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OPINION

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Epigenome-Wide Association Studies Provide Insight into the Pathogenesis of Non-alcoholic Fatty Liver Disease and Non-alcoholic Steatohepatitis

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ABSTRACT

Non-alcoholic Fatty Liver Disease (NAFLD) and its progressive form or NASH with fibrosis, are rapidly increasing in conjunction with the growing rates of obesity and type II diabetes. Novel technologies such as epigenome-wide association studies (EWAS) may provide opportunities to get valuable insight into not only the mechanisms of disease, but to also discover potential biomarker targets with clinical relevance to disease stage.

Key words. NASH, NAFLD, EWAS.

OPINION

Non-alcoholic Fatty Liver Disease (NAFLD) is a metabolic disorder characterized by the presence of ectopic fat in hepatocytes.[1] It is estimated that NAFLD affects 24.5% of the World population.[2] Since the prevalence of NAFLD increases proportionally with the presence of diabetes and obesity, this global epidemic is projected to double by 2030.[1] The NAFLD spectrum ranges from hepatic steatosis to steatosis with hepatocellular inflammation and damage (nonalcoholic steatohepatitis: NASH) which is the subtype that can progress with the development of fibrosis, in some rare cases, to hepatocellular carcinoma (HCC).[3] NASH is rapidly becoming one of the most common indication for liver transplantation.[4] The most important clinical predictor of adverse outcome is presence of diabetes mellitus (DM), while stage of hepatic fibrosis is the most important histologic predictor of mortality.[2,5-7] In addition to its clinical impact, NAFLD has a substantial economic impact to the society.[8]

Many pathways have been implicated in the pathogenesis of NAFLD, such as insulin resistance and enhanced oxidative stress. These studies suggest that NASH is a phenotype that can involve multiple environmental, genetic and epigenetic factors.[5] Despite increased awareness, it remains challenging to quantify the true prevalence of NASH in the general population. While the use of liver ultrasound can accurately establish hepatic steatosis, liver biopsy remains the most reliable "gold standard" for diagnosis of NASH and to stage hepatic fibrosis.[9] Thus, the growing epidemic of NAFLD and most importantly, NASH with fibrosis, urgently necessitates reliable and accurate non-invasive biomarkers that have a high degree of disease stage specificity.

In the current study *Epigenome-Wide Association Study Identifies Methylation Sites Associated With Liver Enzymes and Hepatic Steatosis,* Nano, *et al.* assess the differential DNA methylation levels and their association with circulating levels of liver enzymes and hepatic steatosis, as assessed by ultrasound, in several cohorts of the Rotterdam Study.

The authors conduct an epigenome-wide association study (EWAS) using whole blood to determine liver enzyme levels: gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). The same markers are assessed in two separate cohorts, the "*discovery set*" (n = 731) and the "replication set" n = 719. The study analyzes significant DNA methylation changes and evaluates their relation with hepatic steatosis by using generalized linear mixed effects models that include the following factors: age, sex, smoking history, and

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whole blood cells proportions, body mass index, alcohol consumption, coffee intake, hypertension medication, lipid levels, glucose levels and liver enzymes.

Additionally, the authors determine the expression levels of the genes identified in the previous experiment in 9 human hepatoma cell lines (Hep3B, Huh6, Huh7, PLC/PRF/5, SNU182, SNU398, SNU449, HepG2 and HepaRG) and correlate these with potential targets associated with lipid traits. They confirm the cellular effects of these candidate genes by knock-down studies using lentiviral vectors expressing small hairpin RNAs.

The results of this study provide some interesting associations and insight about NAFLD. Briefly, the authors found 8 CpG sites annotated to 7 unique loci (SLC7A11, SLC1A5, SLC43A1, PHGDH, PSORS1C1, SREBF1, ANKS3) associated with GGT but only 1 probe annotated to SLC7A11, associated with ALT. Interestingly, no probe was identified for AST. In the replication group 4 probes associated with GGT levels (SLC7A11, SLC43A1, SLC1A5 and PHGDH), and 1 for ALT levels (SLC7A11). Importantly, the authors found an association between DNA methylation at SLC7A11 with reduced risk of hepatic steatosis in participants as measured by ultrasound. The cell culture experiments showed SLC7A11 to be highly expressed in human hepatoma cell lines with a positive correlation with the expression of a panel of lipid-associated genes. However, it must be noted that elevated levels of SLC7A11 were found in HCC patients with shorter overall survival time,[10] suggesting that the levels of SLC7A11 seen in this cell culture study may be a feature of the cell lines, rather than the mechanism of lipid deregulation seen in hepatic steatosis.

Based on these observations the authors suggest a significant role for SLC7A11 in both lipid metabolism and hepatic steatosis. SLC7A11 is a member of a heteromeric, sodium-independent, anionic amino acid transport system, highly specific for cysteine and glutamate. SLC7A11 has been shown to be differentially methylated in correlation with trigylceride levels,[11] but mostly studied in gliomas and aggressiveness of glioblastomas.[12,13] In fact, SLC7A11 has been implicated in numerous cancers via the enhancement of cancer cell glucose dependence.[14]

However, more relevant to the field of NASH, novel studies have shown SLC7A11 to be involved in a type of iron and ROS dependent regulated cell death, ferroptosis.[15] This process has been observed in both cancer and normal cells (notably T-cells and fibroblasts) and functions via the activation of mitochondrial voltage-dependent anion channels and mitogen-activated protein kinases, upregulation of endoplasmic reticulum stress, and inhibition SLC7A11, as well as accumulation of lipid peroxidation products and lethal reactive oxygen species (ROS) derived from iron metabolism.[16] Interestingly, p53 inhibits SLC7A11 expression and induces ferroptosis following ROS-induced stress, potentially explaining the involvement of this gene in both cancer etiologies and lipid metabolism.[17] Within the context of NAFLD, lipid peroxidation has been suspected to be involved in causing hepatocyte injury and fibrosis.[18] These lines of research suggest an intricate role for SLC7A11 in NAFLD and possibly a novel mechanism in which the deregulation of a cell-death pathway may contribute to NAFLD progression. Regrettably, the use of ultrasound rather than liver tissue biopsy in this study, limits the conjecture one can make with regard to the role of SLC7A11 in the development of NASH and related fibrosis.

Despite the intriguing results in this EWAS, one must remember that DNA methylation was performed using the whole blood. Extensive reporting has shown substantial differences between CpG methylation of blood and tissue cells[19]and therefore these results must be interpreted with caution.

Although this study provides some novel insights for investigation, it does not address the most important predictor of long term outcomes in NAFLD, i.e. stage of hepatic fibrosis. Non-invasive biomarkers that can reliably predict stage of fibrosis and its progression over time can have significant prognostic and therapeutic implications. While this study provides insights about the pathogenesis of NAFLD using novel technologies, to be clinically relevant and meet the challenge of this growing epidemic, future studies must focus on NASH and its associated fibrosis.

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