The development of metabolomics-based predictors of nonalcoholic fatty liver disease

1. Study Design & Methods
   Pre-clinical study in GNMt-KO mice. (left) Glucose-N-methyltransferase (GNMT) deletion leads to steatosis and fibrosis. At 4 months of age micro- and macrovesicular steatosis was observed through the hepatic lobule in GNMt-KO mice. Collagen deposition (pink red staining) indicates moderate fibrosis. (M. L. Martinez-Chantar et al. Hepatology (2008), (right) Principal components analysis of serum metabolic profile (J. Barr et al. J. Prot. Res. (2010)). An obstacle to the development of metabolome-based human NAFLD predictors has been the lack of large cohort data from biopsy-proven patients matched for key metabolic attributes such as obesity. Guided by the results of the pre-clinical study, we examined the serum metabolic profile of 467 biopsy patients.

2. NAFLD Serum Metabolic Profile
   The heat map representation of the serum metabolic profile obtained from patients included in the study. (a) and (b) and (c) metabolic ion abundance ratios in NAFLD versus lean/normal (left), obese class I-IV (middle), and obese class V (right) comparing metabolic groups. steatosis/normal liver, NAFLD steatosis, and NAFLDfibrosis respectively. For each component, log transformed ion abundance ratios are displayed, as represented by the scales (d), where pronounced colors correspond to significant (p≤0.05) - two-tailed Wilcoxon Rank Sum Test changes, and (e), where light colors correspond to nonsignificant (p>0.05) - two-tailed Wilcoxon Rank Sum Test changes.

3. Obesity Dependent NASH Biomarkers
   Obesity dependent NASH biomarkers. Mean percent ion abundance deviations of (a) sphingolipids, and (b) Oxidized fatty acids in patients diagnosed with NASH compared to isolated steatosis. Most sphingolipid species were significantly (* denotes p<0.05, two-tailed Wilcoxon Rank Sum Test) reduced in lean NASH patients sera. Oxidized fatty acids, including both eicosapentaenoic (15- and 12-hydroxyeicosatetraenoic acid, pro-inflammatory (12-hydroxyoctadecatrienoic products) and non-oxidative (9-, 11- and 12-hydroxyoctadecatrienoic acid) oxidation products of metabolic acid were found elevated in obese NASH patients sera.

4. Conclusions
   The serum metabolic profile of 467 liver biopsied individuals had a significant association with liver histology that was dependent on BMI, an observation which indicates that the mechanism of NAFLD pathogenesis may be dependent on an individual’s level of obesity.

   Groups of NASH serum metabolite biomarkers include altered ether phospholipids, and elevated levels of proinflammatory eicosanoids in obese patients. Lean NASH patients had significantly reduced levels of serum sphingolipids.

   The present data, indicating that a BMI-dependent serum metabolic profile may reliably distinguish NASH from steatosis patients, have significant implications for the development of NASH biomarkers and potential novel targets for therapeutic intervention.