The Role of Genetic Assessments in Non-Alcoholic Fatty Liver Disease

2nd International Workshop on NASH Biomarkers, Washington DC, USA, May 2017

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Newcastle University, UK.
Quentin M. Anstee

Research Grant Funding: Abbvie, GlaxoSmithKline, Allergan/Tobira, Vertex.

Are there heritable factors that can be used to **risk stratify** patients into prognostic ‘high risk’ or ‘low risk’ groups **before** they develop fibrosis?
Do Genetic Factors Contribute to Inter-Individual Variation of NAFLD Progression?

• **Familial aggregation**
  
  Struben, 2000; Wilner, 2001; Schwimmer, 2009

• **Inter-ethnic differences in susceptibility**
  
  Caldwell, 2002; Browning, 2004; Weston, 2005; Mohanty, 2009; Bambha, 2012

• **Twin studies**
  
  – 145 twin-pairs (57MZ, 88DZ)
  – Heritability of ALT and serum Insulin levels

  Makkonen, 2009

  – 60 twin-pairs (42MZ, 18DZ)
  – Heritability of:
    
    • **Steatosis** 0.52 (95%CI 0.31-0.72) and
    
    • **NAFLD-fibrosis** 0.50 (95%CI 0.28-0.72)

  Loomba, 2015
Heritability of Hepatic Fibrosis and Steatosis Based on a Prospective Twin Study

Loomba et al

42 MZ, 18 DZ sets of twins

Steatosis: MRI-PDFF

Steatosis heritability 0.52 (95%CI 0.31-0.72)

Fibrosis heritability 0.50 (95%CI 0.28-0.72)

Fibrosis: MR-E
Genomic Variation & Control of Gene Expression

DNA Sequence Variation

DNA Methylation

“Transcriptional Control”

Histone Modification

Transcription

RNA

“Translational Control”

MicroRNA (miRNA)

Translation

Protein

“Post-Translational Control”

Protein Phosphorylation
Genomic Variation & Control of Gene Expression

DNA Sequence Variation

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“Post-Translational Control”

DNA

RNA

Protein

Transcription

Translation

MicroRNA (miRNA)

Protein Phosphorylation
Identified Biomarker Needs in NAFLD by FDA BEST Classification

• **Diagnostic** (BIPED ‘Burden/Severity of disease’ and ‘Diagnostic’)
  – Degree of steatosis,
  – Grade of steatohepatitis,
  – Discriminating Steatosis (NAFL) vs. Steatohepatitis (NASH),
  – Stage of fibrosis

• **Prognostic** (BIPED ‘Prognostic’).
  – Stratify individuals by fibrosis progression risk,
  – Discriminate “fast” vs. “slow” progressors,
  – Predict long-term outcomes and hard endpoints

• **Predictive** (BIPED ‘Efficacy of Intervention’)
  – Selection of patients for a specific treatment

• **Monitoring “Dynamic”** (BIPED ‘Efficacy of Intervention’)
  – Track progression and/or regression of disease severity
  – Response to therapy & efficacy of intervention
Candidate Gene Studies in NAFLD

Genetic Modifiers of Fatty Acid Flux & Triglyceride Levels
- e.g. SLC27A5, LIPN1, MTTP, PEMT, ADIPOQ, ADIPOR2, ApoC3, TCF7L2, ApoE, NR1I2/PXR, PPARA, FADS1, [PNPLA3, TM6SF2, MBOAT7]*

Genetic Modifiers of Oxidative Stress
- e.g. HFE, SOD2, GCLC, MRP2 (ABCC2), MTHFR

Genetic Modifiers of Fibrogenesis
- e.g. AGT, ATGR1, KLF6, TGFB1, COL13A1, CDKN1A, [PNPLA3, TM6SF2, MBOAT7]*

Genetic Modifiers of Endotoxin Response
- e.g. TLR4, CD14

Genetic Modifiers of Cytokine Activity
- e.g. TNF, sTNFr-2, FDFT1, IL6

Genetic Modifiers of Insulin Sensitivity/Resistance
- e.g. IRS-1, ENPP1, GCKR, PPARG, TCF7L2, SLC2A1

Genetic Modifiers of Fibrogenesis
- e.g. AGT, ATGR1, KLF6, TGFB1, COL13A1, CDKN1A, [PNPLA3, TM6SF2, MBOAT7]*

* Identified by GWAS not candidate gene studies
Genome-Wide Association Study (GWAS)

- Require large sample sizes (>1000): a problem for histologically defined diseases like NAFLD.
- **Good** for detecting
  - Common (>5%) variants that confer modest disease risk (OR ~ 1.2-1.5)
- **Not Good** for detecting
  - Multiple, rare, high-risk variants,
  - Very low effect size (OR <1.1)
- Associations still require independent replication
- Finds loci, not genes: this can complicate identification of the true pathogenic variation
<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Modality</th>
<th>N</th>
<th>Number of SNPs</th>
<th>Modifier Genes Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Romeo et al, 2008</td>
<td>USA (Mixed ethnicity)</td>
<td>$^3$H-MRS Steatosis</td>
<td>2,051*</td>
<td>9,229</td>
<td>Chr 22: PNPLA3</td>
</tr>
<tr>
<td>Yuam et al, 2008</td>
<td>European (Mixed ethnicity)</td>
<td>Clinical Biochemistry (ALT)</td>
<td>12,419</td>
<td>-</td>
<td>Chr 22: PNPLA3; CPN1-ERLIN1-CHUK,</td>
</tr>
<tr>
<td>Chalasani et al, 2010</td>
<td>USA (All Female, European Caucasian)</td>
<td>Histology</td>
<td>236</td>
<td>324,623</td>
<td>FDFT1, COL13A1, EFCAB4B, PZP</td>
</tr>
<tr>
<td>Speliotes et al, 2011</td>
<td>USA &amp; Europe (Meta-analysis of previous studies)</td>
<td>CT Steatosis (with histological 'candidate gene' validation set)</td>
<td>7,176</td>
<td>Range 329k-618k before imputation</td>
<td>Chr 22: PNPLA3; Chr 19: NCAN; GCKR, LYPLAL1, (PPP1R3B)</td>
</tr>
<tr>
<td>Chambers et al, 2011</td>
<td>European (Mixed ethnicity)</td>
<td>Clinical Biochemistry (ALT)</td>
<td>61,089</td>
<td>~2.6 million genotyped and imputed</td>
<td>Chr 22: PNPLA3; TRIB1, HSD17B13, CPN1</td>
</tr>
<tr>
<td>Kawaguchi et al, 2012</td>
<td>Japan</td>
<td>Histology</td>
<td>529</td>
<td>484,751</td>
<td>Chr 22: PNPLA3</td>
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<td>Kozlitina et al, 2014</td>
<td>USA (Mixed ethnicity)</td>
<td>$^3$H-MRS Steatosis</td>
<td>2,736*</td>
<td>138,374</td>
<td>Chr 22: PNPLA3; Chr 19: TM6SF2</td>
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* Both based on Dallas Heart Study cohort
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<td>$^3$H-MRS Steatosis</td>
<td>2,051* (B: 1,032; W: 636; H: 383)</td>
<td>9,229</td>
<td>Chr 22: PNPLA3</td>
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<td>Yuam et al, 2008</td>
<td>European (Mixed ethnicity)</td>
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<td>-</td>
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<td>Kozlitina et al, 2014</td>
<td>USA (Mixed ethnicity)</td>
<td>$^3$H-MRS Steatosis</td>
<td>2,736* (B: 1,324; W: 882; H:467; O: 63)</td>
<td>138,374 (Exome)</td>
<td>Chr 22: PNPLA3; Chr 19: TM6SF2</td>
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</tbody>
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* Both based on Dallas Heart Study cohort
Genetic variation in *PNPLA3* confers susceptibility to nonalcoholic fatty liver disease

Stefano Romeo¹,₈, Julia Kozlitina²,³,⁸, Chao Xing¹,², Alexander Pertsemlidis¹, David Cox⁴, Len A Pennacchio⁵, Eric Boerwinkle⁶, Jonathan C Cohen¹ & Helen H Hobbs¹,⁷

- USA Study population: (Black n=1032, White n=636, Hispanic n=383).
- 9229 non-synonymous exonic SNPs.
- Steatosis defined non-invasively by $^1$H-MRS.

*PNPLA3* rs738409 c.444 C>G p.Ile167Met (I148M)

PNPLA3 (I148M) – Carriage of G allele associated with increased steatosis (p=5.9 x 10⁻¹⁰)
Presence of NASH
OR 1.5 (1.12-2.04)

Fibrosis >F1
OR 1.5 (1.09-2.12)
Chromosome 22: PNPLA3

<table>
<thead>
<tr>
<th>Index SNP</th>
<th>NAFLD P-value</th>
<th>Steatosis P-value</th>
<th>NASH P-value</th>
<th>Fibrosis P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs738409*</td>
<td>6.634e-68</td>
<td>2.99e-64</td>
<td>5.818e-72</td>
<td>3.336e-65</td>
</tr>
</tbody>
</table>

* Direct genotyping of rs738409 by TaqMan allelic discrimination plus imputation.
Carriage of the *PNPLA3* rs738409 C>G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma


*Journal of Hepatology 2014* vol. 61 75–81

### Carriage of the *PNPLA3* rs738409 C>G polymorphism

#### Carriage of the *PNPLA3* rs738409 C>G polymorphism

**Variables**

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>PNPLA3</em> rs738409</td>
<td>2.26 (1.23-4.14)</td>
<td>0.0082</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>1.24 (1.17-1.32)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Gender (Male)</strong></td>
<td>11.11 (4.17-33.33)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI</td>
<td>0.94 (0.87-1.02)</td>
<td>0.148</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.33 (0.93-5.81)</td>
<td>0.070</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>9.37 (3.82-23.00)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

#### NAFLD-HCC vs. NAFLD

<table>
<thead>
<tr>
<th>Genotype</th>
<th>NAFLD-HCC n(%)</th>
<th>NAFLD Cohort n(%)</th>
<th>Unadjusted OR (95% CI)</th>
<th>P-value</th>
<th>Adjusted OR (95% CI)</th>
<th>P-value</th>
<th>UK Pop n(%)</th>
<th>Unadjusted OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>28 (0.28)</td>
<td>125</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>871 (0.59)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GG</td>
<td>43 (0.43)</td>
<td>117</td>
<td>1.64 (0.96-2.81)</td>
<td>0.072</td>
<td>2.35 (0.90-6.13)</td>
<td>0.082</td>
<td>531 (0.36)</td>
<td>2.52 (1.55-4.10)</td>
<td>0.0002</td>
</tr>
<tr>
<td>GG</td>
<td>29 (0.29)</td>
<td>33 (0.12)</td>
<td>3.92 (2.06-7.48)</td>
<td>&lt;0.0001</td>
<td>5.05 (1.47-17.29)</td>
<td>0.01</td>
<td>74 (0.08)</td>
<td>12.19 (6.89-21.58)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
**PNPLA3 Associated with Increased HCC Risk**

- Morbid obesity (Burza, 2012)
- ‘Mixed aetiology’ cohort (Hassan, 2013)
- Alcoholic liver disease (Nischalke, 2011; Trepo, 2012 & 2014; Guyot, 2013)
- Chronic hepatitis C (Valenti, 2011; **borderline** Trepo, 2014; **not** Nischalke, 2011)
- NAFLD (Liu, 2014)
- Meta-analysis (Singal, 2014)

**Effect of PNPLA3 genotype on HCC risk greatest in Steatotic liver diseases (NAFLD, ALD) vs. Non-steatotic diseases (HCV)**
PNPLA3 - Adiponutrin

- Recombinant I148M has reduced acylglycerol hydrolase activity \textit{in vitro}
- Purified I148M adiponutrin has reduced enzymatic activity
- I148M reduces VLDL secretion \textit{in vitro} and \textit{in vivo}
- I148M alters lipid remodeling, accumulates on LD

- Knockout \textit{in vivo} does not cause steatosis
- I148M overexpression causes steatosis \textit{in vivo}
- WT has no effect
- I148M knockin causes steatosis on high sucrose diet \textit{in vivo}
- \textit{PNPLA3} I148M accumulates on LD

- Adiponutrin has lysophosphatidic acid acetyltransferase activity increased by I148M to increase TAG synthesis

\begin{itemize}
\item Recombinant I148M has reduced acylglycerol hydrolase activity \textit{in vitro}
\item Purified I148M adiponutrin has reduced enzymatic activity
\item I148M reduces VLDL secretion \textit{in vitro} and \textit{in vivo}
\item I148M alters lipid remodeling, accumulates on LD
\item Knockout \textit{in vivo} does not cause steatosis
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\item I148M knockin causes steatosis on high sucrose diet \textit{in vivo}
\item \textit{PNPLA3} I148M accumulates on LD
\item Adiponutrin has lysophosphatidic acid acetyltransferase activity increased by I148M to increase TAG synthesis
\end{itemize}
Chronic overexpression of PNPLA3<sup>1148M</sup> in mouse liver causes hepatic steatosis

John Zhong Li, Yongcheng Huang, Ruchan Karaman, Pavlina T. Ivanova, H. Alex Brown, Thomas Roddy, Jose Castro-Perez, Jonathan C. Cohen, and Helen H. Hobbs

Departments of Molecular Genetics and Internal Medicine, Eugene McDermott Center for Human Growth and Development, University of Texas Southwestern Medical Center, Dallas, Texas, USA. Departments of Pharmacology and Chemistry, Vanderbilt University, Nashville, Tennessee, USA. Atherosclerosis Merck Research Laboratories, Rahway, New Jersey, USA. Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, Texas, USA.

Hepatic PNPLA3<sup>1148M</sup> over-expression

Liver

- TAG (mg/g)
- Cholesteryl ester (mg/g)
- Free cholesterol (mg/g)
- Phosphatidylcholine (mg/g)

* indicates statistical significance.
“PNPLA3 plays a role in remodelling TAG in lipid droplets”

Increased formation of fatty acids and TAG,
Impaired hydrolysis of TAG,
Relative depletion of TAG long-chain PUFA.
But no evidence of inflammation or fibrosis.....

Does progressive damage require a ‘Second hit’?

OR

Does PNPLA3 have independent effects on cells involved in inflammation/fibrosis/cancer?
The PNPLA3 I148M variant modulates the fibrogenic phenotype of human hepatic stellate cells

PNPLA3 is required for HSC activation and its genetic variant I148M potentiates the pro-fibrogenic features of HSCs

PNPLA3 I148M confers a pro-inflammatory and pro-fibrotic profile
The PNPLA3 I148M variant modulates the fibrogenic phenotype of human hepatic stellate cells

PNPLA3 I148M confers a pro-inflammatory and pro-fibrotic profile in LX-2 cells

PNPLA3 is required for HSC activation and its genetic variant I148M potentiates the pro-fibrogenic features of HSCs

Bruschi et al, 2017, in press
Genome-Wide Association Analysis Identifies Variants Associated with Nonalcoholic Fatty Liver Disease That Have Distinct Effects on Metabolic Traits

Speliotes et al, 2011: PLOS Genetics (7), e1001324

A.

-5 0 5 10 15 20 25 30
-log_{10} p-value

LYPLAL1 GCKR

PNPLA3

PPP1R3B

NCAN

Stage 1 Discovery

Stage 2 Pathology

Stage 3 Other Analyses

GOLD Meta-analysis

NAFLD Histology

NASH CRN/iCONT

Metabolic phenotypes eQTL

AGES

Amish Family Heart Study
Framingham Heart Study
(n=7,176)

CT hepatic steatosis

45 loci

p<5x10^{-3}

NASH CRN/MiGen

n=592/1405

NASH fibrosis

5 loci

NASH CRN/iCONT

Metabolic phenotypes eQTL
1000G SNP Density: Chromosome 19 ‘19p13.11 Region’

Anstee et al, EASL 2013
Exome-wide association study identifies a **TM6SF2** variant that confers susceptibility to nonalcoholic fatty liver disease

**TM6SF2 rs58542926 c.449 C>T p.Glu167Lys (E167K)**

**TM6SF2 (E167K) minor allele carriage associated with increased steatosis** (p=5.7 x 10^{-8})
Two histologically characterized NAFLD cohorts (n=349+725, Total=1,074)

TM6SF2 rs58542926 C>T (E167K) associated with presence of NAFLD vs. healthy population sample ($X^2$ for trend $p=0.0008$)

Uncommon variant: MAF 7% in healthy population, MAF 12% in NAFLD (cf PNPLA3 rs738409 MAF 28% in healthy population, MAF 38% in NAFLD)
**TM6SF2 rs58542926 INFLUENCES HEPATIC FIBROSIS PROGRESSION IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE**


Two histologically characterized European Caucasian NAFLD cohorts (n=349+725, Total=1,074)

### Table 1 | Multivariate analysis of association between TM6SF2 rs58542926 genotype and fibrosis stage F0-1 (mild) versus F2-4 (advanced).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Discovery cohort (n = 349)</th>
<th></th>
<th>Validation cohort (n = 725)</th>
<th></th>
<th>Combined cohort (n = 1,074)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>P-value</td>
<td>OR (95%CI)</td>
<td>P-value</td>
<td>OR (95%CI)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>TM6SF2 genotype</strong></td>
<td>2.94 (1.76-4.89)</td>
<td>3.44 x 10^-5</td>
<td>1.46 (1.03-2.09)</td>
<td>0.0362</td>
<td>1.88 (1.41-2.5)</td>
<td>1.63 x 10^-5</td>
</tr>
<tr>
<td><strong>PNPLA3 genotype</strong></td>
<td>1.57 (1.21-2.19)</td>
<td>0.0086</td>
<td>1.32 (1.05-1.66)</td>
<td>0.0183</td>
<td>1.40 (1.16-1.69)</td>
<td>4.84 x 10^-4</td>
</tr>
<tr>
<td>Age</td>
<td>1.03 (1.01-1.06)</td>
<td>0.0045</td>
<td>1.02 (1.01-1.04)</td>
<td>0.0041</td>
<td>1.03 (1.01-1.04)</td>
<td>1.57 x 10^-5</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>0.94 (0.57-1.56)</td>
<td>0.8297</td>
<td>1.81 (1.30-2.50)</td>
<td>4.50 x 10^-4</td>
<td>1.43 (1.09-1.89)</td>
<td>0.0096</td>
</tr>
<tr>
<td>BMI</td>
<td>1.05 (1.00-1.10)</td>
<td>0.0368</td>
<td>1.03 (1.01-1.05)</td>
<td>9.80 x 10^-4</td>
<td>1.04 (1.02-1.05)</td>
<td>3.78 x 10^-5</td>
</tr>
<tr>
<td>T2DM</td>
<td>2.39 (1.49-3.84)</td>
<td>0.0003</td>
<td>2.73 (1.93-3.88)</td>
<td>1.68 x 10^-8</td>
<td>2.57 (1.95-3.39)</td>
<td>1.78 x 10^-11</td>
</tr>
</tbody>
</table>

BMI: body mass index; CI: confidence interval; OR: odds ratio; T2DM: type 2 diabetes mellitus.

Additive model including age, gender, BMI, T2DM and PNPLA3 rs738409 genotype as covariates. Discovery/validation/combined cohorts: stage F0-1 (mild) n=198/439/637, stage F2-4 (advanced) n=151/286/437.

Multivariate analysis (additive model) incorporating Age, Gender, BMI, T2DM and PNPLA3 rs738409 genotype.

i.e. OR per copy of the minor allele carried

Conditional analysis confirms that it is **TM6SF2 not NCAN** that drives the association
<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Study Design</th>
<th>Phenotype</th>
<th>Cohort size (n)</th>
<th>Ethnicity</th>
<th>Conclusions</th>
</tr>
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<tr>
<td>Speliotes, 2011</td>
<td>GWAS</td>
<td>CT</td>
<td>7,176 combined (+592 histology)</td>
<td>Mixed</td>
<td>NCAN: ▲ HTGC, ▼ NASH</td>
</tr>
<tr>
<td>Gordon, 2013</td>
<td>Candidate-Gene</td>
<td>Histology</td>
<td>1,092</td>
<td>Mixed</td>
<td>NCAN: ▲ Steatosis, ▼ NASH, ▼ Fibrosis</td>
</tr>
<tr>
<td>Kozlitina, 2014</td>
<td>GWAS</td>
<td>MRS</td>
<td>2,736 Population sample</td>
<td>Mixed</td>
<td>TM6SF2: ▲ HTGC</td>
</tr>
<tr>
<td>Liu, 2014</td>
<td>Candidate-Gene</td>
<td>Histology</td>
<td>1,074 NAFLD EUR</td>
<td></td>
<td>TM6SF2: ▲ NAFLD, ▼ NASH, ▼ Fibrosis</td>
</tr>
<tr>
<td>Dongiovani, 2015</td>
<td>Candidate-Gene</td>
<td>Histology</td>
<td>1,201 NAFLD EUR</td>
<td></td>
<td>TM6SF2: ▲ Steatosis, ▼ NASH, ▼ Fibrosis</td>
</tr>
<tr>
<td>Sookoian, 2015</td>
<td>Candidate-Gene</td>
<td>Histology</td>
<td>226 NAFLD (+ 135 controls)</td>
<td>-</td>
<td>TM6SF2: ▲ NAFLD (not fibrosis)</td>
</tr>
<tr>
<td>Wong, 2014</td>
<td>Candidate-Gene</td>
<td>MRS/TE</td>
<td>922</td>
<td>CHI</td>
<td>TM6SF2: No association</td>
</tr>
<tr>
<td>Wang, 2015</td>
<td>Candidate-Gene</td>
<td>USND</td>
<td>384 NAFLD (+384 controls)</td>
<td>CHI</td>
<td>TM6SF2: ▲ NAFLD</td>
</tr>
<tr>
<td>Surakka, 2015</td>
<td>GWAS</td>
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# Chromosome 19 loci, *TM6SF2* rs58542926 & NAFLD

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Validates results of Holmen et al GWAS, showing that the TM6SF2 (E167, major allele) is associated with increased cholesterol and cardiovascular disease
A potential master-regulator of Metabolic Syndrome outcome?

Modified from Kahali, Day, Anstee & Speliotes, Gastroenterology 2015
TM6SF2 is a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content


Effect of TM6SF2 siRNA on Gene Expression

Gene expression analysis of TM6SF2 siRNA treatment in Huh7 cells: TM6SF2 siRNA increases lipid droplet size and TG content.

Overexpression of WT TM6SF2 decreases hepatic TG content.

eQTL analysis of Chr 19 region identifies TM6SF2

Localised to ER and ER-Golgi intermediate compartment

Effect of TM6SF2 siRNA on Gene Expression
Pathophysiology of TM6SF2 in NAFLD & Dyslipidaemia

- TM6SF2 preferentially expressed in liver and small intestine.  
  Smagris et al, JBC 2016

- TM6SF2 E167K carriage confers a lipid profile beneficial for CVD risk.  
  Holmen et al, Nat Genetics 2014; Dongiovanni et al, Hep 2015; Luukonnen et al, J Hep 2017, in press; O’Hare et al, Hep 2017, in press

- E167K carriers exhibit significantly lower postprandial serum triglycerides, suggestive of a role for TM6SF2 in the small intestine.

- Carriage alters lipid processing in both liver and small intestine leading to increased ER stress.  
  O’Hare et al, Hep 2017, in press.

- Knockout mice have a 3-fold reduced rate of VLDL-TG secretion rate.

- TM6SF2 required to mobilize neutral lipids for VLDL assembly, but is not required for secretion of ApoB-containing lipoproteins.  
  Smagris et al, JBC 2016

- TM6SF2 level is an important determinant of VLDL metabolism and variants may affect protein stability.  
  Ehrhardt et al, 2017
TM6SF2 may effect: transport of neutral lipids from lipid droplets to the particle by transferring lipid to MTTP [1], to neutral LD in the ER lumen [2], or directly to the nascent VLDL particle [3]. Alternatively, TM6SF2 could participate in the transfer of lipid to the particle en route to or within the Golgi complex [4] [5].
A genome-wide association study confirms *PNPLA3* and identifies *TM6SF2* and *MBOAT7* as risk loci for alcohol-related cirrhosis

Buch et al

Membrane bound O-acyltransferase domain-containing 7 (*MBOAT7*) rs626283 & rs641738
Mancina et al

Genotyped rs641738 C>T in two cohorts:

- Dallas Heart Study (H1-MRS) n=2,736
- Italian/Finnish Liver Biopsy Cohort n=1,149
The **MBOAT7-TMC4 Variant rs641738 Increases Risk of Nonalcoholic Fatty Liver Disease in Individuals of European Descent**

Mancina et al

---

*MBOAT7* encodes lysophosphatidylinositol acyltransferase 1, an enzyme involved in the phospholipid acyl-chain remodeling. It catalyzes desaturation of the second acyl-chain of phospholipids and specifically transfers polyunsaturated fatty acids, such as arachidonoyl-CoA, to lysophosphatidylinositol and other lysophospholipids.

---

**Validation:** Luukkonen et al, *J Hep* 2016
Targeting the Patient...?
Targeting the Patient...?
Factors Associated with NAFLD Progression

Only **Association Studies** – causality has not yet been established

No studies examining **prognostic** value of any of these factors
Clinical Application of PNPLA3 Genetic Associations?

• **Diagnosis of NAFLD**
  – AST, AST/ALT, fs-Insulin, T2DM, PNPLA3 (Kotronen, 2009)

• **Presence of NASH**
  – ALT, fs-C-Peptide, USnd: AUROC 0.85/0.82 (Francque, 2012)
    CK18 and PNPLA3 did not improve AUROC
  – CK18, Glucose, CRP, PNPLA3 (Guichelaar, 2013)
  – AST, fs-Insulin, PNPLA3: AUROC 0.74/0.76 (Hyysalo, 2014)

• **Presence of Fibrosis**
  – AST, Waist circumference: AUROC 0.84/0.85 (Francque, 2012)
    CK18 and PNPLA3 did not improve AUROC
Genetic Odds Ratio and Positive Predictive Value

- False positive fractions at 80% Sensitivity:
  - OR 1.5 >75%
  - OR 10 ~25%
  - OR 50 <10%

Manolio, Nat Rev Genetics, 2014
The \textit{MBOAT7-TMC4} Variant rs641738 Increases Risk of Nonalcoholic Fatty Liver Disease in Individuals of European Descent

Mancina et al

Combined effects of \textit{PNPLA3}, \textit{TM6SF2} \& \textit{MBOAT7} on $\text{H}_1$-MRS Hepatic TG Content
NAFLD-Associated HCC

- Increasing proportion of NAFLD-related HCC.
  Dyson et al, 2014

- Of 87 biopsy characterized NAFLD-HCC patients
  - 49% of NAFLD-HCC were in non-cirrhotic patients
  - [28% F0-2; 21% F3]
  Yasui, 2011

- Retrospective study of patients with NAFLD.
  - Median follow-up 5.6 years
  - 16 cases HCC, annual rate 0.046%
  - Increased with T2DM and APRI
  Kawamura et al, 2012
PNPLA3 in HCC Surveillance: use in the clinic?

• Relative to the Newcastle NAFLD cohort
  - GG vs CC: PPV 47% NPV 82% Sens 51% Spec 79%
  - CG/GG vs CC: PPV 32% NPV 82% Sens 72% Spec 45%

• Relative to the background UK population
  - GG vs CC: PPV 28% NPV 97% Sens 51% Spec 92%
  - CG/GG vs CC: PPV 10% NPV 97% Sens 72% Spec 59%

High NPV – Possible utility to focus HCC surveillance, if validated.

Liu et al, J Hepatology 2014; Anstee et al, J Hepatology 2015
Informing *Precision-Medicine* Therapeutics

• Individualized therapy through tailored selection of drug and lifestyle interventions based on **predicted response profile**.

• PNPLA3 (I148M) minor allele carriage associated with:
  – Less beneficial effect with Omega-3 fatty acid supplementation (DHA enrichment).
  – Less beneficial effect with statin therapy.
  – Greater response to lifestyle intervention.
  – Trend towards enhanced effect of Elafibranor in GOLDEN-505.

  Scorletti et al, 2015
  Dongiovani et al, 2015
  Shen et al, 2015
  Ratziu et al, EASL 2016
## Potential Relevance of Genetics & Epigenetics by FDA BEST Classification

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<th>Research Outputs</th>
<th>Potential Application</th>
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<td>Insights into <em>pathophysiology</em></td>
<td>• Identify potential novel therapeutic targets</td>
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<tr>
<td><strong>Diagnostic</strong> Association with <em>disease presence</em> and/or <em>category of severity</em></td>
<td>• Risk stratification of patients for <strong>NAFLD presence</strong></td>
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<td>• Identify patients for <strong>disease severity</strong> e.g. NAFL vs. NASH, Stage of Fibrosis</td>
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<td><strong>Prognostic</strong> Informs our understanding of <em>inter-patient variation in disease natural history</em></td>
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<td>• Risk stratification for late-stage disease complications, e.g. NAFLD-associated <strong>Hepatocellular carcinoma (HCC) risk</strong>.</td>
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<td>• Risk stratification into “<em>fast</em>” vs. “<em>slow</em>” <em>fibrosis progression</em>.</td>
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<td><strong>Predictive</strong> Insights into <em>individual response to therapeutics</em></td>
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<td><strong>Monitoring</strong> Track disease severity and/or treatment response</td>
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DNA Methylation: Transcriptional Repression

**Unmethylated gene promoter**

![DNA structure with unmethylated promoter](image)

**Methylated gene promoter**

![DNA structure with methylated promoter](image)

**CpG island**

**DNA**

**Gene**

**TRANSCRIPTION**

**TRANSCRIPTION REPRESSED**

**Cytosine**

![Cytosine structure](image)

**5’ Methyl-cytosine**

**DNMT**

**SAM**

**SAH**

Multigenerational epigenetic adaptation of the hepatic wound-healing response

Müjdat Zeybel¹, Timothy Hardy¹, Yi K Wong², John C Mathers², Christopher R Fox¹, Agata Gackowska¹, Fiona Oakley¹, Alastair D Burt¹, Caroline L Wilson¹, Quentin M Anstee¹, Matt J Barter¹, Steven Masson¹, Ahmed M Elsharkawy¹, Derek A Mann¹,³ & Jelena Mann¹,³
PPARγ a negative regulator of HSC activation. CpG hypermethylation proportional to fibrosis.
DNA Methylation Changes Observed in Pro-fibrotic Genes

*In normal liver: no significant impact on DNA methylation pattern of age, gender or anatomical location.*

Zeybel et al, Clinical Epigenetics, 2015
Hypermethylation of PPARγ promoter CpGs in plasma ‘cell-free’ DNA in advanced fibrosis.
Conclusions

• NAFLD is a complex disease trait with pathogenesis and progression determined by combinations of genetic and environmental factors.

• Several genetic factors have been robustly identified as disease modifiers.
  – **PNPLA3 rs738409 C>G (I148M)** remains the single most consistently validated genetic modifier of NAFLD identified to date.
  – **TM6SF2 rs58542926 C>T (E167K)** a variant which significant clinical relevance that dissociates NAFLD from CVD.
  – **MBOAT7 rs641738 C>T** is a more recently described variant first identified in ALD that appears to also have an effect on NAFLD severity

• **Epigenetic factors have a major influence on disease severity**
  – DNA methylation provides new insights into a novel form of trans-generational modifier for NAFLD risk and a now offers a potential non-invasive biomarker.
  – Despite at times conflicting data, microRNA signatures offer another potential form of non-invasive biomaker.

• **Much work remains to identify further genetic and epigenetic modifiers of disease risk and to explore how this knowledge may be applied in a clinical setting.**
Acknowledgements

Prof Chris Day,
Prof Ann Daly,
Dr Olivier Govare,
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Dr Rebecca Darlay,
Dr Helen Reeves,
Dr Dina Tiniakos,
Mrs Elsbeth Henderson.