REVIEW ARTICLE

Genetics of non-alcoholic fatty liver disease and associated metabolic disorders

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Abstract Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world. Whereas insulin resistance and obesity are considered major risk factors for the development and progression of NAFLD, the genetic underpinnings remain to be established. Before 2008, numerous candidate gene studies, based on prior knowledge of the pathophysiology of fatty liver, published numerous papers with conflicting results. In 2008, Romeo et al. published the first genome wide association study (GWAS), reporting the strongest genetic signal for fatty liver (PNPLA3, patatin-like phospholipase domain containing 3; rs738409). Most of the candidate genes and GWAS associations, however, have not been replicated and, therefore, its significance remains undetermined. Moreover, the associations between candidate genes and metabolic traits have yielded mixed results. This review summarizes the current understanding of genetic epidemiology of NAFLD and associated metabolic traits, namely, insulin resistance, adiposity and lipid disorders.

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PALABRAS CLAVE
Estudios genéticos de asociación;
Enfermedad grasa del hígado no alcohólica;
Hígado graso;
Insulino resistencia;
Obesidad;
Revision

Genética de la enfermedad del hígado graso no alcohólica y trastornos metabólicos asociados

Resumen La enfermedad del hígado graso no alcohólica es la enfermedad crónica del hígado más frecuente en el mundo. Considerando que la resistencia a la insulina y la obesidad son los factores de riesgo importantes para el desarrollo y progresión del hígado graso no alcohólico, las bases genéticas aún no se han establecido. Antes de 2008, se realizaron numerosos estudios de genes candidatos basándose en el conocimiento previo de la fisiopatología del hígado graso. Sin embargo, los resultados han sido contradictorios. En 2008, Romeo et al. publicó el primer estudio de asociación genómica amplia, descubriendo la señal genética más robusta para el hígado graso (PNPLA3, patatin-like phospholipase domain containing 3; rs738409). La mayoría de las asociaciones de genes candidatos y estudios de asociación genómica amplia, sin embargo, no se han reproducido y, por tanto, su importancia sigue siendo indeterminada. Por otra parte,
Brief epidemiology and genetic basis of non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD), the most common chronic liver disease,1 represents a wide spectrum of diseases characterized by the presence hepatic steatosis in the absence of significant alcohol consumption or other causes of liver disease.2,3 The pathogenic processes leading to steatosis, steatohepatitis (NASH) and fibrosis are multifactorial and involve both environmental and genetic factors.4

Obesity and type 2 diabetes mellitus (T2DM)/insulin resistance are the most common risk factors for the development and progression of NAFLD.5 A recent meta-analysis showed a direct association between waist circumference and NAFLD (odds ratio (OR) ~ 1.10-1.13 per 1 cm increase in waist circumference) and between intra-abdominal fat and NAFLD (OR = 1.50 per 1 mm increase in visceral fat thickness).6 In addition, between 47% and 71% of NAFLD patients have metabolic syndrome,7 and conversely, the presence of metabolic syndrome is a strong predictor for NAFLD.8 Consequently, NAFLD is considered the hepatic component of the metabolic syndrome.9 As expected, patients with T2DM have shown a prevalence of NAFLD ranging from 40% to 80%,10,11 and the presence of T2DM is associated with more severe liver disease.12 Since both obesity and T2DM continue to rise, it is expected that NAFLD will reach epidemic proportions worldwide. The prevalence of ultrasound-defined NAFLD ranges from 14 to 45%.13 In Spain, Caballeria et al., in a recent cross-sectional study, showed a prevalence of 26% in individuals aged 15 and 85 years randomly selected from 25 Primary Healthcare Centers,14 consistent with previous estimates.

A genetic underpinning for NAFLD has been suggested by familial aggregation studies,15,16 heritability studies,17,18 candidate gene studies,4 genome-wide scans19-22 and expression studies.23-27 The presence of a genetic basis in NAFLD is important in order not only to identify, individuals at risk, but also to dissect NAFLD pathogenesis and the development of new therapies.

The ultimate goal of this review is to provide the most recent studies in the genetic epidemiology of NAFLD and its association with metabolic traits, namely, adiposity/obesity, insulin resistance, and dyslipidemia. The review is structured in two parts: first, the author will provide some background concepts in genetic epidemiology and the current understanding of NAFLD pathophysiology; and, secondly, the reviewer will show the most up-to-date findings in humans in candidate gene studies and genome wide association studies (GWAS) and their association with metabolic traits. This paper, however, will not assess other forms of fatty liver disease, expression studies or animal studies.

Concepts in genetic epidemiology of NAFLD

NAFLD is considered a complex disease, that is, there is no simple Mendelian pattern of single-gene inheritance such as maturity onset diabetes of the young. In NAFLD, multiple genes, environmental factors, age effects, and their interactions, may be involved.18

Genetic epidemiology studies can be divided in two broad categories: (a) according to the relationships between participants (family-based versus population-based); and, (b) according to the knowledge of the genetic marker(s) used (hypothesis-driven or candidate gene study versus hypothesis free or genome wide association study). A candidate gene is defined as a gene in which its product is considered to play a role in disease pathogenesis.19 It is important to bear in mind that all studies provide key information and are not mutually exclusive. For instance, family-based studies are typically the first step in determining whether a condition is genetic in nature, the model of inheritance, and to understand the proportion of phenotypic variance due to shared genetic factors (heritability). Family-based studies may use linkage analysis to identify the location of a major gene or, association analyses such as the transmission-disequilibrium test, candidate gene approach and a genome-wide association analysis.

Population-based studies, on the other hand, are the mainstream of studies in NAFLD and, until recently, the candidate gene study was the most common design using the “two hit” hypothesis as a framework.30 Both candidate gene and GWAS rely on the statistical association of genetic markers and the disease (phenotype) of interest. The most commonly used genetic markers are the single nucleotide polymorphisms or SNPs, defined as a change in a single base pair of the DNA common in more than 1% of the population.31

The major problem in both candidate gene and GWAS is the presence of false negative and false positive associations. The interpretation of a negative study in genetics is challenging, but most of the time, the problem is a lack of statistical power, that is, true associations are not detected in the study. Consequently, power calculation will provide a robust answer whether the association is indeed possible or not. In GWAS, where there is no hypothesis behind the statistical analysis, the problem we encounter is the opposite,
and we commonly find spurious associations due to chance, even after statistical correction for multiple comparisons. As a result, most of the genetics journals in the same paper need to replicate newly associated genes in different, independent samples (known as 'replication'). The final step is gene fine sequencing to understand what the mechanism is for the association of this particular gene, and use of animal models (knock-outs) and in vitro studies to characterize the metabolic effect of that particular mutation.

Current understanding of NAFLD pathogenesis

The key pathological finding in fatty liver disease is the accumulation of triglycerides in the hepatocytes due to a disproportion between triglycerides acquisition and removal.\(^{32,33}\) The key component of triglyceride is the long-chain fatty acid. The major routes of free fatty acids (FFA) are (a) dietary intake; (b) lipolysis from fat reservoirs, and (c) de novo lipogenesis. Animal and human inherited diseases have shown increased hepatic steatosis with increased triglyceride dietary intake, increased lipolysis from peripheral tissues, increased de novo lipogenesis, or prevention of triglyceride removal from the liver (i.e. decreased efflux or oxidation).\(^{32}\) The exact contributions of each pathway to the development of hepatic steatosis have been studied by Donnelly et al.,\(^{34}\) who showed that 59% of hepatic fat derived from circulating FFAs (mainly lipolysis), 26% from de novo lipogenesis and 15% from diet. The latter mechanism is particularly interesting in the context of obesity and frequent consumption of refined sugars. Increased glucose intake has been associated with hepatic steatosis due to the de novo synthesis of free fatty acids by increased insulin (up-regulator of the transcription factor sterol regulatory element-binding protein-1c, SREBP-1c), availability of substrate and the activation of the transcription factor carbohydrate responsive element-binding protein (ChREBP), involved in the expression of lipogenic genes.\(^{32,33}\)

Insulin resistance is an essential key etiopathogenic factor in the development and progression of fatty liver. Whether insulin resistance is a cause of hepatic steatosis, or vice versa, is a matter of debate and has been reviewed recently.\(^{32}\) According to Cohen et al.,\(^{32}\) current evidence suggests that insulin resistance comes first and leads to hepatic steatosis, as shown by animal models and human diseases where hepatic steatosis is not associated with insulin resistance and, conversely, human diseases with inherited insulin resistance lead to hepatic steatosis (e.g. AKT2 mutation). Opposite views, however, suggested that hepatitis steatosis, possibly originated by impaired mitochondrial β-oxidation of fatty acids, leads to hepatic insulin resistance which, in turn, increases de novo fatty acid synthesis, impairs gluconeogenesis, hepatic glucose uptake, transport of lipoproteins perpetuating increase peripheral insulin resistance.\(^{13,33}\)

A "two-hit model" for the development of steatohepatitis was proposed by Day and James in 1998. In their view, the first step was the accumulation of free fatty acids in the liver, and, from there, further insults induced inflammation, fibrosis and, eventually, cirrhosis.\(^{30}\) Nevertheless, Dr. Day’s group, based on current evidence from animal models, updated their own hypothesis and suggested that "steatosis may be early adaptive response to hepatocyte stress through which potentially lipotoxic FFAs are partitioned into relatively stable intracellular triglyceride stores".\(^{35}\) NAFLD is now seen "as a combination of effects of several fundamental biochemical and immunological mechanisms of liver injury rather than adhering to a sequential "two-hit" paradigm".\(^{35}\)

In summary, based on Day et al., "insulin resistance promotes increased hepatic FFA flux (lipolysis, diet and lipogenesis) leading to hepatic steatosis, and is driven by (1) direct hepatocyte lipotoxicity, (2) hepatocellular oxidative stress secondary to free radicals produced during b- and FFAs oxidation, (3) endotoxin/TLR4-induced inflammation, (4) cytokine release, and (5) endoplasmic reticulum (ER) stress", finally leading to inflammation, cellular damage, and progression to cirrhosis.

A systematic approach to reviewing the genetic epidemiology of NAFLD

In order to identify most of the available published literature on the genetics of NAFLD, the author performed a systematic search using PUBMED, by applying the following engine: (polymorphism OR SNP) AND ('steatosis' OR "steatohepatitis" OR NAFLD OR NASH) without language restriction and up to July 26th, 2011. Studies were included and analyzed if they were performed on humans. The search yielded 224 references, of which 60 corresponded to candidate gene studies and are summarized in Table 1\(^{34}\), and three genome wide association studies (Table 2). This search engine did not use the more ‘non-English language friendly’ database EMBASE, and was limited by the published papers (risk of publication bias since negative studies usually remain unpublished). Genes (or nearby genes) are named using the HUGO Gene Nomenclature Committee (HGNC) and reported identification number.\(^{36}\)

Candidate genes studies in human NAFLD: hepatic lipid metabolism, insulin resistance and oxidative stress

Numerous candidate gene studies applying this ‘multiple-hit’ hypothesis, have studied the effects of genes on NAFLD presence and progression. The reviewer has simplified the previous hypothesis into the following mechanisms (not mutually exclusive): genes involved in hepatic lipid metabolism (synthesis, storage, export, oxidation), genes implicated in insulin signaling (insulin resistance), and finally, genes involved in oxidative stress and inflammation (and therefore, most likely involved in progression to cirrhosis) (Table 1). The reviewer included, when appropriate, other data from different metabolic traits to illustrate the direction of association for a particular gene (in parenthesis). Interestingly, the strongest signals for other well-known risk factors, such as obesity or diabetes (e.g. FTO, TCF7L2) did not associate with fatty liver disease, suggesting that fatty liver disease, on its own, has other genetic underpinnings.
<table>
<thead>
<tr>
<th>Gene (HGNC), SNP</th>
<th>Fatty liver</th>
<th>Inflammation/ fibrosis</th>
<th>Insulin resistance (*)</th>
<th>Adiposity (*)</th>
<th>Lipids (*)</th>
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</thead>
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<td><strong>Hepatic lipid metabolism</strong></td>
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<tr>
<td>PEMT (8830),43-46 phosphatidylethanolamine N-methyltransferase; rs7946</td>
<td>☐↑↑</td>
<td>↑</td>
<td>☐</td>
<td>☐</td>
<td>↑</td>
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<tr>
<td>MTPP (7467),47-51 microsomal triglyceride transfer protein; rs1800591</td>
<td>☐☐</td>
<td>☐↑↑↑↑</td>
<td>☐↑↑</td>
<td>☐↑ (↓52)</td>
<td>☐↑↑</td>
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<tr>
<td>APOC3 (610),53,54 apolipoprotein C-III; rs2854161/2854117</td>
<td>☐↑</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>↓52</td>
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<tr>
<td>FABP2 (2556),56,57 fatty acid binding protein 2, intestinal; rs1799883</td>
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<td></td>
<td>☐(↑58)</td>
<td>☐(↓59)</td>
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<tr>
<td>DGAT (2843),61 acyl-CoA:diacylglycerol acyltransferase; rs1944438</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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<td>ADRB3 (288),65 adrenergic, beta-3-, receptor; rs4994</td>
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<td>↑</td>
<td>(↑66)</td>
<td>(↑67)</td>
<td>(↑68)</td>
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<td>ADRB2 (286),69 adrenergic, beta-2-, receptor, surface; rs1042714</td>
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<td>☐</td>
<td>☐(↑70)</td>
<td>☐(↑71)</td>
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<td>APOE (613),76-79 apolipoprotein E; N/A</td>
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<td>↑</td>
<td>(↑80)</td>
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<td>CLOCK (2082),81 clock homolog (mouse); rs11932595</td>
<td>↑</td>
<td>↑</td>
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<td><strong>Insulin sensitivity</strong></td>
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<td>ENPP1 (3356),67,83 ectonucleotide pyrophosphatase/phosphodiesterase 1 or PC-1; rs1044498</td>
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<td>☐☐↑</td>
<td>☐☐(↑84,85)</td>
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<td>IRS1 (6125),83 insulin receptor substrate 1; rs1801278</td>
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<td>☐</td>
<td>☐(↑87,88)</td>
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<td>☐</td>
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<tr>
<td>ADIPOQ (13633),89-92 adiponectin, C1Q and collagen domain containing; rs2241766</td>
<td>☐☐☐↑↑</td>
<td>☐↑</td>
<td>↑↑(↑93-95)</td>
<td>☐↑(↑96)</td>
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<td><strong>PPAR sensitivity</strong></td>
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<td>PPARA (9232),97,98 peroxisome proliferator-activated receptor alpha, rs1800206</td>
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<tr>
<td>PPARG (9236),43 peroxisome proliferative activated receptor, gamma; rs3856806</td>
<td>☐</td>
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### Table 1 (Continued)

<table>
<thead>
<tr>
<th>Gene (HGNC), SNP</th>
<th>Fatty liver</th>
<th>Inflammation/fibrosis</th>
<th>Insulin resistance (*)</th>
<th>Adiposity (*)</th>
<th>Lipids (*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARG, 97,99 rs1801282</td>
<td>⊙⊙</td>
<td>⊙⊙⊙⊙</td>
<td>↓↓ (↑100-104)</td>
<td>↑(↑103)</td>
<td>↑(↑105)</td>
</tr>
<tr>
<td>PPARGC1A (9237), 43 peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; rs8192687</td>
<td>⊙</td>
<td>⊙⊙⊙⊙</td>
<td>(↑106)</td>
<td>(⊙106)</td>
<td></td>
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<tr>
<td>TCF7L2 (11641), 107 transcription factor 7-like 2 (T-cell specific, HMG-box); rs7903146</td>
<td>⊤</td>
<td>⊤</td>
<td>↑(↑108-111,102)</td>
<td></td>
<td>⊤</td>
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<tr>
<td>GCKR (4196), 112 glucokinase (hexokinase 4) regulator; rs780994</td>
<td>⊤</td>
<td></td>
<td>(↑113,114,110,115-117)</td>
<td></td>
<td>↑(↑113,114,110,115-117)</td>
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<tr>
<td>MC4R (6932), 118 melanocortin-4 receptor; rs17782313</td>
<td>⊙</td>
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<tr>
<td>SPINK-1 (11244), 125 Serine protease inhibitor Kazal-1; N/A</td>
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<tr>
<td>LEPR (6554), 126 leptin receptor gene; N/A</td>
<td>⊤</td>
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<tr>
<td>LEP (6553), 43 leptin; rs7799039</td>
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</table>

**Genes influencing generation of reactive oxidant species, or cytokine genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fatty liver</th>
<th>Inflammation/fibrosis</th>
<th>Insulin resistance (*)</th>
<th>Adiposity (*)</th>
<th>Lipids (*)</th>
</tr>
</thead>
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<tr>
<td>TNF factor-alpha; rs180062</td>
<td>⊙⊙⊙⊙</td>
<td></td>
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<tr>
<td>IL-6 (6018), 47 rs1800795</td>
<td>⊤</td>
<td></td>
<td></td>
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<tr>
<td>CD14 (1628), 139 rs2569190</td>
<td>⊙⊙⊙⊙</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD2 (11180), 50,51,141 superoxide dismutase 2, mitochondrial; rs4880</td>
<td>⊤</td>
<td></td>
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<td></td>
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<tr>
<td>HFE (4886), 37 hemochromatosis; rs1800562, rs1799945</td>
<td>⊙⊙⊙⊙</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ABCB11 (42), 142 ATP-binding cassette, subfamily B, member 11; rs2287622</td>
<td>⊙⊙⊙⊙</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CFTR/MRP (53), 143 ATP-binding cassette, sub-family C, member 2; rs17222723</td>
<td>⊤</td>
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<td></td>
<td></td>
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<tr>
<td>rs8187710</td>
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</table>

Parenthesis denotes studies done with metabolic traits but not with liver steatosis
Finally, other appealing genes latent transforming growth factor beta. They studied 9,299 non-synonymous SNPs, including 3,599 SNPs in the platelet-derived growth factor alpha, collagen, type XIII, alpha 1, rs1227756, neurocan, rs2228603, EF-hand calcium binding domain 4B, rs887304, glucokinase regulatory protein, rs780094, and protein phosphatase 1, regulatory subunit 3b, rs4240624. These SNPs were associated with increased adiposity and lipids.}

### Hepatic lipid metabolism

The alteration in the management of triglycerides handling would likely lead to the development of hepatic steatosis. One of the most promising candidate genes was the microsomal triglyceride transfer protein (MTTP, 7467) central in lipoprotein role assembly and its inhibition in familial hypercholesterolemia was shown to increase liver steatosis. Unfortunately, this review showed inconsistent association with fatty liver disease and a marginal effect on related metabolic traits, suggesting that it is not clearly implicated in the development of fatty liver disease, or that the studies lack power to detect any differences. Other natural metabolic pathways in lipid metabolism, such as lipoprotein metabolism and hepatic lipase activity, have yielded similar inconsistent associations with hepatic steatosis (Table 1).

### Insulin sensitivity

NAFLD has been considered the liver component of the metabolic syndrome and, therefore, insulin resistance is supposed to be a key etiopathogenic factor. Adiponectin is a cytokine with insulin-sensitizing, antifibrogenic and anti-inflammatory roles and, in histology, hypoadiponectinemia, have been associated with advanced NAFLD. As shown in Table 1, the association with steatosis is inconsistent with adiponectin polymorphisms.

Iron overload, commonly associated with progression of liver disease, insulin resistance and oxidative stress, have been suggested to play a role in the development and progression of NAFLD. Our recent meta-analysis has shown that the hemochromatosis gene (HFE) has no association with the NAFLD or advanced NASH. Finally, other appealing genes such as PPARG or TCF7L2 have been associated with either liver steatosis and metabolic traits, however, the bulk of the literature shows minimal association, and warrants more research. Recently, a GWAS consortium have shown that, except for the GCKR, none of the reported candidate genes in NALFD has been associated with levels of A1c highlighting that fat accumulation may represent a different type of individual with diabetes.

### Oxidative stress

The association with polymorphisms associated with oxidative stress would help in understanding the progression of NAFLD to NASH/cirrhosis and, through oxidation of lipoproteins, its association with cardiovascular disease. Up to date, there is no strong evidence to pinpoint a particular polymorphism associated with progression of liver disease or associated with oxidation of lipoproteins (Table 1).

### Genome wide association studies in fatty liver disease

In 2008, Romeo et al. were the first to apply the GWAS method using a phenotype based on magnetic resonance spectroscopy. They studied 9,299 non-synonymous sequence variations and identified a missense mutation [Ile148→Met148 (I148M)] in a patatin-like phospholipase domain-containing (PNPLA3) gene PNPLA3. The association with polymorphisms associated with progression of liver disease or associated with oxidation of lipoproteins (Table 1).
in animal studies.\textsuperscript{39} This mutation is, by far, the strongest genetic signal up to date and showed a increased odds ratio for fatty liver of 3.26 in the original report.\textsuperscript{20} In addition, the PNPLA3 gene could also be responsible for the difference in prevalence of fatty liver disease between ethnic groups. For instance, Mexican-Americans have a higher prevalence of the high risk allele, whereas African Americans, where fatty liver is known to be less frequent, had a protective variation for such.\textsuperscript{39} Further studies confirmed the association between this gene and the presence of fatty liver disease, including GWAS with liver enzymes\textsuperscript{22} and multiple case-control studies\textsuperscript{40} (Table 2).

Chalasani et al.\textsuperscript{19} described, in 236 white female biopsy-proven NAFLD patients, five new genetic variants associated with inflammation and fibrosis: a variant in the FDFT1 (farnesol diphosphate farnesyl transferase 1, HGNC: 3629) was correlated with NAFLD; in addition, they found an association with lobular inflammation for the collagen gene \textit{COL13A1} (collagen, type XIII, alpha 1, HGNC: 2190), a SNP near the \textit{PGDFB} gene (platelet-derived growth factor alpha polypeptide, HGNC: 8799); the \textit{LTBP3} (latent transforming growth factor beta-binding protein 3, HGNC: 6716), and the \textit{EFCAAB} (EF-hand calcium binding domain 4B, HGNC: 28657), Table 2).

Most recently, we showed, in the Genetics of Obesity-related Liver Disease (GOLD) Consortium, four additional genetic variants in addition to the PNPLA3 (HGNC: 18590), namely, \textit{NCAN} (neurocan, HGNC: 2465), \textit{LYPLAL1} (lysophospholipase-like 1, HGNC: 20440); \textit{GCKR} (glucokinase regulatory protein, HGNC: 4196); and the \textit{PPP1R3B} (protein phosphatase 1, regulatory subunit 3b, HGNC: 14942), Table 2. \textit{GCKR} and \textit{PPP1R3B} are key enzymes in de novo lipogenesis from glucose and \textit{LYPLAL1} related protein, and make them plausible for having a role in consecutive steps in triglyceride breakdown. The role of \textit{NCAN} remains to be determined and has been associated with lipid traits.\textsuperscript{41} Interestingly, we found that \textit{PNPLA3} had a modest role in lipid metabolism, suggesting that these genes, if they are involved in lipid metabolism, exert their liver effects within the liver through different mechanistic pathways than those observed by conventional laboratory lipid measurement. In addition, \textit{PNPLA3} has been shown to have a null effect on insulin sensitivity, or, surprisingly an insulinsensitizing effect in obese carriers,\textsuperscript{42} consistent with the current hypothesis that fat in the liver does not lead to insulin resistance and may be a protective stage. A recent GWAS showed, however, that A1c levels were associated with \textit{GCKR} mutations and, therefore, this would be a common link in why patients with NAFLD have increased levels of A1c compared to the general population.\textsuperscript{28}

**Conflict of interests**

The author declares no conflict of interests.

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**References**

Genetics of non-alcoholic fatty liver disease and associated metabolic disorders


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