AASLD THE LIVER MEETING® NOVEMBER 8-12 2019 BOSTON

Novel non-invasive cell-free nucleic acid-based diagnostic test for liver fibrosis in patients with type II diabetes

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Disclosure

Chenlu Hou

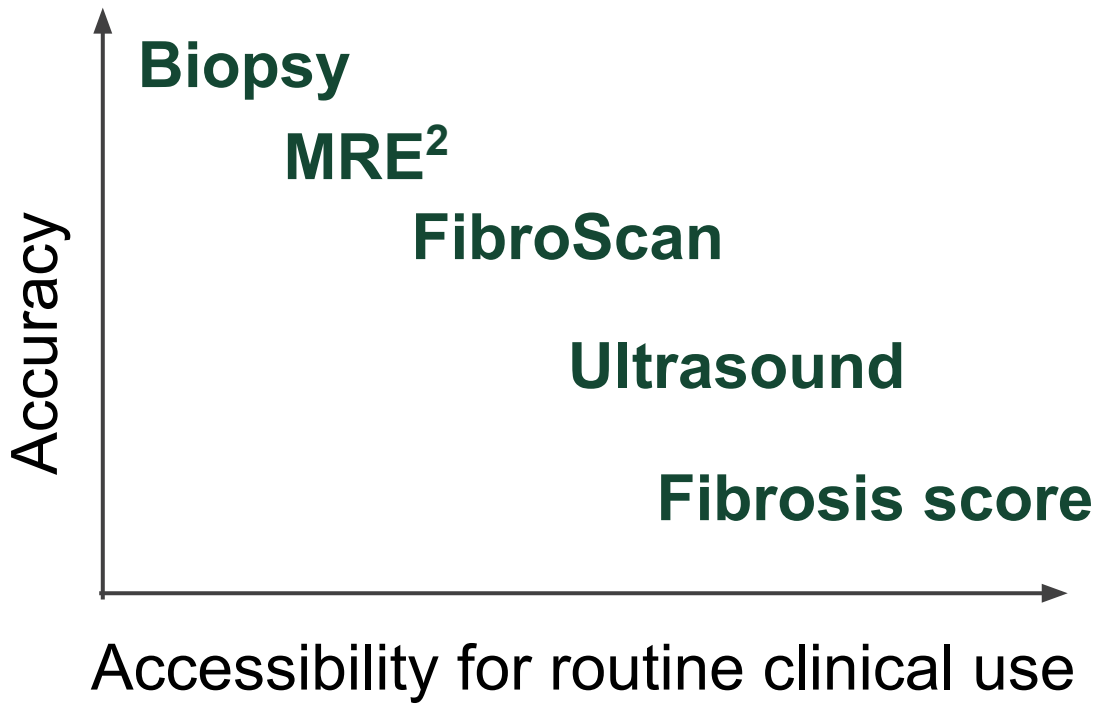
I disclose the following financial relationship(s) with a commercial interest: employee of GRAIL and shareholder of GRAIL Inc. and Illumina

Opportunity to Improve Fibrosis Diagnostics in NAFLD

Fibrosis is the Most Significant Prognostic Feature for NAFLD

- Mortality of NAFLD patients (*Eksedt 2015*)
  - 4x worse prognosis for F3 - F4
  - No increase for high NAS[1] (5-8) without severe fibrosis
- Fibrosis stage instead of NAS or other features of NASH associate with mortality (*Angulo 2016*)

No Accurate and Easily Accessible Solution for Routine Clinical Use



1. NAFLD activity score 2. Magnetic reasonance enterography

Cell-free Nucleic Acids for Non-Invasive Diagnostics

Tissues Shed Nucleic Acids into Blood

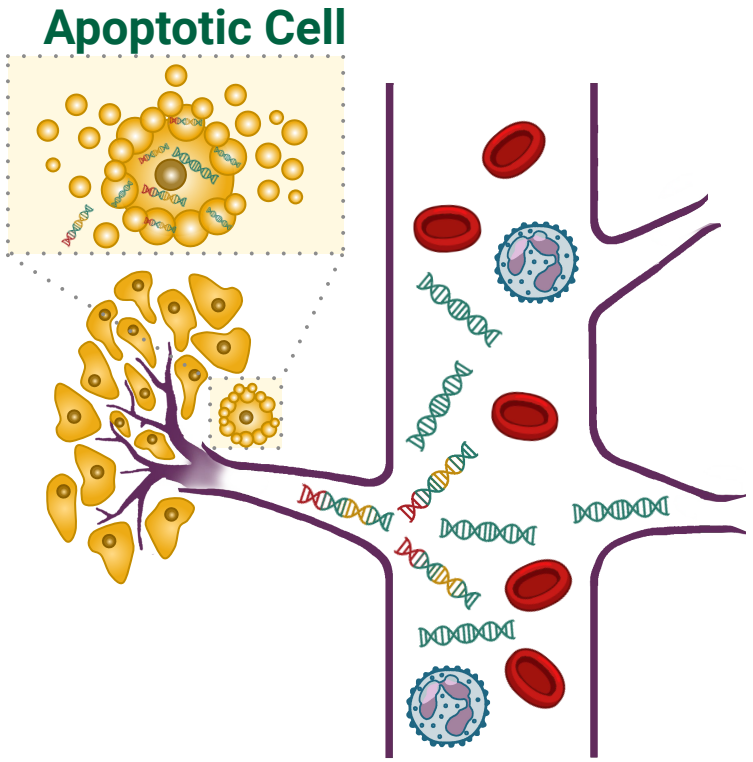


Image adapted from Crowley 2013.

Tissue Signatures are Observed in Cell Free Nucleic Acids (cfNA)

- cfNA tissue of origin could be predicted by
  - Methylation patterns (*Sun 2015; Oxnard 2019**)
  - Fragment endpoint distribution (*Snyder 2016*)
- Presence of cancer mutations in cfNA has been extensively studied

*Oxnard GR et al. Proffered paper at: ASCO Breakthrough Meeting; Oct 11, 2019; Bangkok, Thailand. Abstract 44.

Aim

To examine the association between cell-free nucleic acids and stage of fibrosis in diabetes with or without NAFLD

Objective: Explore Fibrosis Signal in Plasma

Biology	Hypothesis	Analysis Approach
Increased liver cell injury and death from fibrosis	Higher level of liver-derived nucleic acids in plasma	Use liver specific methylation markers to determine liver contribution to cfDNA
Epigenetic changes associated with fibrosis progression	• cfDNA contains abnormal methylation • cfRNA has increased expression in specific genes	Quantify the presence of fibrosis-related abnormal methylation and gene expression in plasma

Study Design

Study Samples

- 37 patients with Type II diabetes and varying level of steatosis and fibrosis:
  - MRI-PDFF[1] and MRE imaging
  - Tissue biopsy not available
- 226 controls (BMI < 25)

cfNA Assays

- Whole genome bisulfite sequencing (30x):
  - Plasma cfDNA
  - Matched PBMC[2] DNA
- Whole Transcriptome RNA sequencing

Analysis

- Liver contribution to cfDNA
- Abnormal cfDNA methylation
- cfRNA expression

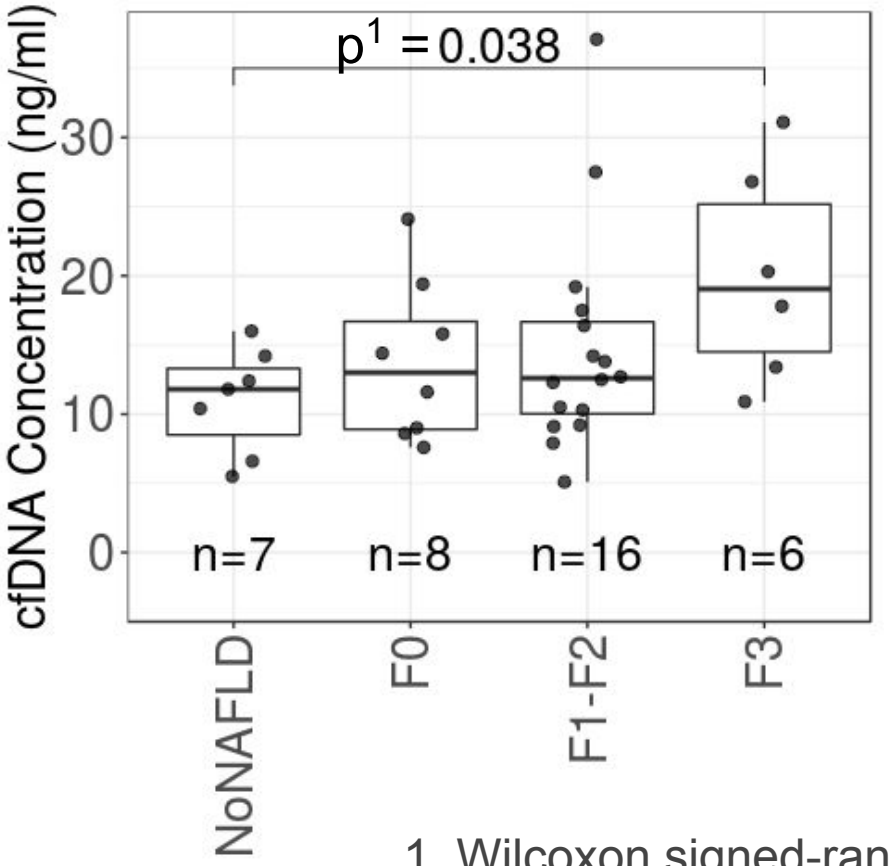
1. Magnetic Resonance Imaging Proton Density Fat Fraction
2. Peripheral blood mononuclear cell

Increased Total cfDNA Concentration in F3+ Patients

Patient Classification by Imaging

Stage	MRI-PDFF	MRE (kPa)	N
No-NAFLD	< 5%	< 2.55	7
Steatosis/no fibrosis (F0)	≥ 5%	< 2.55	8
F1-2		2.55-3.62	16
≥ F3		> 3.62	6

cfDNA Yield / Plasma Volume



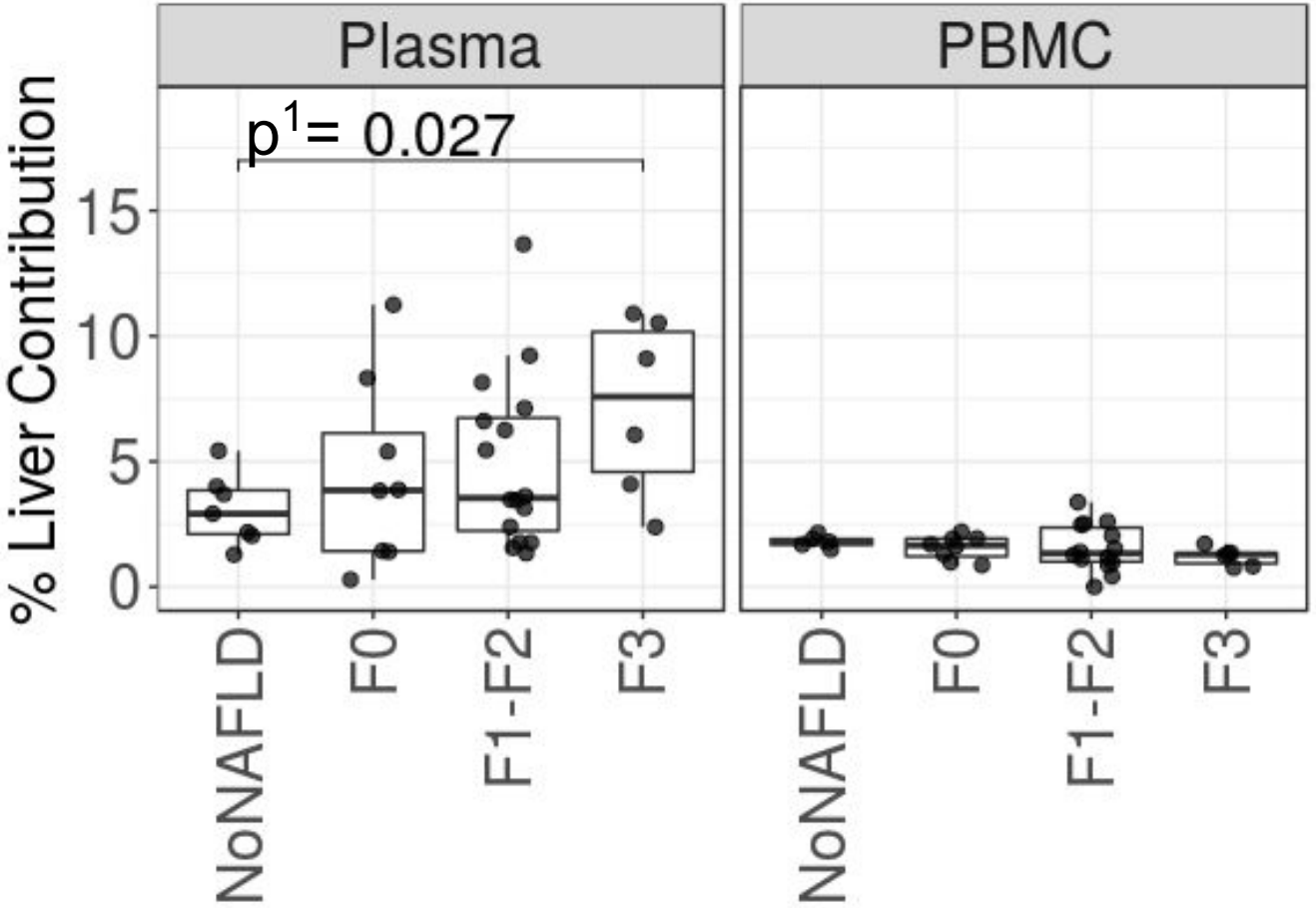
1. Wilcoxon signed-rank test

Increased Liver cfDNA Fraction in F3+ Patients

Liver cfDNA Fraction Measurement Approach

Identified liver specific methylation patterns from literature reports

Analyzed WGBS data for % methylation at liver-specific sites in plasma and PBMC



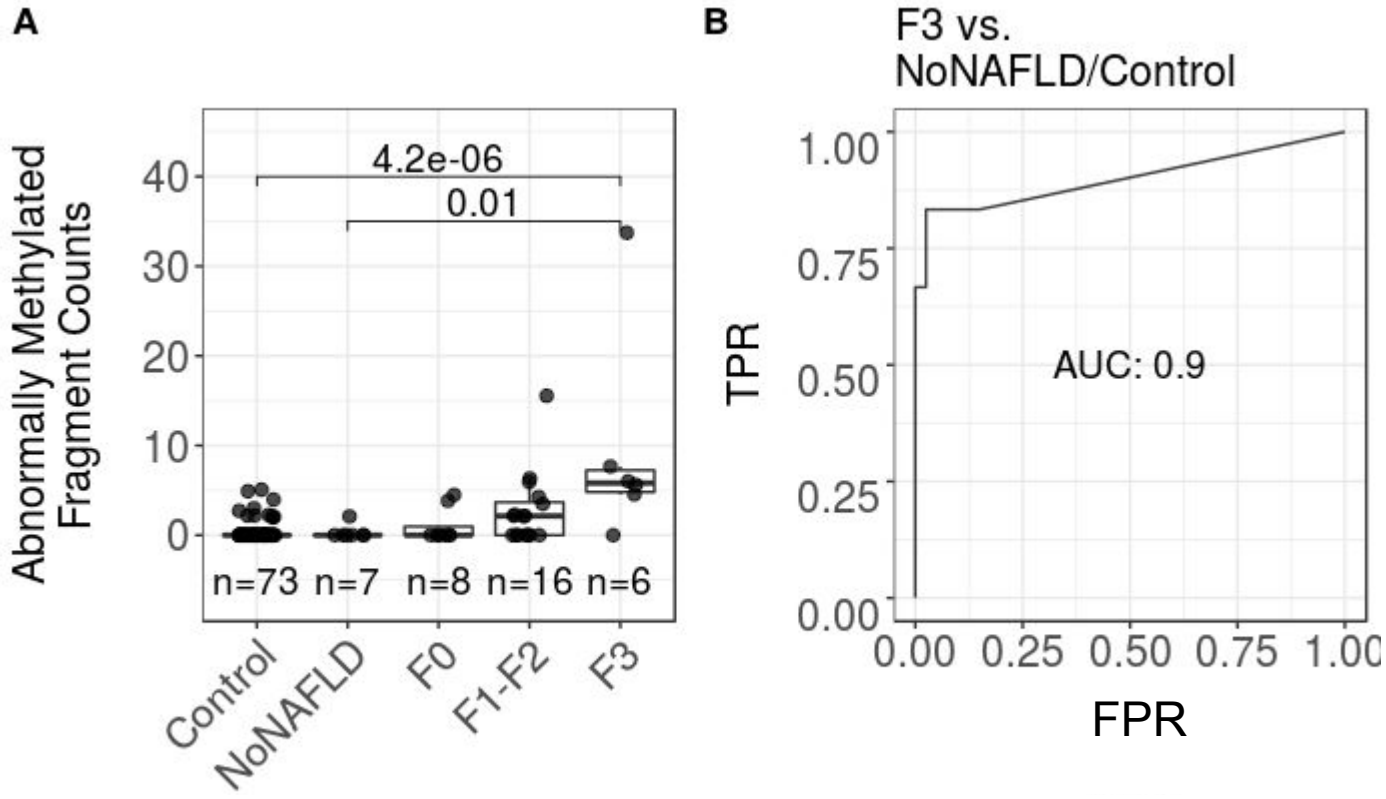
1. Wilcoxon signed-rank test

Abnormal Methylation Patterns Observed in F3+ Patients

Abnormal Methylation Criteria

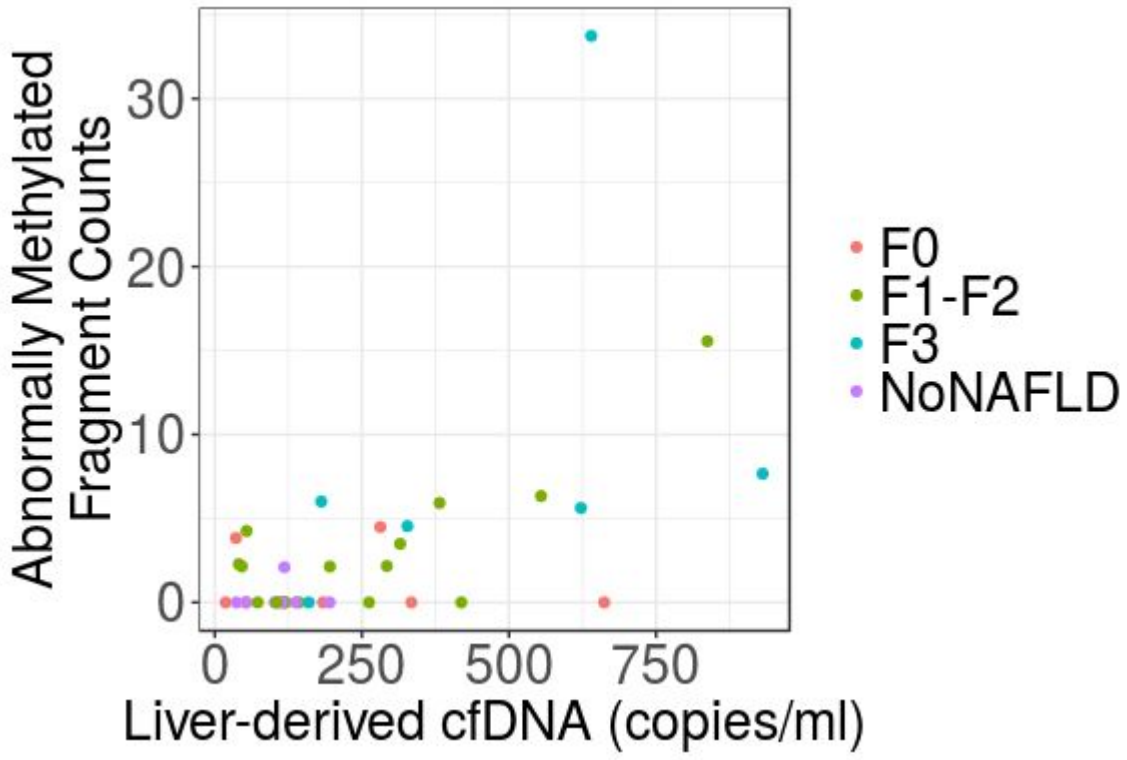
- Methylation patterns (fragment level) rarely found ($p<0.01$) in controls → could be specific to fibrosis or other diabetes-related phenotype
- Methylation patterns (CpG level) matched those associated with fibrosis severity in tissue studies

Fibrosis-Specific Abnormal Methylation Fragments could Differentiate ≥F3

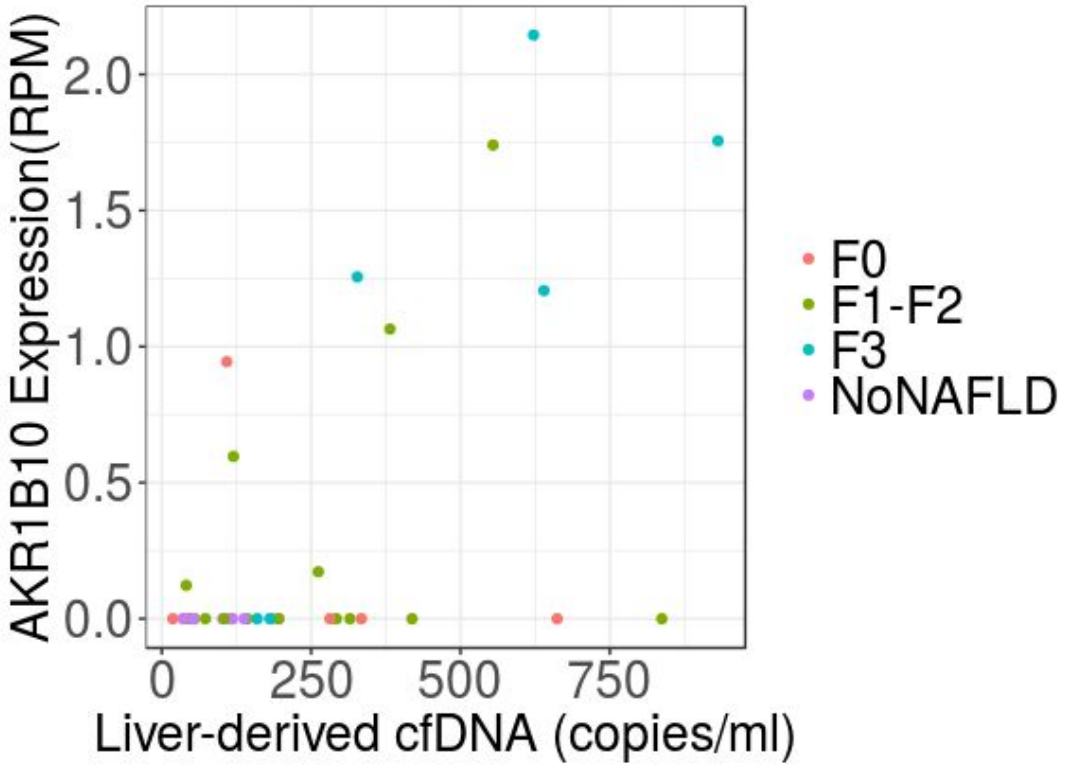


Correlation of Disease- and Liver-specific Signatures

Higher Abnormal Methylation Observed in Samples with Higher Liver-Derived DNA



F3+ Samples with High Methylation Signal have Higher AKR1B10 Expression



Key Take-Aways

- Liver- and fibrosis-specific signals observed in plasma indicate potential feasibility of non-invasive detection of fibrosis in NAFLD patients
  - Increased liver DNA in plasma from patients with severe fibrosis
  - Abnormal plasma cfDNA methylation at regions identified from tissue studies could distinguish F3+ patients from those without NAFLD
  - Correlation between quantity of liver-derived DNA and abnormal methylation / gene expression in patients with severe fibrosis strengthens the conclusion that cfNA signals represent true liver biology