Profiles of circulating mRs as non-invasive liquid biopsies for investigating hepatotoxicity in human subjects

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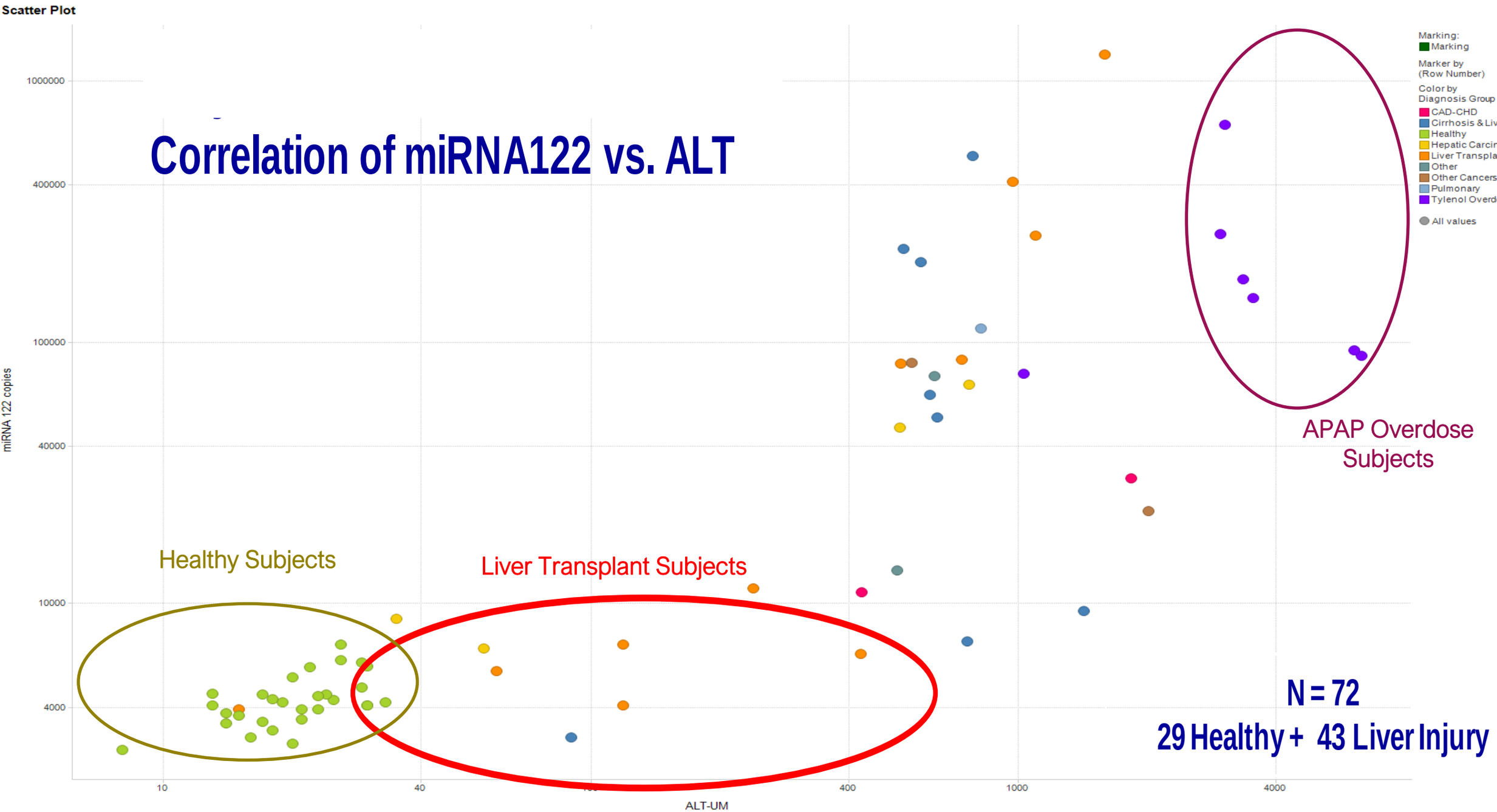
miR122 - potential biomarker of Liver Injury

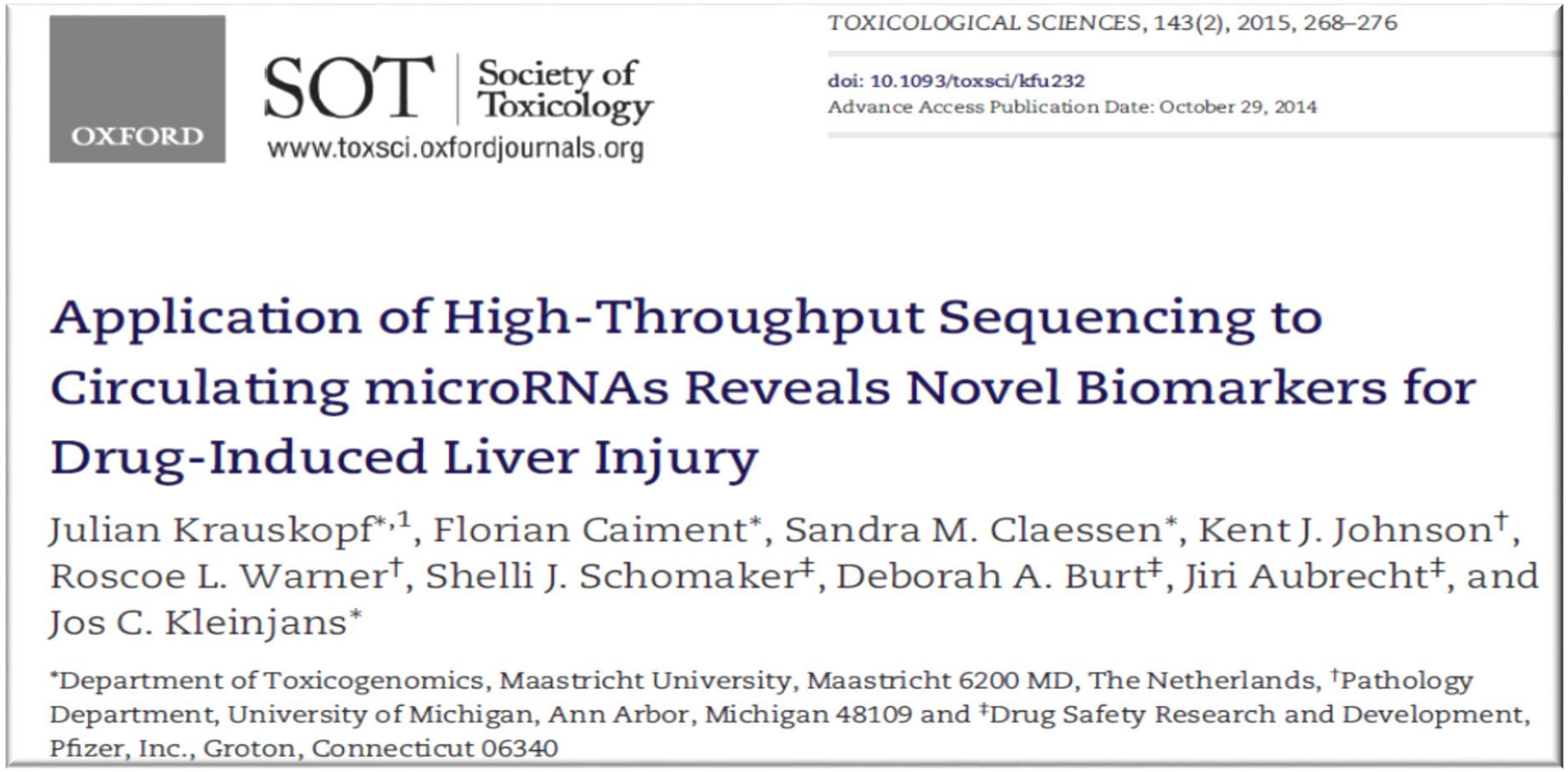


Hypothesis

- Profiles (signatures) of circulating miRs reflect mechanistic information about toxicity, disease
- miR signatures might be useful for:
  - understanding tox effect
  - Diagnosis of disease
  - Susceptible populations
  - Patient stratification

Proof of concept studies

1. miR signature of APAP overdose



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www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 143(2), 2015, 268–276

doi: 10.1093/toxsci/kfu232

Advance Access Publication Date: October 29, 2014

Application of High-Throughput Sequencing to Circulating microRNAs Reveals Novel Biomarkers for Drug-Induced Liver Injury

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2. miR signatures of liver diseases

Serum microRNA signatures as “liquid biopsies” for interrogating hepatotoxic mechanisms and liver pathogenesis in humans.
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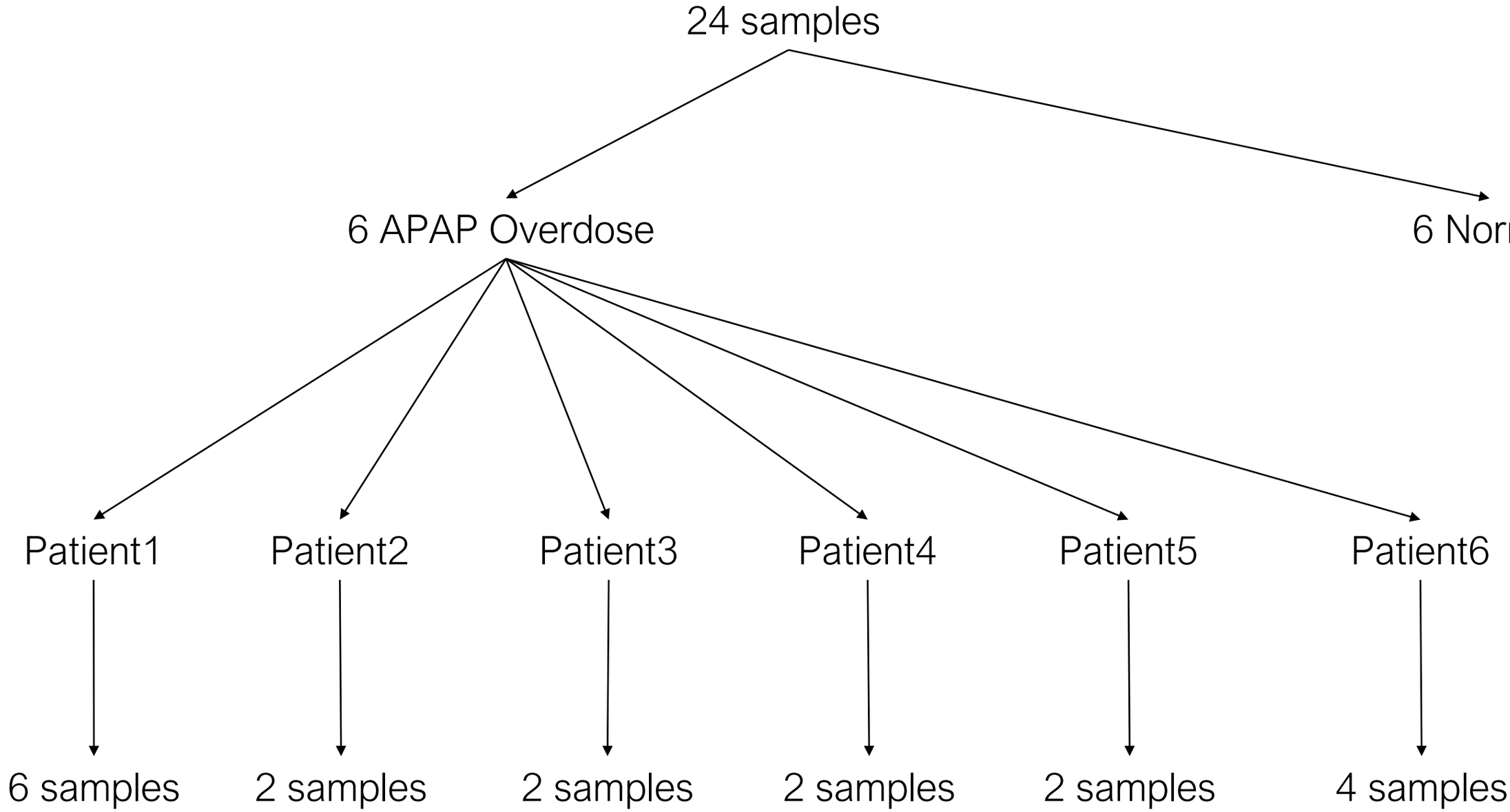
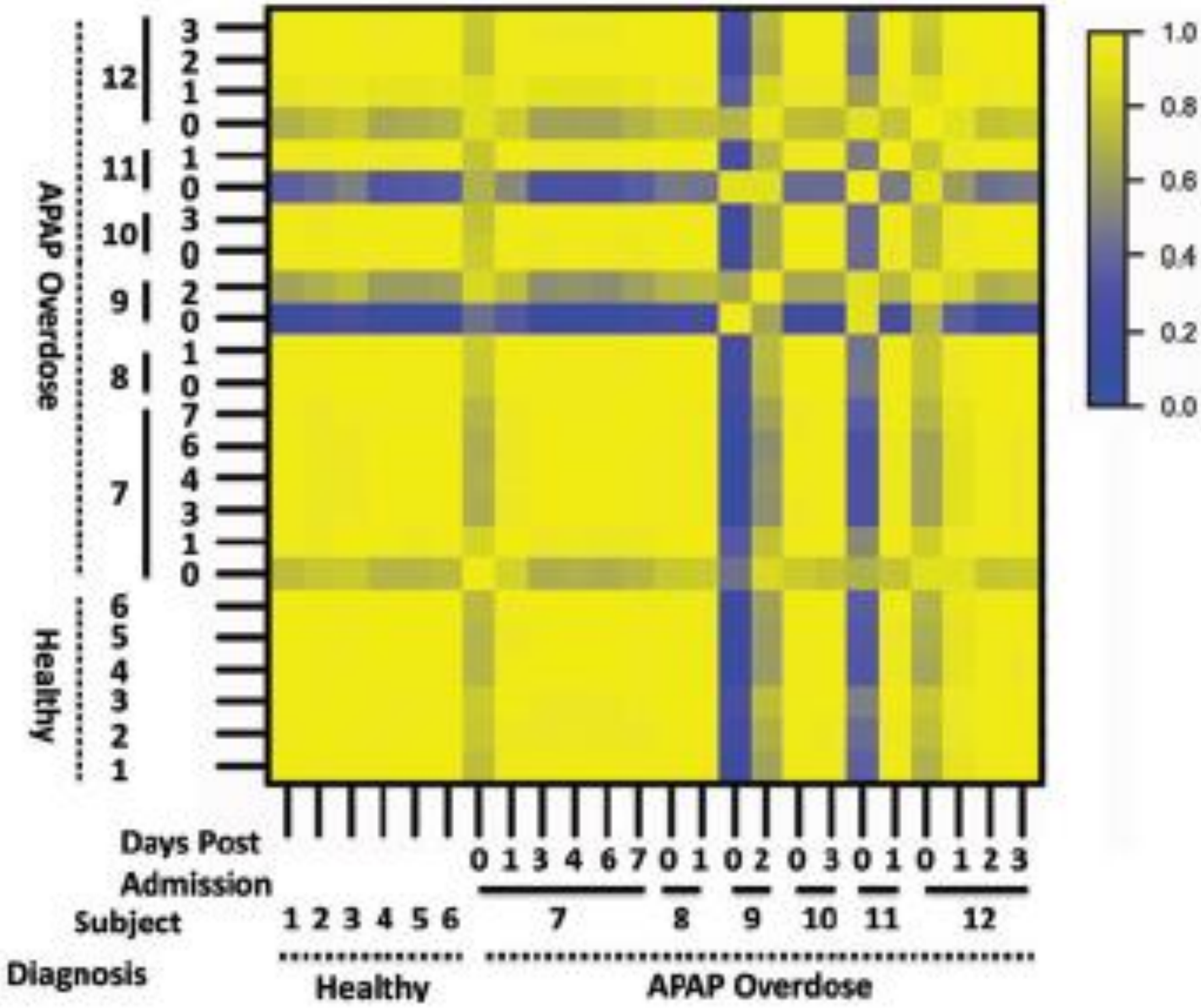
PLoS ONE 2017

3. Differentiate outcome of lethal APAP poisoning

1. miR signatures of APAP Overdose– Study design

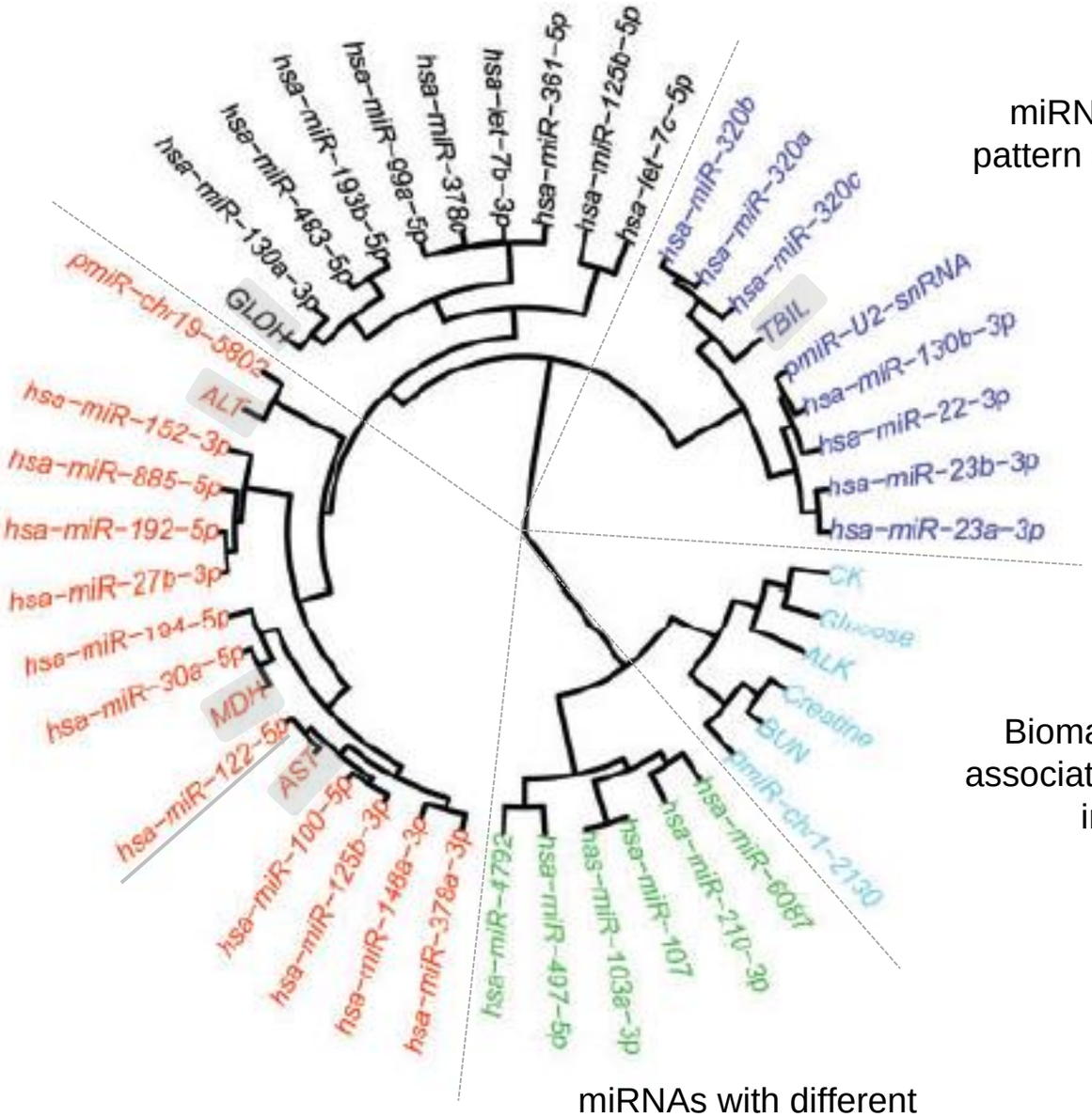


Circulating miR profiles differentiate APAP-induced liver injury



miRs time course patterns cluster with conventional biomarkers

miRNAs with a similar pattern of response as GLDH



miRNAs with a similar pattern of response as Tbil

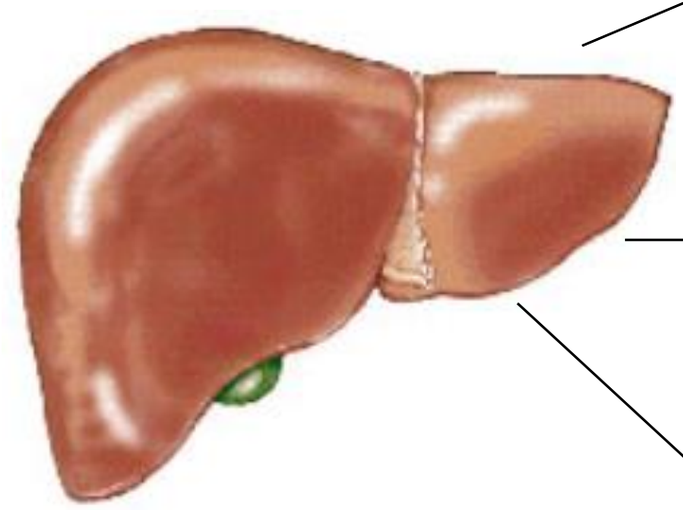
miRNAs with a similar pattern of response as ALT and AST

Biomarkers not associated with liver injury

miRNAs with different pattern

Hierarchical Clustering Based on Spearman Distance

Biological significance of observed miRs



Liver-specific Biological Function	miRNAs
Cancer/proliferation	
Liver regeneration	let-7b, miR-27b
Liver proliferation/cancer	let-7c, miR-100, miR-122, miR-130b, miR-148, miR-152, miR-192, miR-193, miR-22, miR-23, miR-30, miR-483, miR-497
Wnt pathway	miR-130a
TGF-beta	miR-23a
Metabolism	
Glucose homeostasis	miR-103, miR-107, miR-22
Insulin sensitivity/signaling	miR-130, miR-320a
Lipid metabolism	miR-122, miR-27b, miR-99a
PPAR pathway	let-7c, miR-27b
PXR, CYP3A	miR-148, miR-378
Iron metabolism	miR-122
Fibrosis	miR-483
Hepatobiliary development	miR-30
Oxidative stress	let-7b
ER stress	miR-107
Not reported function in liver	miR-125, miR194, miR361, miR-6087, miR-885

- Liver specific processes indicated by miRs are consistent with molecular mechanism of APAP toxicity

2. miR signatures of liver impairments

PLOS ONE

RESEARCH ARTICLE

Serum microRNA signatures as "liquid biopsies" for interrogating hepatotoxic mechanisms and liver pathogenesis in human

Julian Krauskopf[1]*, Theo M. de Kok[1], Shelli J. Schomaker[2], Mark Gosink[2], Deborah A. Burt[2], Patricia Chandler[3], Roscoe L. Warner[4], Kent J. Johnson[4], Florian Caiment[1], Jos C. Kleinjans[1], Jiri Aubrecht[2]

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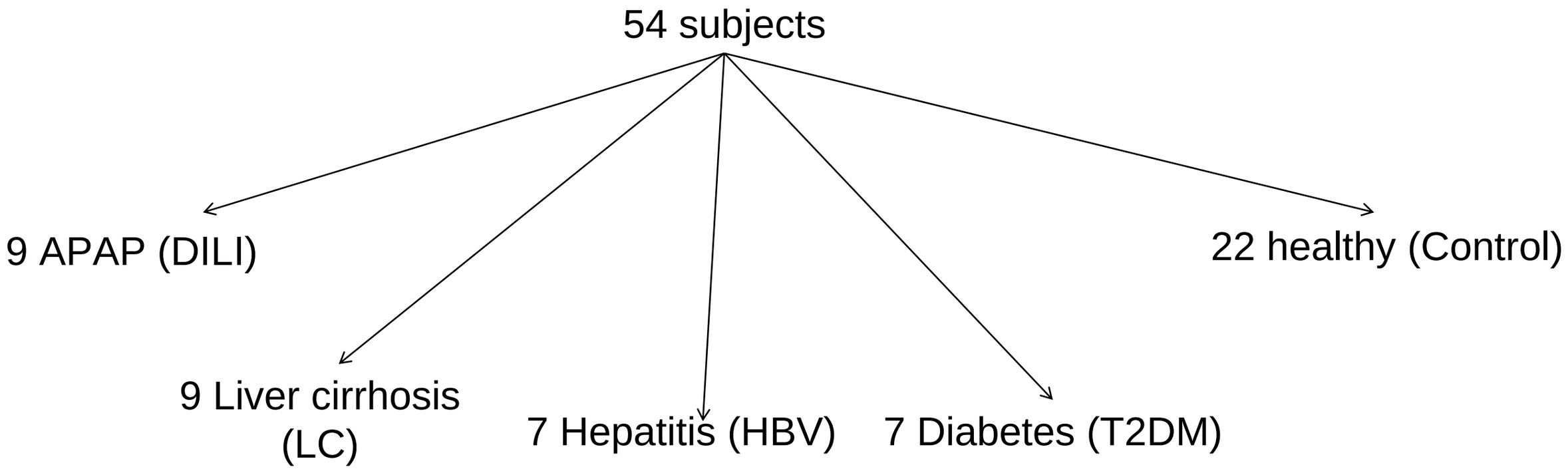
Hypothesis:

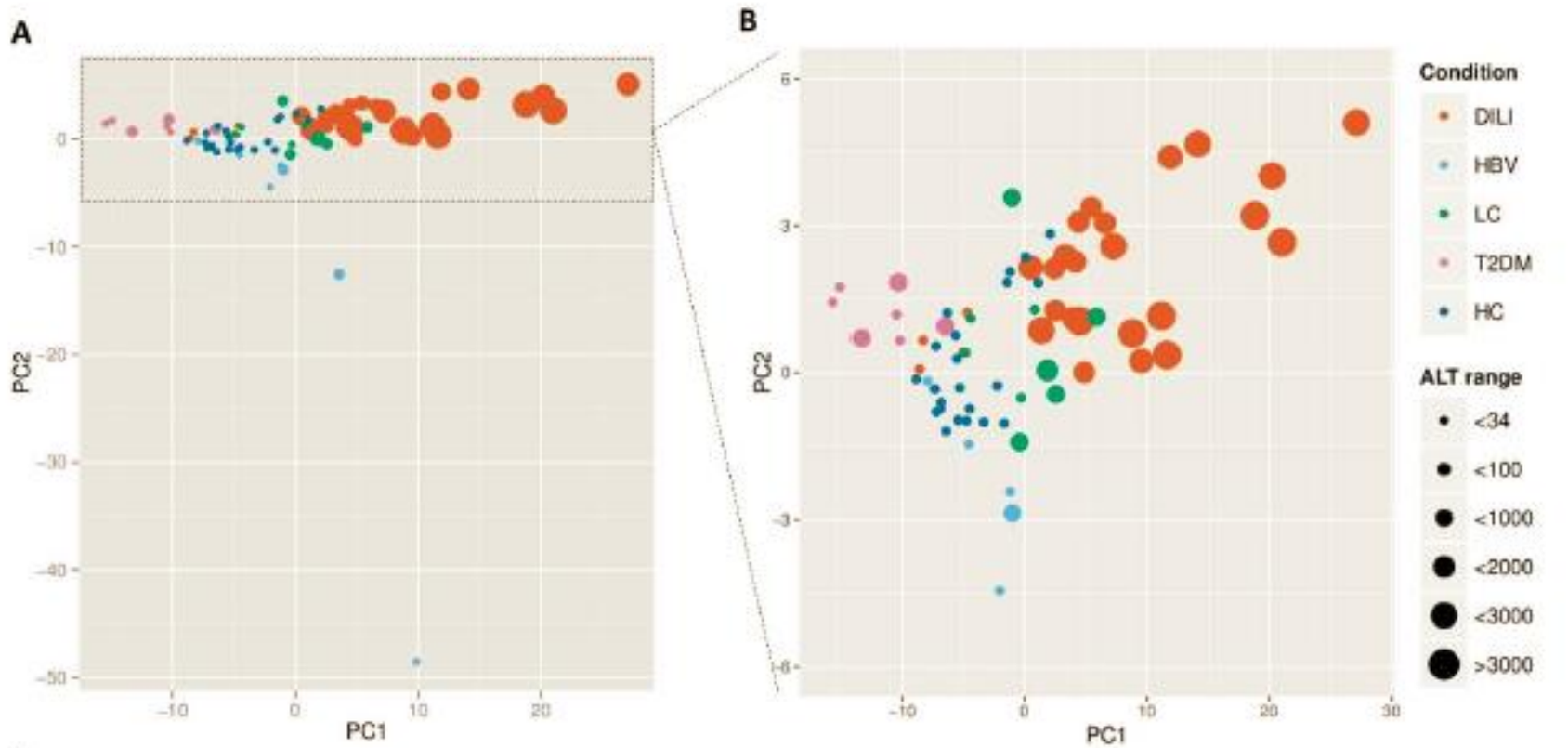
miR “signatures” in serum can differentiate among variety of liver impairments including providing insights into pathophysiology of disease

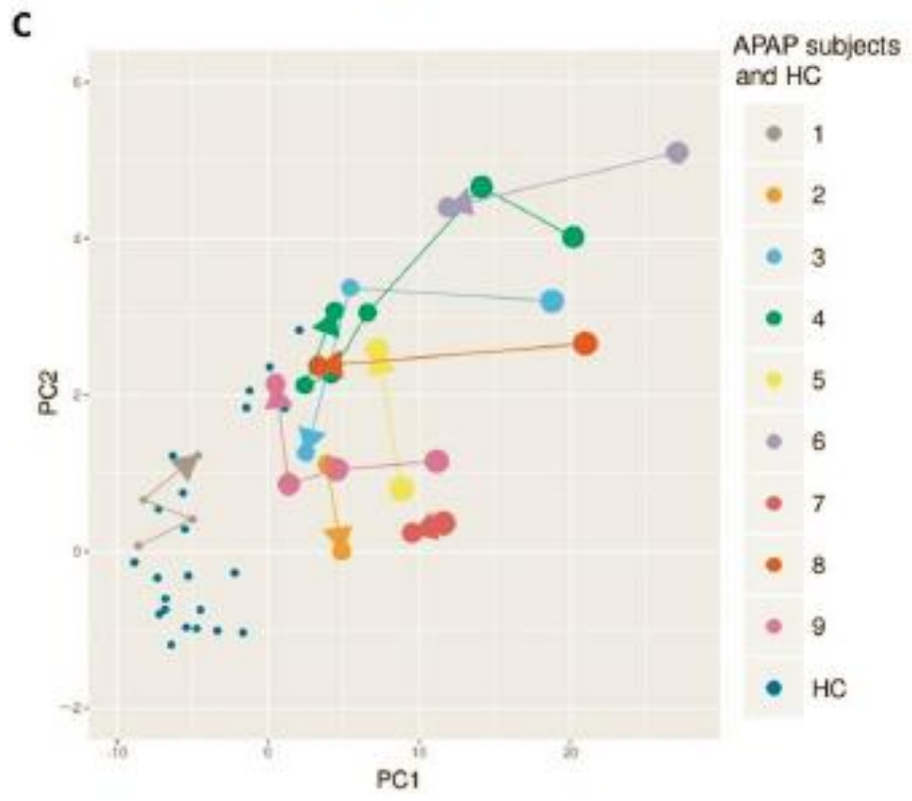
Study design

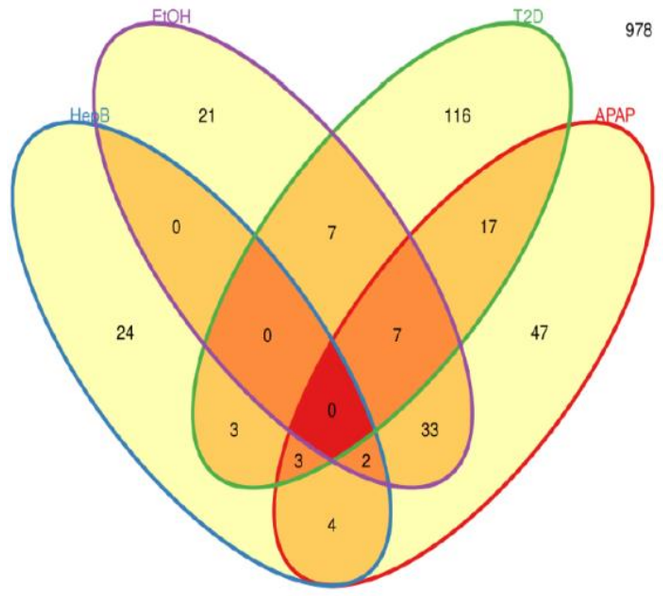


- Age and gender matched groups of subjects selected based on adjudication of medical records
- NextGen sequencing analysis of serum samples
- Bioinformatic anlysis

miR profiles differentiate among variety of liver impairments







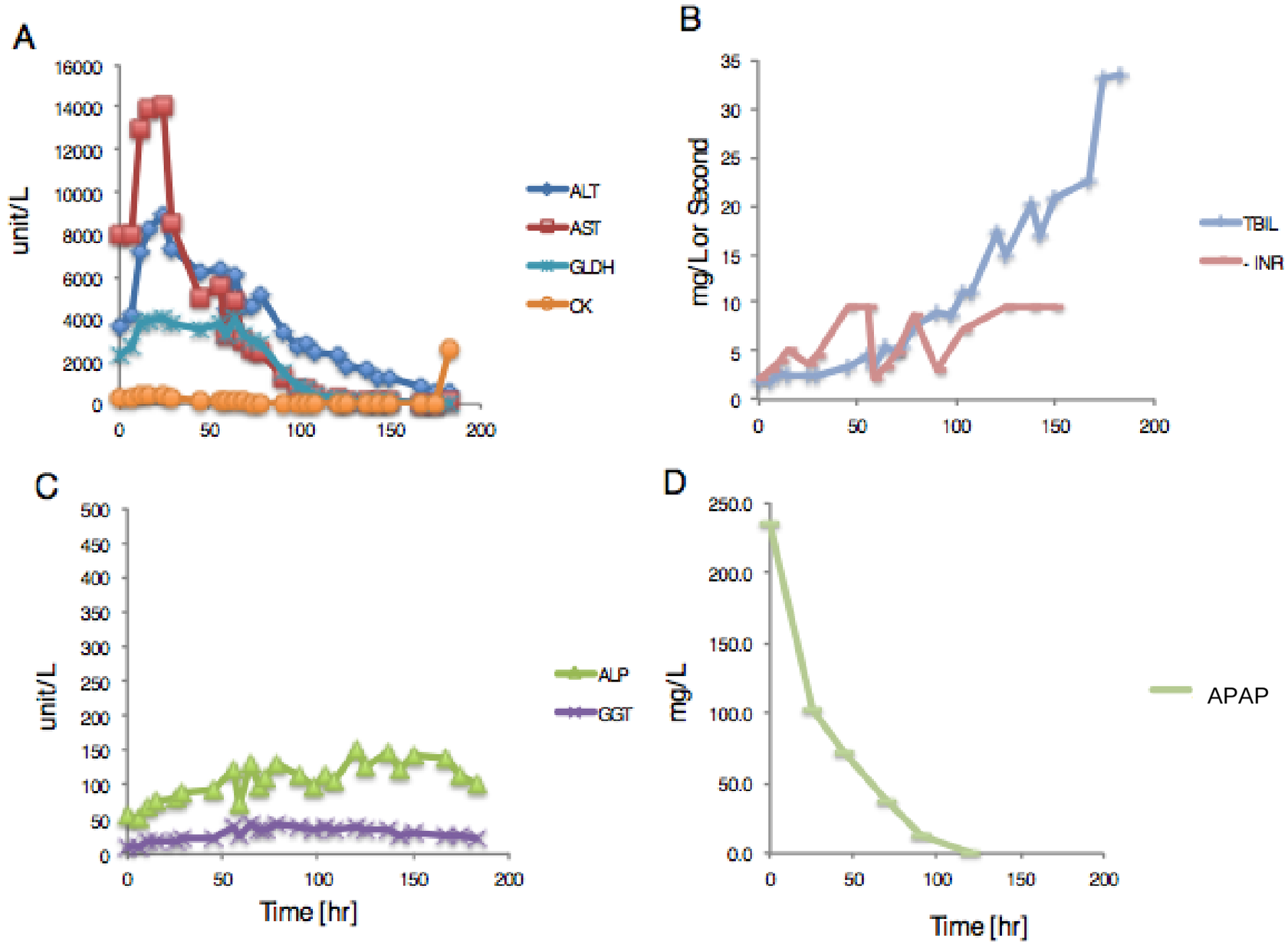
miR signatures reveal relevant pathways and mechanisms

Table 1. Comparison of pathways derived from literature analysis with pathways derived from miRNA target genes.

Cirrhosis Pathways	Cirrhosis pathways from PubMed	Cirrhosis pathways associated with identified miRNAs
Bladder Cancer Signaling	3.29E-14	8.25E-16
Regulation of the Epithelial-Mesenchymal Transition Pathway	7.06E-15	8.25E-16
Agranulocyte Adhesion and Diapedesis	5.55E-15	8.25E-16
Granulocyte Adhesion and Diapedesis	5.55E-15	8.25E-16
Glioblastoma Multiforme Signaling	1.47E-11	8.25E-16
STAT3 Pathway	2.40E-12	8.25E-16
Antiproliferative Role of TOB in T Cell Signaling	3.16E-08	8.51E-15
DILI Pathways	**DILI pathways from PubMed**	**DILI pathways associated with identified miRNAs**
NRF2-mediated Oxidative Stress Response	9.02E-15	5.75E-16
Apoptosis Signaling	8.33E-13	5.75E-16
Acetone Degradation I (to Methylglyoxal)	6.13E-09	1.20E-08
Bupropion Degradation	6.13E-09	2.38E-08
Estrogen Biosynthesis	6.74E-08	8.81E-08
Hepatitis B Pathways	**HBV pathways from PubMed**	**HBV pathways associated with identified miRNAs**
Antigen Presentation Pathway	8.02E-15	8.30E-06
Interferon Signaling	8.02E-15	7.59E-10
Type-2 Diabetes Pathways	**T2D pathways from PubMed**	**T2D pathways associated with identified miRNAs**
AMPK Signaling	2.54E-13	5.96E-16
Insulin Receptor Signaling	1.88E-13	5.96E-16
TR/RXR Activation	7.76E-11	5.96E-16
eNOS Signaling	1.23E-09	5.96E-16
Type II Diabetes Mellitus Signaling	1.20E-14	5.96E-16
Leptin Signaling in Obesity	1.20E-09	5.96E-16
Role of NFAT in Cardiac Hypertrophy	1.19E-07	5.96E-16

Significance values for pathways predicted to be the most specific for each individual disease state were calculated based on published literature or known miRNA target genes. Pathways in the table are arranged by relative specificity determined from the literature for individual disease state.

3. Differentiate outcome of lethal case of APAP poisoning

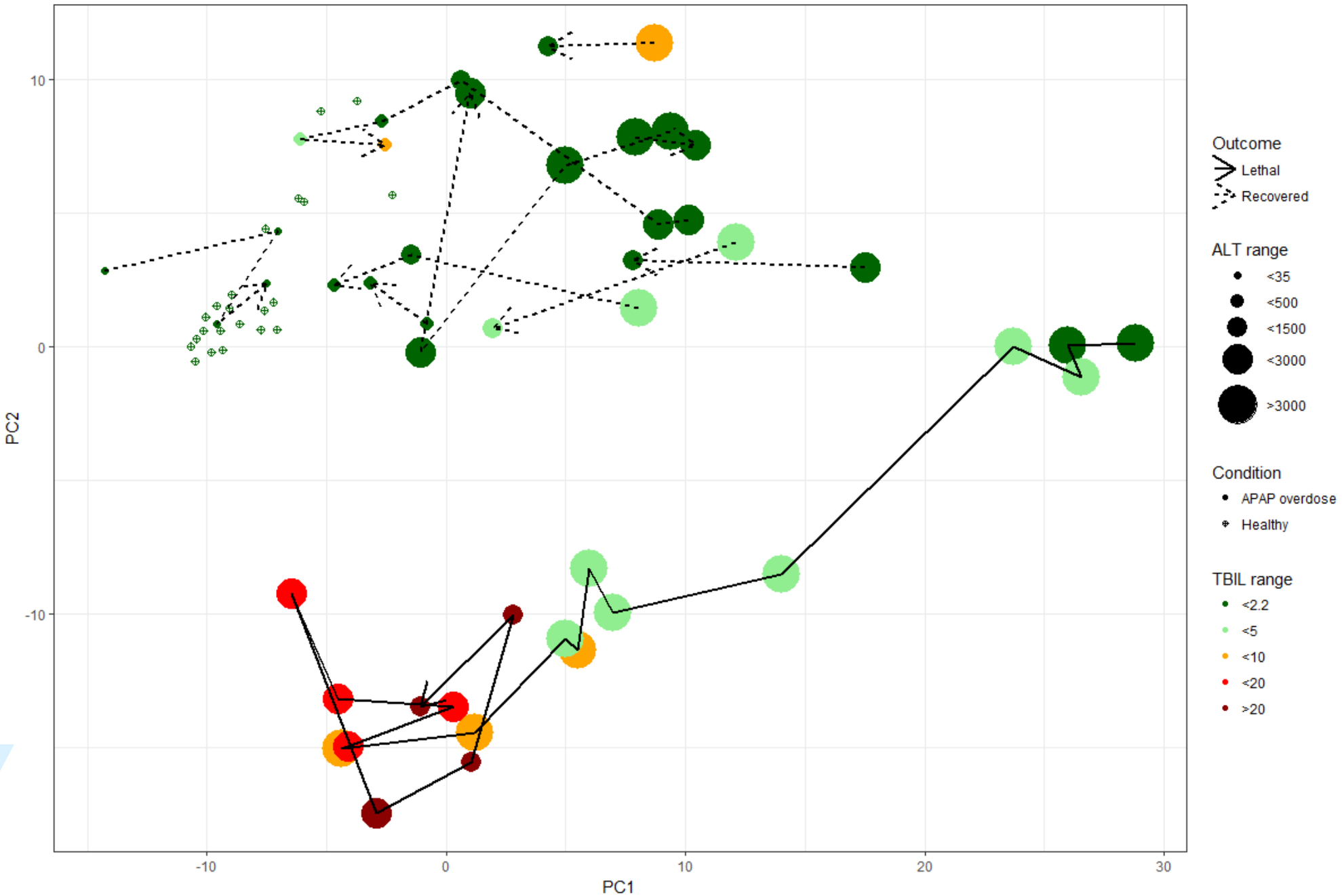


Female, 46 years, admitted for APAP overdose, progressed to liver failure and death

miR signatures predict clinical outcome of APAP overdose

Cellular stress, autophagy….

Prothrombin activation pathway, fibrosis…



Conclusions

- miR signatures have a potential to provide a fundamental advancement (paradigm shift) as a non-invasive tool for studying molecular mechanisms with impact on:

  - Understanding of disease process, efficacy and safety of new therapies

  - Stratification of subjects

  - Reverse translation

- miR based approach has a potential to translate across species and in vitro models to clinic – future direction

Acknowledgments

Pfizer

- Shelli Schomaker
- Deborah Burt
- Patricia Chandler
- David Potter
- Mark Gosink

University of Michigan

- Kent Johnson
- Roscoe Warren

University of Maastricht

- Julian Krauskopf
- Jos Kleinjans