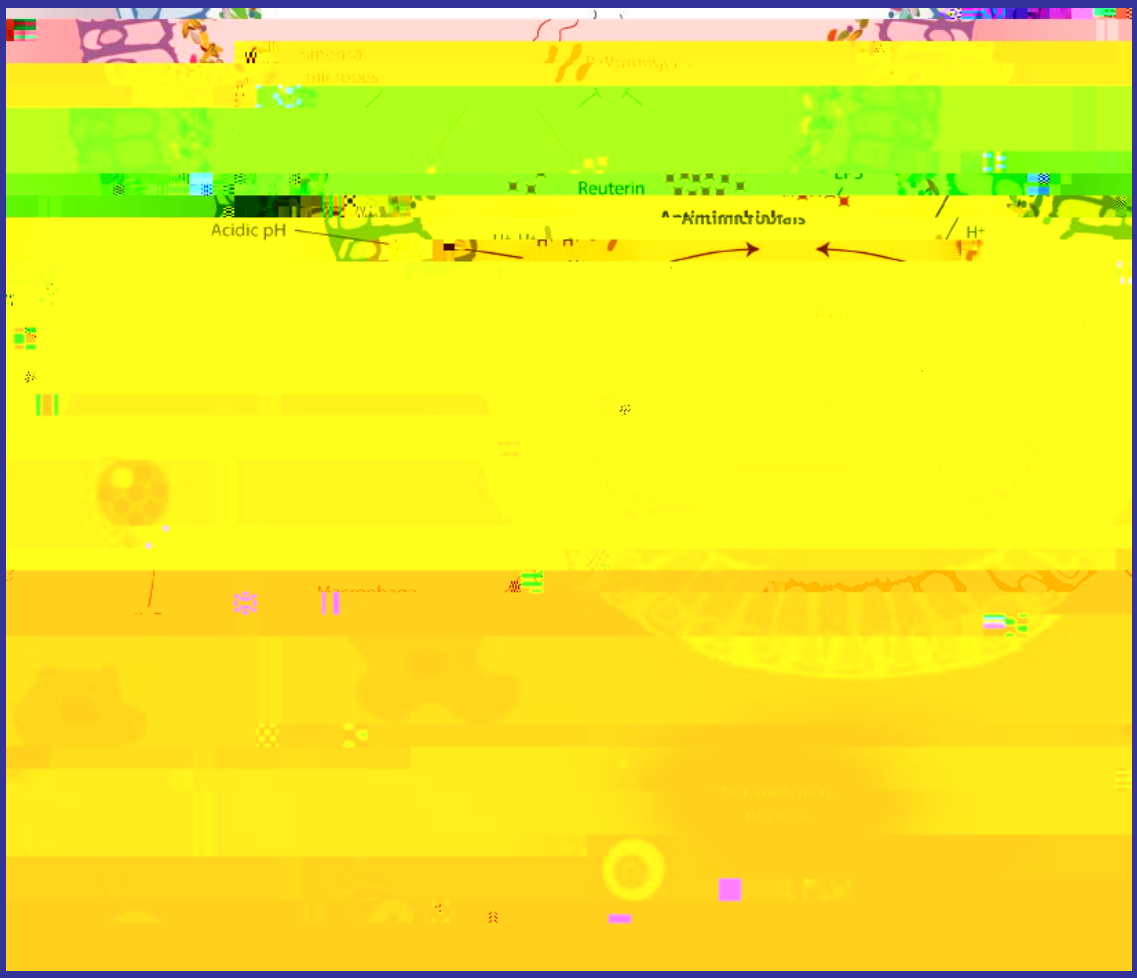
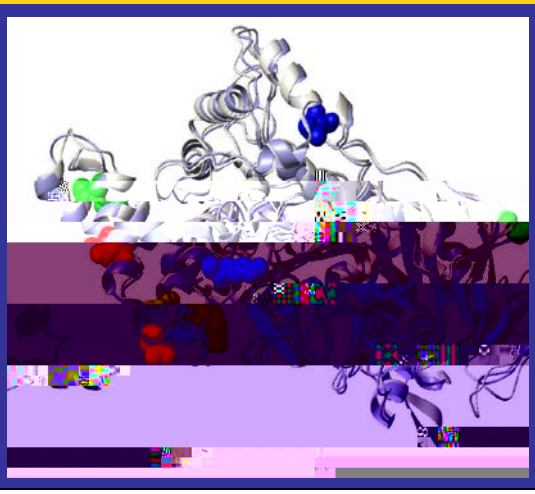
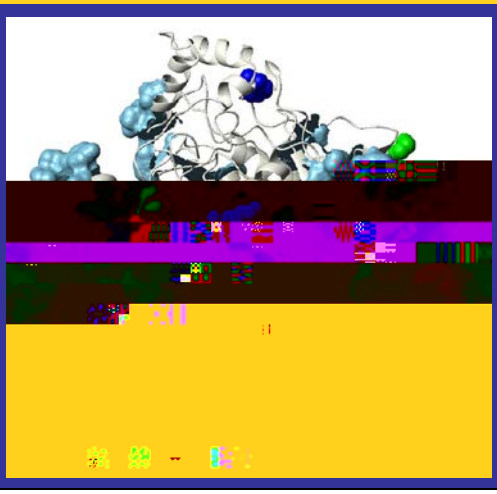
Texas Medical Center Digestive Diseases Center
5th Annual *"Frontiers in Digestive Diseases"* Symposium:
The Gut Microbiome in Health & Disease







Saturday, February 8, 2014
MD Anderson – Onstead Auditorium
Houston, Texas

Texas Medical Center Digestive Diseases Center presents the 5th Annual
Frontiers in Digestive Diseases Symposium: The Gut Microbiome in Health & Disease

Saturday, February 8, 2013
MD Anderson - Mitchell Research Building
Onstead Auditorium
6767 Bertner, Houston, Texas

On the cover:

Human-derived L. reuteri strains each differentially promote acidic environments, produce the antimicrobial reuterin, synthesize essential vitamins, and generate immunomodulatory compounds that effect immune signaling in the host. The differences associated with each ecotype are illustrated here.

(2) P-34. Numan Oezguen. Ribbon representation of the structural alignment of human neuroligin 3 (NLGN3) model (in gray) and human NLGN4-X linked crystal structure (in light blue, PDB code 2xb6). The NLGN3 model is based on rat NLGN1 (PDB code 3vkf). Highlighted in spheres are autism spectrum disorders (ASD) associated mutation sites. In yellow is the R451C NLGN3 site and in red, blue, and green are other mutation sites reported for NLGN4-X linked.

(3) P-34. Numan Oezguen. Human NLGN3 model is shown in ribbon representation. ASD associated mutations in NLGN3 and NLGN4-X linked are shown in spheres. Additionally, NLGN3 predicted protein-protein interface patches are highlighted.

Texas Medical Center Digestive Diseases Center presents the 5th Annual
***Frontiers in Digestive Diseases Symposium*: The Gut Microbiome in Health & Disease**

A G E N D A
Saturday, February 8, 2014
MD Anderson - Mitchell Research Building
Onstead Auditorium
6767 Bertner
Houston, Texas

7:45 am ***Coffee and Continental Breakfast***

Session I. Theme: Gut Microbiome: Basic Systems Biology

8:15- 8:45 am ***Welcome.***

L I S T O F A B S T R A C T S

21.	David Y. Graham, MD DDC Clinical Research Core Director Professor, Medicine-GI BAYLOR	Triple Bacteroides fecal replacement therapy for of relapsing Clostridium difficile diarrhea (fecal transplantation sans feces).
22.	Diane Hutchinson Graduate Student BAYLOR	Norwalk virus infection and the gut microbiome
23.	Joseph M. Hyser, PhD Assistant Professor BAYLOR	Activation of Store-operated and Voltage-activated Calcium Channels by Rotavirus NSP4-mediated Release of ER Calcium Stores
24.	Baijun Kou Research Associate BAYLOR	Characterization of cross-reactive norovirus-specific monoclonal antibodies
25.	Lenard Lichtenberger, PhD DDC Associate Director Director Integrative Biology Core UTHealth	NSAID injury to the small intestine is dependent upon bile and is associated with overgrowth of enterococci
26.	Yuying Liu, PhD Assistant Professor UTHealth	Lactobacillus reuteri DSM 17938 prolongs the survival of Treg-deficient Scurfy mice
27.	Janielle P Maynard Research Assistant BAYLOR	Dysregulation of purinergic signaling in Hepatocellular Carcinoma
28.	Sharada Mokkapati, PhD Postdoctoral Associate MDACC	

Denotes past DDC Pilot/Feasibility awardees

Oral Administration of Pulmonary Surfactant Protein-A Reduces Pathology in an Experimental Model of Necrotizing Enterocolitis

Hector D. Quintanilla, MD, Yuying Liu, PhD, Nicole Y. Fatheree BS, Constance L. Atkins, BS, Syed S. Hashmi, PhD, Joanna Floros, PhD, Francis X. McCormack, MD, Jon M. Rhoads, MD and Joseph L. Alcorn, PhD

OBJECTIVES: Necrotizing enterocolitis (NEC) frequently results in significant gastrointestinal tract morbidity and mortality in premature infants. Others reported that mice deficient in pulmonary surfactant protein-A (SP-A) born and raised in a nonhygienic environment succumb to a pathology resembling NEC, and enteral administration of purified SP-A significantly reduced mortality. We hypothesized that oral administration of purified SP-A can ameliorate pathology in an experimental model of neonatal NEC.

METHODS: NEC was induced in newborn Sprague-Dawley rat pups by daily formula gavage and intermittent exposure to hypoxia (FH). Purified human SP-A (5 g/day) was administered orally via gavage (FHS). After 4 days, surviving pups were sacrificed and NEC was assessed by histological examination of distal terminal ileal sections. Intestinal levels of inflammatory cytokines (IL-1 IFN-γ and TNF- were assessed by ELISA. Intestinal toll-like receptor 4 (TLR4) levels were assessed by western analysis.

RESULTS: Exposure of rat pups to hypoxia (FH) significantly increased mortality and the incidence and severity of NEC. Oral administration of SP-A (FHS) significantly reduced mortality and assessment of experimental NEC. Intestinal levels of pro-inflammatory cytokines (IL-1 TNF-α and IFN-γ) were significantly increased in FH pups. Administration of SP-A significantly reduced IL-1 and TNF-α levels, but had little effect on elevated levels of IFN-γ. Expression of intestinal TLR4, key in NEC pathogenesis, was significantly reduced in the FHS group as compared to the FH group.

CONCLUSIONS: In an experimental rat model of NEC, oral administration of SP-A reduces intestinal levels of pro-inflammatory cytokines and TLR4 protein, and ameliorates adverse outcomes associated with NEC.

Evaluation of P2Y2 Purinergic Receptor Function in the Pathogenesis of Sepsis.

Athis Arunachalam, Bryan Tackett and Sundararajah Thevananther

Department of Pediatrics, Baylor College of Medicine/Texas Children's Hospital

Background: Severe sepsis is a major cause of morbidity and mortality. Cellular stress triggers ATP release into extracellular milieu. Extracellular ATP, via activation of P2Y2 purinergic receptors, plays a pivotal role in inflammation and immunomodulation. However, the functional significance of P2Y2 receptor activation in the modulation of inflammation, multi organ injury, and death is not well understood. The overall goal is to test the **hypothesis** that P2Y2 purinergic receptor activation is essential for the induction of inflammatory cascades and multi-organ injury secondary to microbial infection.

Methods: Sepsis was induced by cecal ligation and puncture (CLP) in adult (12-16 weeks) wild type (WT; C57BL6/J) and P2Y2-/- (KO; C57BL6/J) mice. Tissues (liver, lungs) and blood were collected at 3,6,12, 21h and 7 days after CLP. Tissues were analyzed for leukocyte infiltration, apoptosis, and pro-inflammatory cytokine/chemokine protein and RNA (quantitative RT-PCR) expression.

Results: WT mice subjected to CLP developed symptoms of severe sepsis and were moribund (100%) between 21-48 hr. However, KO mice survived longer (70% survival at 7 days). In response to CLP, induction of serum cytokines/chemokines was significantly attenuated in KO mice (IL-6, 67%; IFN , 91%; MCP-1, 96%; MIP-2, 75%), as compared to WT mice at 21 hr. CLP induced massive liver injury in the WT mice, with elevated serum ALT, leukocyte infiltration into the hepatic parenchyma, hepatocyte injury, and hemorrhagic necrosis at 21 hr. Liver injury was attenuated in the KO mice in response to CLP at 21 hr, with decreased induction of TNFα (58%), IL-6 (72%), IL-1β (49%) and MCP-1 (90%) mRNA expression, as compared to WT livers. Additionally, attenuation of markers of lung inflammation and kidney injury were noted in the KO mice as compared to WT.

Conclusions: P2Y2 receptor function is critical for sepsis-induced mortality in mice. Early induction of hyper-inflammatory phase is dependent on intact P2Y2 receptor expression. These findings highlight the functional significance of purinergic signaling in the pathogenesis of sepsis and eventually will lead to development of novel therapeutics.

Delayed Initiation of Enteral Formula Feeding Reduces the Incidence of Necrotizing Enterocolitis (NEC) inPpreterm Piglets

Caroline Bauchart-Thevret[1], Nada Ghoneim[1], Barbara Stoll[1], Madhulika Kulkarni[1], Berthe Oosterloo[1], Miguel Saenz de Pipaeon[1], Oluyinka Olutoye[1], Irving Zamora[1], Brian Berg[2], Anja Wittke[2], Douglas G. Burrin[1].

[1] USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas. [2] Mead Johnson Pediatric Nutrition Institute, Evansville, Indiana.

Background: Necrotizing enterocolitis (NEC) is a major complication of enteral feeding in premature infants with a high morbidity and mortality. Early enteral feeding of fortified human milk is considered optimal nutrition for the preterm infant. However, human milk is not always available, and commercial formulas are needed that mimic human milk as closely as possible. **Objective:** Our aim was to test the effects of early vs. late enteral feeding of an intact vs. partially hydrolyzed protein formula on NEC incidence in a preterm piglet model being developed to closely simulate clinical practice.

Design/Methods: Moderately preterm pigs (at 90% of gestation) were randomized to either an early (EA) or late (LA) feeding protocol. The EA and LA groups received 2 d and 5 d of total parenteral nutrition (TPN), and orogastric formula feeds (50% full intake) began on d of life 3 and 6, respectively and PN continued. Pigs in the EA and LA groups were also randomized to one of two formulas containing either intact or hydrolyzed protein. All four groups were euthanized due to NEC onset or after 5 d formula feeding. NEC severity and incidence was assessed based on macroscopic and histological scoring in the stomach, proximal jejunum, distal ileum, and colon.

Results: Twenty-eight of 40 pigs in the EA group developed NEC (70%) as compared to 9 of 22 pigs in the LA group (41%) (p=0.033). The mean total clinical NEC severity score was significantly greater in the early vs late group (12.50 vs. 7.27; p=0.003). There was no significant difference in the incidence of NEC in pigs that received hydrolyzed protein formula (67% in EA, 45% in LA) and those that received intact protein formula (77% in EA, 36% in LA).

Conclusions: Although TPN has been associated with impaired gut development, this study provides evidence that delayed initiation of enteral feeding at 5 d vs. 2 d is protective and reduced both the incidence and severity of NEC in preterm pigs. The formula containing intact or hydrolyzed protein had no effect on NEC development. Future studies will explore whether more gradual rather than an abrupt introduction of feeds impacts NEC incidence.

Impact of Parenteral Lipid Emulsions on Metabolomic Phenotype in Preterm TPN-Fed Piglets

Madhulika A. Kulkarni[2], Hester Vlaardingerbroek[4], Barbara Stoll[1], Olga Ilkayeva[5], Christopher Newgard[5], Oluyinka Olutoye[3], Johannes B. Van Goudoever[6], Douglas Burrin[1].

[1]USDA-ARS Children's Nutrition Research Center, [2]Newborn Section, [3]Department Surgery, Baylor College of Medicine, Houston, TX; [4]Erasmus MC - Sophia Children's Hospital, Rotterdam, Netherlands; [5]Duke University Medical Center, Durham, NC; [6]VU University Medical Center, Amsterdam, Netherlands.

BACKGROUND: Lipids in parenteral nutrition provide essential fatty acids and are a major source of energy for hospitalized neonates. Intralipid (IL) is the only approved lipid emulsion in the U.S, but new generation emulsions including Omegaven (OV) and SMOFlipid (SL) have been approved in Europe and are being considered by the FDA. Our studies in TPN-fed preterm piglets given IL lipid emulsion leads to hepatic cholestasis and steatosis implicating metabolic dysfunction in the liver. There are no studies describing the metabolite profile in neonates comparing IL with new generation lipid emulsions.

OBJECTIVE: Our objective was to perform metabolomic profiling of liver and muscle tissue in total parenteral nutrition (TPN) fed piglets given different lipid emulsions.

DESIGN/METHODS: Preterm pigs were assigned to 4 groups (7-14 pigs/group; equal daily macronutrient intake with 5 g/kg lipid): PN+IL, PN+OV, PN+SL and an enteral group fed milk formula (EN). These emulsions are based on soybean oil (IL), fish oil (OV), or a blend of lipids soy, fish, olive, medium chain fatty acids (SL). Cesearean-derived pigs received their treatments beginning at birth until 14 d of age. Plasma, muscle and liver tissue were collected after 14 d and subjected to analysis of fatty acid, amino acid and citric acid cycle metabolites by various HPLC and GC/MS methods.

RESULTS: We found marked increase in hepatic steatosis in IL vs EN pigs that was prevented in both new generation emulsions (OV and SL). The difference in hepatic steatosis was associated with insulin resistance in IL vs EN and SL pigs. In liver tissue, acyl-CoA species were most abundant for the dominant fatty acids in the respective lipid emulsions. Tissue free carnitine in EN pigs was 4-fold higher than TPN groups and this resulted in a reduced liver, but not muscle acyl-carnitine levels. The acyl-CoA: acyl-carnitine ratios were higher in liver tssue, 4cyl-cari6x in.9TJ0 lnm is resu009 tit fatty acto

Vitamin E Added to Intralipid Positively Impacts Hepatic Bile Acid and Fatty Acid Homeostasis in TPN-Fed Preterm Pigs

K. Ng[1]; B. Stoll[1]; M Sáenz de Pipaón[2], D. Burrin[1]

1. Department of Pediatrics, Division of Pediatric Gastroenterology, Hepatology, and Nutrition, and USDA Children's Nutrition Research Center, and Division of Pediatric Surgery, Baylor College of Medicine, Houston, TX, United States; Department of Neonatology, La Paz University Hospital, Madrid, Spain.

Background: Prolonged total parenteral nutrition (PN) may lead to cholestasis and parenteral nutrition associated liver disease (PNALD). The etiology of PNALD is unknown, but constituents of lipid emulsions may positively or negatively impact nuclear receptors involved in bile acid homeostasis (BAH) and steatosis. Plant phytosterols present in soybean oil-based lipid emulsions (e.g. Intralipid) have been suggested to negatively impact BAH by antagonizing the bile acid sensing farnesoid X receptor (FXR) and in turn its downstream targets. The fish oil-based lipid emulsion Omegaven, abundant in vitamin E and docosahexaenoic acid (DHA) yet devoid of phytosterols, may positively impact the nuclear receptors pregnane X (PXR) and peroxisome proliferator-activated receptor-alpha (PPARα) and their downstream targets thus protecting hepatocytes against bile acid and fatty acid homeostatic dysregulation. We investigated the serum

Interaction of BMP, Apelin and PTHrP pathways in pancreatic duct ligation-induced chronic pancreatitis

Y. Cao,[1,2] C. Rastellini,[2] S. Han,[2] V. Bhatia,[2] M. Falzon,[2] G. Greeley, Jr [2] and T.C. Ko[1,2]
[1]Dept. of Surgery, UTHSC-Houston; [2]UTMB at Galveston, TX

Background: Our laboratories have shown that pancreatic bone morphogenetic protein (BMP), apelin and parathyroid hormone-related protein (PTHrP) signaling pathways are up-regulated by cerulein-induced chronic pancreatitis (CP) in mice. Furthermore, BMP and apelin are anti-fibrogenic, while PTHrP is pro-fibrogenic. It is unclear whether these pathways interact in CP.

Objective: To examine potential interaction of BMP, apelin and PTHrP in pancreatic duct ligation (PDL)-induced CP model.

Methods: CP was induced by PDL in adult male C57/BL6 mice (n=5). The ligated and non-ligated lobes (control) of the pancreas were harvested for histology, protein and mRNA analyses. For *in vitro* experiment, cultured mouse PSCs were treated with BMP2 (50 ng/ml) or PTHrP (10^{-7} M). The effect of apelin gene knockout (APKO) on pancreatic PTHrP levels was examined.

Results: In the ligated lobes, PDL-induced CP results in inflammation, acinar degeneration and fibrosis, while the control lobes appear histological normal. Fibronectin protein levels are elevated in the ligated lobes (control 0.27$\pm$0.03 vs ligated 1.73$\pm$0.30). Pancreatic mRNA levels of BMP2 and apelin are elevated in the ligated lobes (7- and 4 to 10-fold vs control respectively, $p<0.05$). *In vitro*, BMP2 induces apelin mRNA expression (vehicle 1.1$\pm$0.01 vs BMP2 1.4$\pm$0.10), whereas PTHrP inhibits apelin mRNA expression (vehicle 1.0$\pm$0.01 vs PTHrP 0.28$\pm$0.03). Pancreatic PTHrP mRNA levels are elevated 8-fold in APKO mice compared to wildtype.

Discussion: PDL-induced changes in BMP2 and apelin levels replicate those measured in cerulein-induced CP. BMP2 and PTHrP influence apelin expression implying that BMP2 and PTHrP regulate apelin signaling during CP. Results from APKO imply a feedback axis between apelin and PTHrP.

Conclusion: BMP and apelin interact in CP that may form an endogenous network protecting the pancreas during CP. PTHrP may suppress BMP's and apelin's protective effects.

Texas Medical Center Digestive Diseas es Center presents the 5th Annual
Frontiers in Digestive Diseases Symposium : The Gut Microbiome in Health & Disease

Targeting TGF- regulated telomerase: a novel therapeutic approach for liver and gastrointestinal cancers

Jian Chen[1], Jiun-Sheng Chen[1], Zhixing Yao[1], Wilma Jogunoori[2], Bibhuti Mishra[2], Lopa Mishra[1]

[1]Department of Gastroenterology, Hepatology & Nutrition, University of Texas MD Anderson Cancer Center
[2]Institute of Clinical Research, Veterans Affairs Medical Center, Washington DC

Background: A hereditary human cancer stem cell syndrome, Beckwith-Wiedemann syndrome (BWS) is currently linked to deregulated imprinting at chromosome 11p15, IGF2, CDKN1C and others, and develops multiple cancers, including liver and gastrointestinal cancers. Yet, causal molecular defects and genetic models of this overgrowth syndrome have remained elusive to date in the majority of cases. CCCTC-Binding Factor CTCF is a highly conserved zinc finger protein that has diverse regulatory functions, including transcriptional activation/repression/imprinting of molecules such as TERT, IGF-2 and c-Myc. Recent studies demonstrate a high frequency of TERT

TGF-β Regulated E3 Ligases are Novel Therapeutic Targets for Primary Liver Cancer

Jian Chen[1], Jiun-Sheng Chen[1], YoungJin Gi[1], Lior H Katz[1], Ji-Hyun Shin[1], Liem Phan[1], Wilma Jogunoori[2], Vivek Shukla[3], Bibhuti Mishra[2], Shulin Li[1], Milind Javle[1], Mien-Chie Hung[1], Lopa Mishra[1]

[1]Department of Gastroenterology, Hepatology & Nutrition, University of Texas MD Anderson Cancer Center, Houston, TX
[2]Institute of Clinical Research, Veterans Affairs Medical Center
[3]Surgery Branch, National Cancer Institute

Background: Hepatocellular Cancer (HCC) is lethal and difficult to treat due to late diagnosis, few viable targeted therapeutics and unclear molecular profiling of each stage of tumor development, from cirrhosis to nodule formation to carcinoma. A large number of studies have demonstrated that the TGF-β pathway plays a fundamental role in the biology of the GI tract, and as such, several components of the pathway are commonly targeted in various GI cancers. TGF-β signaling is regulated by the ubiquitin–proteasome pathway, in which E3 ubiquitin ligases recognize and target proteins for degradation by the proteasome. Numerous E3 ubiquitin ligases have been identified as negative regulators of different components of the TGF-β pathway. More recently, Praja and Keap1 have been identified as E3 ligases that ubiquitinates β2-spectrin (β2SP) in a TGF-β-dependent manner. Accordingly, Praja E3 ligase activity regulates TGF-β signaling by controlling β2SP abundance through ubiquitin-mediated degradation.

Therefore, we **hypothesis** is that Specific E3 ligases, which disrupt TGF-β/β2SP/Smad3 tumor suppressor pathway lead to uncontrolled activation of chromatin and tumor formation. Therefore,

The Pathophysiology of Lipid Metabolism During Rotavirus Infection

Sue E. Crawford and Mary K. Estes

Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX.

Rotavirus, the causative agent of viral gastroenteritis in children and young animals, requires the formation of lipid droplets for assembly of viroplasms that are sites of genome replication and particle assembly. However, the precise mechanism of rotavirus-induced formation of lipid droplets

Texas Medical Center Digestive Diseases Center presents the 5th Annual

Microfluidic isolation of bacteria from diverse specimens for metagenomics analysis

Lorenzo D'Amico[1,2], Nadim Ajami[3], Javier A Adachi[4], Joseph F Petrosino[3], Peter RC Gascoyne[2]

[1]UT Austin Department of Biomedical Engineering, Austin, Texas; [2]UT MD Anderson Cancer Center, Department of Imaging Physics, Houston, Texas; [3]Baylor College of Medicine, Alkek Center for Metagenomics and Microbiome Research, Department of Molecular Virology and Microbiology; [4]UT MD Anderson Cancer Center, Department of Infectious Diseases, Infection Control, and Employee Health, Houston, Texas

Metagenomic analysis of the human microbiome has revealed a multiplicity of microorganisms and their previously unsuspected involvement in human health. The prevailing metagenomic paradigm attempts to decipher host-microbe interactions by analyzing massive quantities of genetic sequence data *in silico*. An important limitation of this strategy, especially in low bacterial biomass samples, is that host nucleic acids in the specimen interfere with the interpretation of DNA sequence data. To address this limitation our multidisciplinary team of bioengineers, clinicians and biologists is developing a microfluidic apparatus to isolate and concentrate bacterial cells prior to extracting nucleic acids. We showed that gram-positive and gram-negative bacteria could be isolated from small volumes of resuspended stool, saliva and skin specimens with >75% efficiency at a processing rate of 100 µL/min, and could be released from the apparatus in a new fluid medium. Comparative metagenomic analyses between input samples and system isolates were carried out using 16S ribosomal subunit RNA genes and revealed that isolates represent the diversity of microorganisms present in the original specimen. Whole genome sequencing of processed skin specimens demonstrated enrich of microbial DNA against the background of host DNA. These data demonstrate feasibility for applying this approach to enhance metagenomic analyses by depleting host DNA and enriching low-level bacteria. The microfluidic approach requires little sample preparation and exploits the intrinsic biophysical properties of viable microorganisms to accomplish isolation, thereby obviating the need for expensive biochemical labels and bioengineered tags. Upon completion of this project we expect that this technology will enhance microbiome research as a preparative and potentially analytical tool to rapidly purify and profile bacteria present in clinical specimens. This project may have further applications in screening transfusion products and diagnosing bloodstream infections.

Human Organoid and Jejunal Enteroid Cultures as a Functional Model of Human Small Intestine to Study Infection with Human Enteric Microbes

Khalil Ettayebi[1], Xi-Lei Zeng[1], Sue E. Crawford[1], Joseph M. Hyser[1], Umesh Karandikar[1], James Broughman[1], Sarah Blutt[1], Kapil Saxena[1], Lin Qu[1], Richard E. Lloyd[1], Antone R. Opekun[2], David Y. Graham[2], Vadim Sherman[3], Nicholas C. Zachos[4], Olga Kovbasnjuk[4], Hugo R. De Jonge[5], Mark Donowitz[4] and Mary K. Estes[1]

(1) Department of Molecular Virology and Microbiology, (2) Department of Medicine, Baylor College of Medicine, Houston, Texas; (3) Department of Surgery at Methodist Hospital, Houston, Texas; (4) Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; (5) Department of Pediatric Gastroenterology, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands.

A significant limitation in translational research is the absence of reliable pre-clinical models that mimic relevant human physiology and disease pathology. This is particularly true in the field of

Attenuation of Colonic Inflammation by Probiotic *Lactobacillus Reuteri* via Histamine Production

Chunxu Gao[1,3]; Carissa Thomas[3]; Jennifer K. Spinler[3]; Amrita Sontakke[3]; Vanessa Jackson[3]; Monica Lugo[3]; Angela Major[3]; Caterina Kaffes[4]; David A Rendon[4]; Mostafa (Waleed) Gaber[4]; James Versalovic[1,2,3]

Departments of Molecular Virology & Microbiology[1] and Pathology & Immunology[2], Baylor College of Medicine,
Department of Pathology[3] and Small Animal Imaging Facility[4], Texas Children's Hospital, Houston, TX

Supplementation with probiotic *Lactobacillus reuteri* strains that naturally colonize the gut of mammals has been effective at ameliorating intestinal inflammation in rodent colitis models, but the underlying mechanisms are unknown. Pangenomic studies showed that *L. reuteri* strains with anti-inflammatory properties contain a complete *hdc* gene cluster which is responsible for syn7 T(p5a5.5(h0 TCmw7(

Texas Medical Center Digestive Diseases Center presents the 5th Annual
***Frontiers in Digestive Diseases Symposium*: The Gut Microbiome in Health & Disease**

Norwalk virus infection and the gut microbiome

Hutchinson DLS; Ajami NJ; Neil FH; Opekun AR; Finkbeiner SR; Graham DY; Petrosino JF; Atmar RL; Estes MK

The intestinal microbiome has recently been shown to play a role in the pathogenesis of viral enteric infections by enhancing viral infectivity. Norovirus (NoV) pathogenesis is not fully understood, and the effect of the gut microbiota in the context of NoV infection has not been described thoroughly. To assess the interaction between NoV, the intestinal microbiota, and the human host, we used fecal samples collected from the Norwalk virus (NV) challenge study carried out at Baylor College of Medicine. The study population consisted of 57 individuals who participated in an experimental challenge with NV. Longitudinal fecal samples were collected prior to, during, and subsequent to infection. NV infection was defiÞ

Characterization of cross-reactive norovirus-specific monoclonal antibodies

Baijun Kou[2], Sue E.Crawford[2], Nadim J. Ajami[2], Rita Czakó[2], Frederick H. Neill[2], Tomoyuki N.

NSAID injury to the small intestine is dependent upon bile and is associated with overgrowth of enterococci

AS Mayo, Y Song, MR Cruz, TM Phan, KV Singh, DA Garsin,
BE Murray, EJ Dial, LM Lichtenberger

Texas Medical Center Digestive Diseases Center presents the 5th Annual

Dysregulation of purinergic signaling in Hepatocellular Carcinoma

Maynard, Janielle P.[1], Johnson, Randy L.[2], Lee, Ju-Seog[2], Lopez-Terrada, Dolores[1], Goss, John A[1]. Thevananther, Sundararajah[1]

1

Liver Cancer In Mice With Wnt Pathway Activation in Unique Fetal Liver Progenitors.

Sharada Mokkapati[1], Katharina Genreith[1], Le Huang[1,2], Kegan J. Cunniff[1], E. Cristy Ruteshouser[1], Mark deCaestecker[4], Milton J. Finegold[5], Vicki Huff[1,2,3]

SOX9 and NF-Y converge on the promoter regions of cell cycle regulatory genes

Zhongcheng Shi, Chi-I Chiang, Chun Shik Park, Toni-Ann Mistretta,
Daniel Lacorazza, and Yuko Mori-Akiyama

Pathology & Immunology, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas

The transcription factor SOX9 plays critical roles on cell lineage specification by directly regulating target genes in various tissues. Aberrant SOX9 expression is observed in various cancers and SOX9 has also been shown to regulate cellular proliferation; however, the mechanism underlying this regulation has not been well studied. Here, we report a novel mechanism of SOX9-mediated regulation of cell division. Our genome-wide analysis of SOX9 binding to the chromatin of HT-29 human colorectal cancer cells revealed that SOX9 binds to same site of the nuclear factor Y (NF-Y), a histone-like CCAAT-binding trimer. The NF-Y complex plays a central role to support proliferation by regulating the basal transcription of regulatory genes responsible for cell cycle progression particularly in the G2/M checkpoint. In addition to the SOX9 consensus binding sequences, a motif analysis revealed a novel SOX9-binding motif (CCAAT) on the cell cycle genes that are also regulated by NF-Y, including *CCNB1*, *CCNB2*, *CDK1*, and *Topo IIa*. The peaks of SOX9 chromatin affinity on the genome overlapped with NF-Y binding to regulatory sequences of genes involved in cell cycle regulation. Interestingly, lowering the levels of SOX9 resulted in increased affinity of NF-Y proteins to its target genes. *SOX9* knockdown resulted in elevated cellular proliferation by an accumulation in the G2/M phase and increased expression of genes involved in the G2/M transition in human colorectal cancer cell lines. Collectively, our results suggest that SOX9 interferes with NF-Y binding to its target genes and that SOX9 suppresses cell proliferation via inhibition of NF-Y mediated activation of cell-cycle regulatory genes. Therefore, SOX9 levels are critical for the control of cellular proliferation and differentiation.

Screening of candidate proteins for norovirus co-receptores

Kosuke Murakami[1, 2], Lin Qu[1], Kazuhiko Katayama[2] and Mary Estes[1]

[1] Baylor College of Medicine, Houston, USA
[2] National Institute of Infectious Diseases, Tokyo, Japan.

Human norovirus (HuNoVs) are a major cause of acute non-bacterial gastroenteritis; however, the mechanism of cell binding is not clear. Histo-blood group antigens (HBGAs) have been implicated in the initial binding of NoVs to cells. To determine the involvement of HuNoVs and HBGAs in cell binding, we previously investigated the localization of HuNoV virus-like particles (VLPs) and HBGAs in a human intestinal cell line, Caco-2, by immunofluorescence microscopy. Following incubation of Caco-2 cells with GII.6 VLPs, we found that VLP-cell binding depended on the state of cell differentiation, but not on the presence of type-H1, -H2 and -Le^b HBGAs. This result suggested that VLPs utilize molecule(s) other than HBGAs

Microbiome and host factors communicate protection against acute murine colitis following omega-6 fatty acid induced transient pediatric obesity

Dorottya Nagy-Szakal[1,2], Sabina Mir[1], R. Alan Harris[3], Scot E. Dowd[4], Takeshi Yamada[5], H. Daniel Lacorazza[5], Nina Tatevian[6], Richard Kellermayer[1,2]

[1]Section of Pediatric Gastroenterology, Baylor College of Medicine, Texas Children Hospital, Houston, TX USA; [2]USDA/ARS Children's Nutrition Research Center, Houston, TX, USA, [3]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; [4]Research and Testing Laboratory, Lubbock, TX, USA;[5]Department of Pathology, Baylor College of Medicine, Houston, TX, USA; [6]Department of Pathology and Laboratory Medicine, The University of Texas Health Science Center, Houston, TX, USA

Background: Dietary influences may affect microbiota composition and host immune responses at the intestinal surface. These nutritionally induced biologic component changes may modulate

The length of newly diagnosed Barrett's esophagus has been decreasing over time

Theresa Nguyen, BS[1], Abeer Alsarraj, BS[1], Hashem B. El-Serag, MD, MPH[1,2,3]

[1]Houston VA HSR&D Center of Excellence and Departments of [2]Medicine, Michael E. DeBakey Veterans Affairs Medical Center and Sections of [3]Gastroenterology and Hepatology, Department of Medicine, Baylor College of Medicine, Houston, Texas, USA.

Statin use may decrease the risk of Barrett's esophagus: A case-control study of US veterans

Theresa Nguyen, BS[1,4], Natalia Khalaf, MD[4], David Ramsey, PhD[1], Hashem B. El-Serag, MD, MPH[1-4]

[1]Houston VA HSR&D Center for Innovations in Quality, Effectiveness and Safety, and Departments of [2]Medicine and Pathology, Michael E. DeBakey Veterans Affairs Medical Center and Sections of [3]Gastroenterology and Hepatology, [4]Department of Medicine and Department of Pathology, Baylor College of Medicine, Houston, Texas, USA.

Objectives: Statins have been associated with a reduced risk of esophageal adenocarcinoma, however their possible effect on the risk of developing Barrett's esophagus (BE) is unknown. This study evaluated the association between statin use and the risk of BE.

Methods: We conducted a case-control study among eligible patients scheduled for elective esophagogastroduodenoscopy (EGD) and a sample of patients eligible for screening colonoscopy recruited from primary care clinics at a single VA center. We compared 303 patients with definitive BE with two separate frequency matched control groups: 303 patients from the primary care group ("primary care controls") and 606 patients from the elective EGD group ("endoscopy controls") with no endoscopic or histopathologic BE. Index date was the earliest BE diagnosis date for cases, and the study EGD date for controls. Use of statins and other lipid lowering medications was ascertained by reviewing filled prescriptions in electronic pharmacy records during a 10-year period before index date, and use before the index date was compared between cases and controls. We calculated odds ratios (OR) and 95% confidence intervals (95% CI) using multivariable logistic regression models adjusting for age, sex, race, GERD symptoms, *H. pylori* infection, and waist-to-hip ratio (WHR).

Results: Most in the cohort were men (97.7%) and white (75.5%). Dispensed prescriptions for statins were identified in 64.6% of subjects, most of whom (94.3%) used simvastatin. The proportion of BE cases (57.4%) with filled statin prescriptions was significantly less than in endoscopy controls (64.9%; p=0.029) and primary care controls (71.3%; p<0.001). Most of the difference was related to a higher proportion of controls having statin prescriptions that were first filled earlier than 5 years before the index date than cases (p=0.001) and prescriptions that lasted for 3-10 years (33.9% vs. 28.1%; p=0.011). Longer durations of statin prescriptions were filled by the combined groups of control subjects than BE cases; mean duration (28.6 months vs. 22.1 months, p=0.001). In multivariable analysis, statin use significantly reduced the risk of BE (adjusted OR, 0.70; 95% CI, 0.52-0.93) compared with the combined control groups. The risk of BE is particularly lower with statin use among patients who were obese (OR, 0.49; 95% CI, 0.31-0.78), or had a high WHR (OR, 0.65; 95% CI, 0.48-0.89). Importantly, we found no significant association between BE and non-statin lipid lowering medications (p=0.452). Most study subjects (91.3%) reported the VA as the primary source for most medications, and 84.6% reported receiving all prescriptions from the VA pharmacy with no significant differences between cases and controls (p=0.618).

Conclusion: Statin use may decrease the risk for BE, especially among patients who are obese or have a high WHR.

This study was supported in part with resources at the VA HSR&D Center for Innovations in Quality, Effectiveness and Safety (#CIN 13-413, at the Michael E. Debakey VA Medical Center, Houston, TX, as well as by NIH Grant 5T32DK083266-04, which supports the Texas Medical Center Digestive Diseases Center.

Texas Medical Center Digestive Diseases Center presents the 5th Annual

NoroGLuc: a cell-based human norovirus protease reporter system

Lin Qu, Sompong Vongpunsawad, and Mary K. Estes

Department of Molecular Virology and Microbiology,
Baylor College of Medicine, Houston, Texas

Human noroviruses, a group of positive strand RNA viruses in the *Caliciviridae* family, are the leading cause of epidemic viral gastroenteritis worldwide. Our understanding of their replication and pathogenesis is limited by the inability to propagate these viruses in cell culture. Although genomic RNA isolated from Norwalk virus (NV), the prototype of human noroviruses, is able to replicate in several cell lines, a reporter system for monitoring viral RNA replication is lacking. Here we report a cell-based human norovirus protease reporter system in which the NV protein p41 is fused with *Gaussia* luciferase (NoroGLuc) through a cleavage site for the viral protease (Pro). The fusion of GLuc with membrane-associated NV protein p41 not only retains GLuc within the cells, but also renders the intracellular GLuc enzyme inactive. *Trans* cleavage of NoroGLuc by NV Pro or Pro precursors results in release and secretion of an active GLuc into the cell culture medium as a readout. Using this system, we first demonstrated that the ORF1 region of NV, which encodes the entire nonstructural polyprotein including Pro, and its processing intermediates p22VpgProPol and p22VPgPro have the most potent protease activities. We further showed that transfection of NV stool RNA resulted in measurable increase of secreted GLuc, validating that this system can detect viral RNA replication. Although designed for NV (genogroup GI.1), this system is also suitable for viral proteases of noroviruses in other genogroups. We thus have developed a cell-based, wide-spectrum human norovirus protease reporter system that can serve as a platform for detection of viral replication, identification of host factors that regulate viral replication, and validation of antiviral drugs.

This work is supported by the NIH Pedi GI

16S rRNA and Whole Metagenome Shotgun Analysis using the Genboree Workbench

Kevin Riehle, Emily B. Hollister, Andrew R. Jackson, Sameer Paithankar, Matt Roth, Robert J. Shulman, James Versalovic, Aleksandar Milosavljevic

The decreasing cost, accessibility, and increasing volume of sequencing data provides unprecedented opportunities for scientific discovery. However, next-generation sequence data sets pose non-trivial data management and analysis challenges to individual investigators. There exists a need for an infrastructure that provides leading "omics" analysis tools without the overhead, cost, and learning curve required to develop and manage such a system. We have integrated a suite of analytical tools and pipelines on a web-based platform, the Genboree Workbench (http://genboree.org), to bring large data management and analysis capabilities to the individual investigator. The Genboree Workbench constitutes a cloud-based platform for collaborative sequence-centric research for web-based data analysis.

To address the needs of the metagenomics community, we have developed, integrated, and combined leading open-source tools for researchers to analyze their data without requiring programming or scripting of the tools themselves. Using a web browser, investigators can upload and analyze either 16S rRNA or whole metagenome shotgun (WMS) sequencing data. The Genboree Workbench contains tools for analyzing 16S rRNA data to explore diversity (alpha and beta diversity), taxonomic abundances, and perform analyses using machine learning methods (feature selection, classification, etc.).

We are currently developing and integrating into the Genboree Workbench a pipeline to support WMS data and analysis. The computational and hardware requirements necessary for processing WMS sequence data is dramatically higher than with 16S rRNA sequence data, which can pose significant challenges. Additionally, there is a scarcity of polished, peer-reviewed tools available for WMS experiments as compared to the availability of 16S rRNA toolsets. We have crafted a novel WMS pipeline using open-source tools that allow users to explore taxonomic abundances, produce phylogenetic trees, normalize sequence data, assemble metagenomes (contigs), predict genes, map predicted genes onto the KEGG database and derive functional annotations (orthologous genes, modules, and pathways).

Texas Medical Center Digestive Diseases Center presents the 5th Annual
***Frontiers in Digestive Diseases Symposium*: The Gut Microbiome in Health & Disease**

Lactobacillus reuteri supplementation is a viable option for preventative treatment of *Clostridium difficile*-associated diarrhea

Jennifer K. Spinler[1,2], Amrita Sontakke[1,2], Sara Dann[3], Alex Peniche[3], James Versalovic[1,2], Tor Savidge[1,2]

[1]Texas Children's Microbiome Center, Department of Pathology, Texas Children's Hospital, 1102 Bates Ave., Houston, Texas, USA; [2]Department of Pathology & Immunology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas, USA; [3]Department of Microbiology & Immunology, University of Texas Medical Branch, Galveston, TX

The most prolific cause of bacterial-induced diarrhea in the U.S. is infection by *Clostridium difficile.* Up to one million cases are reported annually at a cost >$3.5 billion, with rates in some hospitals approaching 40%. In the last 15 years, the incidence of *C. difficile* infection (CDI) has more than doubled. Despite a known inverse correlation between the protective gut microbiota and the development of symptoms in infected patients, there is still a major gap in our understanding of how host bacteria protect against this pathogen. Vertical transmission of *Lactobacillus* spp. from mother to child at birth is important in establishing a healthy microbiome during the first years of life. Nourishment and secondary inoculation of the infant GI microbiota from breast milk provides another maternal source of microbes rich in lactic acid bacteria. Metagenomic studies have indicated >10-fold difference in the abundance of *Lactobacillus* in the GI tract of healthy preadolescent children compared to healthy adults. Incidence of CDI increases with age and is rare in children even in the presence of this pathogen. Our metagenomic data analysis of stool specimens from adults with recurrent CDI showed the relative abundance of *Lactobacillus* is significantly decreased. We propose that lactobacilli isolated from the infant GI tract represent candidate probiotic strains that naturally interfere with *C. difficile* cytotoxicity, and repopulation of known lacto-deficient GI microbiomes with such strains may serve as an efficient means to protect against recurrent CDI. Our central hypothesis is that the Achilles heel of *Clostridium difficile* pathogenesis is its susceptibility to secreted antimicrobials by the host microbiota. The rationale for this hypothesis is based on (1) the established concept that *C. difficile* pathogenesis occurs when the normal gut microbiota is disrupted by antibiotic use, and (2) restoration of an intact intestinal microbiota by transplantation represents the most successful clinical practice to date to prevent severe, recurrent CDI. We screened probiotic *Lactobacillus* ssp. already shown to be safe in humans, and identified *L. reuteri* as an intrinsically antibiotic-resistant strain which possessed greater cytotoxicity than currently FDA-approved vancomycin and fidaxomicin. *L. reuteri* was also shown to be resistant to three antibiotics typically used to treat CDI: vancomycin, metronidazole, and fidaxomicin. We have confirmed reuterin production by *L. reuteri* is required for *C. difficile* growth inhibition using targeted mutagenesis. Finally, we demonstrated *C. difficile* killing in the gut lumen of mice following oral synbiotic *L. reuteri* and glycerol administration, allowing development of a prototypic therapeutic concept targeting microbial infection. In keeping with our long-term goal of understanding microbiota-protective mechanisms in gut inflammation, our project aims to characterize this previously unappreciated antimicrobial mechanism, and to exploit this novel finding to develop prototypic therapeutic concepts for CDI. The impact of achieving this outcome would be to provide a novel adjunct treatment for a global epidemic that is becoming one of the major public health threats of the 21st century.

This study was conducted in part with the support of Texas Children's Hospital, supported in part by the Institute for Translational Sciences at the University of Texas Medical Branch, supported in part by a Clinical and Translational Science Award (8UL1TR000071-04) from the National Center for Research Resources, now at the National Center for Advancing Translational Sciences, National Institutes of Health.

From prediction to function using evolutionary genomics: Human-specific ecotypes of *Lactobacillus reuteri* have diverse probiotic functions

Jennifer K. Spinler[1,2,], Amrita Sontakke[1,2], Emily B. Hollister[1,2], Susan Venable[1,2], Phaik Lyn Oh[3], Miriam A. Balderas[4], Delphine M. A. Saulnier[1,2,†], Toni-Ann Mistretta[1,2], Sridevi Devaraj[1,2], Jens Walter[3], James Versalovic[1,2,4], Sarah K. Highlander[4,5]

[1]Texas Children's Microbiome Center, Department of Pathology, Texas Children's Hospital, 1102 Bates Ave., Houston, Texas, USA; [2]Department of Pathology & Immunology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas, USA; [3]Department of Food Science and Technology,

Texas Medical Center Digestive Diseases Center presents the 5th Annual

Early activation of P2Y2 purinergic signaling is essential for efficient hepatocyte proliferation in response to partial hepatectomy.

Bryan Tackett[1,2], Hongdan Sun[1], Sayuri Cheruvu[1], Yu Mei[1], Arunmani Mani[1], Andres Hernandez-Garcia[3], Nadarajah Vigneswaran[4], Saul J. Karpen[1,2], & Sundararajah Thevananther[1,2].

[1]Department of Pediatrics, Division of Gastroenterology, Hepatology & Nutrition, Texas Children's Liver Center, [2]Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston TX, [3]Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX and [4]Department of Diagnostic Sciences, University of Texas Dental Branch in Houston, Houston, TX.

Background & Aims. tyte in response tiial fon 0 ge.0onseng liver Tfm-1.140.9 Tc

Texas Medical Center Digestive Diseases Center presents the 5th Annual

Low Abdominal NIRS Values and Elevated Serum Intestinal Fatty Acid-Binding Protein Predict Necrotizing Enterocolitis in a Premature Piglet Model

Irving J. Zamora, MD[a]; Barbara Stoll, PhD[b]; Cecilia G. Ethun, BA[a]; Fariha Sheikh, MD[a] ; Ling Yu[a] ; Doug G. Burrin, PhD[b]; Oluyinka O. Olutoye, MD, PhD[a]

[a]Division of Pediatric Surgery, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Texas Children's Hospital, [b]Department of Pediatrics, Baylor College of Medicine, USDA/ARS Children's Nutrition Research Center, Houston, TX

Purpose: To identify early markers of necrotizing enterocolitis (NEC), we hypothesized that continuous abdominal near-infrared spectroscopy (A-NIRS) measurement of splanchnic tissue oxygen saturation and serum intestinal fatty-acid binding protein (*s*I-FABP) can detect NEC prior to onset of clinical symptoms.

Methods: Premature piglets received parenteral nutrition for 48-hours after delivery, followed by enteral feeds every three hours until death or euthanasia at 96-hours. Continuous A-NIRS, oxygen saturation, and heart rate were measured while monitoring for clinical signs of NEC. Blood samples obtained at 6-hour intervals were used to determine *s*I-FABP levels by ELISA. Presence of NEC was assessed by a validated clinical score and confirmed by histology. Data were analyzed using Student's *t*-test and receiver operating characteristic curves.

Results: Of 43 piglets, 49% developed NEC and 51% were No-NEC littermate controls. A-NIRS values within the first 3-hours of life were lower in the NEC group (71±4.4%) compared to littermate controls (79±1.9%; p=0.003) and remained lower throughout the study. A-NIRS $\leq$75% predicted NEC with 94% sensitivity and 94% specificity. Mean *s*I-FABP was higher in animals that developed NEC (0.66±0.62ng/ml) compared to littermate controls (0.09±0.05ng/mL; p<0.001). In the NEC group sI-FABP increased from 0.04±0.06ng/mL on parenteral nutrition to (0.7±0.76ng/mL; p<0.001) after feeds. *s*I-FABP levels increased in parallel with disease progression and a value $\geq$0.25ng/mL identified animals with NEC (71% sensitivity and 95% specificity).

Conclusions: In premature piglets, low A-NIRS in the early neonatal period predicts NEC and *s*I-FABP increases with disease progression. These modalities may help identify neonates with NEC prior to clinical manifestations of disease.

L I S T O F P A R T I C I P A N T S

Shailesh Advani
Graduate Research Assistant
MDACC
Shailesh.M.Advani@uth.tmc.edu

Texas Medical Center Digestive Diseases Center presents the 5th Annual

Texas Medical Center Digestive Diseases Center presents the 5th Annual

ACKNOWLEDGEMENTS

The Texas Medical Center Digestive Diseases Center

Mary K. Estes, PhD, Director
Hashem El-Serag, MD, MPH, Co-Director

Associate Directors

Lenard M. Lichtenberger, PhD
Lopa Mishra, MD

Internal Advisory Committee

Douglas Burrin, PhD
Milton Finegold, MD
David Y. Graham, MD
Claudia Kozinetz, PhD
Sundararajah Thevananther, PhD
James Versalovic, MD, PhD

Clinical Liaison Committee

Marc Rhoads, MD

Administrator

Terrlyn Bosshard