

Identification and verification of functional biomarkers for early detection of NASH-induced fibrosis

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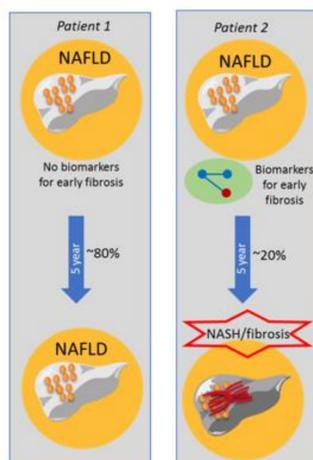
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Background

The number of subjects with non-alcoholic fatty liver disease (NAFLD) is rising, hepatic steatosis which progresses to non-alcoholic hepatic steatohepatitis (NASH) and hepatic fibrosis are considered as a "ticking time bomb". Currently, a liver biopsy is required for both the clinical diagnosis and assessment of a treatment response. Since liver biopsies are invasive, and occasionally associated with serious complications there is an urgent need to develop blood based biomarkers.



Our objective is to gain time-resolved insight in liver functionalities during NAFLD/NASH development and identify candidate biomarkers that detect the development of fibrosis already at an early stage of the process in humans.

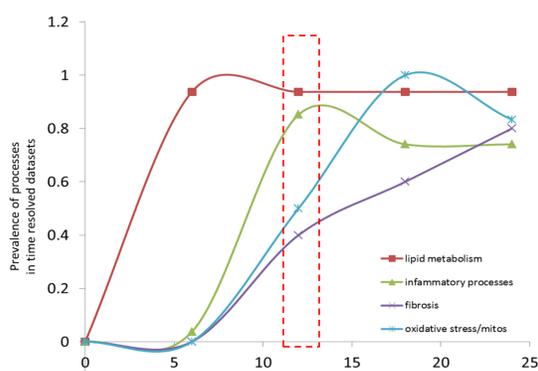
Approach

- Identification of early fibrosis signature in pre-clinical study, **Figure 1** (Ldlr^{-/-} Leiden)
- Verification of early fibrosis signature in (a limited) existing human data, gene analysis in RNA isolated from fresh frozen liver biopsies, **Figure 2** (n=60)
- Test suitability of formalin fixed paraffin embedded material for further verification of the signature in larger (human) sample sets, not shown
- Verification of signature in human FFPE samples with well defined fibrosis stages, **Figure 3** (n=71)
- Translation of the gene signature into a circulating protein signature, not shown
- Analyse circulating protein signature in serum and plasma samples from patients with different fibrosis stages, same as Figure 3, ongoing

References

- Uncovering a predictive molecular signature for the onset of NASH-related fibrosis in a translational NASH mouse model. van Koppen A, Verschuren L, et al. Cell Mol Gastroenterol Hepatol, 2017 Oct 14;5(1):83-98.e10.
- Key Inflammatory Processes in Human NASH Are Reflected in Ldlr^{-/-} Leiden Mice: A Translational Gene Profiling Study. Morrison MC, Kleemann R, van Koppen A, Hanemaaijer R, Verschuren L., Front Physiol. 2018 Feb 23;9:132

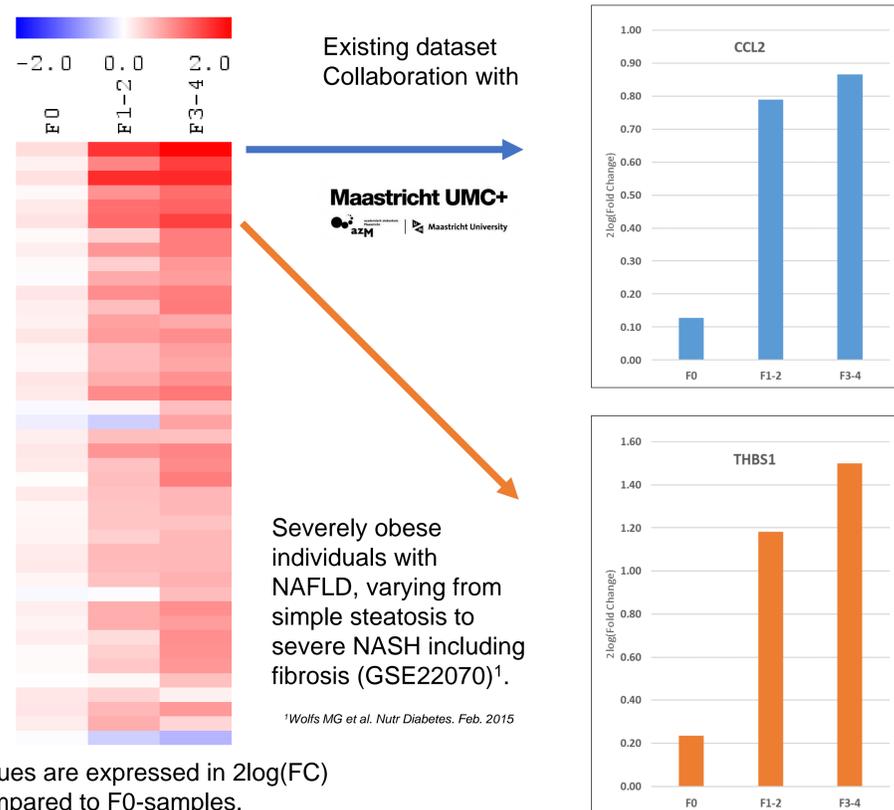
Functional molecular signature



Multi-omics analysis from liver of HFD-fed male LDLr^{-/-} Leiden mice show time-resolved evolution of processes related to NAFLD/NASH and fibrosis development

Figure 1: Data integrative approaches using transcriptome and dynamic proteome (incl. D₂O labeling) elucidate the dynamic molecular processes involved in development of NASH and fibrosis. Systems biology approaches enabled selection of a functional molecular signature related to formation of new collagen/ fibrosis **already seen after 12 weeks** [1,2].

Signature expression in human liver



Values are expressed in 2log(FC) compared to F0-samples. F0: n=36; F1-2, n=18; F3-4, n=6.

Figure 2: Heatmap representation of genes from the molecular signature and their expression in human liver biopsies with different amount of fibrosis as scored by an independent pathologist. 37 genes correlate with the severity of the disease.

Signature expression in human FFPE samples

FFPE section samples including plasma or serum samples from the same patients were collected by the collaborating medical centers. F0: n=9, F1: n=19, F2: n=27, F3: n=10, F4: n=6

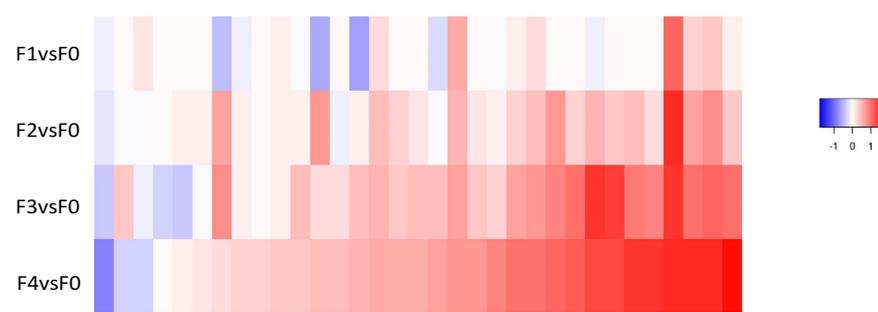


Figure 3: Expression of signature genes (same genes, not the same order as Figure 2) in human FFPE samples. Some of the genes show a significant correlation with severity of fibrosis in NASH patients.

Conclusions

- A gene signature related to new collagen/fibrosis formation (=Function) was identified in liver from preclinical studies;
- Human biopsy study (microarray array data from fresh frozen samples) verifies 55 genes from the signature to be regulated in liver tissue; 37 genes correlate with severity of fibrosis;
- The signature is already differentially expressed in mild fibrosis stages F1-F2, indicating the potential for early detection of fibrosis;
- FFPE sections (71) with a good distribution of the fibrosis severity grades were collected and and RNA sequenced;
- The potential of the signature for early detection of fibrosis was confirmed in an independent cohort based on FFPE samples;
- Translation of signature into a circulating protein panel is ongoing;
- Plasma samples from same patients as FFPE samples will be analysed.