Hepatic molecular signature in a translational NASH model as an early screening tool for novel NASH therapeutics

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Introduction

Non-alcoholic steatohepatitis (NASH) is the most rapidly growing liver disease that is nevertheless without approved pharmacological treatment. Despite great effort in developing novel NASH therapeutics, many have failed in clinical trials.

Aim

The present study was designed to select promising compound(s) in terms of efficacy on preventing NASH and fibrosis as early as possible, using a preclinical translational in vivo model.

For this we identified and applied a molecular signature which represents early processes in fibrosis development, even before pathology becomes manifest.

Method

- Ldlr-/-.Leiden mice, a well-established model for hyperlipidemia that develop NASH with advanced fibrosis and atherosclerosis when fed a high fat diet (HFD) were used.
- Ldlr-/-.Leiden mice were pre-fed a HFD (without cholesterol supplementation) for 14 weeks to induce obesity and NASH.
- Mice were subsequently treated for 4 weeks with various novel therapeutic compounds and obeticholic acid (OCA) as reference.
- As additional controls, mice on the HFD left untreated or mice on a healthy chow diet were added.

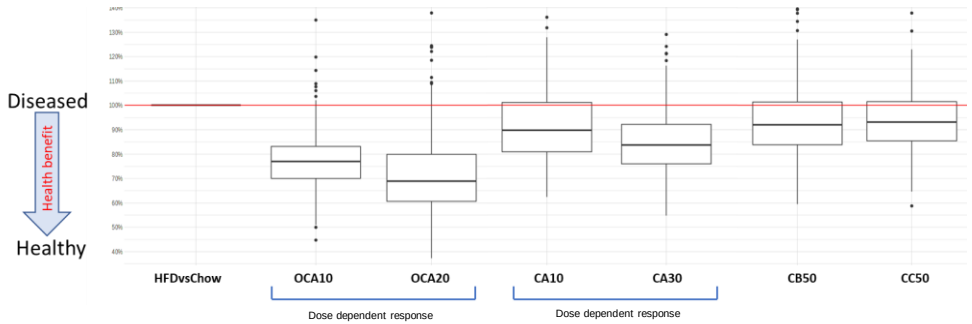
Ldlr-/-.Leiden mice developed obesity, insulin resistance and hyperlipidemia

Several characteristics of the Metabolic Syndrome are induced after 18 weeks of HFD feeding in the model.
As well as NASH with early start of fibrosis (F2).

Parameter	Chow	HFD
Body weight (g)	40.1 ± 0.7	52.1 ± 1.2*
Blood glucose (mM)	8.1 ± 0.3	8.7 ± 0.3
Plasma insulin (ng/mL)	1.0 ± 0.2	9.8 ± 2.1*
Plasma cholesterol (mM)	9.3 ± 0.7	35.9 ± 2.2*
Plasma triglycerides (mM)	1.9 ± 0.2	7.3 ± 0.9*
Steatosis (%)	3.8 ± 3.1	65.3 ± 3.5
Inflammation (# of infl. foci/mm^2)	0.2 ± 0.1	9.1 ± 1.3*
Fibrosis (% SR staining)	1.0 ± 0.1	1.8 ± 0.2*

* P<0.05 vs chow
Values are means ± SEM

The fibrosis signature (212 genes) allows for the detection of efficacy after 4 weeks of treatment



The degree of impact on the gene signature is related to the magnitude of reduction in fibrosis, as evidenced by a stronger effect observed towards 0%.

Significance was calculated based on the average logFC in treatment versus chow using 212 genes from the fibrosis signature. CA[dose]=compound A, CB[dose]=compound B, CC[dose]= compound C.

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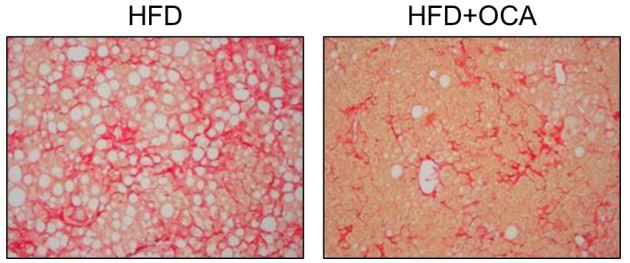
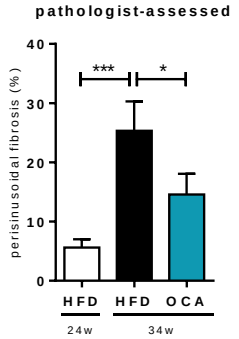
OCA attenuated hepatic fibrosis after 10 weeks of treatment in 2nd independent study in line with molecular signature prediction





In a 2nd study Ldlr-/-.Leiden mice were pre-fed the HFD for 24 weeks to induce NASH with more pronounced fibrosis, and either sacrificed (reference group for start treatment), left untreated (HFD control group) or treated with 10 mg/kg/d OCA for 10 weeks.
Effects on histological end-point of fibrosis was assessed and found to be attenuated with OCA treatment (as predicted by molecular signature).

* P<0.05, *** P<0.001
Values are means ± SEM

Conclusions

The present study reveals the **potency of a novel hepatic molecular signature as an early screening tool for NASH therapeutics** when used in a translational model, even when no pathology is detected, yet. Instead of using a longer study duration to evaluate the effect on hard end-points like fibrosis, a short intervention was used to evaluate the effect on hepatic molecular signature relevant for fibrosis in humans.