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Genetics of nonalcoholic fatty liver disease

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ABSTRACT

Epidemiological, familial, and twin studies indicate that non-alcoholic fatty liver disease, now the leading cause of liver damage in developed countries, has a strong heritability. The common I148M variant of *PNPLA3* impairing hepatocellular lipid droplets remodeling is the major genetic determinant of hepatic fat content. The I148M variant has a strong impact on the full spectrum of liver damage related to fatty liver, encompassing non-alcoholic steatohepatitis, advanced fibrosis, and hepatocellular carcinoma, and influences the response to therapeutic approaches. Common variants in *GCKR* enhance *de novo* hepatic lipogenesis in response to glucose and liver inflammation. Furthermore, the low-frequency E167K variant of *TM6SF2* and rare mutations in *APOB*, which impair very low-density lipoproteins secretion, predispose to progressive fatty liver.

Conclusions. These and other recent findings reviewed here indicate that impaired lipid handling by hepatocytes has a major role in the pathogenesis of non-alcoholic fatty liver disease by triggering inflammation, fibrogenesis, and carcinogenesis. These discoveries have provided potential novel biomarkers for clinical use and have revealed intriguing therapeutic targets.

1. Non-Alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD) is defined by fat content exceeding 5% of liver weight in the absence of excessive alcohol intake. The major risk factor of NAFLD is insulin resistance associated with overweight, physical inactivity, and development of type 2 diabetes mellitus [1]. In susceptible individuals, hepatic fat deposition, also known as steatosis, may result in hepatocellular damage related to oxidative stress, inflammation, and cell death, that is non-alcoholic steatohepatitis (NASH) [2]. NASH is associated with activation of hepatic stellate cells and of the fibrogenic program, and can progress towards cirrhosis in 20% of cases and hepatocellular carcinoma [3]. Paralleling the "Diabesity" epidemic, NAFLD is now the most frequent liver disease and the leading cause of hepatic damage in developed countries [4], while NASH is projected to become the leading cause of end-stage liver disease, liver transplantation and hepatocellular carcinoma within the next decades.

Abbreviations: APOB, apolipoprotein B; APOC3, apolipoprotein C3; ATGL, adipocyte triglyceride lipase; CGI-58, 1-acylglycerol-3-phosphate O-acyltransferase ABHD5; ENPP1, ectonucleotide pyrophosphate phosphodiesterase-1; FATP5, fatty acid transport protein 5; FFA, free fatty acid; FFAR4, free fatty acid receptor 4; GCKR, glucokinase regulator; GPR120, G protein coupled recptor-120; GWAS, genomewide association study; HSL, hormone sensitive lipase; IFN, interferon; IL28B, interleukin 28B; IRS1, insulin like receptor substrate 1; LYPLAL1, lysophospholipase-like 1; MTTP, microsomal triglyceride transfer protein; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PLIN1, perilipin-1; PNPLA3, patatin-like phospholipase domain-containing-3; SNP, single nucleotide polymorphism; SOD2, manganese superoxide dismutase; TM6SF2, transmenbrane-6 superfamily member 2; TNFα, tumor necrosis factor α.

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The main purpose is to provide an overview of NAFLD genetics. Indeed, recent advances in this field have contributed to clarify the pathogenesis of this condition and may translate into therapeutic advances [5]. Newly identified risk variants could prove useful biomarkers for the clinical management and prognosis of patients with NAFLD. They may also lead to the identification of new targets for pharmacological treatment NASH.

2. Pathogenesis

Hepatic fat accumulation results from an imbalance between triglycerides acquisition, synthesis, utilization and secretion [6,7]. Triglycerides accumulate within intracellular lipid droplets, representing the safest way for the body to store free fatty acids (FFAs) during insulin resistance limiting lipotoxicity [8]. The FFAs that accumulate in NAFLD derive from increased peripheral lipolysis [9] caused by adipose tissue insulin resistance [10], increased *de novo* lipogenesis due to hyperinsulinemia, and an excess in food intake via chylomicrons. Systemic insulin resistance is the major driver of this process [1]. Impaired ability to secrete neutral lipids through very low-density lipoproteins (VLDLs) [7] and to perform beta-oxidation due to mitochondrial damage is also involved in hepatic fat accumulation. Steatosis may in turn worsen hepatic insulin resistance, thereby contributing to metabolic disturbances and cardiovascular damage [11,12].

Lipid droplets are highly regulated intracellular organelles that undergo a complex metabolism and exert many functions beyond lipid storage. Lipid droplets are comprised of a core of triglycerides and sterol esters, surrounded by a phospholipid monolayer. They are also involved in the regulation of metabolism and of signaling pathways [13]. Many proteins participate in the development, maintenance, and metabolism of lipid droplets [13]. Derangement of their activity is involved in the pathogenesis of hepatic and metabolic complications of NAFLD.

The development of NASH is currently explained by the occurrence of multiple parallel "Hits" in the background of fatty liver, which trigger inflammation and fibrogenesis [14,15]. These insults are represented by a) direct hepatic lipotoxicity and endoplasmic reticulum stress; b) hepatocellular oxidative stress secondary to free radicals produced during β- and ω-oxidation of FFAs; c) inflammation triggered by endotoxin engaging Toll-like receptor-4 in Kupffer cells and hepatocytes due to increased intestinal permeability and to qualitative and quantitative changes in the gut microbiota [16,17]; d) insulin resistance and an altered profile of adipokines [18] e) activation and senescence of hepatic stellate cells [19].

3. Heritability

Although environmental risk factors contribute to development of steatosis, the variability in phenotypic penetrance and expression in persons with similar risk factors implicates a genetic contribution. A strong heritability of NAFLD susceptibility is supported by converging evidence from epidemiological, familial and twin studies, and from clinical cases [5,20–22].

Indeed, there is a huge inter-ethnic variability in the predisposition towards NAFLD [4,23]. Two large multi-ethnic population studies conducted in the US detected higher risk of NAFLD in Hispanics than individuals of European descent, whereas African-Americans were protected irrespectively of diabetes, excess in body weight, and socioeconomic factors, consistent with a major role of heritability [21,24].

Familial aggregation studies in overweight children and individuals with combined hyperlipidemia showed that liver fat fraction, alanine aminotransferases (ALTs), and fatty liver are strongly heritable traits [20,25]. In individuals who do not abuse alcohol and are not infected with viral hepatitis, serum ALT levels mostly reflect liver fat content, and have been used as a surrogate index of steatosis severity. Twin studies indicated that ALT variability is 35%–60% explained for by genetic factors, suggesting that hepatic fat content is highly heritable [26,27]. Furthermore, serum gamma-glutamyl peptidase levels, another liver enzyme frequently induced by fat accumulation, were 50% explained for by inherited factors, and clustered with NAFLD risk factors including insulin resistance, lipid levels, and diastolic blood pressure [28].

The genetic determinants of steatosis are now beginning to be defined by genome-wide association studies. The rs738409 C > G sequence variant in *Patatin-like phospholipase domain-containing 3* (*PNPLA3*), encoding for the I148M protein variation, has been identified as a major determinant of inter-individual and ethnicity-related differences in hepatic fat content [29,30]. The *Transmembrane 6 Superfamily Member 2* (*TM6SF2*) E167K variant has recently been shown to increase susceptibility to progressive NAFLD [31,32]. Furthermore, case–control studies demonstrated a role of other genetic variants implicated in inflammation, insulin signaling, oxidative stress, and fibrogenesis in NAFLD progression.

4. *PNPLA3* I148M is the Main Common Genetic Determinant of Liver Fat

The rs738409 C > G single nucleotide polymorphism in the *PNPLA3* gene, encoding for the Isoleucine to Methionine substitution at position 148 (I148M), was linked to hepatic fat content and the risk of NAFLD in 2008 by a genome wide association study evaluating common mutations determining protein sequence variants [29]. The association was independent of insulin resistance and body weight, and contributed to explaining the inter-ethnic susceptibility to NAFLD. Indeed, the risk allele is more prevalent in Hispanics than in Europeans, and less frequent in African Americans.

PNPLA3, also called *Adiponutrin*, encodes a 481 amino acid membrane protein localized in the endoplasmic reticulum and at the surface of lipid droplets. PNPLA3 protein is expressed in the retina, hepatic stellate cells, and hepatocytes. In mice, Pnpla3 is up-regulated in the liver after feeding and during insulin resistance by fatty acids and the master regulator of lipogenesis SREBP-1c [33]. Although the mechanism linking the I148M variant with the development of liver disease remains an area of active research, PNPLA3 has a triglyceride and retinyl-palmitate esterase activity. It is involved in lipid droplets remodeling in hepatocytes and the