
Article:
Moore, JB orcid.org/0000-0003-4750-1550 and Thorne, JL orcid.org/0000-0002-3037-8528 (2019) Predicting and reducing hepatic lipotoxicity in non-alcoholic fatty liver disease. Lab Animal, 48 (5). pp. 143-144. ISSN 0093-7355

https://doi.org/10.1038/s41684-019-0291-0

News & Views

SYSTEMS BIOLOGY

Predicting and reducing hepatic lipotoxicity in non-alcoholic fatty liver disease

Utilising a systems genetics approach, a recent study identifies genetic variants and proteins associated with plasma and hepatic lipid abundance and hepatic lipotoxicity.

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Non-alcoholic fatty liver disease (NAFLD) is a complex phenotype that results from interactions between genetic, dietary, and lifestyle factors.[1] Closely associated with obesity, NAFLD is now a significant public health concern worldwide, with ~25-30% of the general population in many countries having fatty liver (steatosis) and ~5% having clinically meaningful fibrosis. Although several genetic variants have been identified that influence susceptibility to NAFLD, as well as liver disease more generally, these do not account for all of the estimated heritability of liver fat and fibrosis.[1] Challenging to diagnose and treat, there are unmet clinical and public health needs for non-invasive biomarkers for NAFLD, and a critical research question is how to predict who is at significantly higher risk for developing hepatic lipotoxicity and advanced liver disease.

Capitalising on a genetic reference panel of 107 inbred mouse strains, a recent study has taken a systems biology approach integrating plasma and liver lipidomics with proteomics and quantitative trait loci (QTL) mapping, allowing predictions to be made of genetic variants involved in hepatic lipotoxicity.[2] Utilising replicate (n=2-3) male mice, ~60 days old, from the Hybrid Mouse Diversity Panel (HMDP) strains of mice that exhibit substantial diversity in metabolic traits relevant to human disease, Parker and colleagues integrate systems genetics and correlation analyses to identify genetic variants and co-regulated networks that influence hepatic proteome and lipidome abundance across the HMDP (Fig 1a., excerpted from Parker et al.). This work makes several significant contributions to the field through identifying novel lipid-regulatory proteins, as well as novel candidate circulating biomarkers for diagnosis and determining prognosis in liver disease.

The paper exploits the significant inter-strain variation for both liver and plasma lipid abundance, with triacyclglycerols, for example, varying by 27- and 16-fold respectively. Correlation analyses between 190 plasma and liver lipid species, in combination with machine learning models, were used to identify plasma lipid signatures that had high predictability for the abundance of hepatic lipids associated with pathology (e.g. ceramides, diacylglycerol, triacylglycerol). Notably, two of these signatures of plasma lipid ratios were found to correlate to human liver triacylglycerol and diacylglycerol levels in a cohort of obese humans with hepatic steatosis (n=58). Integration of QTL mapping for hepatic protein (pQTLs), plasma and hepatic lipid (lQTLs), and protein:lipid correlation data, identified 281 single nucleotide polymorphisms mapping to 17 genetic loci that contained a pQTL, an lQTL, and a direct correlation between the mapped protein and lipid in the liver. For example, the GLO1 locus was identified as having both a significant cis-pQTL and having lQTLs for multiple hepatic triacylglycerol species; while the GLO1 protein (glyoxalase 1) correlated with 9 triacylglycerol species in liver (Fig 1b, excerpted from Parker et al.). These data corroborate those of Spanos and workers,[3] who had, through proteomic screening, previously identified altered hepatic GLO1 expression, which they confirmed in paediatric patients with NAFLD. This independent finding further implicates GLO1 disruption as a causative factor in hepatic lipotoxicity and cardiometabolic disease. Targeting GLO1 remains largely unexplored in the therapeutic setting. Although initial results from a pilot clinical trial of a glyoxalase 1 inducer (a combination of the dietary bioactive compounds trans-resveratrol and hesperetin), in participants (n=29) with overweight and obesity,

suggested improved glycaemic control, insulin resistance and vascular function with GLO1 induction.[4]

Global correlations between the lipidomic and proteomic datasets, in combination with pathway enrichment, identified proteins associated with the proteasome and proteolysis as strongly correlated with hepatic lipid abundance. The authors follow up on PSMD9, a relatively unstudied protein, which they find strongly correlated to multiple hepatic and plasma lipid species. A proteosomal chaperone protein, PSMD9 (also known as p27) is released during assembly of the 26S proteasome.[5] Intriguingly PSMD9 has previously been shown to be associated with lipid droplets under conditions of caloric restriction and intermittent fasting[6]. While early work suggested PSMD9 also has transactivating activity influencing glucose-dependent insulin production and glucose metabolism.[7] Parker et al. identify a significant cis-pQTL within the PSMD9 locus that also co-mapped to multiple lQTLs for plasma triacylglycerols. In impressive proof-of-concept work, antisense oligonucleotides were used to silence PSMD9 in two strains of mice that vary significantly in abundance of liver and plasma lipids (DBA/2J and C57BL/6J), and were fed a western diet for 28 days. Silencing of PSMD9 reduced plasma lipids and prevented hepatic steatosis preferentially in DBA/2J mice mediated in part through reductions in fatty acid synthesis by de novo lipogenesis.

Strengths of this work include the integration of intermediate phenotypes (proteomics, lipidomics) with high-resolution genomic association mapping of the HDMP and the examination of plasma and liver lipid abundance as quantitative traits. Quantifying >4,000 proteins in more than 50 strains of mice along with 311 lipid species across 3 classes is not a trivial experimental accomplishment, and the associated datasets of Parker et al. are a rich bioinformatics resource. Exploitation of the high inter-strain differences in lipid traits observed within the HDMP was a key strength; without such variation, the associated genetic variant loci would not have been identified. The authors rationally target their investigation a priori to a subset of known pathogenic lipid subclasses, and then again to those that were most strongly correlated (ceramides, triacylglycerols and diacylglycerols). Nonetheless, it is probable there are other pathogenic lipid profiles in liver, along with their associated plasma signatures, which the application of unsupervised data-centric approaches could help identify. Similarly, while the authors followed up on PSMD9 and highlighted the independent GLO1 data, other loci identified are also worth detailed investigation and examination of translation to the human disease. For example, it is interesting to note the enzyme serine hydroxymethyltransferase 1 (SHMT1), was also among loci identified having both a pQTL, lQTL and correlation between protein and lipid in the liver. Previous systems work utilising a genome scale metabolic network in tandem with transcriptomic data from NAFLD patients suggested serine deficiency in NAFLD and proposed SHMT1 as possible target.[8]

A key question will be in the translation to human disease. The differential effect of PSMD9 knockdown observed in the DBA/2J and C57BL/6J strains, underscores caution that a single target for NAFLD is likely to suffice. There are, and will be further, genetic and environmental mediators of hepatic lipotoxicity, particularly in outbred humans. Only male animals were profiled here; although

the majority of subjects in the human lipidomic dataset used in validation were female. However, it should be noted these participants were undergoing bariatric surgery (thereby making the matching plasma and liver lipidomics possible), and had BMIs 40-50 that further restricts the extrapolation of these results. Additionally, measuring the plasma lipids represented in the ratios identified here is not routine clinically, preventing the examination of existing larger scale or population datasets retrospectively. Nonetheless, Parker et al provide multiple avenues for future novel cell biology and their data underscore how much we have to learn about the proteasome, its role in lipid metabolism and the roles of its moonlighting chaperone and component proteins.

Fig. 1 **Systems genetic analysis of hepatic lipid metabolism**. Study overview (A) and back-to-back Manhattan plots showing a locus significantly associated with liver triacylglycerol abundance and liver GLO1 protein (B).

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