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Insights from Genome-Wide Association Analyses of Nonalcoholic Fatty Liver Disease

Bratati Kahali, PhD[1], **Brian Halligan, PhD**[1], and **Elizabeth K. Speliotes, MD, PhD, MPH**[1]

[1] Division of Gastroenterology, Department of Internal Medicine, Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, Michigan

Abstract

Nonalcoholic fatty liver disease (NAFLD) is caused by hepatic steatosis, which can progress to nonalcoholic steatohepatitis, fibrosis/cirrhosis, and hepatocellular carcinoma in the absence of excessive alcohol consumption. Nonalcoholic fatty liver disease will become the number one cause of liver disease worldwide by 2020. Nonalcoholic fatty liver disease is correlated albeit imperfectly with obesity and other metabolic diseases such as diabetes, hyperlipidemia, and cardiovascular disease, but exactly how having one of these diseases contributes to the development of other metabolic diseases is only now being elucidated. Development of NAFLD and related metabolic diseases is genetically influenced in the population, and recent genome-wide association studies (GWASs) have discovered genetic variants that associate with these diseases. These GWAS-associated variants cannot only help us to identify individuals at high risk of developing NAFLD, but also to better understand its pathophysiology so that we can develop more effective treatments for this disease and related metabolic diseases in the future.

Keywords

nonalcoholic liver disease; nonalcoholic steatohepatitis; gene; genetics; polymorphism

With the rise in obesity, we are seeing an increase in nonalcoholic fatty liver disease (NAFLD) which is reaching epidemic proportions. Nonalcoholic fatty liver disease is expected to become the number one cause of liver disease worldwide by 2020.[1] Nonalcoholic fatty liver disease is correlated with the presence of other metabolic diseases including obesity, diabetes, dyslipidemia, hypertension, and cardiovascular disease, but imperfectly so.[2,3] The extent to which these correlated metabolic diseases contribute to the development of NAFLD or to which NAFLD contributes to the development of these correlated metabolic diseases is currently being investigated. Like obesity, NAFLD is very common in the United States, with an overall prevalence of approximately 30%.[3–5] Nonalcoholic fatty liver disease varies in prevalence across ancestries, however.[4–6] In the United States, the prevalence of NAFLD in individuals of Hispanic ancestry is higher (34–58%) than in individuals of European ancestry (28–45%) than in individuals of African

Address for correspondence Elizabeth K. Speliotes, MD, PhD, MPH, Division of Gastroenterology and Hepatology, University of Michigan Health System, 3912 Taubman Center, 1500 E. Medical Center Drive, SPC 5362, Ann Arbor, MI 48109-5362 (espeliot@med.umich.edu).

ancestry (19–35%).[4–6] The majority of this difference in the prevalence of disease across ancestries is due to genetic differences in the predisposition to develop this disease.[7,8]

Nonalcoholic fatty liver disease is a spectrum of disease that includes steatosis (fat deposition in hepatocytes), nonalcoholic steatohepatitis (NASH; inflammation around the hepatic fat), and fibrosis/cirrhosis (scarring of the liver) in individuals that do not drink significant amounts of alcohol.[9] In some individuals, NAFLD can predispose to the development of hepatocellular carcinoma (HCC).[10] Steatosis is the hallmark of the disease, but can be absent in advanced stages of the disease. As a consequence, many causes of cryptogenic cirrhosis may actually be due to NAFLD that was not previously recognized as NAFLD.

To develop NAFLD there must be an imbalance in hepatic lipid homeostasis between fatty acid inputs and outputs. Inputs include fatty acids from dietary sources, adipose breakdown, de novo lipogenesis, and diet, while outputs include fatty acid oxidation and fatty acid export in the form of very low-density lipoprotein (VLDL; reviewed in [11]). Some studies have shown that lipotoxicity and mitochondrial dysfunction may contribute to hepatocyte toxicity and death.[11] Furthermore, there is evidence that activation of Kupffer cells and stellate cells can initiate the inflammatory and fibrotic processes, respectively, that lead to progressive liver disease in the form of NASH and fibrosis. Although it was thought in the past that hepatic steatosis was “benign,” more evidence suggests that hepatic steatosis can cause hepatocyte toxicity and trigger development of advanced liver disease. Indeed, HCC can develop in patients who do not have cirrhosis[12] and some cases of HCC develop in the presence of just “simple steatosis,”[13,14] suggesting that even hepatic steatosis may merit intervention to prevent development of subsequent liver disease.

NAFLD Measurement

Nonalcoholic fatty liver disease can be measured using various modalities. It can be measured using liver histology, which is the gold standard for the diagnosis of the disease. With histology, the complete range of the disease can be measured including steatosis, inflammation, and fibrosis/cirrhosis. Because liver biopsies carry a risk of complications, they are not usually performed on normal individuals unless there is an indication. Indications for biopsy may include many risk factors for development of advanced liver disease, including advanced age and concomitant metabolic disease. Thus, studies based on using histology may not accurately reflect population-based NAFLD. Hepatic steatosis can also be measured using noninvasive imaging, but these imaging modalities are not very sensitive for detection of NASH or early fibrosis. Magnetic resonance spectroscopy (MRS) is the most sensitive and specific for measuring triglycerides in liver, but requires specialized expertise to implement and is not widely used in clinical practice.[15] Magnetic resonance imaging (MRI), computed tomography (CT), or ultrasound imaging are affordable, widely available, and confer minimal risk to the patient; thus, they are most often used in clinical practice and in the population for evaluating for the presence of hepatic steatosis.[15] The sensitivity and specificity of MRI is greater for quantifying hepatic steatosis than CT, which is greater than ultrasound.[15] Finally, elevations in serum ala-nine aminotransferase (ALT) can be seen with NAFLD, but this biomarker is not sensitive or specific for NAFLD[16] (e.g.,

it can fail to be elevated in people with NAFLD or be elevated without NAFLD as a consequence of many other liver diseases) . Therefore, they require further follow-up to confirm that the elevations are due to NAFLD and not some other liver disease.

Heritability Estimates for NAFLD

Population-based NAFLD has been found to be heritable, or genetic, in various twin and family studies. In individuals of Hispanic ancestry, the heritability of population-based NAFLD has been found to be 31 to 38%.[17,18] In the first study, 44 children (probands) and their parents were characterized for the presence of hepatic steatosis using MRI and histology; the heritability of NAFLD was found to be 38%.[17] In the second, 843 individuals from the Insulin Resistance Atherosclerosis Study (IRAS) family study were measured for hepatic steatosis using CT-measured liver attenuation and heritability determined to be 31%.[18] In 6,629 individuals from three European ancestry family-based studies (Family Heart Study, The Old Order Amish, and The Framingham Heart Study) liver steatosis was measured using computed tomography liver attenuation and heritability was calculated to be 26 to 27%.[19] Finally, in individuals of African ancestry from the Insulin Resistance and Atherosclerosis Study, Family Heart Study, and Genetic Epidemiology of Arteriopathy (GENOA) Study the heritability was found to be 22 to 34%.[7] Thus in all ancestries examined there is a substantial heritable component to the disease, ranging from 22 to 38%. Because there is statistical variation in these heritability measures, it cannot be determined that the heritability is more in one ancestry than the other. A recent study of 60 pairs of twins revealed that the heritability of hepatic steatosis and fibrosis using MRI proton-based fat fraction was 52% and 50%, respectively.[20] The slightly higher estimate of steatosis heritability in this study compared with the family-based studies noted above is likely due to an overestimate of heritability in twin studies. In particular, heritability estimates based on phenotypic resemblance that may be due to unmeasured environmental variables which are more likely to be shared by monozygotic twins (because they often do live in the same environment and may be treated more similarly) than more distantly related individuals often inflate the heritability calculated for traits in twin studies.[21]

Candidate Gene Studies

Many studies have tested genetic variants in or near candidate genes for association with NAFLD measures.[22] These include associations with single nucleotide polymorphisms (SNPs) in or near *IRS1, ENPP1, SOD2, p21, USF1, KLF6, INFL4, IL28B*, and *APOC3*.[22–29] These associations have not been reproduced in completely independent studies to date; thus, their validity remains to be determined. Some associations have, however, already turned out to be false-positives. In 2010 Peterson et al reported an association with variants near *APOC3*,[29] but reports by others since then have not substantiated this association.[30,31] Similarly, the association of variants in or near *IL28B* with NAFLD has been challenged.[32] In this latter case, authors of the original study suggest that the *IL28B* allele may have a larger effect in individuals that do not have many other NAFLD predisposing risk factors as the reason for discrepancy between studies,[33] but this remains to be substantiated.

GWASs for Nonalcoholic Fatty Liver Disease

To identify specific genetic elements that associate with NAFLD in an unbiased way, several genome-wide association studies (GWASs) of NAFLD have been performed (►**Table 1**). Romeo et al used a custom chip of over 9,000 nonsynonymous variants across the genome to genotype individuals of European, Hispanic, and African American ancestries who had liver fat measured by proton MRS.[8] They found that one variant in the gene *PNPLA3*, the G-allele at rs738409, encoding an I148M missense mutation, increased hepatic fat levels across European American, African American, and Hispanic American ancestries. The frequency of this variant was highest in individuals of Hispanic ancestry, followed by those of European and African American ancestries, respectively,[8] a finding consistent with the known prevalence of NAFLD in these ethnic groups.[5] Since the discovery of this variant, other groups have replicated the association of this variant with hepatic steatosis as well as other measures of NAFLD. Specifically, associations have been noted with elevated serum ALT levels,[34–38] imaging-based steatosis,[4,19,39] or with histologic NAFLD including steatosis, NASH, and fibrosis/cirrhosis (►**Table 2**).[19,40–44] A recent meta-analysis of up to 2,937 individuals with NAFLD measured by histology revealed that individuals with GG at rs738409 in PNPLA3 had 73% higher lipid fat content, 3.24-fold greater risk of higher necroinflammatory scores, 3.2-fold greater risk of developing fibrosis, and 3.44 higher odds of developing NASH compared with CC individuals.[45] More than 70% of the difference in prevalence between individuals of diverse ancestries is likely due to genetic influence—and most of that is due to variation in the prevalence of fatty liver promoting the PNPLA3 allele.[7,8]

Two different GWASs of liver function tests identified variants at four loci that associated with elevated ALT levels. These are in or near the PNPLA3 (rs2281135, rs738409)–SAMM50 (rs2143571) genomic locus,[34,35] the CPN1–ERLIN1–CHUK gene cluster (rs10883437, rs11597390, rs11591741, rs11597086),[34,35] TRIB1 (rs2954021),[34,35] and near HSD17B13/MAPK10 (rs6834314).[35] The SNPs in the genomic region of PNPLA3–SAMM50 are correlated with each other, suggesting that they may represent the same genetic signal (e.g., are in moderate to high linkage disequilibrium [LD] with each other with a r^2 [correlation]= 0.34 between rs738409 and rs2143571, r^2 = 0.63 between rs2281135 and rs2143571 and r^2 = 0.86 between rs2281135 and rs738409). Follow-up studies by independent groups found that variants in *PNPLA3*-rs738409 (see last paragraph) and near *TRIB1* (rs2954021) reproducibly affect the development of NAFLD.[49] Specifically, variants (rs2954021) near *TRIB1* have been found to associate with histologic NAFLD in a Japanese population.[49] Further, a SNP (rs6982502) in an enhancer near TRIB1 was significantly ($p = 9.39 \times 10^{-7}$) associated with ultrasonographically diagnosed NAFLD in a population of 5,570 individuals.[50] These two SNPs that are located in or near TRIB1 are highly correlated with each other with an LD r^2 = 0.94.

Another GWAS on NAFLD focused on 236 non-Hispanic white women that were genotyped with the Illumina CNV370 platform and assessed for various histologic parameters related to NAFLD.[51] After correcting for multiple hypothesis testing, however, none of the SNPs managed to pass the genome-wide significance threshold of a p value less than 5×10^{-8}.

One of the largest GWASs was a meta-analysis across four groups, all of European ancestry, that were genotyped either on the Affymetrix or Illumina platforms, imputed to the 2.8 million SNPs in HapMap.[19] These SNPs were tested for associations with hepatic steatosis measured by CT in each of the four groups separately and combined by meta-analysis for a total of 7,176 assayed individuals[19] controlling for age, gender, and principal components. Top associating variants from this meta-analysis were taken forward for assessment of effects on 592 cases of histology proven NASH/ fibrosis genotypically matched to 1405 controls, all of European ancestry.[19] Variants in or near the genes *PNPLA3, LYPLAL1, PPP1R3B, NCAN/TM6SF2*, and *GCKR* were found to be associated with hepatic steatosis.[19] Variants that increased hepatic steatosis at all loci except *PPP1R3B* were also found to be associated with NASH/fibrosis (►**Table 2**).[19] The associations of variants at PNPLA3 have been confirmed in subsequent studies as noted above. The association of variants at GCKR and NCAN/TM6SF2 with NAFLD/NASH/fibrosis have also been confirmed by independent subsequent studies.[48,49,52]

The most significant variants at *PNPLA3* and *GCKR* either were themselves missense variants or in high-linkage disequilibrium with missense variants in those genes.[19] Indeed, fine mapping of these loci across ancestries suggests that the *PNPLA3(I148M)* and *GCKR(P446L)* are the variants most likely to be causally related to development of NAFLD.[7] Variants at *NCAN/TM6SF2* have been fine mapped to likely a missense variant in *TM6SF2(E167K)* as possibly the causal variant in promoting NAFLD in a recent study of exonic variants (variants in the coding parts of genes). These were assayed using the Illumina Human Exome chip for association with NAFLD measured using MRS in the Dallas Heart Study.[48] This variant can account for the association signal with NAFLD seen at this locus, which in the literature also goes by the name *NCAN, CILP2*, and the 19p13.11 locus.[48] The *TM6SF2* variant rs58542926 that causes a nucleotide change of C to T, is a nonsynonymous change causing a glutamate to lysine amino acid substitution at residue 167 (E167K); the glutamate is highly conserved across mammals.[48] The putative causal variant is suggested to cause a decreased function in *TM6SF2* to promote NAFLD.[48] The NAFLD-associated variants at *LYPLAL1* or *PPP1R3B* fall in noncoding regions near these genes. The best associating NALFD promoting variant at *PPP1R3B* increases *PPP1R3B* expression, suggesting that this could be a functional expression quantitative trait locus (eQTL) variant.[19]

Pleiotropies

Effects on Related Metabolic Traits and Diseases

Some but not all NAFLD-associated variants affect the risk of developing related metabolic diseases (►**Table 2**). The G allele at rs738409 in *PNPLA3* has not been found to associate with measures of obesity, impaired fasting glucose, diabetes, low-serum high-density lipoprotein cholesterol (HDL-C), high-serum triglycerides, and hypertension.[7,34] One study did find some associations of this allele with decreased total cholesterol levels in a study of > 100,000 individuals,[53] but this remains to be replicated. The fatty liver promoting allele at TM6SF2/NCAN associates not only with decreased serum low-density lipoprotein cholesterol (LDL-C), serum triglycerides, and increased serum HDL-C levels, but also with

decreased risk of coronary artery disease.[14,54] The fatty liver increasing allele at GCKR associates with increased serum LDL-C, increased serum triglycerides, serum gamma-glutamyl transferase, and decreased fasting glucose and homeostatic model assessment insulin resistance levels.[14,35] The fatty liver increasing variant near PPP1R3B exhibited similar behavior to GCKR in associating significantly with increased serum LDL-C, increased serum HDL-C, and decreased fasting glucose,[14] but also associated with increased alkaline phosphatase.[35] The serum ALT level increasing allele at TRIB1 (rs2954021) significantly associates with increased serum levels of total cholesterol, serum LDL-C, serum triglycerides, and serum alkaline phosphatase, but decreased levels of serum HDL-C.[35,53] Variants near TRIB1 have also been associated with adiponectin.[55]

The finding that some, but not all genetic variants that predispose to development of NAFLD predispose to related metabolic disease, suggests that genetics may help explain in part the imperfect correlation of these traits with each other. That is, some of the correlation seen between developing metabolic disease may be due to having shared genetic elements that predispose to more than one metabolic abnormality. The finding that not all genetic variants that predispose the NAFLD equally affect related metabolic disease suggests, however, that depending on the sets of genetic variants individuals carry, they may have different risks of developing NAFLD and concomitant related metabolic diseases. The exciting thing is that now with this new genetic information we may in the future be able to make more precise predictions on who will not only develop NAFLD but also related metabolic diseases. Because some genetic changes result in patterns of effects across traits that are similar to each other, but different from those of other variants, we may in the future also be able to use these genetic changes to subclassify individuals into disease types. These pleiotropy data are also interesting and useful from a therapeutic standpoint because they suggest that interfering with the function of some of these genes may lead to pleiotropic effects, whether desired or undesired, if targeted for medical intervention. For example, if one were to decrease the function of TM6SF2 globally to decrease serum lipid levels to prevent heart attacks, there is ample data for these studies to suggest that this would cause hepatic steatosis and advanced liver disease. Thus, this approach to reducing cardiovascular disease risk may not be optimal. The other striking finding from these genetic data is that most genes, when targeted, will have pleiotropic effects (i.e., side effects). Thus, a better strategy suggested from the genetic work is to make weak agonists and antagonists (which is what nature has done in creating these variants of small effect) that when used together, may maximally affect the desired phenotype, but have low if not canceling effects on related traits and thus can maximally affect the targeted phenotype while minimizing side effects.

Effects in Other Liver Diseases

Alcoholic liver disease (ALD), more than any other liver disease, has a similar spectrum of histopathologic features as NAFLD. This includes the development of alcoholic steatosis, alcoholic hepatitis, alcoholic fibrosis, alcoholic cirrhosis, and a predisposition to the development of HCC.[64,65] The inciting event is the development of alcoholic fatty liver, which is a pathologic condition that shares many features with NAFLD.[66] To try to distinguish ALD from NAFLD, cutoffs of drinking > 21 drinks a week for men and > 14 for women have been suggested as a guideline for determining what constitutes significant

alcohol consumption.[67] Nevertheless, given the high prevalence of metabolic disease in the population and the high prevalence of alcohol consumption in the population, it is likely that in clinical practice the two diseases often coexist, even though by formal definition they are mutually exclusive. Indeed, there is evidence to suggest that ALD can be exacerbated by the presence of obesity and that some of the pathophysiology of the two conditions may overlap.[66,68,69]

To date, the I148M at PNPLA3 has been most extensively studied for effects on ALD. A recent meta-analysis shows that this allele does affect development of ALD.[56] More than 4,000 cases of ALD and 4,000 controls without ALD across 10 studies[45,49,50,56,67–72] were analyzed.[56] The PNPLA3 G-allele at rs738409 has been found to increase the risk of alcoholic liver injury with an odds ratio (OR) of 1.45 (95% confidence interval [CI] 1.24–1.69) and increase the risk for development of alcoholic cirrhosis 2.09 (95% CI 1.79–2.44).[56] There was not a clear association of the I148M PNPLA3 allele with the development of alcoholic fatty liver, however.[56] This could be because the allele may not have an effect in alcoholic fatty liver, but could also be due to this analysis being underpowered (with only 239 individuals being assessed) or to fatty liver being so prominent in ALD that the genetic change was not of large enough magnitude to be statistically significant across groups. A recent GWAS study has also found that variants at PNPLA3 and TM6SF2 affect development of alcoholic cirrhosis at genome-wide significance levels.[145]

Infection with both hepatitis B and C virus can lead to hepatitis, fibrosis, and cirrhosis and can predispose to the development of HCC (reviewed in [70,71]). Association of hepatic steatosis with hepatitis C infection is well documented and most pronounced for hepatitis C genotype 3, which induces hepatic steatosis more than the other subtypes.[72] Hepatic steatosis has been found to promote liver disease progression from hepatitis C, but not from hepatitis B (reviewed in [73]). Hepatic steatosis has been found to exacerbate liver disease progression from nongenotype 3 hepatitis C.[57] Obesity and insulin resistance seems to also exacerbate hepatitis C disease progression in most studies examined (reviewed in [73]). Steatosis is not a prominent feature of hepatitis B infection,[74] but concomitant presence of metabolic syndrome with hepatitis B infection does lead to exacerbated liver disease development.[75] For hepatitis C, it has also been shown that hepatic steatosis can affect the ability of patients to attain sustained virologic response in nondiabetic, but not diabetic patients.[76] Given the exacerbation of hepatitis C- and hepatitis B-caused liver disease by metabolic disease, genetic variants that affect NAFLD may have effects on liver disease in patients with hepatitis B and C.

The first study to assess NAFLD-associated genetic variants for effects in hepatitis C was of 819 patients from Italy with chronic hepatitis C.[57] They found that the rs738409 GG genotype was associated with steatosis independently of age, sex, body mass index (BMI), diabetes, alcohol intake, and viral genotype (OR = 1.90, 95% CI 1.4–2.7; $p < 0.001$).[57] The association with rs738409 genotype was confirmed for severe steatosis, was independent of serum liver function test levels for ALT and gamma-glutamyl transferase (GGT), and was observed in all viral genotypes but genotype 3.[57] There was a trend toward the same pattern of effect also in genotype 3 hepatitis C, which was their smallest subgroup, and thus may have been underpowered to see a statistical significance. The rs738409 GG genotype was

also associated with fibrosis stage and cirrhosis (OR = 1.47, 95% CI 1.2–1.9; $p = 0.002$) and with worse treatment response in terms of attaining sustained virologic response ($n = 470$; OR = 0.63, 95% CI 0.4–0.8; $p = 0.006$). Around the same time, another large study from Belgium, Germany, and France ($n = 537$) of mostly genotype 1 hepatitis C patients showed that after adjustment for age, sex, BMI, alcohol consumption, and diabetes, rs738409 mutant G allele homozygote carriers remained at higher risk for steatosis (OR = 2.55, 95% CI 1.08–6.03; $p = 0.034$), fibrosis (OR = 3.13, 95% CI 1.50–6.51; $p = 0.002$), and fibrosis progression (OR = 2.64, 95% CI 1.22–5.67; $p = 0.013$).[77] They found that rs738409 was not independently associated with treatment failure (OR = 1.07, 95% CI 0.46–2.49; $p = 0.875$) and did not influence clinical or biologic variables.[77] More recent studies have confirmed that the PNPLA3 I148M variant is a risk factor for the development of severe steatosis or fibrosis progression in chronic hepatitis C in individuals of European ancestry.[78,79] Similarly, in 276 Japanese patients with chronic hepatitis C, the GG genotype for rs738409 was independently associated with the presence of steatosis (OR = 2.58, 95% CI 1.37–4.84; $p = 0.003$), severe necroinflammatory activity (OR = 2.16, 95% CI 1.12–4.16; $p = 0.02$), and advanced fibrosis (OR = 2.10, 95% CI 1.07–4.11; $p = 0.03$), after adjustment for age, sex, BMI, and diabetes.[80] The effect of the I148M allele on sustained viro-logic response, however, is controversial. Valenti et al[57] noted that the G allele at rs738409 was significantly associated with a lower sustained virologic response (SVR) rate in patients with genotype 1 and 4 hepatitis C virus (HCV) infection.[57] In a subsequent study, they noted that the I148M variant associated with SVR in patients with genotype 1 and 4 HCV and bridging fibrosis, but not in genotypes 1 and 4 HCV without bridging fibrosis or in those with genotypes 2 and 3 HCV.[81] Trepo et al,[77] as well as various other groups,[53,82] have not found an association of the I148M allele and SVR, however. Whether these differences are due to power or to uncorrected confounders remains to be determined.

The E167K variant at TM6SF2 has also been associated with histologic liver disease in chronic hepatitis C. One study of 148 consecutive patients from southern Italy with biopsy proven anti-HCV/HCV-RNA-positive chronic hepatitis, mostly of genotype 1 and 2, naive for antiviral therapy, were genotyped for TM6SF2 E167K.[54] The liver steatosis score was higher in the 18 patients with TM6SF2 E167K variant than in the 130 homozygotes for TM6SF2 167E allele.[54] There was no difference in necroinflammatory or fibrosis scores found between the two groups.[54] In another study of 815 Italian patients mostly of genotype 1 and 2 with replication in 645 Swiss/German patients, the authors found that TM6SF2 E167K was associated with histologic severity of steatosis, necroinflammation, and fibrosis.[83] After adjustment for steatosis severity, the E167K variant associated with cirrhosis (OR = 2.22, 95% CI 1.20–4.03; $p = 0.010$). The association with clinically significant fibrosis (F2-F4) was replicated in 645 Swiss/German patients (OR = 1.81, 95% CI 1.12–3.02; $p = 0.016$). A third recent study of 694 chronic hepatitis C patients from Italy, however, did not see an association with steatosis severity after adjustment for age, gender, BMI, and homoeostasis model assessment score with TM6SF2 rs58542926 (OR = 1.48, 95% CI 0.82–2.69; $p = 0.19$) or with fibrosis (OR = 0.75, 95% CI 0.34–1.63; $p = 0.47$).[78] It is not clear whether the differences in effects between studies are due to differences in power, to differences in the population, or to the presence of unaccounted confounders.

The PNPLA3 I148M allele has also been associated with hepatic steatosis in 235 hepatitis B patients (OR = 1.62, 95% CI 1.00–7.00) after correction for BMI, age, and diabetes/impaired fasting glucose.[58] A similar result has been reported in a separate study of 99 individuals with hepatitis B in which the I148M homozygotes had an OR = 13.9 (95% CI 2.2–86.9; p = 0.005) for the presence of severe steatosis compared with individuals lacking this allele.[55] This allele has not been associated with fibrosis or cirrhosis in multiple studies.[55,59,60]

Effects on Graft Outcomes after Liver Transplantation

Liver transplantation provides a unique situation in which the effects of genetic variants within and outside the liver can be evaluated (►**Table 3**). After transplantation the liver genotype may not match the recipient's genotype and thus forms a genetic mosaic. In one study of 101 hepatitis C-infected individuals who underwent transplantation, the time to Ishak stage 3 fibrosis or HCV-related mortality/graft loss was analyzed using Cox regression modeling adjusting for HCV-Donor Risk Index, warm ischemic time, pretransplant Model for Endstage Liver Disease (MELD), and viral load in 620 days of follow-up after transplantation.[63] The rs738409 donor GC or GG variants had 2.53 times the risk of developing fibrosis (95% CI 1.25–5.02; p = 0.008) compared with CC variants.[63] In the alternative endpoint: stage 3 fibrosis or all-cause mortality/graft loss, the effect of donor genotype was attenuated but remained significant at 1.98 (95% CI 1.11–3.53).[63] This result is interesting as it suggests that PNPLA3's effect is to influence liver disease development and progression. The authors did not observe an association between the PNPLA3 I148M variant, neither in donors nor recipients, with posttransplant hepatic steatosis, however.[63] Another larger study, however, did not see an association of the I148M PNPLA3 allele with development of advanced fibrosis in 176 individual with hepatitis C that underwent liver transplantation.[84] Also, unlike Dunn et al, Finkenstedt et al found that the PNPLA3 I148M variant in recipients, that is, recipients who carried rs738409-GG, had a higher risk of graft steatosis than recipients who carried rs738409-CC, independent of recipient age or weight gain after liver transplantation.[85] This latter result is interesting because it suggests that PNPLA3 may have effects outside of the liver that may be important for development of graft steatosis. Further work will be needed to determine whether these observations are reproducible; if so they may become important to help physicians establish a fibrosis progression management plan after transplantation.

Effects on Hepatocellular Carcinoma

Hepatocellular carcinoma, like obesity and NAFLD, is rising in prevalence and has now become the number one cause of obesity-related deaths in middle-aged men (reviewed in[10]) (►**Table 3**). Hepatocellular carcinoma can develop from many liver insults, although in the United States major causes are hepatitis C, ALD, NAFLD, and hepatitis B.[65] The development of HCC was previously thought to require liver cirrhosis as a precursor, but more recent work suggests otherwise. In particular, 10 to 75% of HCC can develop in patients who do not have cirrhosis.[12] It has indeed been reported that HCC can develop in patients with simple steatosis, or more specifically, patients who do not have any signs of inflammation or fibrosis.[13,14] Because steatosis is a clear risk factor for the development of

HCC, it is not surprising that variants that affect hepatic steatosis have been tested for effects on development of HCC.

In obese individuals from the Swedish Obese Subjects Cohort, the G allele at rs738409 in PNPLA3 increased risk of developing HCC with a hazard ratio = 5.9 (95% CI 1.5–23.8) in individuals that did not have bariatric surgery to treat their obesity.[86] Further, in individuals with HCC from NAFLD compared with 275 controls with histologically characterized NAFLD, each G allele at rs738409 in multivariate analysis adjusted for age, gender, diabetes, BMI, and presence of cirrhosis, conferred an additive risk for HCC (OR = 2.26, 95% CI 1.23–4.14; $p = 0.0082$), with GG homozygotes exhibiting a fivefold (95% CI 1.47–17.29; $p = 0.01$) increased risk over CC homozygotes.[61] When compared with the UK general population (1958 British Birth Cohort, $n = 1476$), the risk-effect was more pronounced (GC vs. CC: unadjusted OR = 2.52, 95% CI 1.55–4.10; $p = 0.0002$; GG vs. CC: OR = 12.19, 95% CI 6.89–21.58; $p < 0.0001$).[61]

Multiple groups reported associations of I148M with HCC in the setting of alcoholic- and hepatitis C-caused liver disease as well.[57,59,77,87–90] A meta-analysis of 1,374 individuals with ALD and 945 individuals with chronic hepatitis C found that each I148M allele incurred a higher risk of developing HCC from ALD (OR = 2.20, 95% CI 1.80–2.67) than from chronic hepatitis C (OR = 1.55, 95% CI 1.03–2.34) although both were statistically significant.[62] In another meta-analysis of 357 individuals the odds of developing HCC in alcoholic cirrhotics was 2.87 (95% CI 1.61–5.10) for GC versus CC individuals and 12.41 (95% CI 6.99–22.03) for GG versus CC individuals,[56] which is similar to what was calculated in the study by Trepo et al.[62] This suggests that the pathophysiology of ALD, which is known to have significant steatosis, may in conjunction with a problem in lipid handling incurred by the PNPLA3 mutation, lead to more advance liver pathology than simply having the I148M genetic variant along with concomitant liver disease from hepatitis C.

Improved Understanding of the Biology of Common NAFLD

Following the GWASs for NAFLD, studies into the function of several genes implicated by these GWASs has given us new insights into the pathophysiology of NAFLD (►**Fig. 1,** ►**Table 4**).

PNPLA3

PNPLA3 stands for patatin-like phospholipase domain containing 3, but has also been called adiponutrin (ADPN) and acylglycerol O-acyltransferase or calcium-independent phospholipase A2-epsilon (iPLA2-epsilon).[108] The exact biochemical mechanism by which PNPLA3 acts to cause NAFLD is not yet clear. PNPLA3 is found on the surface of lipid droplets in liver.[92] There are data to suggest that wild-type PNPLA3 can break down triglycerides and thus act as a triglyceride hydrolase,[92] but others have found that it has lysophosphatidic acid acyltransferase activity also.[109] Numerous studies suggest that altering PNPLA3 function can affect lipid trafficking in hepatocytes. Overexpression of the PNPLA3 (I148M) mutant allele in human hepatocyte culture results in lipid accumulation, but overexpression of the wild type of allele does not,[92] suggesting a possible gain of

function (new function compared with what it does normally) phenotype caused by the I148M variation. Consistent with this result, mice knocked out for *PNPLA3* do not develop hepatic steatosis,[110,111] whereas mice carrying a PNPLA3 (I148M) knock in mutation and fed a high sucrose diet develop NAFLD confirming that this allele can cause fatty liver disease.[103] Human PNPLA3 (I148M) and the mouse equivalent Pnpla3$^{47A/A}$ both produce proteins that are catalytically inactive for TG hydrolysis, but as noted above, a knockout of the gene alone is not sufficient to cause steatosis. The presence of the catalytically inactive protein is required to develop the fatty liver phenotype, implying that its presence may interfere with normal lipid metabolism by binding to and increasing the size of lipid droplets and/or blocking lipolysis.[103] PNPLA3 mRNA levels are increased by carbohydrate feeding and this increase is mediated by SREBP-1c.[112] PNPLA3 degradation is also reduced with the addition of fatty acids to hepatocytes without affecting mRNA levels.[112]

TRIB1

TRIB1 stands for tribbles pseudokinase 1, but has also been called phosphoprotein regulated by mitogenic pathways, g protein coupled receptor-induced protein, tribbles homolog 1, tribbles-like protein 1, and tribbles homolog 1.[113] TRIB1 has been found to affect hepatic lipogenesis and VLDL export via interacting proteins to mediate its NAFLD-promoting effects.[50,114,115] Overexpression of *Trib1* in mouse livers decreased serum lipids, whereas knockdown increased liver triglyceride content and glycogen as well as serum\plasma glucose, TG, and cholesterol levels.[40,88] Knockdown of *Trib1* in two separate studies increased the levels of multiple genes involved in lipogenesis, repressed multiple genes involved in fatty acid oxidation, and also increased secretion of VLDL from liver although the exact levels of these genes varied between the two studies.[40,88] Pull-down and mammalian two-hybrid analyses revealed novel molecular interaction between TRIB1 and a hepatic lipogenic master regulator, MLXIPL (also known as carbohydrate response element binding protein [ChREBP]).[50] MLXIPL binds to MLX to form a heterodimeric complex that binds and activates, in a glucose-dependent manner, carbohydrate response element (ChoRE) motifs in the promoter of lipogenic enzymes. Presumably some of the effects of knocking down *Trib1* are due to activation of MLXIPL. TRIB1 was also found to interact with Sin3A-associated protein (SAP18), which when knocked down increased decreased microsomal triglyceride transfer protein (MTTP) levels and led to increasing hepatic lipid levels while decreasing serum lipid levels.[87] Because MTTP transfers lipids onto APOB as part of VLDL formation, presumably this results in decreased VLDL export from liver to cause the increased hepatic steatosis while simultaneously causing reduced serum levels of lipids.[50] Because variants near TRIB1 that associate with NAFLD increase serum levels of triglycerides, it remains to be seen whether any of the effects of this variant is via SAP18. TRIB1 is also known to be upregulated during inflammatory events such as chronic inflammation of atherosclerotic arteries or chronic antibody-mediated rejection of transplanted organs.[116] Recently Akira et al, working with *Trib1* knockout mice, demonstrated that mice lacking *Trib1* in hematopoietic cells exhibited severe lipodystrophy due to increased lipolysis; while in a high-fat diet, these mice exhibited hypertriglyceridemia, insulin resistance, together with increased proinflammatory cytokine production.[117] They suggested that *Trib1* is critical for adipose tissue maintenance and suppression of metabolic disorders by controlling the differentiation of tissue-resident anti-

inflammatory-like macrophages. Thus, we cannot rule out the possibility that decreased TRIB1 function outside of liver contributes to the development of insulin resistance and higher levels of circulating triglycerides that can secondarily lead to the development of hepatic steatosis.

GCKR

GCKR stands for glucokinase regulator, but has also been called hexokinase 4 regulator and glucokinase regulatory protein.[118] GCKR is a regulator of the cellular location of glucokinase (GK). When low levels of glucose are present, GCKR binds to and keeps GK localized to the nucleus where it is not active.[119] High glucose concentrations lead to the release of GK from GCKR, allowing GK to be released into the cytoplasm where it can mediate phosphorylation of glucose to glucose 6-phosphate.[120] Glucose 6-phosphate can be used either in glycogen synthesis or converted via the pentose phosphate shunt to the precursors required for de novo lipogenesis.[121] Variants in GCKR may promote NAFLD by resulting in glucose to triglyceride shifts; this model is being tested in cell biologic systems presently. Indeed, the *GCKR* P446L variant that associates with NAFLD results in increased activity of glucokinase which phosphorylates glucose[122] and in this way[121] may promotes glucose to triglyceride shifts.

TM6SF2

TM6SF2 stands for transmembrane 6 superfamily 2 and is also known as KIAA1926.[123] TM6SF2 is a membrane protein with 7 to 10 predicted transmembrane domains whose function is not known.[48] Kozlitina et al showed that abundance of the mutant form of TM6SF2, (E167K) is lower than the wild-type TM6SF2 protein due to mis-folding and degradation, consistent with this being a loss of function allele.[48] They showed that knockdown of mouse *Tm6sf2* resulted in increased liver lipid accumulation and decreased serum TG and LDL. They also found that knockdown of mouse *Tm6sf2* resulted in decreased VLDL secretion. In a separate study employing confocal microscopy, TM6SF2 was localized to the endoplasmic reticulum (ER) and the ER-Golgi compartment.[102] By siRNA knockdown studies, it was found that *TM6SF2* is associated with the secretion of triglyceride-rich lipoproteins and inhibition of *TM6SF2* leads to increased triglyceride and lipid droplet accumulation.[102] From these data, it appears that *TM6SF2* may play a role in VLDL excretion.[102]

PPP1R3B

PPP1R3B stands for protein phosphatase 1, regulatory subunit 3B and is also known as PP1 subunit R4, hepatic glycogen-targeting protein phosphatase 1 regulatory subunit GL, protein phosphatase 1 regulatory subunit 4 (PPP1R4), and hepatic glycogen-targeting subunit, G(L).[124] *PPP1R3B* regulates protein phosphatase-1 (PP-1) catalytic subunit (PPP1CA) and increases PP-1 dephosphorylation of glycogen synthase and phosphorylase kinase.[98,100] Glycogen synthase is activated by dephosphorylation and phosphorylase kinase is inactivated by dephosphorylation.[125] Thus PPP1R3B promotes glycogen synthesis and inhibits glycogen breakdown to glucose 1-phosphate, which can be converted to glucose 6-phosphate by phosphoglucomutase.[126] As discussed in the section on GCKR, glucose 6-

phosphate can be converted to precursors of de novo lipid synthesis via either the TCA cycle or the pentose phosphate shunt[121]

LYPLAL1

LYPLAL1 stands for lysophospholipase-1.[127] As has been seen with some genes linked to NAFLD, how LYPLAL1 promotes the development of NAFLD is not fully understood. The *LYPLAL1* gene encodes a protein with sequence similarity to APT1, a member of the lysophospholipase family of proteins that have acyl protein thioesterases activity.[128] The most well-characterized member of the family is APT1, which has been shown to depalmitoylate proteins such as Ras and other Gα signaling proteins.[129] Unlike other lipid modifications of proteins, palmitoylation, also known as *S*-acylation, like phosphorylation, is a covalent protein modification that can be enzymatically reversed. The cycle of palmitoylation and depalmitoylation of proteins such as N-Ras and HRas has been demonstrated to change the cellular localization of the protein, from the plasma membrane to the Golgi apparatus.[129] Although there were suggestions that LYPLAL1 might function as a TG lipase,[130] more recent biochemical and X-ray crystallographic studies indicate that it functions as a lysophospholipase.[128] Studies of insulin regulated protein palmitoylation have identified several candidates for palmitoylation, but not a linkage to steatosis.[131]

Clinical Applications

The effect sizes of *PNPLA3* and three of the four new NAFLD-associated variants have some of the largest ORs to date for affecting NASH/fibrosis per allele (1.37–3.26)[19] (►**Table 2**), making them common variants with relatively strong effect sizes. These also increase the risk of developing HCC, again with ORs that range from 1.45–2.53 (►**Table 3**). These variants not only promote the development of advanced liver disease in patients with NAFLD, but also in patients with other liver diseases. Thus, it may not be too long before we may have specific recommendations by genotype for care of patients that carry these genetic variants. Already, these variants alone or in combination have been incorporated into clinical algorithms where they do have significant effects on predicting advanced liver disease.

One of the limits of using already diagnosed metabolic disease (obesity, dyslipidemia, hypertension, and dysglycemia) to predict prevalent NAFLD is that disease is already present (i.e., the individual has developed metabolic disease that is being used to predict more metabolic disease). Ideally, we should inform people of their risk of developing disease while they are still disease free, and make recommendations to prevent or minimize future disease. Here, genetics can be quite useful, as genetic variation can be determined at birth and predates all disease development. One group has determined that the rs738409 variant in *PNPLA3* is able to statistically predict prevalent NAFLD above and beyond traditional risk factors; however, in the model alone it did not increase the c statistic very much compared with associated traditional metabolic risk factors.[132] However, this study had a high proportion of individuals with metabolic disease; thus, it may not be as generalizable to more population-based samples and may underestimate the true power of genetic studies. Another study used a combination of variants in or near *PNPLA3, TM6SF2, GCKR,*

LYPLAL1, and *PPP1R3B* and found that this genetic risk score improved prediction of NAFLD in individuals of Mexican ancestry with morbid obesity.[133] The combined genetic risk score was significantly associated with higher hepatic triglyceride and total cholesterol content ($p = 1.0 \times 10^{-4}$ and 0.048, respectively), steatosis stage ($p = 0.029$), and higher ALT levels ($p = 0.002$). Subjects with a genetic risk score ≥ 6 showed a significantly increased risk of NASH (OR = 2.55, $p = 0.045$) compared with those with a score ≤ 5. However, the genetic risk score did not predict NASH status, as area under the receiver operating curve was 0.56 ($p = 0.219$). Overall then, these markers are able to provide information above and beyond traditional risk factors, but alone are not (yet) predictive of developing NAFLD.

A key part to using genetics to predict and more importantly prevent disease is understanding what factors in the environment these genes interact with to cause disease. In particular, identifying environmental factors that interact with the genetic variant to not only increase risk but increase it in an exponential (gene–environment interaction effect) versus additive manner are especially important to find, as these can substantially affect outcomes. Toward that end, PNPLA3 I148M has been found to interact with obesity,[38] abdominal fat,[110,134,135] excessive alcohol consumption,[136] chronic hepatitis B and C,[57,58] or liver iron overload[137] to trigger progressive liver disease (►**Fig. 2**). The magnitude of increase in liver enzymes in I148M carriers is correlated to high dietary carbohydrate, sugar,[138–140] and increased omega6/omega3 polyunsaturated fatty acid ratio[141,142] intake (►**Fig. 2**). In the future, incorporating not only one's genetic, but also environmental exposures such as this may help us to not only predict development of NAFLD better than we can presently but in addition to make tailored recommendations on how to mitigate this increased risk.

The above findings suggest that by avoiding obesity and alcohol and other disease-promoting triggers we may be able to prevent liver disease in those that carry the I148M allele at *PNPLA3*. This is the premise behind the precision medicine initiatives that are currently underway, where, after knowing a person's genetic risk we can make more directed recommendations to prevent disease (i.e., *PNPLA3* I148M carriers may have more benefit from losing weight than noncarriers[143]). Prospective studies should be done soon to evaluate the effects of various recommendations on outcomes especially for alleles that confer substantial increased risk of developing advanced liver disease such as *PNPLA3*. At some loci, however, such as *TM6SF2*, the non-steatosis predisposing allele predisposes to development of elevated serum lipids and cardiovascular disease,[46] suggesting that interventions that increase TM6SF2 function may not be completely benign. In particular, augmenting or decreasing TM6SF2 function may predispose to dyslipidemia/cardiovascular disease or to NAFLD/advanced liver disease/HCC, respectively, so the patient's overall risk of developing these diseases would have to be assessed using not only this allele, but also many others before advising patients on the best course of action.[144]

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Abbreviations

ADPN	adiponutrin
ALD	alcoholic liver disease
ALT	alanine aminotransferase
BMI	body mass index
ChoRE	carbohydrate response element
ChREBP	carbohydrate response element binding protein
CI	confidence interval
CT	computed tomography
eQTL	expression quantitative trait locus
ER	endoplasmic reticulum
GCKR	glucokinase regulator
GENOA	Genetic Epidemiology of Arteriopathy
GK	glucokinase
GWASs	genome-wide association studies
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDL-C	high-density lipoprotein cholesterol
LD	linkage disequilibrium
LDL-C	low-density lipoprotein cholesterol
LYPLAL1	lysophospholipase-1
MELD	Model for Endstage Liver Disease
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MTTP	microsomal triglyceride transfer protein
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
OR	odds ratio
PNPLA3	patatin-like phospholipase domain containing 3

PPP1R3B	protein phosphatase 1
SNPs	single nucleotide polymorphisms
SVR	sustained virologic response
TM6SF2	transmembrane 6 superfamily 2
TRIB1	tribbles pseudokinase 1
VLDL	very low-density lipoprotein

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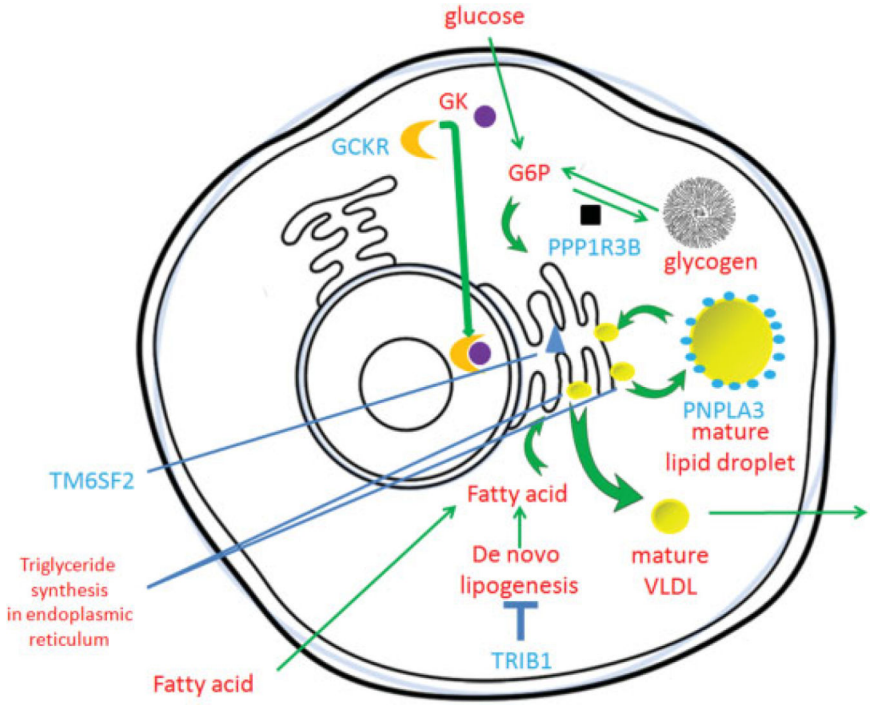

Fig. 1.

Cellular localization of some genes/gene products associated with nonalcoholic fatty liver disease (NAFLD) from genome-wide association studies (GWASs). Genes implicated by GWASs in blue. Glucokinase regulator protein (GCKR) binds to and inactivates glucokinase (GK) by bringing it into the nucleus, thus decreasing phosphorylation of glucose to glucose 6-phosphate, which is a precursor for both glycogen and triglyceride synthesis. Loss of GCKR thus might promote triglyceride synthesis and hepatic steatosis. Protein phosphatase 1 (PPP1R3B) promotes glycogen synthesis and inhibits glycogen breakdown to phosphorylated glucose which can be used to make triglycerides and promote hepatic steatosis; patatin-like phospholipase domain-containing protein 3 (PNPLA3) is present on lipid droplets, but its exact biochemical function is debated; the NAFLD promoting I148M mutation, however, results in increased triglyceride storage in lipid droplets and hepatic steatosis. Transmembrane protein 6 (TM6SF2) is in the perinuclear endoplasmic reticulum-interference with its function results in impaired very low-density lipoprotein cholesterol (VLDL) excretion, accumulation of triglycerides in the cell, and hepatic steatosis. Tribbles 1 homolog (TRIB1) inhibits de novo lipogenesis so its loss thus promotes fatty acid production, triglyceride (TG) synthesis, and hepatic steatosis. G6P, glucose 6-phosphate.

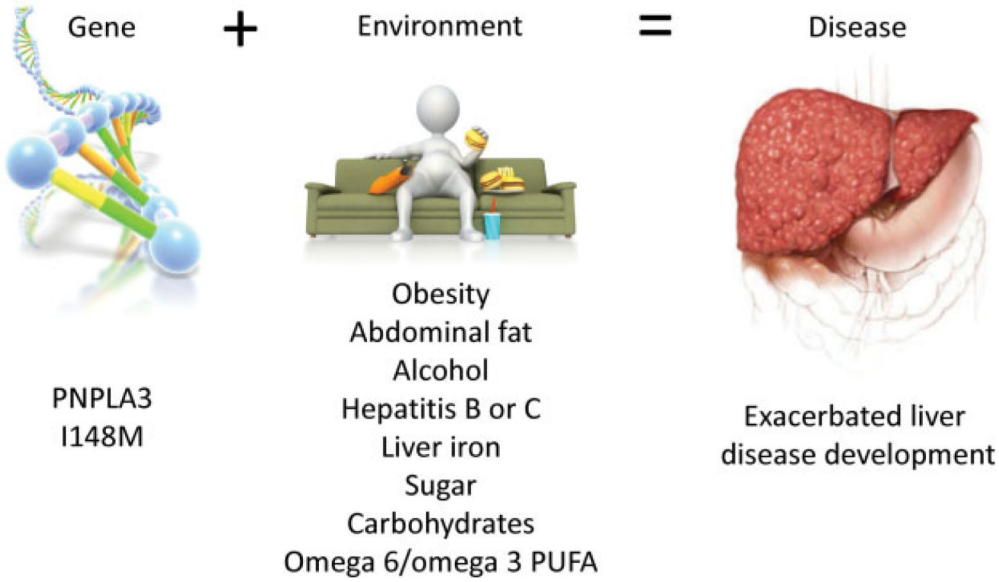

Fig. 2.

Factors that interact with PNPLA3 I148M to exacerbate liver disease development.[22] PUFA, polyunsaturated fatty acid.

Table 1

Genome-wide association studies (GWASs) of nonalcoholic fatty liver disease (NAFLD)

Study	Primary phenotype	Ancestry	Genotyping array	Discovery panel size (Cases/ controls)	Replication panel size (Cases/ controls)	Loci/genes	Scientific impact
Yuan et al (2008)[34]	Clinical biochemistry (ALT levels)	European	Affymetrix 500/Illumina HumanHap 550/Illumina HumanHap 300/Perlegen Sciences custom array	7,715	4,704	*PNPLA3-SAMM50* genomic locus, *CPN1-ERLIN1-CHUK* genomic locus	First GWAS identifying multiple loci/genes influencing plasma levels of liver enzymes
Romeo et al (2008)[8]	MRS measured hepatic steatosis	Hispanic, African American, European	Perlegen Sciences custom array (12k)	2,111 individuals with MRS measured hepatic steatosis	none	*PNPLA3*	First exome analysis of hepatic steatosis. Frequency differences of *PNPLA3* effect allele across ancestries account for much of the differences in NAFLD population prevalance
Chalasani et al (2010)[51]	Hepatic histology	European American	Illumina CNV370	236 non-Hispanic white women	none	none genome-wide significant	First GWAS of NAFLD histology phenotypes
Speliotes et al (2011)[19]	CT measured hepatic steatosis	European American	Affymetrix 550/Illumina CNV370/Illumina 1 million SNP chip/Affymetrix 500K	7,126 with CT measured hepatic steatosis	592/1405 histology	*PNPLA3, NCAN, PPP1R3B, CCKR, LYPLAL1*	Largest GWAS of hepatic steatosis. Multiple loci/genes identified and characterized, including pleiotropies that suggest disease subtypes
Chambers et al (2011)[35]	Clinical serum chemistry phenotypes (ALT levels)	European	Affymetrix, Illumina, and Perlegen Sciences arrays	61,089	none	*PNPLA3, TRIB1, CPN1, loci near HSD17B13 and MAPK10*	Largest GWAS identifying multiple loci/genes influencing plasma levels of liver enzymes
Kozlitina et al (2014)[48]	MRS measured hepatic steatosis	Hispanic, African American, European	HumanExome BeadChip, Illumina	2,736	none	*PNPLA3, TM6SF2*	First exome chip association study of liver fat content. Identified *TM6SF2* as a probable causal gene for NAFLD in chr19p13.11 locus

Abbreviations: ALT, alanine aminotransferase; MRS, magnetic resonance spectroscopy; CT, computed tomography; SNP, single nucleotide polymorphism.

Table 2

Association with nonalcoholic fatty liver disease (NAFLD) and related metabolic traits

Gene SNP (effect allele, EAF)	CAD/MI (OR 95% CI)[46]	Fasting glucose (effect SE) mmol/L[19]	HOMA-IR (effect SE)[19]	LDL-C (effect SE) mg/dL[47]	TG (effect SE) mg/dL[47]	TC (effect se) mg/dL[47]	ALT (effect 95% CI) IU/L[35]	ALP (effect 95% CI) IU/L[35]	GGT (effect 95% CI) IU/L[35]	Fatty liver (effect SE)[a,19]	NASH/fibrosis (OR 95% CI)[19]
PNPLA3 rs738409 (G, 0.23)	NA	NS	NS	NS	NS	NA	6.0 (5.0–7.0)	NS	NS	0.26 (0.02)	3.26 (2.11–7.21)
TM6SF2 rs58542926 (T, 0.07)[b]	0.87 (0.79–0.95)	NA	NA	–3.11 (0.38)	–7.83 (0.82)	–6.6 (1.2)[46]	NA	_ [c,48]	NS	0.24 (0.03)[d]	1.88 (1.41–2.5)
GCKR rs780094 (T, 0.39)	NS	–0.03 (0.004)	–0.02 (0.004)	NS	8.76 (0.40)	1.91 (0.19)	NS	NS	3.2 (2.4–4.0)	0.06 (0.02)	1.45 (1.19–1.86)
PPP1 R3B rs4240624 (A, 0.92)	NS	–0.03(0.006)	NS	2.22 (0.29)	NS	3.14 (0.32)	NS	2.7 (1.1–4.4)	NS	0.29 (0.03)	NS
LYPLAL1 rs12137855 (C, 0.79)	NA	NS	NS	NS	NS	NS	NS	NS	NS	0.08 (0.02)	1.37 (1.17–1.57)
TRIB1 rs2954021 (A, 0.52)[e]	NA	NA	NA	–1.84 (0.17)	–5.64 (0.39)	–2.30 (0.19)	1.6 (0.6–2.6)	1.4 (0.5–2.3)	NS	_ [f]	NA

Abbreviations: EAF, effect allele frequency in Europeans; CAD/MI, coronary artery disease/myocardial infarction; effect SE, effect per effect allele with standard error of mean in units given; mmol/L, millimole per liter; HOMA-IR, homeostatic model assessment for insulin resistance; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; TC, total cholesterol; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; NASH, nonalcoholic steatohepatitis; NS, not significant; NA, not available, source of values in table are from the references in the headers except when cited separately.

[a] Effect measured as the increase in inverse normalized fatty liver by computed tomography for per effect allele (from[19]).

[b] TC, LDL-C, TG results are for rs10401969 (LD r^2= 0.98 with rs58542926 in EUR) (from[47]).

[c] Direction of effect on serum alkaline phosphatase is decreased for the T allele of rs58542926 with a p-value = 4.3×10^{-7} (from[48]).

[d] Data for SNP rs2228603 (LD r^2= 0.80 with rs58542926 in EUR) (from[19]).

[e] TG data for rs2954029 (LD r^2= 0.81 with rs2954021 in EUR). TC and LDL-C data for rs2954022 (LD r^2=0.84 with rs2954021 in EUR) (both from[47]).

[f] Effect given as the odds of having >5% fat by histology for each effect allele of rs2954021 and is 1.53 (95% CI 1.25–1.88) (from[52]).

Table 3

Association of PNPLA3 NAFLD-associated variants with other liver diseases

Gene SNP (effect allele, EAF)	Alcoholic steatosis OR (95% CI)	Alcoholic liver injury OR (95% CI)	Alcoholic cirrhosis OR (95% CI)	Hepatitis C steatosis OR (95% CI)	Hepatitis C cirrhosis OR (95% CI)	Hepatitis C SVR OR (95% CI)	Hepatitis B steatosis OR (95% CI)	Hepatitis B cirrhosis OR (95% CI)	NAFLD HCC OR (95% CI)	Alcoholic HCC OR (95% CI)	Hepatitis C HCC OR (95% CI)	Liver fibrosis after transplant
PNPLA3 rs738409 (G, 0.23)	NS[56]	1.45 (1.24–1.69)[56]	2.09 (1.79–2.44)[56]	1.90 (1.4–2.7)[57]	1.47 (1.2–1.9)[57]	0.63 (0.4–0.8)[57]	1.62 (1.00–7.00)[58]	NS[55,59,60]	2.26 (1.23–4.14)[61]	2.20 (1.80–2.67)[62]	1.55 (1.03–2.34)[62]	2.53 (1.25–5.02)[63]

Abbreviations: CI, 95% confidence interval; EAF, effect allele frequency; HCC, hepatocellular carcinoma; NAFLD, nonalcoholic fatty liver disease; NS, not significant; OR, odds ratio; SNP, single nucleotide polymorphism; SVR, sustained virologic response.

Table 4

Properties of proteins encoded by genes associated with NAFLD

Gene	Protein	Function	Cellular location	Catalytic activity	Pathway/biologic function	Chromosome location	Tissue
PNPLA3	Patatin-like phospholipase domain-containing protein 3	Multifunctional enzyme which has both triacylglycerol lipase and acylglycerol O-acyltransferase activities[91]	Lipid droplets[92]	Triglyceride lipase activity and acylglycerol O-acyltransferase[91] Triacylglycerol + H_2O ⇋ diacylglycerol + a carboxylate acyl-CoA + 2-acylglycerol ⇋ CoA + diacylglycerol	Triacylglycerol degradation and in glycero-lipid metabolism	chr22:44,319,619–44,343,448	Liver, gallbladder, kidney, exocrine pancreas, seminal vesicles, intestine, and salivary gland[93]
LYPLAL1	Lysophospholipase-like protein 1	Has depalmitoylating activity. Does not exhibit phospholipase or triacylglycerol lipase activity[94]	Cytoplasm[94]	Lysophospholipase activity Palmitoyl-protein + H_2O ⇋ palmitate + protein[94]	Negative regulation of Golgi to plasma membrane protein transport	chr1:219,347,192–219,386,207	Liver[95] Bone marrow[96] Placenta[97]
PPP1R3B	Protein phosphatase 1 regulatory subunit 3B	Acts as a glycogen-targeting subunit for phosphatase PP1[98]	Glycogen granule[99]	Suppresses the rate at which PP1 dephosphorylates (inactivates) glycogen Phosphorylase and enhances the rate at which it activates glycogen synthase and therefore limits glycogen breakdown[100]	Carbohydrate metabolism, glycogen metabolism	chr8:8,993,764–9,008,220	Highly expressed in the liver, and at lower levels in skeletal muscle[101]
TM6SF2	Transmembrane 6 superfamily member 2	Regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content[6]	Endoplasmic reticulum (ER) and the ER-Golgi intermediate compartment[102]	Regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content[102]	Promotes very low-density lipoprotein export[43,103]	chr19:19,375,174–19,384,074	Liver and intestine[11]
GCKR	Glucokinase regulatory protein	Inhibits glucokinase (GK) by forming an inactive complex with this enzyme. The affinity of GCKR for GK is modulated by fructose metabolites[104]	Cytoplasm, nucleus[105]	Binding of fructose 6-phosphate and fructose 1-phosphate[104]	Carbohydrate metabolism	chr2:27,719,706–27,746,550	Liver and pancreas[106]
TRIB1	Tribbles homolog 1, G-protein-coupled receptor-induced gene 2 protein	Interacts with mitogen-activated protein kinases (MAPKs) and regulates activation of MAPKs. May not display kinase activity[107]	Cytoplasm, nucleus[105]	Protein kinase inhibitor[107]	Inhibits lipogenesis[40,88]	chr8:126,442,563–126,450,644	Expressed in most human tissues including liver with the highest levels in skeletal muscle, thyroid gland, pancreas, peripheral blood leukocytes, and bone marrow[107]