

# IDENTIFICATION AND VERIFICATION OF FUNCTIONAL BIOMARKERS FOR EARLY DETECTION OF NASH-INDUCED FIBROSIS



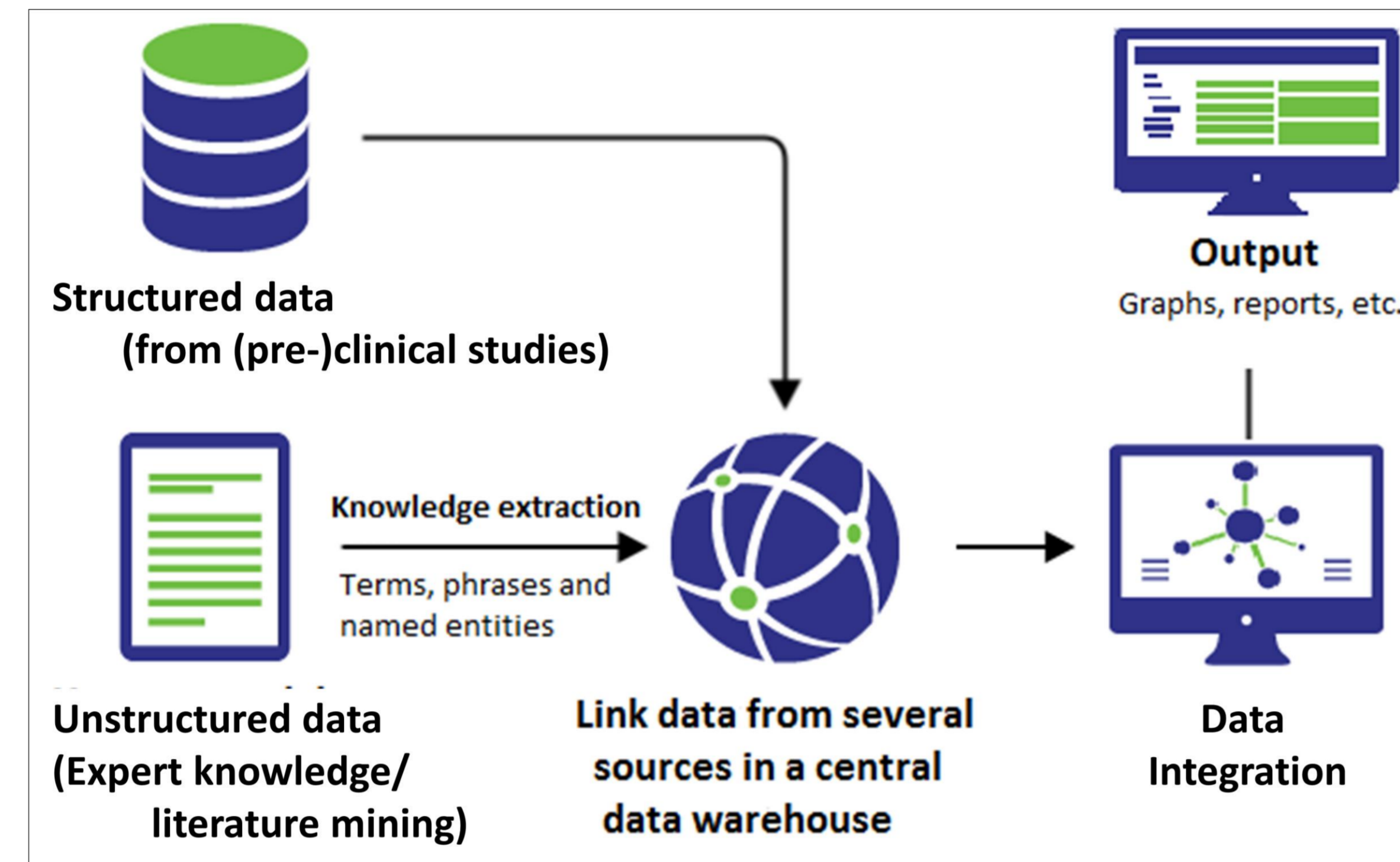
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## Introduction

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". The regulatory authorities (FDA and EMA) have supported the need for the discovery of efficient treatments for this disease.

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.



## Approach

This study identifies candidate biomarkers by *in silico* prediction using structured and unstructured data. We ranked the candidates based on their contribution to a function/pathway and verify their expression in human liver biopsies. This contributes to the ultimate goal, the analysis as circulating biomarkers for NASH-induced fibrosis.

## Methods and references

Preclinical data were obtained from animal studies performed at the facility of TNO, the Netherlands [1]. The *in silico* method is described here [2]. Human gene expression data was obtained in collaboration with Maastricht University.

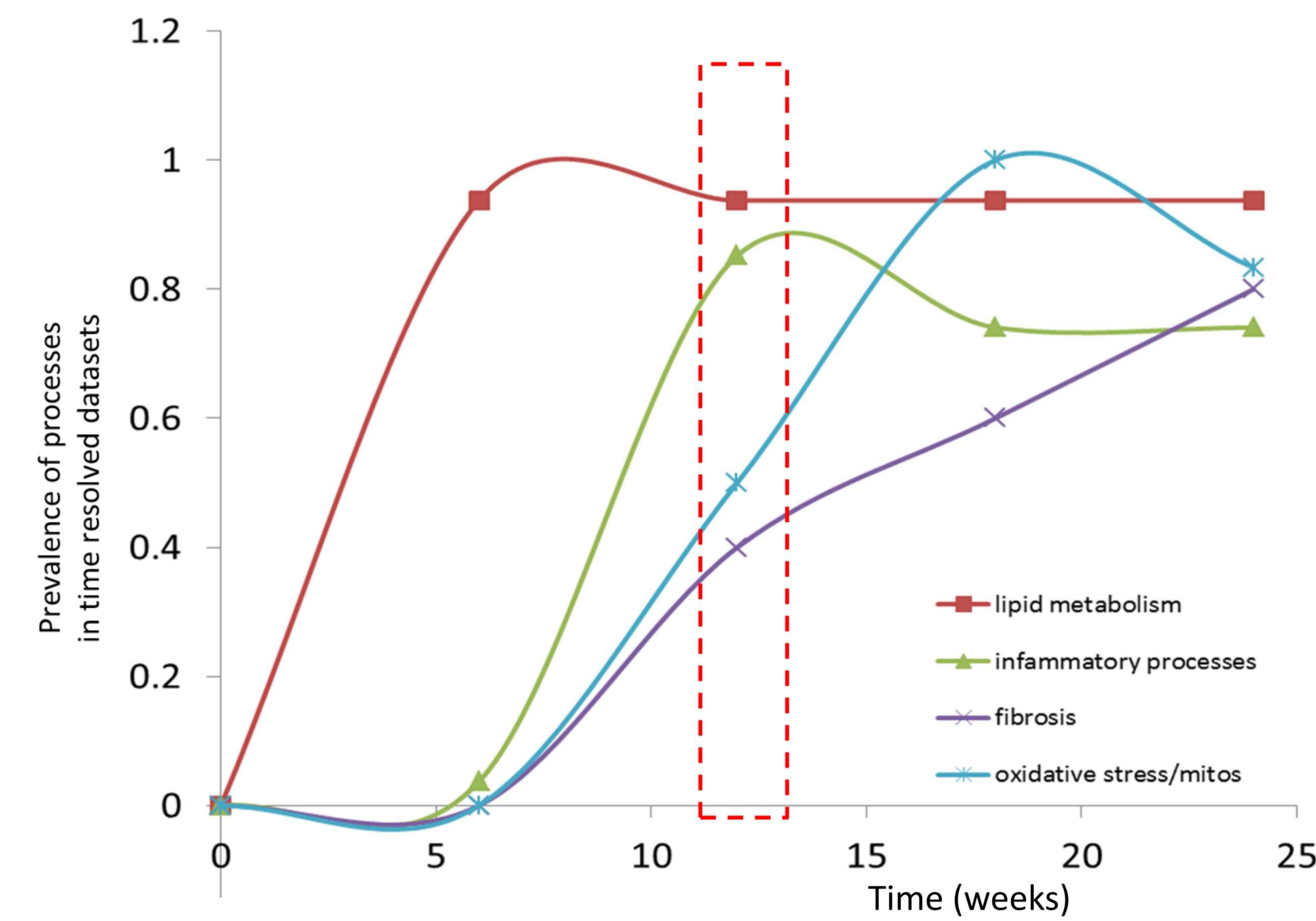
1. Uncovering a predictive molecular signature for the onset of NASH-related fibrosis in a translational NASH mouse model. van Koppen A, Verschuren L, et al. Cell Mol Gastroenterol Hepatol. 2017 Oct 14;5(1):83-98.e10.
2. The future of protein biomarker research in type 2 diabetes mellitus. Tans R, Verschuren L, Wessels HJCT, Bakker SJL, Tack CJ, Gloerich J, van Gool AJ. Expert Rev Proteomics. 2018 Nov 23:1-11.
3. Representing the Process of Inflammation as Key Events in Adverse Outcome Pathways. Villeneuve DL, Landesmann B, Allavena P, Ashley N, et al. Toxicol Sci. 2018;163:1-7.

## Conclusions

- A gene signature (232 genes) related to new collagen/fibrosis formation (=Function) was identified in liver from pre-clinical studies.
- Expert knowledge and literature mining enabled us to select important mechanisms underlying chronic low grade inflammation and fibrosis.
- A ranked list of candidate biomarkers were selected by integrating functional signature with candidate biomarkers from the Integrity database using Network biology approach.
- In total, 55 genes from the signature are expressed in human biopsies of which 42 genes were shown to be regulated in a fibrosis-specific manner.

## Functional molecular signature

Structured multi-omics data from HFD-fed male LDLr-/- .Leiden mice show time-resolved evolution of processes related to NAFLD/NASH and fibrosis development.

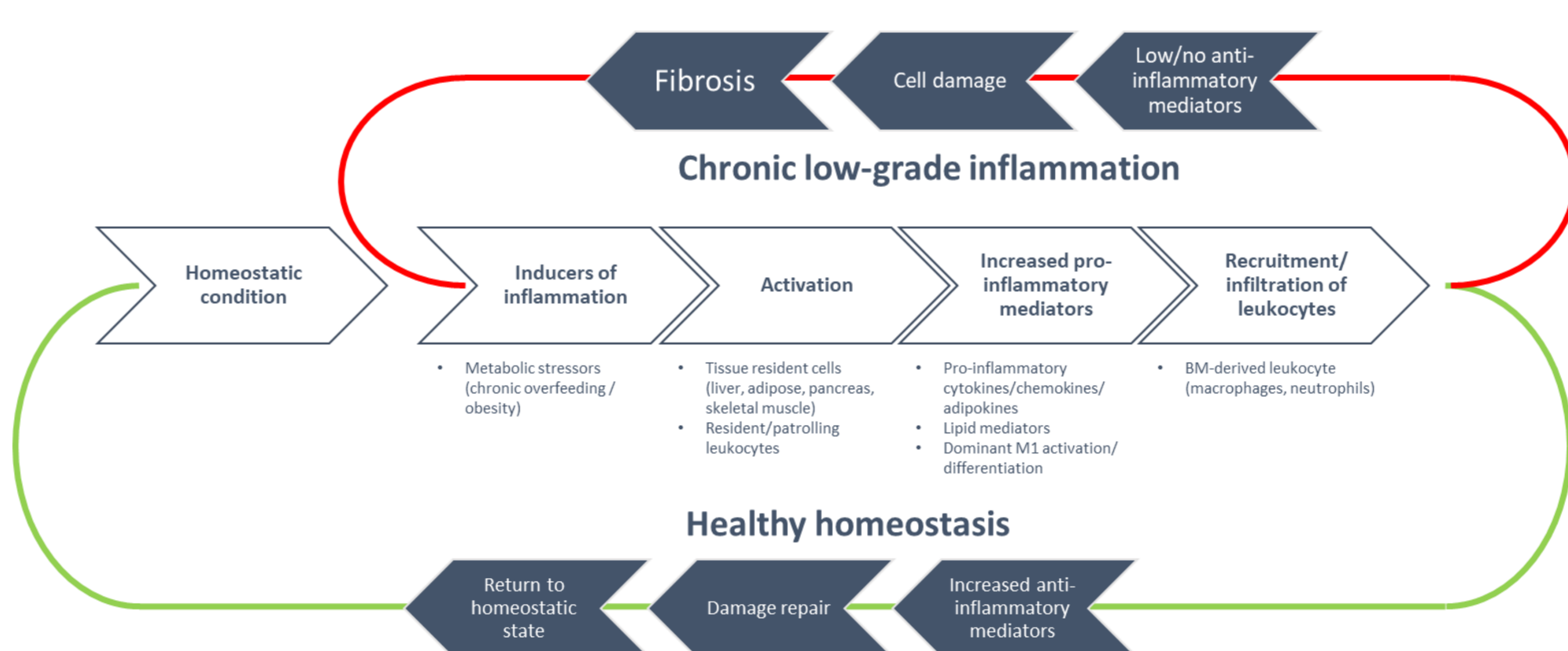


Time-resolved analysis show regulation of lipid metabolism, inflammation, oxidative stress and ECM/fibrosis pathways. Note, pathological fibrosis is detected after 18, 24, and 30 weeks of HFD treatment.

Systems biology approaches using data from D2O-labeled proteome and transcriptome generates a functional signature related to formation of new collagen/ fibrosis [1].

## Literature-derived mechanisms and database-derived biomarkers

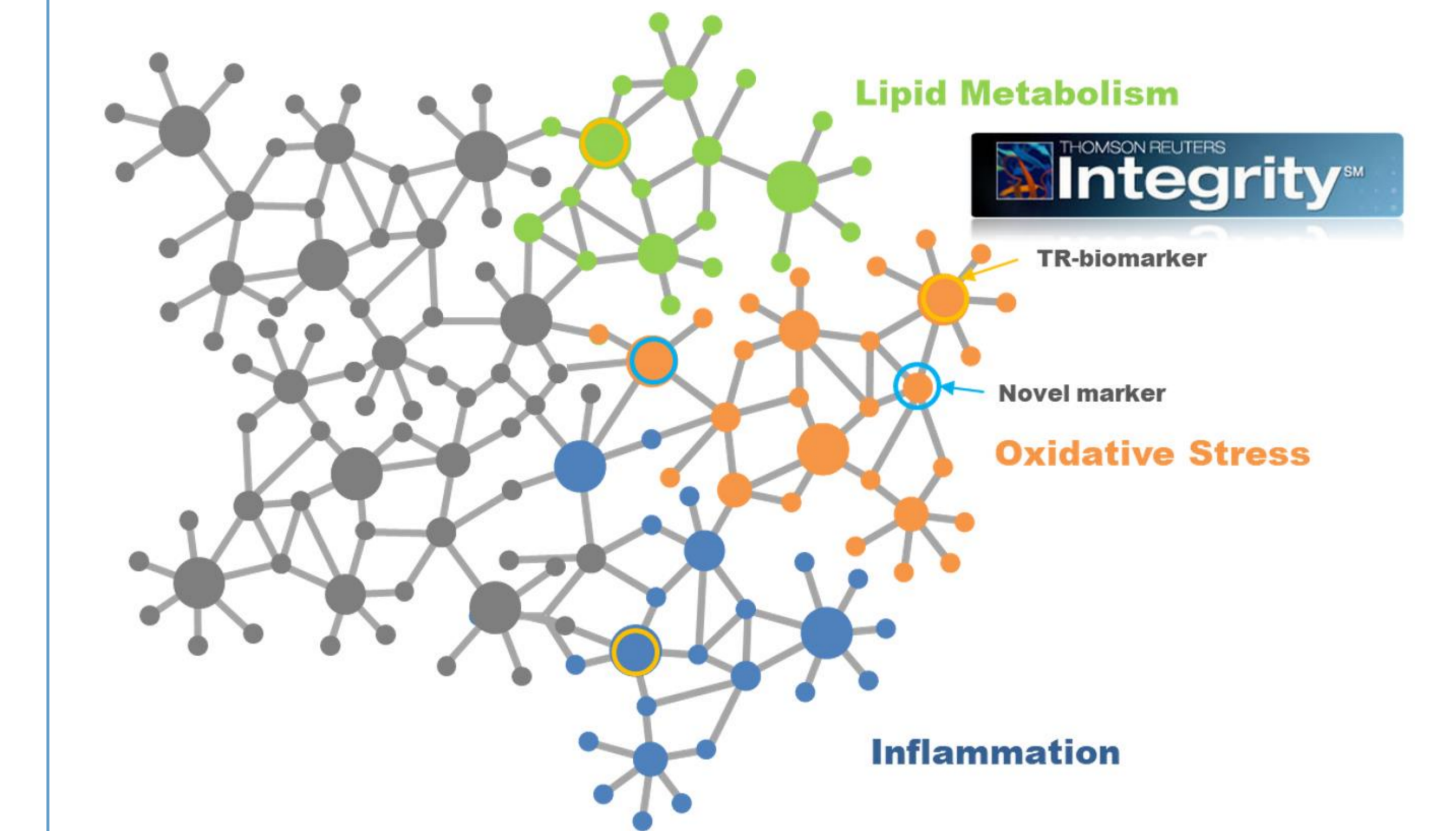
Expert knowledge and literature on mechanisms underlying NASH and fibrosis were collected and known human biomarkers associated were collected from Thomson Reuters Integrity database.



Generalized model of sequential steps involved in the inflammatory response in tissues resulting in chronic low-grade inflammation and fibrosis. Adapted from Villeneuve et al [3].

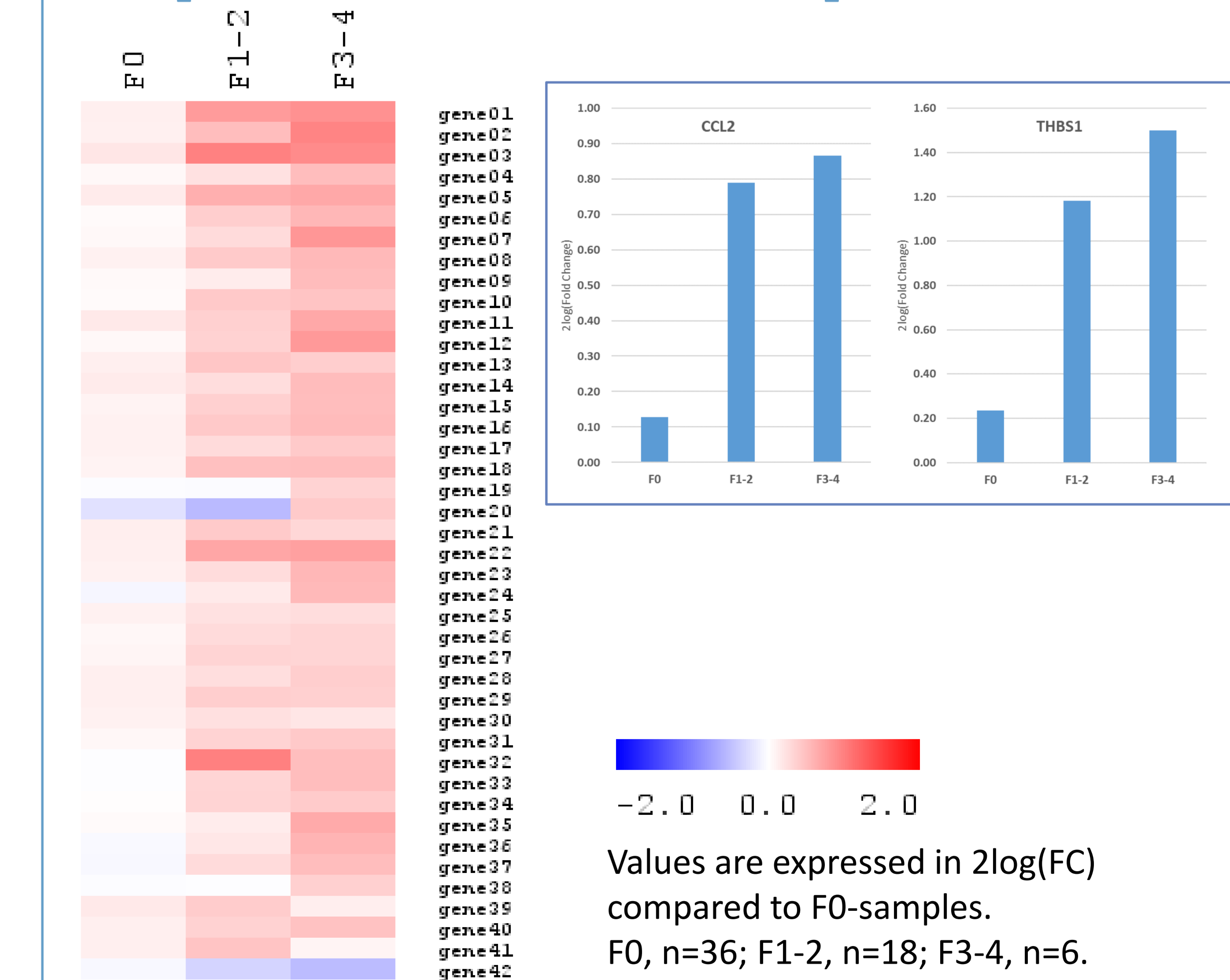
## Network Biology based integration

The inventory of expert knowledge on (novel) candidate biomarkers combined with biomarkers from unbiased literature and database mining are integrated using a network biology approach.



The network is built from the mechanisms involved in NASH and fibrosis development. Thomson Reuters Integrity database is used to select and rank biomarkers that have been applied in human studies.

## Functional signature gene response in human biopsies



Heatmap representation of 42 genes from the Functional signature and their average expression in human liver biopsies. Samples are categorized based on the fibrosis scored by an independent pathologist.