Integrated miR-mRNA Network Underlying Hepatic Fat Accumulation in Humans

Rajneesh Srivastava[1†], Xiaoliang Wang[2†], Jingmei Lin[3], Rongrong Wei[2], Praneet Chaturvedi[1], Naga P. Chalasani[4,5*], Sarath Chandra Janga[1,4,5,6,7,*], Wanqing Liu[2, 4 ,5,*]

[†]Joint co-authors, *Correspondence authors

[1]School of Informatics and Computing, Indiana University-Purdue University Indianapolis, Indiana, Indianapolis, IN 46202, USA.
[2]Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, Purdue University. West Lafayette, IN, 47907
[3]Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN 46202.
[4]Division of Gastroenterology/Hepatology, Indiana University School of Medicine, Indiana University, Indianapolis, IN.
[5]Indiana Fatty Liver Research Group, Indiana University School of Medicine, Indiana University, Indianapolis, IN.
[6]Center for Computational Biology and Bioinformatics, Indiana University School of Medicine, Indianapolis, Indiana
[7]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, Indiana

INDIANACTSI Clinical and Translational Sciences Institute

Abstract

Background

An integrate miRs and mRNAs analysis in the development of Non-Alcoholic Fatty Liver Disease (NAFLD) and Non-Alcoholic Steatohepatitis (NASH) is lacking. We aimed to identify miRs as well as the miR-mRNA regulatory network involved in hepatic fat accumulation and human NAFLD.

Materials and Methods

Hepatic fat content (HFC) was measured, and liver histology was characterized for 73 liver tissue samples. MicroRNAs and mRNAs significantly associated with HFC were identified based on genome-wide mRNA and miR expression profiling data. These miRs and mRNAs were further used to build miR-mRNA association networks in NAFLD and normal samples based on the potential miR-mRNA targeting, as well as to conduct a pathway enrichment analysis.

Results

We identified 62 miRs significantly correlated with HFC ($p<0.05$), with miR-518b and miR-19b demonstrated to be the most significant positive and negative correlation with HFC, respectively ($p<0.008$ for both). Many miRs that were previously associated with NAFLD/NASH were also observed. Integrated network analysis indicated that a few miRs-30b*, 616, 17*, 129-5p, 204, and 20a controlled >80% of HFC-associated mRNAs in this network, and the regulation network was significantly rewired from normal to NAFLD. Pathway analyses revealed that inflammation pathways mediated by chemokine and cytokine signaling, Wnt signaling, Integrin signaling and Natural killer cell mediated cytotoxicity were enriched ($p<0.05$) in hepatic fat accumulation.

Methods

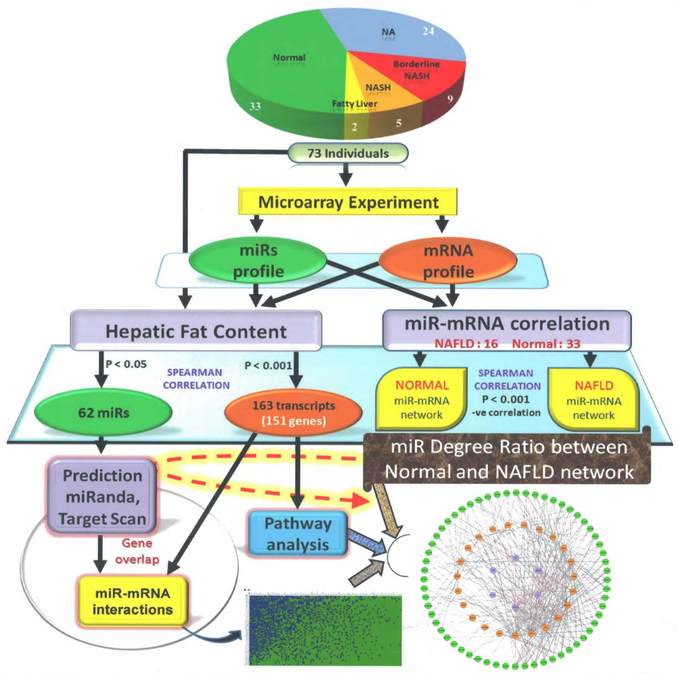

Figure 1. ***Work flow.*** Liver samples were collected from 73 organ transplant donors for our study. MiRnome and transcriptome profiles for these 73 samples were generated by microarray experiment. Further miRnome and transcriptome data was correlated (Spearman Correlation) separately with hepatic fat content of 73 individuals (NAFLD – 16, Normal – 33, NA - 24). HFC associated 163 mRNAs (151 genes) were analyzed for their enrichment in several biological pathways. Additionally 129 targets, predicted by miRanda and TargetScan, for HFC associated 62 miRs were found as an overlapped subset of HFC associated mRNAs (151 genes). HFC affected miR-mRNA interaction network (62 miRs connected to 129 genes with 1972 edges) was generated and a subset of this network was studied for HFC influenced biological pathways. In a subset of 73 liver samples, i.e. 49 samples (33 Normal and 16 NAFLD), miR and mRNA expression data was correlated ($p<0.001$) separately for each group and degree ratio between negatively correlated miR-mRNA interaction for Normal and NAFLD was calculated. These were interpreted for dynamics of post transcriptional regulatory network in NAFLD.

Results

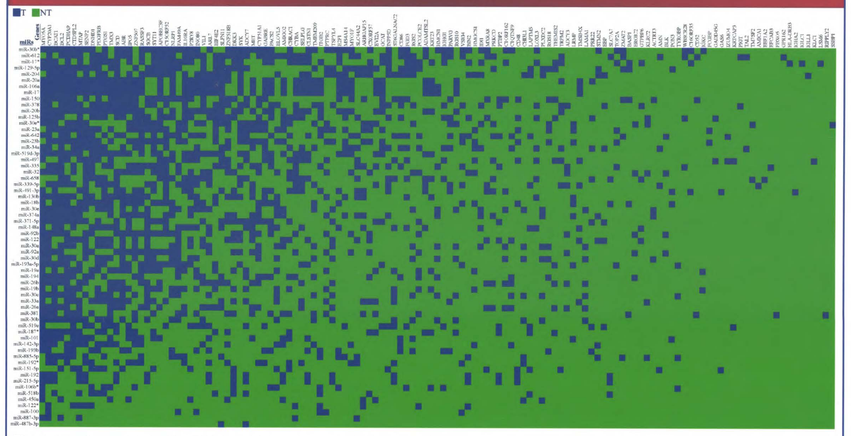

Figure 2. HFC associated miR-mRNA (gene) interaction network. Heat map showing 62 HFC associated miRs and 129 HFC associated target genes with targets shown in blue and non-target shown in green.

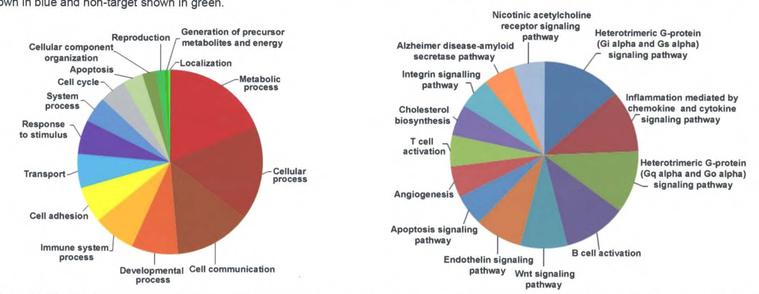

Figure 3. Role of HFC associated genes (mRNA) in several biological processes and pathways using PANTHER- gene ontology and pathway analysis tool.

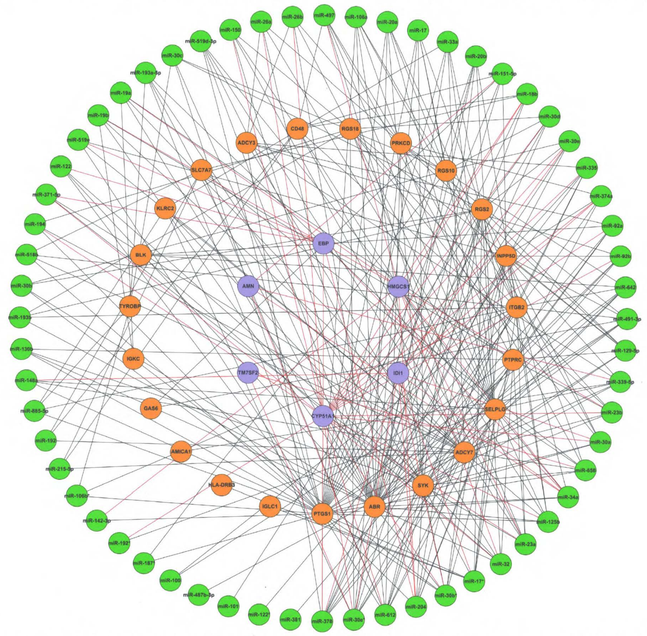

Figure 4. Pathway specific post transcriptional network. HFC associated 62 miRs (outer circle- green) targeting genes, involved in important pathways : 23 genes in immune response and signaling (middle circle- orange) and 6 genes in lipid metabolism (inner circle- violet), are shown as miR-target gene interaction network (355 edges).

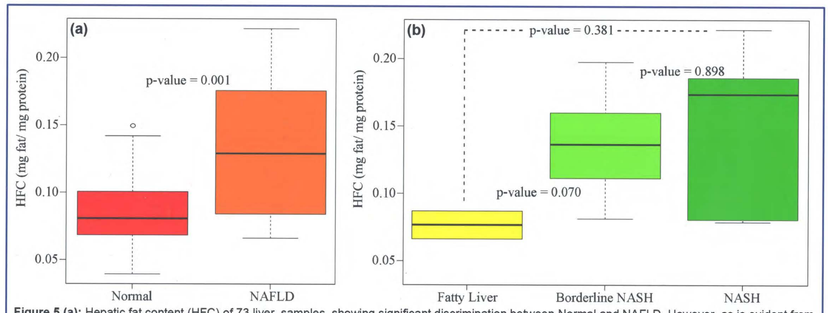

Figure 5 (a): Hepatic fat content (HFC) of 73 liver samples showing significant discrimination between Normal and NAFLD. However as is evident from **figure 5 (b),** HFC fails to significantly discriminate within subclasses of NAFLD.

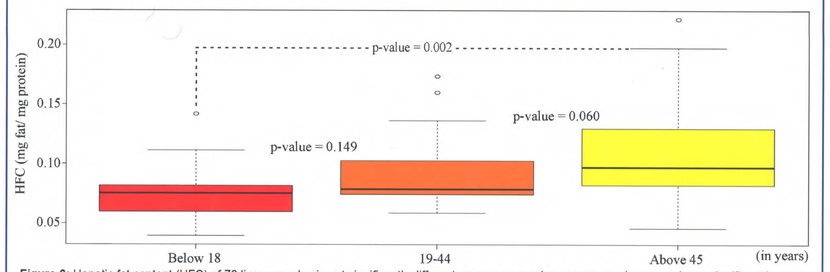

Figure 6: Hepatic fat content (HFC) of 73 liver samples is not significantly different among successive age groups, however shows significant increase in HFC when age groups below 18 and above 45 years were compared.

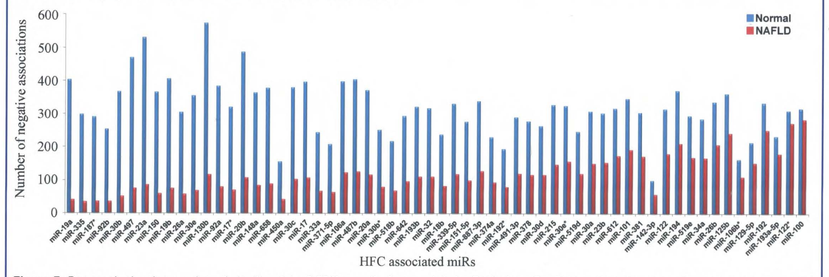

Figure 7. Bar graph showing number of significant ($p<0.001$) negatively associated mRNAs in miR-mRNA association network constructed using HFC associated miRs in each case [normal (33 samples) and NAFLD (16 samples)].

Summary

We identified 62 miRs significantly correlated with HFC of 73 liver donors. We independently associated liver transcriptome of these 73 individuals with their HFC and constructed HFC integrated miR-mRNA post-transcriptional network, which were found affected in several pathways (mainly related to lipid metabolism and immunity). Variation in HFC was found unrelated with sex and race however age factor influenced as slight increase in HFC (with higher in above 45) among 73 individuals. We also found a decrease in HFC associated miR targets (genes) in NAFLD patients.

Conclusion

Our study for the first time provided detailed insights into the HFC-associated miR-mRNA regulatory network that may contribute to the pathogenesis and progression of NAFLD. Our findings further highlighted the important miRs and mRNAs previously identified, and more importantly generated a number of new hypotheses to be investigated in the future.

Correspondence

- **Sarath Chandra Janga (scjanga@iupui.edu)**
- **Naga P. Chalasani (nchalasa@iu.edu)**
- **Wanqing Liu (liu781@purdue.edu)**