APPLICATION OF HUMAN NASH-BASED TRANSCRIPTOME AND METABOLOME PROFILES IN PRECLINICAL MODELS

TRANSLATIONAL STUDY OF DRUG EFFECTS ON LIVER FIBROSIS

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INTRODUCTION

- NASH patients are characterized by complex metabolic disturbances in liver
  - chronic inflammation drives disease progression towards fibrosis
- Recent studies have comprehensively profiled these metabolic and inflammatory disturbances
  - Liver transcriptomics
  - Serum metabolomics
- Identified molecular patterns:
  - That characterize NASH patients
  - That can differentiate between mild and severe pathology

HEPATOLOGY
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Hepatic Gene Expression Profiles Differentiate Presymptomatic Patients With Mild Versus Severe Nonalcoholic Fatty Liver Disease

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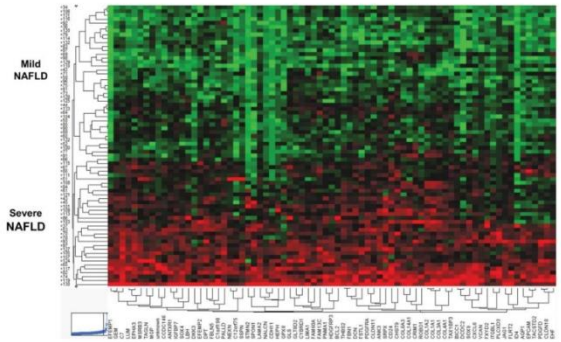


Fig. 1. Hierarchical clustering analysis. Hierarchical clustering of the top 100 differentially expressed probes from the 72 NAFLD patients separated the samples into two main groups: mild NAFLD and severe NAFLD. Data are presented in heat-map format in which patient samples are shown in rows and genes (probes) in columns. Red color corresponds to genes that are up-regulated in severe NAFLD, as compared to the mean, and green color corresponds to genes that are down-regulated in severe NAFLD, as compared to the mean.

Moylan et al. Hepatology. 2014 Feb;59(2):471-82.

Gene profile that associates with severe NASH

INTRODUCTION

- Concerns whether preclinical models sufficiently mimic molecular disease processes of patients
  - translational value in studies of therapeutic interventions for NASH/fibrosis?

Gastroenterology 2016;151:513–525

Comparison of Gene Expression Patterns Between Mouse Models of Nonalcoholic Fatty Liver Disease and Liver Tissues From Patients

Andreas Teufel,[1] Timo Itzel,[1] Wiebke Erhart,[2] Mario Brosch,[3] Xiao Yu Wang,[4] Yong Ook Kim,[4] Witigo von Schönfels,[5] Alexander Herrmann,[2] Stefan Brückner,[3] Felix Stickel,[6] Jean-François Dufour,[6] Triantafyllos Chavakis,[7] Claus Hellerbrand,[1] Rainer Spang,[8] Thorsten Maass,[1] Thomas Becker,[5] Stefan Schreiber,[2] Clemens Schafmayer,[5,§] Detlef Schuppan,[4,9,§] and Jochen Hampe[3,§]

9 NASH models:
Very little overlap between gene expression profiles of mice and patients

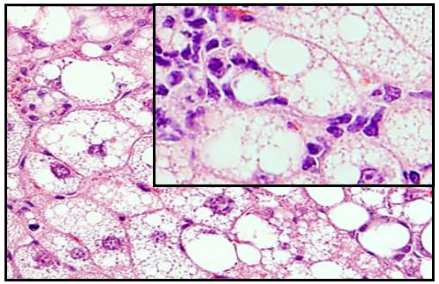
Aim of this study:

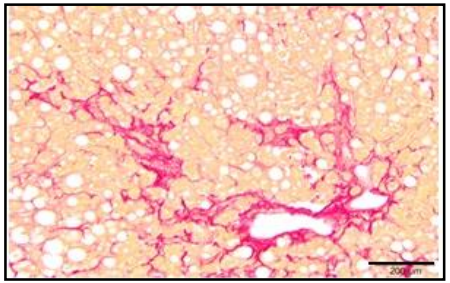
- Representation of human-based disease profiles in Ldlr-/-.Leiden mice
  - Obeticholic acid (OCA) as prototype intervention

LDLR-/-.LEIDEN MICE

Ldlr-/-.Leiden

- Energy-dense high-fat diet (HFD) without added cholesterol
- **Phenotypical** and **histopathological** characteristics of NASH patients
  - Obesity, insulin resistance, hyperlipidaemia
  - Macro- and micro-vesicular steatosis, lobular inflammation, and pronounced fibrosis

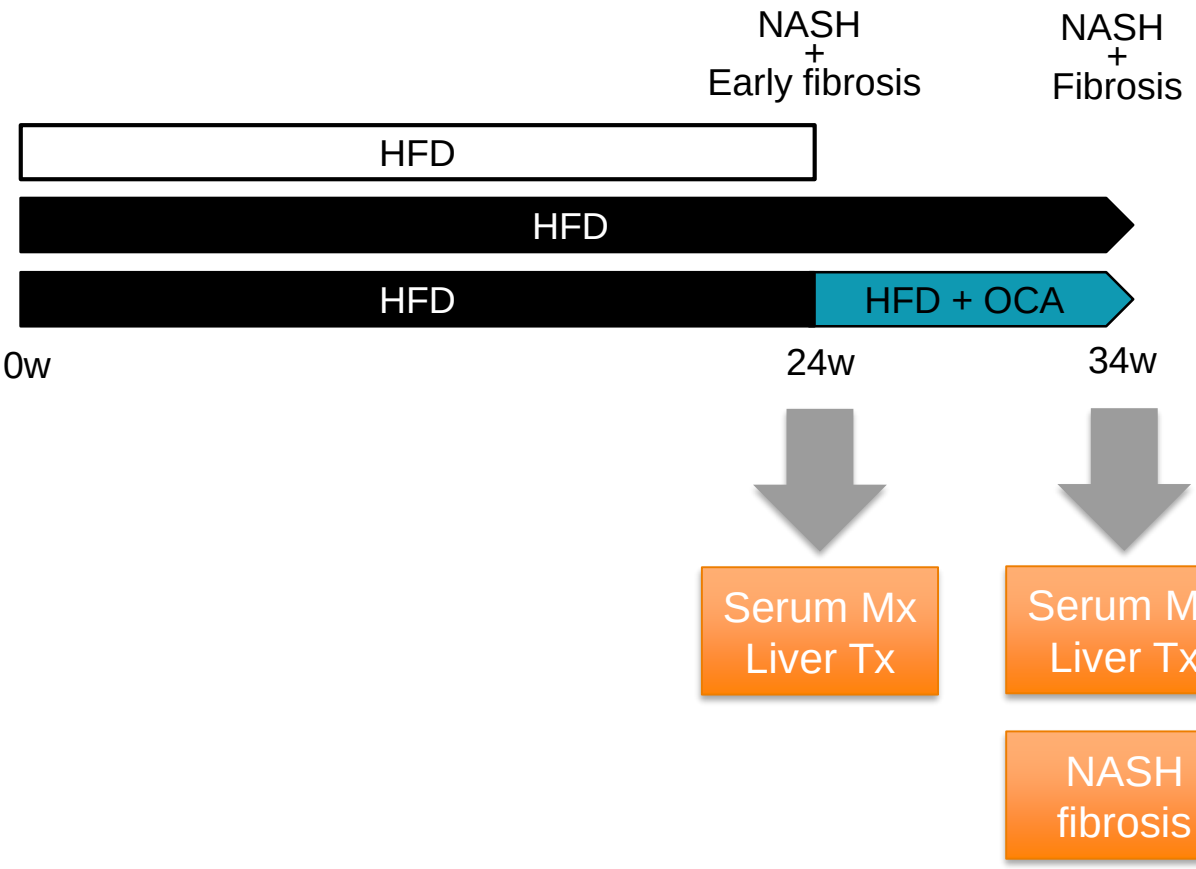




Do they also recapitulate molecular disease signatures observed in patients?

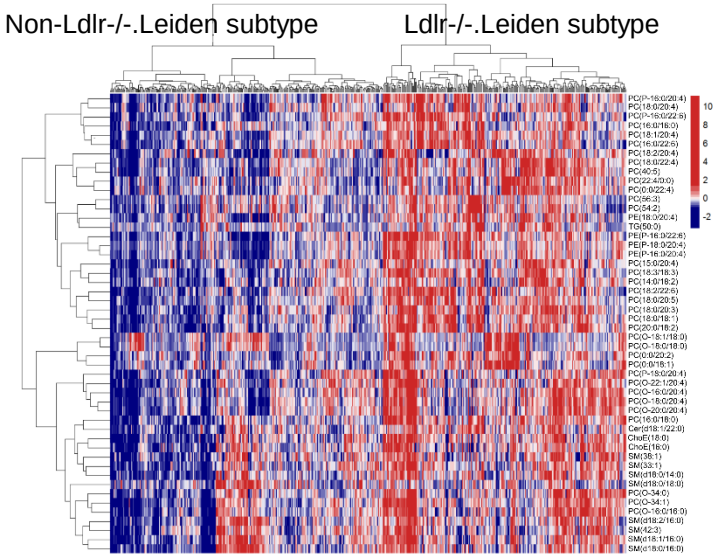
STUDY OUTLINE

Ldlr-/-.Leiden



HUMAN NASH SIGNATURES RECAPITULATED IN LDLR-/-.LEIDEN MOUSE

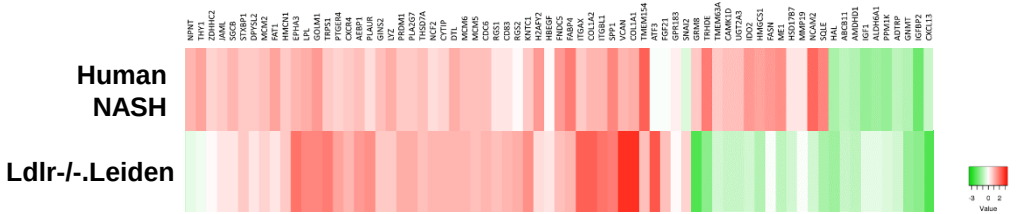
Serum metabolome



Ldlr-/-.Leiden metabolome reflected in substantial proportion of NAFLD/NASH patients

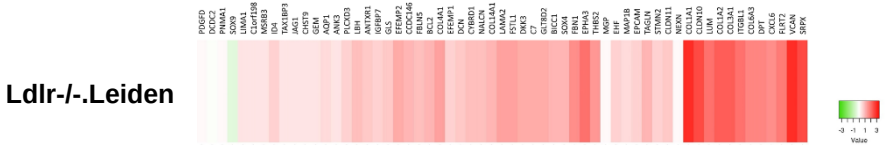
(human cohort described in Alonso et al. Gastroenterology 2016)

Liver transcriptome



Gene profile that characterizes NASH patients

(human dataset described in Teufel et al. Gastrolenterology 2016)

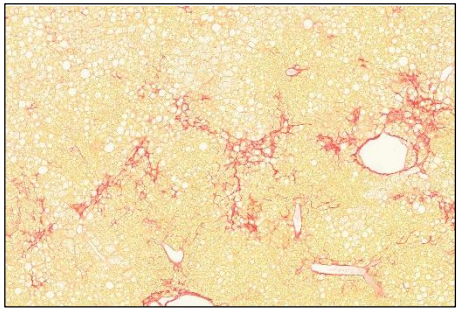


Gene profile that distinguishes between mild and severe NASH patients

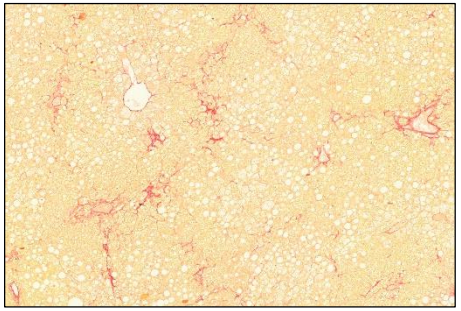
(gene-set described by Moylan et al. Hepatology 2014)

OCA ATTENUATES PROGRESSION OF LIVER FIBROSIS

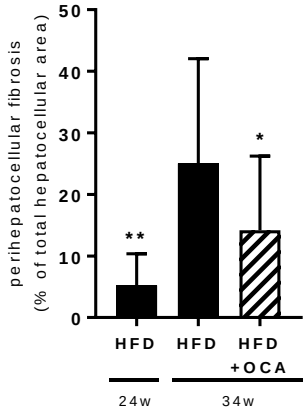
HFD



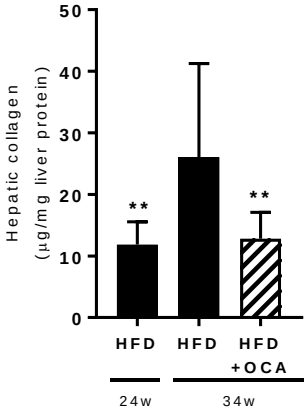
HFD+OCA



Pathologist-assessed

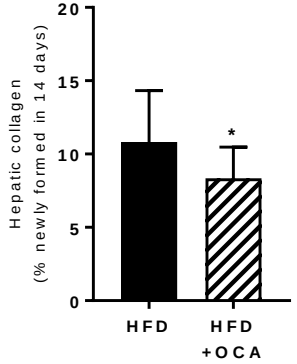


Biochemical analysis (hydroxyproline)



Effect on *de novo* collagen formation?

- Incorporation of deuterium from heavy water (^2H_2O) into newly synthesized collagen
- Measures collagen newly synthesized during labeling period (14 days)

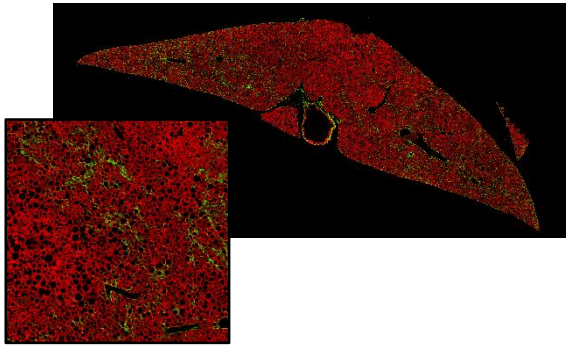


OCA reduces synthesis of new collagen

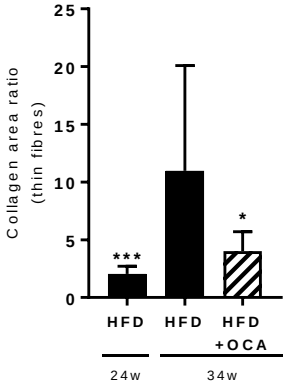
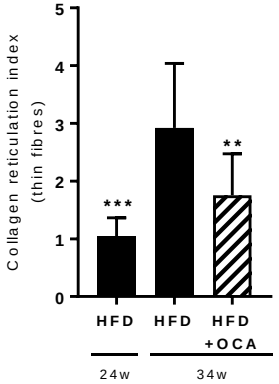
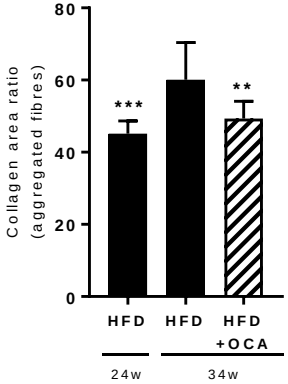
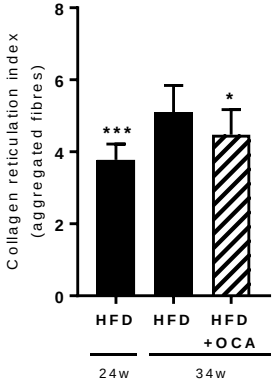
Data are mean ± SD

OCA REDUCED COLLAGEN AREA AND RETICULATION

Multi-photon imaging
and
Second harmonic generation imaging



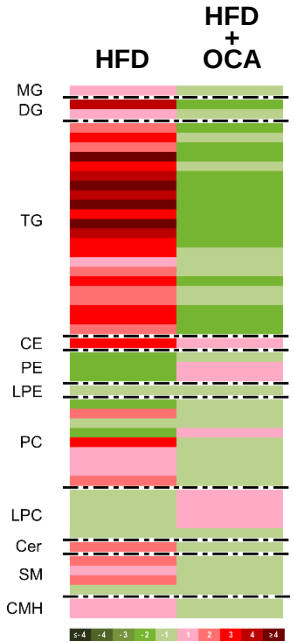
- diameter of collagen fibrils: effect on thin or aggregated fibers?
- Reticulation of collagen fibers: complexity/cross-linkage of collagen network

	area	reticulation
THIN FIBRES		
AGGREGATED FIBRES		

Data are mean ± SD

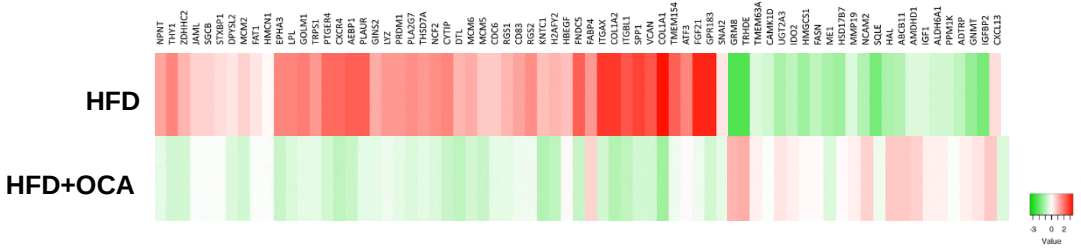
OCA COUNTER-REGULATES HUMAN-BASED OMICS SIGNATURES

Serum metabolome



OCA reversed
HFD-induced alterations
in metabolome

Liver transcriptome



Gene profile that characterizes NASH patients

(human dataset described in
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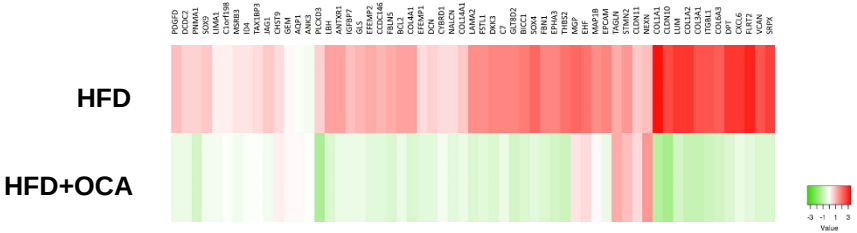

Gene profile that distinguishes between mild and severe NASH patients

(gene-set described by
Moylan et al. Hepatology 2014)

SUMMARY

- Ldlr-/-.Leiden mice recapitulate specific molecular metabolomics and transcriptomics signatures of NASH patients
- Intervention with OCA in developing fibrosis counter-regulates the effects of HFD and normalizes transcriptomics and metabolomics profiles
- OCA reduces collagen deposition and de novo synthesis but does not resolve already manifest fibrosis in the period studied (10 weeks)
- Human molecular signatures may be used to estimate the translational value of preclinical models for NASH

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