The Looming NASH Epidemic: Using –omics for risk stratification and prevention

Cynthia A. Moylan, MD, MHS
23 January 2020
I perceive no conflict of interest with today’s presentation but present companies in the NAFLD field that I have worked with or consulted for:

- Grants (awarded to Duke Health)
  - Medical monitor: TaiwanJ Pharmaceuticals, Madrigal Pharmaceuticals
  - Sub-Investigator: Gilead, Galectin, Intercept, NovoNordisk, Genfit, BMS, Allergan, BI, Astra Zeneca

- Consultant / Advisory Board
  - NuSirt, Gilead
What is Non-Alcoholic Fatty Liver Disease (NAFLD)?

- Excess fat (steatosis) accumulation in the liver of ≥ 5%
- Diagnosed by imaging (ex. ultrasound, MRI, CT) or histology (gold standard)

✓ Not due to alcohol overuse
✓ Not due to another cause
  - Medication
  - Viral hepatitis

**Metabolic Syndrome:**
- Obesity
- Insulin Resistance
- HTN
- Hyperlipidemia
- Sleep apnea, hypothyroidism, PCOS

The NAFLD Continuum

Normal Liver

Steatosis “NAFL”
- Fat infiltration ≥ 5% without ballooning

Steatohepatitis
- Fat infiltration ≥ 5% with necroinflammation and hepatocellular injury (ballooning, hepatocyte degeneration, Mallory bodies, or megamitochondria)

Cirrhosis
- Increasing fibrosis, leading to cirrhosis
- Can be silent and asymptomatic
- Normal liver blood tests

A 54 year-old female with type 2 diabetes and body mass index (BMI) of 37 kg/m² who underwent abdominal ultrasound to evaluate mildly abnormal liver blood tests. The ultrasound showed increased echogenicity consistent with fatty liver.

**Laboratory Data**
- ALT 68 U/L (nmol ~20)
- AST 63 U/L
- Total bilirubin 0.8 g/dL
- Albumin 4 g/dL
- Platelets: 190,000
- INR: 1.0

**Past Medical History:**
- Type 2 Diabetes Mellitus
- Hypertension
- Hypothyroidism

**Social History:**
- No alcohol use, no tobacco.
- Stay at home mom. 3 teenage kids.
- Married.

**Family Hx:**
- Hispanic ethnicity
- Mother: DM
- Father: heart disease

**Medications:**
- metformin, lisinopril, levothyroxine
Important Questions:

1. Does the patient have NAFL or NASH?
2. Does the patient have liver fibrosis? If so, how severe?
3. Will her liver disease impact her liver-related health?
4. Will her liver disease impact her overall health or survival?
Dynamic Nature of NAFLD

- **Normal Liver**
- **Steatosis “NAFL”**
- **Borderline NASH**
- **Steatohepatitis “NASH”**

- **Fibrosis Progression**
  - 5-20% rapid progressors
  - 38% slow progressors

- **Fibrosis Regression**
  - 20% can regress

NAFLD has a Variable Prognosis

Normal Liver

Steatosis “NAFL”

Steatohepatitis “NASH”

Cirrhosis

CVD-related morbidity / mortality

Liver-related morbidity / mortality

The Scope of the Problem

Estimated Number of Americans with NAFLD
80 Million

Estimated Number of Americans with NASH
16.5 Million

Estimated Number of Americans with NASH cirrhosis / adv fibrosis
3.3 million

2030 Estimates²:

- 100 million
- 27 million

USA
- HCV: 3.2 million
- Coronary artery disease: 18 million

Global prevalence of NAFLD = 25% 

North America: 24.13% 
South America: 30.45% 
Europe: 23.71% 
Middle East: 31.79% 
Africa: 13.48% 
Asia: 27.37% 

Important Questions:

1. Does the patient have NAFL or NASH?
2. Does the patient have liver fibrosis? If so, how severe is it?
Source of Heterogeneity in NAFLD

- Obesity
- Hypertension
- Dyslipidemia
- Sleep Apnea
- Diabetes
- Diet & Lifestyle
- Insulin Resistance
- Ethnicity
- Age
- Sex
- Genetics
- Endocrine/Hormonal Factors
- Hypertension
- Diabetes
- Insulin Resistance
- Modifiable factors
- Non-modifiable factors
- NAFLD
- NASH
- Compensated Cirrhosis
- Decompensated Cirrhosis
- Death
- HCC

HCC, hepatocellular carcinoma
Risk Stratification: Current Methods

**Current:**
- NAFL diagnosis
  - Imaging
- NASH is a histologic diagnosis
  - Biopsy required / gold standard
- Fibrosis
  - Biopsy
  - Non-invasive algorithms (FIB4, NFS, APRI, ELF)
  - Fibroscan (liver stiffness and fat measurements)
  - MRI–based (MR-PDFF, MRE)

**Future:**
- Non-invasive, reliable, accurate, safe & inexpensive
- Longitudinal assessments
- Predictive of future events
NAFLD: Can genetics and –omics help?

Epigenetic variation:
- interaction with environmental factors
- disease progression, ?dynamic

Genomic variation:
- present at birth
- small to modest effect size

Proteins:
- diagnosis, prognosis
- circulation
- longitudinal assessment

Coding and non-coding RNAs:
- molecular signatures; tissue or blood
- dynamic, longitudinal assessment

Metabolites:
- diagnosis and prognosis
- circulation
- dynamic, longitudinal assessment

Genetic contributions to NAFLD: Risk stratification

PNPLA3, patatin-like phospholipase domain containing 3; TM6SF2, transmembrane 6 superfamily 2 human gene

- PNPLA3; rs738409; I148M (OR 1.88)

- Other variants:
  - GCKR
  - MBOAT7
  - HSD17B13
  - NCAN

Other variants:
TM6SF2; rs58542926
Genetics and Epigenetics Influence NAFLD Heterogeneity

Variants explain ~10% of heritability of NAFLD
PNPLA3 MAF 26%
TM6SF2 MAF 7%

Genetic

SNPs

Epigenetic

DNA Methylation

Modify the way the DNA code is “read”
= Inherited changes in gene expression
Hepatic Transcriptome & Methylome in NAFLD Fibrosis

Aim: To achieve an unbiased assessment of DNA methylation and hepatic gene expression in patients with varying severities of NAFLD

- 70% Female
- Median age 52.5 years
- Median BMI 33
- Significant between groups:
  - Portal inflammation (p=0.007)
  - Ballooning (p=0.0007)
  - NAFLD Activity Score (p=0.01)

Duke NAFLD Biorepository (Liver Biopsies)

Mild NAFLD  
F0-F1 (N=33)

Advanced NAFLD  
F3-F4 (N=23)

Total RNA and Genomic DNA

Affymetrix Human Genome U133 Plus 2.0 Array

Illumina Infinium HumanMethylation450 BeadChip

Murphy et al. Gastro 2013; 145:1076-1087
DNA Methylation Distinguishes Mild from Advanced NAFLD

- 69,000 differentially methylated (DM) CpG sites
- 76% Hypomethylated in Advanced NAFLD
- 2500 DM and expressed

Epigenetic mechanisms influence the NAFLD fibrosis phenotype

Graphics courtesy of S. Murphy and A. Omenetti
Important Questions:

1. Does the patient have NAFL or NASH?
2. Does the patient have liver fibrosis? If so, how severe is it?
3. Will her liver disease impact her liver-related health?
4. Will her liver disease impact her overall health or survival?
Can transcriptome and methylome signatures predict NAFLD outcomes?

86 patients with GEX and 55 with GEX and DNA met in liver

Outcomes of CVD and liver disease:
- Stroke, MI, death
- Portal Hypertension
- Ascites, PSE, EV bleed, SBP, HCC

Affymetrix arrays

DNA met

450K arrays
Gene expression and methylation profiles may predict future clinical outcomes

Gene profile associated with risk for adverse event

BCAT1 hypomethylated and overexpressed in liver of patients with future adverse event

Validate Larger population Non-invasive Stratify by outcome
DNA methylation as a non-invasive marker of NAFLD and fibrosis

Liver cell death due to ongoing injury

Cell free DNA from plasma
Peripheral blood leukocytes

gDNA extraction
Bisulfite conversion & PCR amplify
Pyrosequencing
Methylation of PPARγ promoter identifies NAFLD fibrosis in cfDNA

Altered DNA methylation can be quantified in blood of NAFLD patients: NAFLD Biomarker?

**p<0.001, *p<0.01, ns=non-significant for non-parametric t-tests
Advanced Fibrosis (n=29); Mild Fibrosis (n=30); Normal (n=9)

## DNA Methylation: NASH or fibrosis biomarker?

**Outcome = Fibrosis**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Model</th>
<th>Covariates</th>
<th>p value</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCAT1</td>
<td>Fibrosis = gene + covariates</td>
<td>age + gender + DM</td>
<td>0.019</td>
<td>0.06</td>
</tr>
<tr>
<td>CASP1</td>
<td>Fibrosis = gene + covariates</td>
<td>age + gender + DM</td>
<td>0.89</td>
<td>0.89</td>
</tr>
<tr>
<td>FGFR2</td>
<td>Fibrosis = gene + covariates</td>
<td>age + gender + DM</td>
<td>0.72</td>
<td>0.89</td>
</tr>
</tbody>
</table>

**Outcome = NAFLD Activity Score (NAS) > 4**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Model</th>
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<th>p value</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCAT1</td>
<td>NAS = gene + covariates</td>
<td>age + gender + DM</td>
<td>0.47</td>
<td>0.57</td>
</tr>
<tr>
<td>CASP1</td>
<td>NAS = gene + covariates</td>
<td>age + gender + DM</td>
<td>0.0008</td>
<td>0.002</td>
</tr>
<tr>
<td>FGFR2</td>
<td>NAS = gene + covariates</td>
<td>age + gender + DM</td>
<td>0.57</td>
<td>0.57</td>
</tr>
</tbody>
</table>

**Model 2**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Model</th>
<th>Covariates</th>
<th>p value</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCAT1</td>
<td>NAS = gene + covariates</td>
<td>age + gender + DM + BMI + fibrosis stage</td>
<td>0.39</td>
<td>0.59</td>
</tr>
<tr>
<td>CASP1</td>
<td>NAS = gene + covariates</td>
<td>age + gender + DM + BMI + fibrosis stage</td>
<td>0.004</td>
<td>0.01</td>
</tr>
<tr>
<td>FGFR2</td>
<td>NAS = gene + covariates</td>
<td>age + gender + DM + BMI + fibrosis stage</td>
<td>0.95</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Moylan CA, et al. *manuscript in preparation*

C\(i\) (clinical) = age, BMI, gender, race, diabetes, alcohol and smoking
Metabolomics and NAFLD

**Epigenetic variation:**
- interaction with environmental factors
- disease progression, dynamic

**Genomic variation:**
- present at birth
- small to modest effect size

**Coding and non-coding RNAs:**
- molecular signatures; tissue or blood
- dynamic, longitudinal assessment

**Proteins:**
- diagnosis, prognosis
- circulation
- longitudinal assessment

**Metabolites:**
- diagnosis and prognosis
- circulation
- dynamic, longitudinal assessment

• Cross sectional analysis of a prospective derivation cohort of 156 patients with biopsy proven NAFLD.

• 2 validation cohorts: 142 patients dx by MR elastography (MRE) and 59 patients with biopsy proven NAFLD

• Performed untargeted metabolite assessment at Metabolon (Durham, NC)
Serum metabolites associate with advanced fibrosis in NAFLD patients

Fold change of top 10 metabolites associated with advanced fibrosis

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>AUROC (95%CI)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV</th>
<th>NPV</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 metabolites</td>
<td>0.94 (0.897 to 0.982)</td>
<td>90</td>
<td>79</td>
<td>43</td>
<td>98</td>
<td>NA</td>
</tr>
<tr>
<td>FIB-4 index</td>
<td>0.78 (0.674 to 0.891)</td>
<td>90</td>
<td>39</td>
<td>21</td>
<td>96</td>
<td>0.002</td>
</tr>
<tr>
<td>FIB-4+10 metabolites</td>
<td>0.94 (0.896 to 0.983)</td>
<td>90</td>
<td>79</td>
<td>43</td>
<td>98</td>
<td>0.836</td>
</tr>
<tr>
<td>NAFLD Fibrosis Score</td>
<td>0.84 (0.752 to 0.929)</td>
<td>90</td>
<td>59</td>
<td>28</td>
<td>97</td>
<td>0.017</td>
</tr>
<tr>
<td>Clinical predictive score†</td>
<td>0.84 (0.724 to 0.945)</td>
<td>90</td>
<td>37</td>
<td>20</td>
<td>95</td>
<td>0.053</td>
</tr>
</tbody>
</table>
Can serum metabolites predict future clinical outcomes?

187 patients with biopsy proven NAFLD and serum collected at biopsy

Untargeted serum metabolites (Metabolon); 683 measured

Outcomes of liver disease:
- Portal Hypertension (ascites, PSE, EV bleed, SBP, HRS)
- Liver transplantation; death

Wegermann K, et al. DDW 2019, May 21, 2019
Results: 11/187 pts suffered 22 events

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic encephalopathy</td>
<td>8</td>
</tr>
<tr>
<td>Ascites</td>
<td>5</td>
</tr>
<tr>
<td>SBP</td>
<td>1</td>
</tr>
<tr>
<td>HCC</td>
<td>1</td>
</tr>
<tr>
<td>Variceal bleeding</td>
<td>1</td>
</tr>
<tr>
<td>Liver-related death</td>
<td>6</td>
</tr>
</tbody>
</table>

- **Mean follow up:** 6.9 ± 3.2 years
- **Time to liver-related event:** 7 months - 9.2 years
- **Well matched cohort:** no differences in age, BMI, DM, smoking
- **EVENTS:** more common in fibrosis stage 3 and 4 (24% with advanced fibrosis)

Multiple metabolites associate with future liver-related events:

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of metabolites</th>
<th>Relevant Pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipids</td>
<td>30</td>
<td>Fatty acid metabolism</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Primary bile acid metabolism</td>
</tr>
<tr>
<td>Amino acids</td>
<td>19</td>
<td>Branched chain AA metabolism</td>
</tr>
<tr>
<td>Peptides</td>
<td>6</td>
<td>Gamma-glutamyl amino acid</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>4</td>
<td>Pentose metabolism</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fructose/mannose/galactose</td>
</tr>
<tr>
<td>Nucleotides</td>
<td>4</td>
<td>Purine metabolism</td>
</tr>
<tr>
<td>Cofactors/Vitamins</td>
<td>4</td>
<td>Tocopherol metabolism</td>
</tr>
</tbody>
</table>

Wegermann K, et al. *manuscript in preparation*
Metabolites associated with Time to Liver-related Events

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Metabolite</th>
<th>Direction</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid metabolism</td>
<td>5-hydroxyindoleacetate</td>
<td>Decreased</td>
<td>0.0007</td>
</tr>
<tr>
<td>Primary bile acid metabolism</td>
<td>taurochenodeoxycholate</td>
<td>Increased</td>
<td>0.0006</td>
</tr>
<tr>
<td>Vitamin E metabolism</td>
<td>gamma-CEHC glucuronide</td>
<td>Decreased</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>gamma-CEHC</td>
<td>Decreased</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

- **Serotonin metabolite (5HIAA)**
  - Signals to liver along gut-brain axis
  - Triggers regeneration (+)
  - Associated with fibrosis progression (-)

- **TCDC=Bile acid composed of chenodeoxycholic acid + taurine**
  - Increased levels associated with:
    - Presence of NASH
    - Cirrhosis (HCV, HBV, NASH, alcohol)
    - HCC

- **Vitamin E metabolites (anti-oxidants)**
  - Improved histology in NAFLD (PIVENS trial)
  - Reduces oxidative stress in animal models

*Controlled for multiple comparisons and age, sex, BMI, DM, HTN, and fibrosis stage*
Could we prevent NAFLD in the future?
Developmental Origins of NAFLD: fetal programming of disease

Aim: To identify early life DNA methylation marks associated with metabolic syndrome and NAFLD in children.

Baker PR and Friedman JE. JCI 2018.
Newborn Epigenetics Study (NEST)
PI: Cathrine Hoyo, Susan Murphy

Eligible Children
Born in Durham County 2005-2009 and 2009-2011

Gave informed consent, blood, umbilical cord blood, DNA methylation analysis on cord blood

~ 500 Eligible children now 6 – 11 yrs of age

Study of Early Life, Epigenetics and Nonalcoholic Fatty Liver Disease
- 90 children and parent enrolled
- Quantified liver fat and fibrosis using MRI technology and FibroScan, PE, bloodwork, risk questionnaires
- DNA methylation in umbilical cord blood collected at birth and DNA and RNA collected at the time of enrollment

*mother granted permission to use pictures of children

FUNDING: ACG Junior Faculty Development Award
### Child Characteristics

<table>
<thead>
<tr>
<th></th>
<th>All (n=90)</th>
<th>Obese/OW (n=46)</th>
<th>Non-Obese/OW* (n=43)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years) (median and IQR)</strong></td>
<td>9.2 (8.1 – 9.8)</td>
<td>9.2 (8.1 - 10.2)</td>
<td>8.8 (8.1-9.7)</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Male Sex, n (%)</strong></td>
<td>42 (46.6)</td>
<td>24 (58.5)</td>
<td>17 (41.5)</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td>White</td>
<td>28 (31.1)</td>
<td>8 (28.6)</td>
<td>20 (71.4)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>58 (64.4)</td>
<td>36 (63.2)</td>
<td>21 (36.8)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4 (4.4)</td>
<td>2 (50)</td>
<td>2 (50)</td>
<td></td>
</tr>
<tr>
<td><strong>Non- Hispanic Ethnicity</strong></td>
<td>81 (90)</td>
<td>41 (51.3)</td>
<td>39 (48.5)</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>Case (Maternal pre-preg BMI &gt; 30)</strong></td>
<td>60 (66.6)</td>
<td>39 (66.1)</td>
<td>20 (33.9)</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Body Mass Index (BMI) (kg/m²)</strong></td>
<td>19.2 (15.8-24)</td>
<td>23.9 (21-28.7)</td>
<td>15.7 (14.7-17.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>BMI %tile</strong>*</td>
<td>88 (45-97.5)</td>
<td>96 (93-99)</td>
<td>44 (18-64)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Obese (&gt; 95%), n (%)</td>
<td>34 (38.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight (&gt; 85th to &lt; 95th), n (%)</td>
<td>12 (13.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (&gt; 5th to &lt;85th), n (%)</td>
<td>39 (43.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight (&lt;5th), n (%)</td>
<td>4 (4.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Waist Circumference (inches)</strong></td>
<td>24 (21.5-29.8)</td>
<td>29.2 (24-31.8)</td>
<td>22 (21-24)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Systolic Blood Pressure (mmHg)%tile</strong></td>
<td>67 (45-88)</td>
<td>75 (47.8-93.2)</td>
<td>54.5 (26.5-85)</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Diastolic Blood Pressure (mmHg)%tile</strong></td>
<td>54 (27.5-74)</td>
<td>54.5 (35.3-82.5)</td>
<td>50.5 (22-66.3)</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Hypertension, n (%)</strong></td>
<td>16 (18)</td>
<td>12 (75)</td>
<td>4 (25)</td>
<td>0.13</td>
</tr>
<tr>
<td>Pre-HTN, n (%)</td>
<td>7 (7.9)</td>
<td>3 (42.9)</td>
<td>4 (57.1)</td>
<td></td>
</tr>
</tbody>
</table>

### Laboratory Data (3 missing)

<table>
<thead>
<tr>
<th></th>
<th>All (n=90)</th>
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<th>Non-Obese/OW* (n=43)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosylated Hemoglobin (%)</td>
<td>5.4 + 0.45</td>
<td>5.5 +/- 0.4</td>
<td>5.4 +/- .5</td>
<td>0.21</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dL) S missing</td>
<td>0.65 + 0.3</td>
<td>0.6 + 0.3</td>
<td>0.7 + 0.3</td>
<td>0.07</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>28 (25-31)</td>
<td>28 (24-29)</td>
<td>27 (25-32)</td>
<td>0.21</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>17 (15-20)</td>
<td>18 (15-22)</td>
<td>15 (13.8-17.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>45 (37-63)</td>
<td>51 (39.5-67)</td>
<td>42 (32.3-49.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>157 (132-166)</td>
<td>151 (123.5-162)</td>
<td>161.5 (145.5-176.3)</td>
<td>0.004</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>55 (44.3-66)</td>
<td>49 (42.5-59)</td>
<td>63.5 (53-70)</td>
<td>0.0001</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>88.5 (75-99.3)</td>
<td>87 (72.5-96.5)</td>
<td>90 (82 – 105.3)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*1 missing height data

- Age 9.2 yrs
- 47% boys
- 64% AA
- 26% pre- or hypertensive
- Obese/OW with more metabolic derangements

Moylan CA, unpublished
# Imaging Characteristics of Children

**Characteristics (median +/- IQR)**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All</th>
<th>Obese/OW (n=42)</th>
<th>Non-Obese (n=42)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FibroScan (kPa) (n=85)</td>
<td>4.4 (3.7-4.9)</td>
<td>4 (3.7-5.0)</td>
<td>4.4 (3.8-4.8)</td>
<td>0.53</td>
</tr>
<tr>
<td>MRE (kPa) (n=81)</td>
<td>2.5 (2.2-2.7)</td>
<td>2.5 (2.2-2.63)</td>
<td>2.5 (2.2-2.7)</td>
<td>0.91</td>
</tr>
<tr>
<td>MRI PDFF (%) (n=79)</td>
<td>1.4 (1.0-2.1)</td>
<td>1.85 (1.2–2.45)</td>
<td>1.1 (0.88-1.53)</td>
<td>0.001</td>
</tr>
<tr>
<td>MR Subcutaneous Fat Area (cm²) (n=78)</td>
<td>51.8 (25.3-145.8)</td>
<td>126 (55.2-197)</td>
<td>29.7 (20.2-46.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MR Visceral Fat Area (cm²) (n=78)</td>
<td>11.2 (6.2–22.5)</td>
<td>19 (9.9–34.9)</td>
<td>7.6 (4.2-11.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MR muscle area (cm²) (n=78)</td>
<td>23.5 (20.2–29.7)</td>
<td>25.7 (22.1–31.7)</td>
<td>21 (15.8-24)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

- 7.6% (7/79) of pre-teen aged children dx with NAFLD

**Examples**

**9.8 yo AA obese female**
- PDFF 13.4%
- ALT 24 U/l

**9.6 yo white female**
- MRE 3.6 kPa
- ALT 15 U/l

Moylan CA, unpublished
Top Differentially Methylated CpG in Cord Blood

Top genes with differentially methylated CpGs in offspring cord blood associated with PDFF, HbA1c% and HDL.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Gene</th>
<th># of CpG</th>
<th>Chr.</th>
<th>max. 𝛽 FDR P-value</th>
<th>GO / Annotation</th>
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<tbody>
<tr>
<td>PDFF</td>
<td>APOBEC3F</td>
<td>5</td>
<td>chr22</td>
<td>0.0143425</td>
<td>cytidine deaminase / RNA binding</td>
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<td>KCNG2</td>
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<td>chr18</td>
<td>0.0125969</td>
<td>voltage gated ion channel activity</td>
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<td>0.0058714</td>
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<td>SH3PXD2A</td>
<td>6</td>
<td>chr10</td>
<td>-0.0091224</td>
<td>adhesion, matrix degradation</td>
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<tr>
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<td>GPR133</td>
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<td>HbA1c%</td>
<td>WNK4</td>
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<td>serine - threonine protein kinase</td>
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</table>

- Completed DNA methylation analysis in blood of children
- Analyzing results
- Comparison between 2 time points
• NAFLD represents a spectrum of phenotypes
• NAFLD affects millions of people
• Accurate risk stratification is difficult but essential for improved patient care
• Genetic and OMICS data will soon improve our ability to care for NAFLD patients
• Differentially methylated DNA in utero associated with liver fat and metabolic syndrome in pre-teen age children and may provide clues for NAFLD PREVENTION

Thank you
cynthia.moylan@duke.edu
Pathogenesis of NAFLD/NASH: Multiple Hits

Kim K and Lee MS. Front in Endocrinology 2018; 9:1-13