Yale Liver Center
Vol. 6, No. 3 | September 2019

Inside this issue:

2019-2020 Pilot Project Awards

Jittima Weerachayaphorn, PhD
Visiting Assistant Professor
Internal Medicine, Digestive Diseases

" Role of inositol 1,4,5-trisphosphate receptors in alcoholic hepatitis"

Alcoholic hepatitis is the most severe form of alcoholic liver disease and presents with high morbidity and mortality. Emerging evidence suggests that cholestasis is a dangerous complication in alcoholic hepatitis. Cholestasis that occurs in alcoholic hepatitis is due in part to cholangiocytes and results from loss of the type 3 inositol trisphosphate receptor (ITPR3) expression in cholangiocytes. Alcoholic hepatitis is associated with a neutrophilic inflammatory infiltrate in hepatic lobules and direct cell-to-cell contact between neutrophils and cholangiocytes prerequisites for the loss of ITPR3 expression. The goal of this project is to determine how neutrophils interact with cholangiocytes to decrease ITPR3 expression and that cause cholestasis and explore the intracellular pathways that decrease ITPR3 expression in cholangiocytes when they interact with neutrophils. This study will increase our understanding of the pathogenesis of cholestasis that occurs in alcoholic hepatitis, may establish a new paradigm for a new role for neutrophils in alcoholic hepatitis, and may define new targets for the treatment of alcoholic hepatitis.

John Onofrey, PhD
Associate Research Scientist
Radiology & Biomedical Imaging

"Automated Hepatic Lesion Detection and LI-RADS Prediction using Deep Learning for Clinical Decision Support"

Dr. John Onofrey, Assistant Professor in the Departments of Radiology and Biomedical Imaging and of Urology, was awarded a 2019 Yale Liver Center Pilot grant to develop novel machine learning methods to automatically diagnose liver cancer. The overarching goal of this project is to develop methods to incorporate both imaging biomarkers and clinical indicators into an automated clinical diagnostic aid. The innovation in this project lies in the use of deep learning to automatically predict the classification of hepatic lesions according to the Liver Imaging Reporting and Data System (LI-RADS). This project, which brings together a multidisciplinary team with expertise in diagnostic and interventional radiology, engineering, biomedical data science, and hepatology, has the potential to improve clinical efficiency and reduce variation in liver cancer diagnosis.

Shi-Ying Cai, PhD
Senior Research Scientist
Internal Medicine, Digestive Diseases

"Role of Ca2+/NFAT signaling pathway in cholestatic liver injury"

Bile acids initiated inflammatory response plays an important role in the pathogenesis of cholestatic liver injury, yet the detailed mechanisms remain to be elucidated. My preliminary data indicates that elevated levels of bile acids cause ER-stress and mitochondrial damage and result in dysregulation of Ca2+ signaling in hepatocytes. Therefore, I hypothesize that activated Ca2+/NFAT (nuclear factor of activated T-cells, a transcription factor) signaling pathway stimulates the expression of hepatic inflammatory cytokines in cholestasis. In this pilot project, I propose to assess NFAT's role in cholestatic liver injury by examining both hepatic expression of NFAT and inflammatory cytokines in vivo in the livers of patients with primary biliary cholangitis and primary sclerosing cholangitis and ARE-Del-/- mice (a novel model of primary biliary cholangitis) and in vitro in primary hepatocyte cultures from humans and mice. Completion of this study may reveal novel mechanisms of cholestatic liver injury and help to develop new strategies for treating cholestasis.

LIVER CENTER THEMES

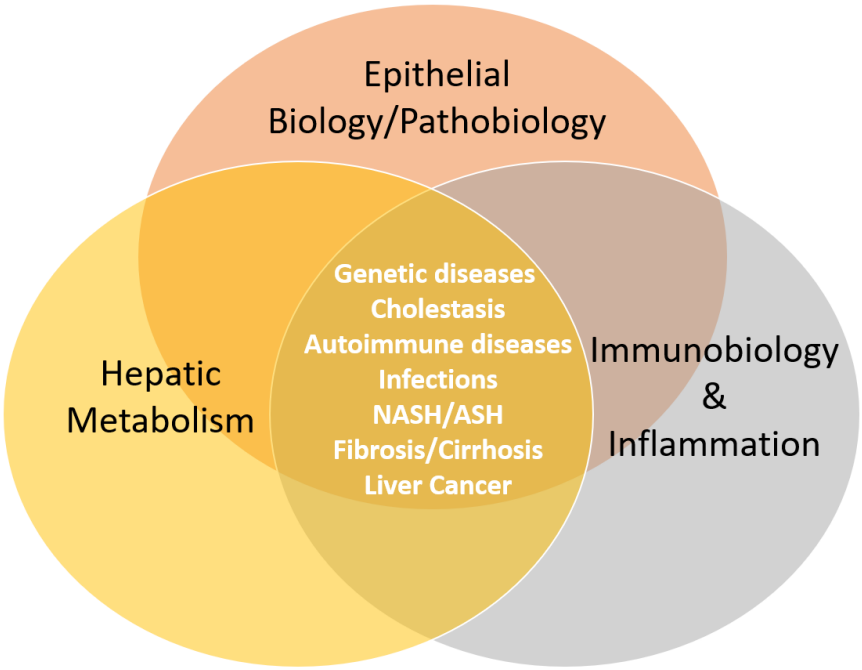
The Research Base of the Liver Center focuses on three broad translational themes. These include:

(1) Immunobiology and inflammation

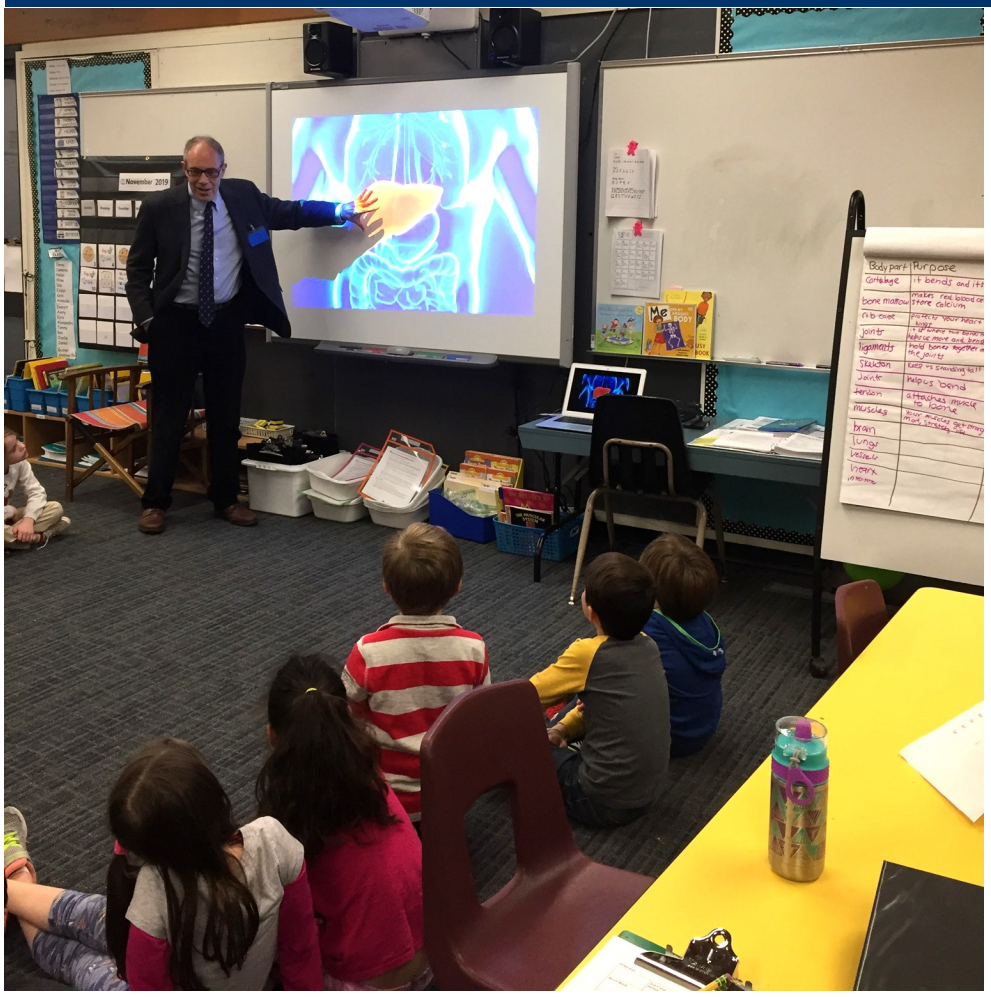
(2) Hepatic metabolism

(3) Epithelial biology and pathobiology

The major areas of liver disease examined within these translational themes include autoimmune diseases, cholestasis, fibrosis/ cirrhosis, genetic diseases, infections, liver cancer, and NASH/ASH. Many of our investigators have research interests that span multiple themes.



IT'S NEVER TOO EARLY TO LEARN ABOUT THE LIVER



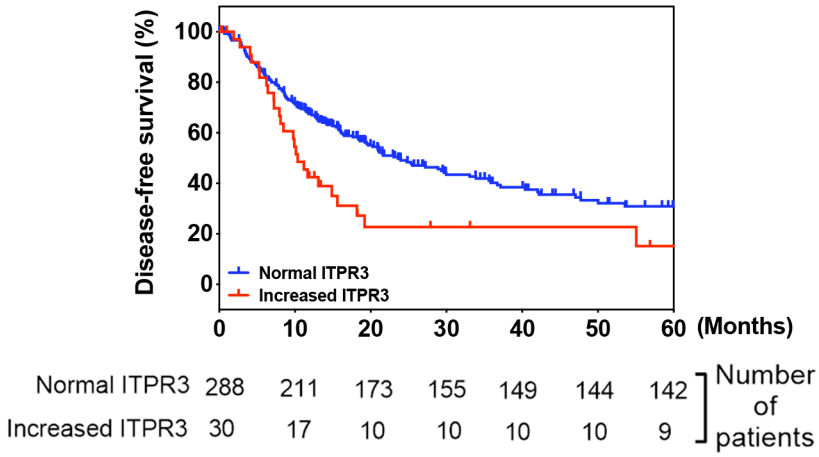
Center Director, Dr. Michael H. Nathanson, recently gave a talk to 1st graders at A.W. Cox Elementary School in Guilford, CT about what the liver does and how to make sure your liver stays healthy.

FEATURED PUBLICATIONS

Expression of the type 3 InsP3 receptor is a final common event in the development of hepatocellular carcinoma

Guerra MT, Florentino RM, Franca A, Lima Filho AC, Dos Santos ML, Fonseca RC, Lemos FO, Fonseca MC, Kruglov E, Mennone A, Njei B, Gibson J, Guan F, Cheng YC, Ananthanarayanan M, Gu J, Jiang J, Zhao H, Lima CX, Vidigal PT, Oliveira AG, Nathanson MH, Leite MF.

Gut. 2019; 68:1676-1687



Hepatocellular carcinoma (HCC) is the second leading cause of cancer death worldwide. Several types of chronic liver disease predispose to HCC, and several different signaling pathways have been implicated in its pathogenesis, but no common molecular event has been identified. Ca2+ signaling regulates the proliferation of both normal hepatocytes and liver cancer cells, so we investigated the role of intracellular Ca2+ release channels in HCC. The type 3 inositol 1, 4, 5-trisphosphate receptor (ITPR3) was absent or expressed in low amounts in hepatocytes from normal liver, but was expressed in HCC specimens from three independent patient cohorts, regardless of the underlying cause of chronic liver disease, and its increased expression level was associated with poorer survival. The ITPR3 gene was heavily methylated in control liver specimens but was demethylated at multiple sites in specimens of patient with HCC. Administration of a demethylating agent in a mouse model resulted in ITPR3 expression in discrete areas of the liver, and Ca2+ signaling was enhanced in these regions. In addition, cell proliferation and liver regeneration were enhanced in the mouse model, and deletion of ITPR3 from human HCC cells enhanced apoptosis. These results provide evidence that de novo expression of ITPR3 typically occurs in HCC and may play a role in its pathogenesis.

CELA2A mutations predispose to early-onset atherosclerosis and metabolic syndrome and affect plasma insulin and platelet activation

Esteghamat F, Broughton JS, Smith E, Cardone R, Tyagi T, Guerra M, Szabó A, Ugwu N, Mani MV, Azari B, Kayingo G, Chung S, Fathzadeh M, Weiss E, Bender J, Mane S, Lifton RP, Adeniran A, Nathanson MH, Gorelick FS, Hwa J, Sahin-Tóth M, Belfort-DeAguiar R, Kibbey RG, Mani A.

Nat Genet. 2019; 51:1233-1243.

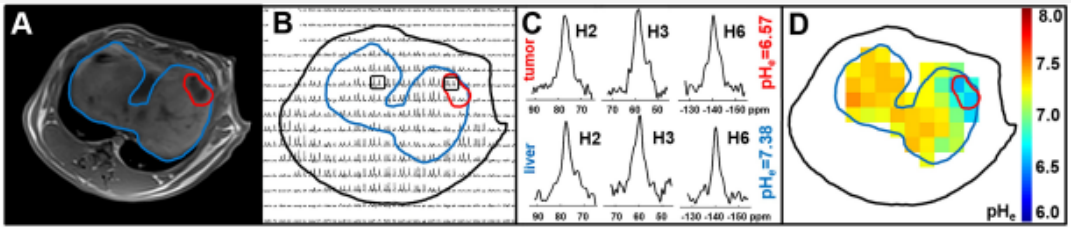
Metabolic syndrome is a cluster of inherited risk factors for coronary artery disease (CAD), which in outlier kindreds with early-onset CAD may be caused by single-gene mutations. In this study, we present a cohort of 30 North European index cases with early-onset CAD and metabolic syndrome. Combined linkage and gene burden analyses led to the identification of multiple independent mutations in CELA2A, which encodes the chymotrypsin-like elastase family member 2A (CELA2A). CELA2A was primarily known as an ‘exocrine’ pancreatic elastase that preferentially cleaves A-acetyl-L-alanyl-L-alanyl-L-alanine/proline methyl-ester and forms a sodium dodecyl sulfate (SDS)-resistant complex with alpha-1-antitrypsin (A1AT). The physiological function of CELA2A outside the exocrine pancreas was not known. Here, we characterize the CELA2A protein in vitro and in vivo, and explore the effects of human mutations on its diverse metabolic functions. Using systems biology, we discovered that CELA2A is a circulating protein that impacts diverse biological processes, including insulin secretion, degradation and sensitivity. Our analyses show that impaired regulation of plasma insulin is a major consequence of disease-inducing CELA2A mutations. The potential to exploit disease pathways makes CELA2A an appealing target for treating diabetes and its complications.

FEATURED PUBLICATIONS

Molecular imaging of extracellular tumor pH to reveal effects of loco-regional therapy on liver cancer microenvironment

Savic LJ, Schobert I, Peters D, Walsh JJ, Laage Gaupp FM, Hamm CA, Tritz N, Doemel LA, Lin M, Sinusas A, Schlachter T, Duncan JS, Hyder F, Coman D, Chapiro J.

Clin Cancer Res. 2019 [Epub ahead of print]



Purpose: To establish MR-based molecular imaging paradigms for the non-invasive monitoring of extracellular pH (pHe) as a functional surrogate biomarker for metabolic changes induced by loco-regional therapy of liver cancer. Experimental Design: Thirty-two VX2 tumor-bearing New Zealand White rabbits underwent longitudinal imaging on clinical 3T-MRI and CT scanners before and up to 2 weeks after complete conventional transarterial chemoembolization (cTACE) using ethiodized oil (Lipiodol) and doxorubicin. MR-spectroscopic imaging (MRSI) was employed for pHe-mapping. Multiparametric MRI and CT were performed to quantify tumor enhancement, diffusion, and Lipiodol coverage post-therapy. Additionally, incomplete cTACE with reduced chemoembolic doses was applied to mimic under-treatment and exploit pHe-mapping to detect viable tumor residuals. Imaging findings were correlated with histopathological markers indicative of metabolic state (HIF-1α, GLUT-1, LAMP-2) and viability (PCNA, TUNEL). Results: Untreated VX2 tumors demonstrated a significantly lower pHe (6.80±0.09) than liver parenchyma (7.19±0.03, p<0.001). Upregulation of HIF-1α, GLUT-1, and LAMP-2 confirmed a hyperglycolytic tumor phenotype and acidosis. A gradual tumor-pHe increase towards normalization similar to parenchyma was revealed within 2 weeks after complete cTACE, which correlated with decreasing detectability of metabolic markers. In contrast, pHe-mapping after incomplete cTACE indicated both acidic viable residuals and increased tumor-pHe of treated regions. Multimodal imaging revealed durable tumor devascularization immediately after complete cTACE, gradually increasing necrosis, and sustained Lipiodol coverage. Conclusion: MRSI-based pHe-mapping can serve as a longitudinal monitoring instrument for viable tumors. As most liver tumors are hyperglycolytic creating microenvironmental acidosis, therapy-induced normalization of tumor-pHe may be used as a functional biomarker for positive therapeutic outcome.

AVAILABLE CENTER CORE SERVICES

ADMINISTRATIVE

PILOT FEASIBILITY PROGRAM
Pilot grants given annually to promote studies of liver disease

ENRICHMENT PROGRAM
Weekly seminar series, annual Klatskin Lectureship, bi-annual Center retreat

CELLULAR-MOLECULAR

ISOLATED CELL PREPARATIONS
Hepatocytes, cholangiocytes, endothelial cells, stellate cells, portal fibroblasts and hepatic lymphocytes, primarily from mice and rates. Human hepatocytes when available.

PROTEIN & GENE EXPRESSION
Quantitative real time PCR and infrared imaging detection . Altering gene expression in these cells using siRNA transfection and adenovirus infection technologies

IPSC/LIVER ORGANOID
On request, PBMCs are transferred to the Yale Stem Cell Center (YSCC) for reprogramming into iPSC. YSCC will generate at least 3 clones of iPSCs for each PBMC sample. iPSCs can be differentiated into liver cells (biliary cells or hepatocytes) and made available. Liver organoids available upon request

CELL CULTURE FACILITIES
Available for short– and long–term cultures and cell lines

MORPHOLOGY

CONFOCAL, SUPER-RESOLUTION, MULTIPHOTON IMAGING
Leica SP5
Swept-field (Opterra II, Bruker)
Zeiss LSM 710 duo
Vutara 252 super-resolution
Leica SP8 gated STED 3X
Zeiss LSM 880 AiryScan Fast
Bruker Luxendo MuVi SPIM

EPIFLUORESCENCE MICROSCOPY INCLUDING QUANTITATIVE & RATIO IMAGING
Zeiss Axio Observer epifluorescence microscope
Olympus BX51 multi-headed brightfield microscope
Dissecting microscope
Zeiss Discovery 8 SteReo

ELECTRON MICROSCOPY
Tecnai 12. biotwinFEI Tecnai TF20 FEG

CLINICAL-TRANSLATIONAL

BIOSTATISTICAL SUPPORT
Two biostatisticians available for expertise in the design, conduct, and analysis of patient-oriented studies, as well as methodological development, education, and training

BIOSPECIMEN & LIVER BIOPSY REPOSITORY
Recruitment of patients and collection of blood samples

PATIENT REGISTRY
Patient databases on diagnoses including: chronic hepatitis C, cirrhosis, chronic hepatitis B, PBC, autoimmune hepatitis, PSC, hepatocellular carcinoma, NAFLD, and cholangiocarcinoma

Members' Original Recent Publications

Pathobiology of inherited biliary diseases: a roadmap to understand acquired liver diseases.
Fabris L, Fiorotto R, Spirli C, Cadamuro M, Mariotti V, Perugorria MJ, Banales JM, Strazzabosco M. Nat Rev Gastroenterol Hepatol. 2019; 16:497-511. Review. PMID: 31165788

Pathophysiology of Cystic Fibrosis Liver Disease: A Channelopathy Leading to Alterations in Innate Immunity and in Microbiota.
Fiorotto R, Strazzabosco M. Cell Mol Gastroenterol Hepatol. 2019; 8:197-207. Review. PMID: 31075352

Clinical utility of genomic analysis in adults with idiopathic liver disease.
Hakim A, Zhang X, DeLisle A, Oral EA, Dykas D, Drzewiecki K, Assis DN, Silveira M, Batisti J, Jain D, Bale A, Mistry PK, Vilarinho S. J Hepatol. 2019; 70:1214-1221. PMID: 31000363

Cholangiocyte pathobiology.
Banales JM, Huebert RC, Karlsen T, Strazzabosco M, LaRusso NF, Gores GJ. Nat Rev Gastroenterol Hepatol. 2019; 16:269-281. Review. PMID: 30850822

Genetic loss of Tmprss6 alters terminal erythroid differentiation in a mouse model of β-thalassemia intermedia.
Stagg DB, Whittlesey RL, Li X, Lozovatsky L, Gardenghi S, Rivella S, Finberg KE. Haematologica. 2019; 104:e442-e446. PMID: 30819909

Hepatic metabolic adaptation in a murine model of glutathione deficiency.
Chen Y, Golla S, Garcia-Milian R, Thompson DC, Gonzalez FJ, Vasiliou V. Chem Biol Interact. 2019; 303:1-6. PMID: 30794799

The microbiome in systemic autoimmune disease: mechanistic insights from recent studies.
Dehner C, Fine R, Kriegel MA. Curr Opin Rheumatol. 2019; 31:201-207. PMID: 30624285

IRF5 Is Required for Bacterial Clearance in Human M1-Polarized Macrophages, and IRF5 Immune-Mediated Disease Risk Variants Modulate This Outcome.
Hedl M, Yan J, Witt H, Abraham C. J Immunol. 2019; 202:920-930. PMID: 30593537

Liver diseases in the dish: iPSC and organoids as a new approach to modeling liver diseases.
Fiorotto R, Amenduni M, Mariotti V, Fabris L, Spirli C, Strazzabosco M. Biochim Biophys Acta Mol Basis Dis. 2019; 1865:920-928. Review. PMID: 30264693

Intrahepatic Cholangiocarcinoma: Continuing Challenges and Translational Advances.
Sirica AE, Gores GJ, Groopman JD, Selaru FM, Strazzabosco M, Wei Wang X, Zhu AX. Hepatology. 2019; 69:1803-1815. Review. PMID: 30251463

Levels of circulating follicular helper T cells, T helper 1 cells, and the prognostic significance of soluble form of CD40 ligand on survival in patients with alcoholic cirrhosis.
Hollister K, Kusumanchi P, Ross RA, Chandler K, Oshodi A, Heathers L, Teagarden S, Wang L, Dent AL, Liangpunsakul S. Liver Res. 2018; 2:52-59. PMID: 30221017

Author response to Letter to the Editor "Post-paracentesis hemoperitoneum - time to become more careful!"
Hung A, Garcia-Tsao G. Liver Int. 2018; 38:1698-1699. PMID: 30145847

Effects of Endotoxin on Type 3 Inositol 1,4,5-Trisphosphate Receptor in Human Cholangiocytes.
Franca A, Carlos Melo Lima Filho A, Guerra MT, Weerachayaphorn J, Loiola Dos Santos M, Njei B, Robert M, Xavier Lima C, Vieira Teixeira Vidigal P, Banales JM, Ananthanarayanam M, Leite MF, Nathanson MH. Hepatology. 2019; 69:817-830. PMID: 30141207

Mitohormesis in Mice via Sustained Basal Activation of Mitochondrial and Antioxidant Signaling.
Cox CS, McKay SE, Holmbeck MA, Christian BE, Scortea AC, Tsay AJ, Newman LE, Shadel GS. Cell Metab. 2018; 28:776-786.e5. PMID: 30122556

Animal models for cystic fibrosis liver disease (CFLD).
Fiorotto R, Amenduni M, Mariotti V, Cadamuro M, Fabris L, Spirli C, Strazzabosco M. Biochim Biophys Acta Mol Basis Dis. 2019; 1865:965-969. Review. PMID: 30071276

Solute Carrier Organic Anion Transporter Family Member 3A1 Is a Bile Acid Efflux Transporter in Cholestasis.
Pan Q, Zhang X, Zhang L, Cheng Y, Zhao N, Li F, Zhou X, Chen S, Li J, Xu S, Huang D, Chen Y, Li L, Wang H, Chen W, Cai SY, Boyer JL, Chai J. Gastroenterology. 2018; 155:1578-1592. PMID: 30063921

Metformin inhibits gluconeogenesis via a redox-dependent mechanism in vivo.
Madiraju AK, Qiu Y, Perry RJ, Rahimi Y, Zhang XM, Zhang D, Camporez JG, Cline GW, Butrico GM, Kemp BE, Casals G, Steinberg GR, Vatner DF, Petersen KF, Shulman GI. Nat Med. 2018; 24:1384-1394. Erratum in: Nat Med. 2019; 25:526-528. PMID: 30038219

Association Between Aldehyde Dehydrogenase 2 Glu504Lys Polymorphism and Alcoholic Liver Disease.
Chang B, Hao S, Zhang L, Gao M, Sun Y, Huang A, Teng G, Li B, Crabb DW, Kusumanchi P, Wang L, Liangpunsakul S, Zou Z. Am J Med Sci. 2018; 356:10-14. PMID: 29779728

Elevated hepatic expression of H19 long noncoding RNA contributes to diabetic hyperglycemia.
Zhang N, Geng T, Wang Z, Zhang R, Cao T, Camporez JP, Cai SY, Liu Y, Dandolo L, Shulman GI, Carmichael GG, Taylor HS, Huang Y. JCI Insight. 2018; 3. pii: 120304. PMID: 29769440

Prototheca zopfii Colitis in Inherited CARD9 Deficiency.
Sari S, Dalgic B, Muehlenbachs A, DeLeon-Carnes M, Goldsmith CS, Ekinci O, Jain D, Keating MK, Vilarinho S. J Infect Dis. 2018; 218:485-489. PMID: 29659908

Translocation of a gut pathobiont drives autoimmunity in mice and humans.
Manfredo Vieira S, Hiltensperger M, Kumar V, Zegarra-Ruiz D, Dehner C, Khan N, Costa FRC, Tiniakou E, Greiling T, Ruff W, Barbieri A, Kriegel C, Mehta SS, Knight JR, Jain D, Goodman AL, Kriegel MA. Science. 2018; 359:1156-1161. Erratum in: Science. 2018; 360: PMID: 29590047

Screening and Surveillance of Varices in Patients With Cirrhosis.
Jakab SS, Garcia-Tsao G. Clin Gastroenterol Hepatol. 2019; 17:26-29. Erratum in: Clin Gastroenterol Hepatol. 2019; 17:1009. PMID: 29551741

Mif-deficiency favors an atheroprotective autoantibody phenotype in atherosclerosis.
Schmitz C, Noels H, El Bounkari O, Straussfeld E, Megens RTA, Sternkopf M, Alampour-Rajabi S, Krammer C, Tilstam PV, Gerdes N, Bürger C, Kapurniotu A, Bucala R, Jankowski J, Weber C, Bernhagen J. FASEB J. 2018; 32:4428-4443. PMID: 29543531

Cholesterol-enriched membrane microdomains are needed for insulin signaling and proliferation in hepatic cells.
Fonseca MC, França A, Florentino RM, Fonseca RC, Lima Filho ACM, Vidigal PTV, Oliveira AG, Dubuquoy L, Nathanson MH, Leite MF. Am J Physiol Gastrointest Liver Physiol. 2018; 315:G80-G94. PMID: 29471671

Digoxin Suppresses Pyruvate Kinase M2-Promoted HIF-1α Transactivation in Steatohepatitis.
Ouyang X, Han SN, Zhang JY, Dioletis E, Nemeth BT, Pacher P, Feng D, Bataller R, Cabezas J, Stärkel P, Caballeria J, Pongratz RL, Cai SY, Schnabl B, Hoque R, Chen Y, Yang WH, Garcia-Martinez I, Wang FS, Gao B, Torok NJ, Kibbey RG, Mehal WZ. Cell Metab. 2018; 27:339-350.e3.Erratum in: Cell Metab. 2018; 27:1156. PMID: 29414684

Acute kidney injury, but not sepsis, is associated with higher procedure-related bleeding in patients with decompensated cirrhosis.
Hung A, Garcia-Tsao G. Liver Int. 2018; 38:1437-1441. PMID: 29393567

Three-Month Randomized Clinical Trial of Nasal Calcitonin in Adults with X-linked Hypophosphatemia.
Sullivan R, Abraham A, Simpson C, Olear E, Carpenter T, Deng Y, Chen C, Insogna KL. Calcif Tissue Int. 2018; 102:666-670. PMID: 29383408