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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Uncovering a Predictive Molecular Signature for the Onset of NASH-Related Fibrosis in a Translational NASH Mouse Model

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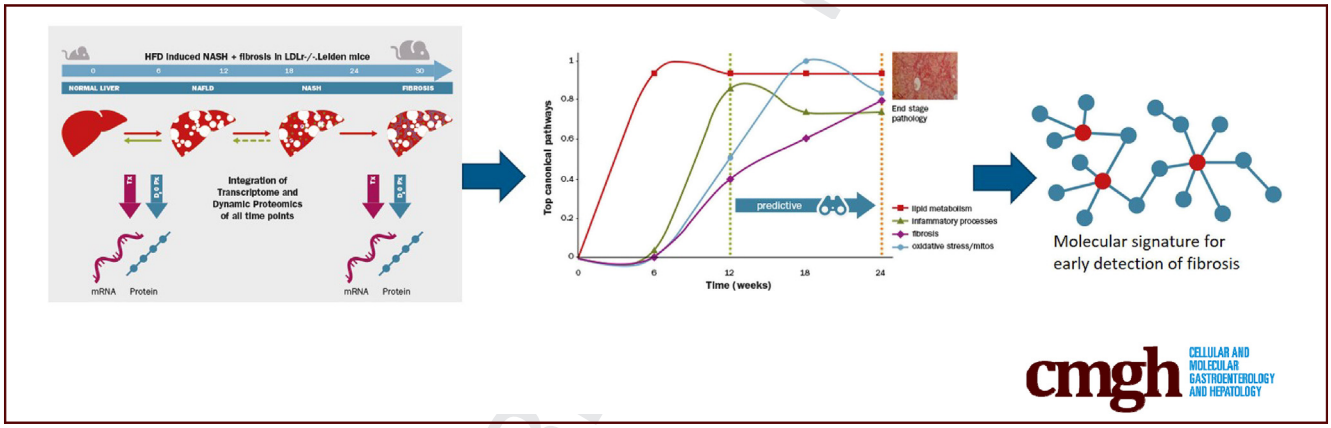

SUMMARY

This article presents a predictive molecular signature that marks the early onset of fibrosis in a translational nonalcoholic steatohepatitis mouse model. Overlap of genes and processes with human nonalcoholic steatohepatitis and a list of top candidate biomarkers for early fibrosis are described.

BACKGROUND & AIMS: The incidence of nonalcoholic steatohepatitis (NASH) is increasing. The pathophysiological mechanisms of NASH and the sequence of events leading to hepatic fibrosis are incompletely understood. The aim of this study was to gain insight into the dynamics of key molecular processes involved in NASH and to rank early markers for hepatic fibrosis.

METHODS: A time-course study in low-density lipoprotein–receptor knockout Leiden mice on a high-fat diet was performed to identify the temporal dynamics of key processes contributing to NASH and fibrosis. An integrative systems biology approach was used to elucidate candidate markers linked to the active fibrosis process by combining transcriptomics, dynamic proteomics, and histopathology. The translational value of these findings were confirmed using human NASH data sets.

RESULTS: High-fat-diet feeding resulted in obesity, hyperlipidemia, insulin resistance, and NASH with fibrosis in a time-dependent manner. Temporal dynamics of key molecular processes involved in the development of NASH were identified, including lipid metabolism, inflammation, oxidative stress, and fibrosis. A data-integrative approach enabled identification of the active fibrotic process preceding histopathologic detection using a novel molecular fibrosis signature. Human studies were used to identify overlap of genes and processes and to perform a network biology-based prioritization to rank top candidate markers representing the early manifestation of fibrosis.

CONCLUSIONS: An early predictive molecular signature was identified that marked the active profibrotic process before histopathologic fibrosis becomes manifest. Early detection of the onset of NASH and fibrosis enables identification of novel blood-based biomarkers to stratify patients at risk,

development of new therapeutics, and help shorten (pre)clinical experimental time frames. *(Cell Mol Gastroenterol Hepatol 2017;■:■–■; https://doi.org/10.1016/j.jcmgh.2017.10.001)*

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Nonalcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease in developed countries.[1] This increasing prevalence is associated closely with the incidence of obesity, insulin resistance, and dyslipidemia, all of which are risk factors for NAFLD.[2–5] NAFLD is associated with 26% higher overall health care costs, mainly from associated cardiometabolic diseases,[6] and is projected to become the primary indication for liver transplantation within the next several years.[7] NAFLD encompasses a spectrum of liver diseases ranging from the relatively benign hepatic steatosis to nonalcoholic steatohepatitis (NASH), the progressive form of NAFLD.

NASH is characterized by the presence of hepatocellular damage and inflammation,[8] which in concert can drive the development of fibrosis.[9] Recently, liver fibrosis was recognized to be strongly associated with long-term overall mortality, independently of other histologic features of NAFLD or NASH.[10,11] There is currently no method to identify which patient will progress from NAFLD and/or NASH to fibrosis. In addition, NASH and liver fibrosis are clinically silent, with hardly any symptoms, which means that detection often does not occur until the advanced stages of disease.

The molecular and cellular mechanisms involved in the pathogenesis of NAFLD and NASH have not been elucidated completely yet, but it is clear that disease progression is the result of complex and dynamic interactions between many processes, such as lipid metabolism, inflammation, oxidative stress, and fibrosis. However, the current body of knowledge relies mostly on results from studies that investigate these processes at a single time point (generally end point pathology) rather than investigating their interplay and dynamics over time. Information on the temporal dynamics and interaction between various molecular and pathologic processes has been shown to provide insight into early disease manifestations and allow detection of the onset of progressive disease.[12]

Animal models of NAFLD and NASH can be used for time-resolved studies and are suitable to provide crucial information on the processes that contribute to disease development. In the current study, we investigated the development of NASH in a time-resolved manner in high-fat-diet–fed low-density lipoprotein-receptor knockout (LDLr-/-.Leiden) mice, which develop NASH and hepatic fibrosis in the context of obesity, dyslipidemia, and insulin resistance, as is typical for NASH patients.[13] Dynamic proteomic analyses that involve deuterated water labeling and tandem mass spectrometry were used to measure the formation of new collagens representing the active fibrosis process.[14–16] RNA sequencing was used to generate a genetic time-resolved profile of processes involved in the development of NASH. This allowed identification of the dynamics of key molecular processes involved in the development of NASH and fibrosis. An integrative systems biology approach was used to investigate the molecular processes involved in the active fibrosis process by combining transcriptomics, dynamic proteomics, and histopathology. To gain insight into the translational value of these findings, the LDLr-/-.Leiden NASH mouse was compared with NASH patients on the molecular level. In addition, network biology-based ranking was performed using databases containing data from human cohort studies to identify candidate markers that represent the early manifestation of fibrosis.

Materials and Methods

Animals and Housing

Animal experiments were approved by an independent Animal Care and Use Committee and were in compliance with European Community specifications for the use of laboratory animals.

Time-Course Study

Twelve-week-old male LDL-receptor knockout mice were obtained from the breeding facility of TNO Metabolic Health Research (Leiden, The Netherlands). Animals received either standard rodent chow (Sniff-R/M-V1530 with 33 kcal% protein, 58 kcal% carbohydrate, and 9 kcal% fat; Uden, The Netherlands) (n = 45) or a high-fat diet (HFD) (D12451; Research Diets, Inc, New Brunswick, NJ; with 20 kcal% protein, 35 kcal% carbohydrate, and 45 kcal% lard fat) (n = 75) for a total of 30 weeks. Mice were group-housed in the SPF animal facility of TNO Metabolic Health Research, in a temperature-controlled room on a 12-hour light/dark cycle with ad libitum access to food and water. All interventions were performed during the light cycle. Groups were killed after 6, 12, 18, 24, and 30 weeks on the diets. Blood samples were collected via the tail vein for EDTA plasma isolation after a 5-hour fast at 6-week intervals. A subset of mice (chow, n = 6; HFD, n = 15) was killed every 6 weeks. This subset was matched to the remaining mice for body weight and the biochemical parameters of plasma cholesterol, triglycerides, blood glucose, and insulin. One group of mice (n = 15) was killed before the start of the diets to define the starting condition (t = 0). In the 18-week and 24-week groups, 1 animal died before killing, which was not included in the analyses (resulting in HFD, n = 14 for these 2 time points). One week before

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Abbreviations used in this paper: **ALT, alanine aminotransferase; AST, aspartate aminotransferase; DEG, differentially expressed genes; ECM, extracellular matrix; HFD, high-fat diet; IPA, Ingenuity Pathway Analysis; LDLr-/-, low-density lipoprotein receptor knock out; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; THBS1, thrombospontin-1.**

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