the mutant alpha-1AT.

**Identification of a Novel Pathway Involved in Alpha-1AT Metabolism**

The researchers next asked whether there were any other, previously unknown pathways that might also be involved in a cell's disposal of mutant, misfolded proteins. They reasoned that, when faced with a potentially toxic accumulation of mutant alpha-1AT, a cell may turn on or off certain genes to regulate various metabolic pathways, some of which would help it dispose of the mutant protein. Thus, the researchers engineered mice to produce mutant alpha-1AT in their livers in an inducible manner, and then analyzed the patterns of gene expression (the extent to which genes are turned on or off) in the mouse livers in the absence and presence of the mutant protein. When the mutant alpha-1AT was produced, the expression of 75 liver genes was increased, and the expression of 131 was decreased. Analysis of these response patterns found that these changes in expression involved genes that play a role in various cellular processes.

One gene whose expression was markedly increased in these mice in the presence of mutant alpha-1AT is the "regulator of G-protein signaling 16," also known as RGS16. G-proteins are important mediators of intracellular signals, so changes in the expression of a gene that modulates G-protein activity could have potentially far-reaching effects on a cell. The increase in RGS16 gene expression was associated strongly with the presence of aggregates of the mutant alpha-1AT in the mouse livers. Similar changes in RGS16 expression were seen in samples of human livers from individuals with Alpha-1.

RGS16 seems to be activated in response to the aggregation of mutant alpha-1AT that characterizes Alpha-1 in individuals with liver disease. Therefore, it may be an excellent marker for the distinct form of metabolic stress seen in these patients. RGS16 may also represent a key player in a novel pathway through which autophagy is regulated, making it a potential target for the development of future therapeutic strategies. Future research will further characterize the role played by RGS16 in modulating cellular metabolism in the presence of mutant alpha-1AT.

**A New Model System To Study Alpha-1**

Dr. Perlmutter next described an innovative series of experiments using a model organism to study Alpha-1, the roundworm *Caenorhabditis elegans*. *C. elegans* is a small (about 1 mm long), transparent worm that is used extensively by biomedical researchers. This organism offers a number of benefits as a disease model, both biological and practical. Its genome has been fully sequenced and its genes and their functions are similar to those of mammals. It is relatively easy to work with, reproducing every 3 days and generating many offspring, and it is transparent—facilitating observation

of its inner workings. There are also substantial existing genetic and molecular tools that researchers can employ when using this organism.

Dr. Perlmutter's collaborators, Drs. Gary Silverman and Stephen Pak, constructed fusions of various alpha-1AT genes with a gene encoding "green fluorescent protein," a marker often used by biologists to allow easy visualization of a protein. When they inserted the normal alpha-1AT gene, fused with green fluorescent protein, into the intestinal cells of worms, they saw green fluorescence in the interior of the intestinal tract, indicating that the protein was being properly secreted out of the cells. (In *C. elegans*, the intestine performs many of the functions of the liver.) In worms that produced fusions with the mutant alpha-1AT gene, the green fluorescence was retained within the cell in globules, indicating a failure to secrete, and intracellular aggregation of the protein. Additionally, worms expressing the mutant gene exhibited arrested development at the larval stage, and did not live as long as normal worms or worms expressing the normal alpha-1AT.

But what is responsible for the physiological manifestation of the mutant alpha-1AT? To answer this question, the researchers used a slightly different mutant of alpha-1AT that is non-functional and accumulates within the cells, but does not form aggregates. When this alternate mutant was inserted into worms, there was no growth arrest at the larval stage in these worms. This finding indicates that some of the biological effects seen in worms with the original mutant alpha-1AT require not only the retention of the protein within the liver cells, but also the formation of protein aggregates within cells.

Dr. Perlmutter outlined the next steps in the research he is doing with Drs. Silverman and Pak: the adaptation of the worm model for high-throughput screening for genetic modifiers of disease severity and for potential drug candidates. He described technology that could automatically sort through and characterize large numbers of these tiny worms. Such an approach would allow the rapid screening of hundreds of potential genetic and/or pharmacologic approaches to address the problems seen in Alpha-1.

**Conclusions**

In a subset of patients with Alpha-1, accumulation of aggregates of the mutant protein in the liver causes damage and increases the risk of cancer. The risk for liver disease is heavily influenced by genetic and/or environmental factors that may impact various degradation pathways and other protective cellular responses. Dr. Perlmutter and his colleagues discovered that the autophagic pathway appears to play a particularly important role in disposing of the mutant protein. Finally, Dr. Perlmutter's development of a novel worm model amenable to high-throughput screening may expedite the identification of genetic modifiers and new therapeutic agents.

*Dr. Perlmutter acknowledged the contributions of his collaborators in research, Drs. Silverman and Pak. Gary Silverman, M.D., Ph.D. is The Twenty Five Club Endowed Professor of Pediatrics, Professor of Cell Biology and Physiology at University of Pittsburgh School of Medicine and Chief of Newborn Medicine at University of Pittsburgh Medical Center. Stephen Pak, Ph.D. is Assistant Professor of Pediatrics at University of Pittsburgh School of Medicine. Drs. Silverman and Pak have worked with Dr. Perlmutter to characterize the* C. elegans *model organism in order to elucidate the role of cellular signaling molecules in regulating cell metabolism.*

[1] *Perlmutter DH, et al: Molecular pathogenesis of alpha-1-antitrypsin deficiency-associated liver disease: a meeting review. Hepatology 45: 1313-1323, 2007.*

## PATIENT PROFILE

# Mariah Watts

*Bariatric Surgery—Weighing the Pros and Cons*

**Mariah Watts**

By the time she was 16 years old, Mariah Watts was five-feet eight-inches tall, suffered from sleep apnea, had pre-diabetes—and weighed over 350 pounds. In addition to being unable to breathe well while sleeping (sleep apnea) and facing a high risk for type 2 diabetes, she also had a difficult time with many ordinary activities, such as sitting in seats at school. "I was always so self conscious," says the now 17-year-old who struggled with being overweight for many years, as well as with some of the serious health conditions that accompany obesity. "I wouldn't wish this on anybody," she says.

In January, 2008, Mariah underwent bariatric surgery, an operation that promotes weight loss. Seven months later, Mariah was down to 234 pounds, no longer had trouble breathing or sleeping, and no longer had pre-diabetes. Both she and her family are delighted with these results. "The surgery has changed Mariah's attitude and given her a whole new lease on life," says her mother, Mazie.

Although bariatric surgery can have dramatic health benefits, researchers caution that it also carries substantial risks.

Today, Mariah is voluntarily participating in the Teen Longitudinal Assessment of Bariatric Surgery, Teen-LABS, an observational study supported by the NIDDK to help determine if bariatric surgery is an appropriate treatment option for extremely overweight teens. Mariah enrolled in the study just before her surgery, enabling researchers to assess her health and quality of life in great detail both before and after the surgery, for comparison. Teen-LABS is being conducted at several medical centers in the U.S. and is led by Dr. Thomas Inge, a pediatric surgeon at the Cincinnati Children's Hospital Medical Center.

*Although bariatric surgery can have dramatic health benefits, researchers caution that it also carries substantial risks.*

Although bariatric surgery is not a common procedure in adolescents, its use has been increasing in clinical practice as a treatment for very severe obesity in this age group. Thus, the NIDDK is supporting the Teen-LABS study to collect health outcome data on adolescents who were already planning to have bariatric surgery, so as to evaluate its risks and benefits. (NIDDK does not pay for the surgery.) With these data, future adolescent patients, their parents, and health care teams will be able to make more informed, evidence-based decisions.

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**Bariatric Surgery and Adolescents**

Bariatric surgery promotes substantial weight loss by restricting food intake and, in some cases, decreasing the amount of calories and nutrients the body absorbs. This surgery can also reduce the risk for—and in some cases even reverse—type 2 diabetes, a devastating disease.

However, the surgery also comes with substantial risks. Early complications may include bleeding, infection, leaks from the site where the intestines are sewn together (in certain operations), and blood clots in the legs that can progress to the lungs and heart. Later complications may include malnutrition, especially in patients who do not take their prescribed vitamins and minerals, hernias, and other health problems.

*Teen-LABS, an observational study supported by the NIDDK, enrolls teens who are already planning to have bariatric surgery. The study collects health outcome data so that future adolescent patients, their parents, and health care teams will be able to make more informed, evidence-based decisions.*

Therefore, recommended criteria for accepting an adolescent as a candidate for bariatric surgery are more stringent than for adults. Teens considered for this surgery have extreme obesity (as defined by body mass index, a measure of weight relative to height). Many of the adolescents who have enrolled in Teen-LABS so far have weighed 300 or 400 pounds, or more. Additionally, the teens who are candidates for this surgery have serious weight-related health problems, such as sleep apnea, type 2 diabetes, or other conditions. Furthermore, they must have tried other approaches to lose weight, without success.

In addition, experts recommend that potential adolescent patients and their parents should be evaluated to see whether they are emotionally prepared for the operation and the lifestyle changes they will need to make. A teen must be motivated and have strong family support, because the fact is bariatric surgery is not an easy way out to control weight. Even after surgery, patients will have to continue follow-up with health care professionals throughout their lives—and they will need to maintain a lifetime of healthy habits, including eating less food and exercising regularly.

Mariah says she is committed to making those life-long changes.

**A Family Decision**

A number of factors contribute to obesity. These include environmental and behavioral factors as well as genetic susceptibility. In Mariah's case, as in most obese individuals, the exact contribution of each is not known. "My father, his sisters and their children are all big people," says Mariah's mother. In addition, she says Mariah struggled with eating as a child. "I ate constantly," adds Mariah. "I'd get full and 30 minutes later would want to eat again."

By age 11, Mariah weighed between 230 and 250 pounds and started dieting. She went to nutritionists and tried different types of diets. But nothing seemed to work. As time passed, Mazie observed her daughter becoming increasingly depressed and lethargic. "I started taking her to doctors and dieticians," says Mazie. "It was painful to watch her go through all of this."

Finally, as a last resort, Mazie encouraged her daughter to consider bariatric surgery. Mariah, desperate to lose weight, was agreeable to the idea—and did her homework. Mariah researched the procedure so thoroughly over the internet "that I could have done the surgery myself," she adds with a smile.

There are several types of bariatric surgery. Mariah underwent the Roux-en-Y gastric bypass version.

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This surgery limits Mariah's food intake because it reduced her stomach to the size of a small pouch. It also decreases the absorption of calories and nutrients by preventing food from contacting most of the stomach and the upper part of her small intestine. The surgery changes the digestive tract so that food is routed directly from the small stomach pouch to a lower part of the small intestine.

According to Mariah, the worst thing about having had the surgery is the fact that she sometimes will eat more than her post-surgery digestive tract can handle, leaving her feeling weak, dizzy, and sweaty. "It's horrible. It feels like you're dying," she says, adding that the feeling lasts for about 25 or 30 minutes. She also adds that she's been learning how to restrict her eating habits further. "When I go to a restaurant now, I know what to eat and what not to eat," says Mariah. And in general, she says she's eating much healthier.

As a result, she has much more energy than she had before. "Before the surgery I used to come home from school, lay around, sleep or watch TV," says Mariah. "Now I find something physical to do every day. My little sister likes when I jump on the trampoline with her. This summer I swam in the family pool all the time and really enjoyed it. And when my friends come over, we go for walks; stuff I didn't do before."

"Mariah is an entirely different person," says her mother. "She's dating for the first time. Life is just more normal. I just see so much joy in her."

**Participating in the Teen-LABS Study**

Shortly after Mariah and her parents decided to go forward with the surgery, Mariah's parents encouraged her to take part in the Teen-LABS study. "The study was something new to us," says Mazie. "We had never experienced anything like this, but we thought it was important for us to get involved so that other parents and their teenage children could make more informed decisions about whether or not to have this type of surgery." Mariah agrees.

*"...We thought it was important for us to get involved [in the Teen-LABS study] so that other parents and their teenage children could make more informed decisions about whether or not to have this type of surgery."*

The Teen-LABS study began in 2007 and is based on the related LABS study, which is assessing risks and benefits of bariatric surgery in adults and is also supported by NIDDK. Over the span of 5 years, Teen-LABS will collect data on teens like Mariah, who had bariatric surgery as adolescents, and will compare this information with data from adult participants in the LABS study who had bariatric surgery as adults, after having been obese since their teen years. Teen-LABS and LABS researchers are collecting information on the pre-operative and 2-year post-operative status of adolescent and adult participants, including measuring body composition, body fat, cardiovascular disease risks, sleep apnea episodes, diabetes indicators, depressive symptoms, quality of life, eating habits, and nutritional status. Additionally, the investigators are storing serum and plasma (components of blood), urine, and genetic samples for future studies.

What would Mariah tell other teens and their families who are considering bariatric surgery? "They need to do their homework first," she says. This homework will become more informative in the future with new data, thanks to her participation in Teen-LABS, along with the other study volunteers. "And," Mariah adds, "they need to commit to eating better and exercising more."

## PATIENT PROFILE

**For additional information about bariatric surgery:**

Bariatric Surgery for Severe Obesity (NIDDK publication)—
*http://win.niddk.nih.gov/publications/gastric.htm*

For more information on the Teen-LABS study—
*http://www.cincinnatichildrens.org/research/project/teen-labs*

As part of its multifaceted research portfolio on the causes, prevention, and treatment of obesity, the NIDDK additionally encourages research to understand how bariatric surgery has its effects. Certain bariatric surgical procedures are associated with remission of diabetes soon after surgery, even before substantial weight loss has occurred. Through increased understanding of potential mechanisms by which alterations in the gut reduce risk for or ameliorate type 2 diabetes in obese individuals, researchers may be able to improve surgical and nonsurgical therapies for obesity-related health conditions.