The Genetics of Nonalcoholic Fatty Liver Disease: Spotlight on *PNPLA3* and *TM6SF2*

Quentin M. Anstee, BSc, MBBS, PhD, MRCP(UK)¹ Christopher P. Day, MD, PhD, FMedSci¹

¹Liver Research Group, Institute of Cellular Medicine, The Medical School, Newcastle University, Framlington Place, Newcastle-upon-Tyne, United Kingdom

Semin Liver Dis 2015;35:270–290.

Address for correspondence Quentin M. Anstee, BSc, MBBS, PhD, MRCP(UK), Institute of Cellular Medicine, The Medical School, Newcastle University, 4th Floor, William Leech Building, Framlington Place, Newcastle-upon-Tyne, NE2 4HH, Great Britain (e-mail: quentin.anstee@ncl.ac.uk).

Abstract

Keywords

- nonalcoholic fatty liver disease
- *TM6SF2*
- *PNPLA3*
- gene
- steatohepatitis
- fibrosis

Nonalcoholic fatty liver disease (NAFLD) encompasses a spectrum that spans simple steatosis, through nonalcoholic steatohepatitis (NASH) to fibrosis and ultimately cirrhosis. Nonalcoholic fatty liver disease is characterized by substantial interpatient variation in rate of progression and disease outcome: Although up to 25% of the general population are at risk of progressive disease, only a minority experience associated liver-related morbidity. Nonalcoholic fatty liver disease is considered a complex disease trait that occurs when environmental exposures act upon a susceptible polygenic background composed of multiple independent modifiers. Recent advances include the identification of *PNPLA3* as a modifier of disease outcome across the full spectrum of NAFLD from steatosis to advanced fibrosis and hepatocellular carcinoma; and the discovery of *TM6SF2* as a potential “master regulator” of metabolic syndrome outcome, determining not only risk of advanced liver disease, but also cardiovascular disease outcomes. In this article, the authors will review the field, discussing in detail the current status of research into these important genetic modifiers of NAFLD progression.

Nonalcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver disease ranging from simple steatosis (hepatic triglyceride content [HTGC] > 5%), through steatohepatitis (fat plus inflammation and hepatocellular ballooning degeneration; NASH), to fibrosis and ultimately cirrhosis, in the absence of excessive alcohol consumption (a threshold of < 20 g/d for women and < 30 g/d for men is typically adopted).¹ Nonalcoholic fatty liver disease is strongly associated with features of the metabolic syndrome including abdominal obesity, insulin resistance or type 2 diabetes mellitus (T2DM) and atherogenic dyslipidaemia.^{2,3} Over the past few decades, lifestyles have become increasingly sedentary and dietary patterns have changed, leading to an increased prevalence of obesity and insulin resistance.³ Against this background, NAFLD has rapidly become the most common cause of abnormal liver biochemistry in many countries and is predicted to be the most common indication for liver transplantation within a decade.^{4–6}

The prevalence of NAFLD and its natural history are reviewed elsewhere in this issue.⁷ A substantial proportion of the population are at risk of progressive NAFLD due to the presence of obesity and insulin resistance. However, it is apparent that only a minority progress to more advanced disease characterized by NASH, advanced fibrosis, and hepatocellular carcinoma (HCC).³ Indeed, studies in cohorts of patients that have undergone serial liver biopsies indicate that approximately 40% of NAFLD cases will develop progressive liver fibrosis while the remaining 60% exhibit stable disease or some degree of regression during long-term follow-up.^{8,9} Rates of fibrosis progression are also nonuniform, with some individuals progressing more rapidly than others.^{8,9} It should also be noted that liver-related mortality occurs in < 5% of NAFLD patients and is the third most common cause of death after cardiovascular disease and extrahepatic malignancy.¹⁰

The reasons for these variations remain incompletely understood, but NAFLD is best considered a complex disease

Issue Theme Nonalcoholic Fatty Liver Disease (NAFLD); Guest Editors, Christopher P. Day, MD, PhD, FMedSci, and Quentin M. Anstee, BSc, MBBS, PhD, MRCP(UK)

Copyright © 2015 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel: +1(212) 584-4662.

DOI <http://dx.doi.org/10.1055/s-0035-1562947>. ISSN 0272-8087.

trait where subtle interpatient variations including host genetic factors and environment interact to produce disease phenotype and determine disease progression.^{11–13} Although the presence of NAFLD is principally determined by environmental factors, it is clear that genetic factors contribute and crucially determine how individuals respond to the challenge of caloric excess and consequent metabolic stressors. Although much work remains to be done, substantial progress in our understanding of genetic modifiers has been made. Here we will summarize the latest developments since we last reviewed the field, in particular focusing on the *PNPLA3* and *TM6SF2* genes.^{13,14}

Evidence Indicative of a Genetic Component to NAFLD

Susceptibility to high-prevalence diseases such as obesity, T2DM, cardiovascular disease, and NAFLD comprises a heritable component variously accounting for up to 30 to 50% of relative risk.¹⁵ These complex traits result from environmental exposures acting on a susceptible polygenic background made up of multiple independent modifiers.^{15,16} Three strands of evidence suggest that there is a significant heritable component to NAFLD: familial aggregation,^{17–19} twin studies,²⁰ and interethnic differences in susceptibility.^{21–25} Although socioeconomic factors can confound analysis of interethnic differences, variations in population prevalence of genetic variants such as the *PNPLA3* rs738409 polymorphism do seem to contribute to this variability.²⁶

Identifying Genetic Modifiers of NAFLD

The allelic frequency of susceptibility loci in common diseases remains a contentious subject.^{27–29} Most studies are founded on the pre-eminent “common disease/common variant” (CD/CV) hypothesis.^{16,28} Broadly, common diseases are of late onset, and so have little impact on reproductive fitness. Therefore, causative mutations are not subject to negative selection pressures, and disease susceptibility is due to the combined effects of multiple relatively common causative polymorphisms (minor allele frequency 1–5%) that are carried by affected individuals.²⁸ Discussion of the relative merits of this and the competing “multiple rare-variant” hypothesis is outside the scope of this review, but are discussed elsewhere. Although one cannot accurately predict the allelic effect-size distribution in NAFLD, it is likely that the majority of modifier loci will each individually have only a small effect, although a minority may have greater effect.²⁸ This seems consistent with evidence from genome-wide association studies (GWASs) in other conditions showing that disease-associated allelic variation is frequently noncoding and perturbs gene expression or exon splicing; subtle changes that in general have modest functional consequences compared with the much less common nonsynonymous coding sequence alterations.^{28,30} Supporting this, the majority of published association studies report allele relative risk ratios of 1.1 to 1.5.^{30–32}

As no single gene is sufficient to determine outcome, clear patterns of inheritance are not seen within kinships and so family-based gene linkage studies lack the necessary power to detect genetic variants as each individually confers only a modest effect.^{15,16}

Hypothesis-Driven Candidate-Gene Association Studies

The principal approach to the study of complex disease traits such as NAFLD has been the case-control disease-association study. These genetic association studies assess correlations between genetic variants and trait differences on a population scale and so have greater power to detect effect.³³ However, selection of candidate genes is founded on an assumed biologically plausible pathogenic role for the encoded protein. As these studies are reliant on there being a valid *a priori* hypotheses for gene selection, candidates are drawn from the limited pool of genes where biological function is already understood and considered relevant to the disease.^{33,34} These studies are therefore unlikely to identify a role for genes that were not already implicated. As will be discussed below, candidate-gene studies have only identified a small number of genes that are robustly and reproducibly associated with NAFLD.

Hypothesis Generation by Genome-Wide Association Studies

Patterns of association among single nucleotide polymorphisms (SNPs) throughout the genome may be characterized in terms of both linkage disequilibrium (LD; correlation between nearby variants on the same chromosome such that alleles are nonrandomly associated) and haplotype (a combination of alleles at multiple linked loci on a chromosome that are transmitted together).³⁵ The use of *tag* SNPs to serve as proxies for other SNPs that are in strong LD, allowed development of genotyping arrays that simultaneously test > 1 million different polymorphisms. These arrays permit the profiling of the majority of common (minor allele frequency [MAF] > 5%) variability in the human genome.^{35–37}

Genome-wide association studies have led to the identification of novel genes contributing to several important diseases.^{34,35} However, due to the nonhypothesis-driven nature of GWAS, the loci identified are frequently novel and would not otherwise have been linked to NAFLD. A corollary of this is that neither biological function nor pathogenic mechanisms are necessarily apparent and so additional study, both to confirm causality and elucidate the pathogenic mechanisms, is required. It is also apparent that a substantial proportion of disease heritability remains elusive and so GWASs are not a panacea.³² The failure to achieve universal success in the study of complex diseases is in part because of the complexity of the phenotypes studied and difficulty in establishing sufficiently large, well-characterized patient cohorts. The latter is especially relevant to NAFLD where histologically characterized patients are required to assess modifiers of steatohepatitis and fibrosis. Current arrays do not yet capture rare variants with more modest effect size (MAF < 0.5%), nor are they effective at assessing disease associations with

structural polymorphisms such as copy number variants, insertions, deletions, and inversions.^{32,35}

GWASs by Phenotype and Chronology

At the time of writing, several genome-scale studies for NAFLD have been reported (summarized in **Table 1**).^{26,38–44} These can be divided into three groups based on disease phenotype studied: (1) radiologically measured steatosis (HTGC), (2) histologically characterized NAFLD, and (3) clinical biochemistry phenotypes.

Radiologically Determined Steatosis-Based GWASs

The first GWAS in NAFLD, published in 2008,²⁶ was of relatively modest size by current standards. It examined 9,229 nonsynonymous SNPs in a North American population of diverse ethnicity (Hispanic, African American, and European ancestry) from the Dallas Heart Study.^{26,45} Hepatic triacylglycerol (TAG) accumulation was measured using non-invasive proton magnetic resonance spectroscopy (¹H-MRS). This study identified a single highly significant association between increased HTGC and the patatin-like phospholipase domain-containing 3 (*PNPLA3*) gene.²⁶ The index single nucleotide polymorphism (SNP) in *PNPLA3* (rs738409 c.444 C > G, p.I148M) is a nonsynonymous cytosine to guanine nucleotide transversion mutation that results in an isoleucine to methionine amino acid change at codon 148. A gene dosage effect for I148M carriage was observed with a stepwise increase in HTGC with increasing carriage of the minor allele and homozygotes I148M carriage being associated with a twofold increase in HTGC. As alluded to earlier, minor-allele frequency correlated with ethnic differences in susceptibility to greater HTGC. I148M was most common in Hispanics (MAF 0.49), lower in those of European ancestry (0.23) and lowest in African Americans (0.17). Although less well characterized, this study also identified a second nonsynonymous SNP in *PNPLA3* (rs6006460, p.S453I) that was associated with reduced HTGC and had the opposite ethnic distribution.^{26,46} The two SNPs in combination accounted for 72% of the ethnic variation in HTGC in the population studied.

In their later study, Speliotes et al employed a two-stage approach.³⁹ The initial exploratory meta-analysis of data from four cohorts (Age/Gene/Environment Susceptibility-Reykjavik Study, Old Order Amish Study, Family Heart Study, and Framingham Heart Study) using 2.4 million SNPs imputed to HapMap in 7,176 individuals of European ancestry remains the largest GWAS meta-analysis for NAFLD to date.³⁹ Adopting a permissive significance threshold ($p < 10^{-3}$), SNPs associated with HTGC were identified and then taken forward in a separate cohort of 592 patients with histologically characterized NAFLD as a candidate gene study. Variants located in or near *PNPLA3* (rs738408), protein phosphatase 1, glucokinase regulator (*GCKR*; rs780094), regulatory (inhibitor) subunit 3B (PPP1R3B; rs4240624), and lysophospholipase-like 1 (*LYPLAL1*; rs12137855) were identified. In addition, a variant in the *NCAN* gene (rs2228603 c.274 C > T, p.Pro92Ser) in a region of chromosome 19 (19p13.11) was also associated with increased HTGC.³⁹ *PNPLA3*, *GCKR*, *LYPLAL1*, and *NCAN* (the

19p13.11 region), but not PPP1R3B were validated for histological steatosis, and additionally associated with lobular inflammation and/or fibrosis. In addition, variants in or near *NCAN*, *GCKR*, and *PPP1R3B* were associated with altered serum lipid levels and variants near *GCKR* and *PPP1R3B* affected glycemic traits. The rs738408 SNP in *PNPLA3* identified in this study is in strong LD with rs738409, previously described by Romeo et al.²⁶

Although further study of each highlighted gene is still warranted to explore the associated pathogenic mechanisms, most were biologically plausible. *GCKR* encodes a regulator of carbohydrate metabolism and so is also highly relevant to NAFLD pathogenesis despite the precise causative variants within the gene remaining ill defined. *LYPLAL1* encodes a protein involved in triglyceride breakdown and so is likely to be relevant. A variant in *PPP1R3B* (rs2126259), which encodes the catalytic subunit of protein phosphatase-1 and so may influence hepatic glycogen synthesis, has since been validated for HTGC in another GWAS.⁴² These contrast with the 19p13.11 locus where attention had focused on the *NCAN* gene that contained the rs2228603 SNP. Although the association was subsequently validated in a separate cohort,⁴⁷ *NCAN* lacked biologically plausible evidence of a functional role in NAFLD while nearby genes had previously been associated with variations in plasma triglyceride and low-density lipoprotein levels and so were more likely candidates.^{48,49} Careful inspection of the LD patterns around rs2228603 and the 19p13.11 region suggested that the variant was in strong LD with a cluster of other SNPs up to 400 kb distant and so tagged > 20 other genes (**Fig. 1**). This led us to question whether *NCAN* was indeed the causative gene.¹⁴

Clarification on this point was provided by a subsequent GWAS based on a genome-wide exome chip in 2,736 individuals drawn from the same North American population as the original Romeo study.⁴³ In addition to again validating the association between *PNPLA3* and NAFLD, Kozlitina et al determined that a nonsynonymous genetic variant within a gene of unknown function called *TM6SF2*, *transmembrane 6 superfamily member 2* (rs58542926 c.449 C > T, p.Glu167Lys) at the 19p13.11 locus was associated with ¹H-MRS quantified HTGC.⁴³ Homozygote *TM6SF2* rs58542926 minor (T) allele carriage was shown to be associated with a modest, but statistically significant increase in ¹H-MRS measured HTGC from $5.86 \pm 0.25\%$ in CC homozygotes to $15.04 \pm 2.23\%$ in TT homozygotes.⁴³ This *TM6SF2* variant is in strong linkage disequilibrium with other SNPs around the 19p13.11 locus, including the rs2228603 SNP previously identified by Speliotes et al.³⁹ Crucially, conditional analyses demonstrated that *TM6SF2* rs58542926, not *NCAN* rs2228603 was the causal variant driving the association with HTGC at this locus.^{43,50}

Histologically Characterized NAFLD-Based GWASs

Reflecting the difficulty establishing large patient cohorts that are characterized using invasive tests such as liver biopsy, most histologically based GWASs published to date have used relatively small cohorts. Four histologically based GWASs have been published to date. It is notable that these have not been as productive in terms of robustly

Table 1 Genome-wide association studies examining phenotypes relevant to nonalcoholic fatty liver disease

	Phenotype	Population	N	Number of SNPs	Modifier loci/genotypes identified
Romeo et al, 2008 ²⁶	¹ H-MRS measured steatosis	USA (mixed ethnicity)	2,051 (B: 1,032; W: 636; H: 383)	9,229	PNPLA3 (rs738409)
Yuan et al, 2008 ⁵⁸	Clinical biochemistry (ALT)	European (mixed ethnicity)	7,715	–	PNPLA3-SAMM50 gene cluster, CPN1-ERLIN1-CHUK gene cluster
Chalasani et al, 2010 ³⁸	Liver histology	USA (all Female, European Caucasian)	236	324,623	FDTT1 (rs2645424) COL13A1 (rs1227756) EFCAB4B (rs887304) PZP (rs6487679)
Speliotes et al, 2011 ³⁹	CT measured Steatosis (with histological 'candidate gene' validation set)	USA & Europe (meta-analysis of previous studies)	7,176	Range 329 k–618 k before imputation	PNPLA3 (rs738409) NCAN (rs2228603) GCKR (rs780094) LYPLAL1 (rs12137855) PPP1R3B (rs4240624)
Chambers et al, 2011 ⁵⁹	Clinical biochemistry (ALT)	European (mixed ethnicity)	61,089	~2.6 million genotyped and imputed	PNPLA3 (rs738409) TRIB1 (rs2954021) HSD17B13 (rs6834314) CPN1 (rs10883437)
Kawaguchi et al, 2012 ⁴¹	Liver histology	Japan	529	484,751	PNPLA3 (rs738409)
Anstee et al, 2012 ^{40,131}	Liver histology	European	1,125	–	PNPLA3 (rs738409)
Kitamoto et al, 2013	Liver histology	Japan	392	261,540	PNPLA3 (rs738409)
Feitosa et al, 2013 ⁴²	CT measured steatosis	USA	2,705	~2.4 million genotyped and imputed	PNPLA3 (rs738409), PPP1R3B (rs2126259), ERLIN1-CHUK-CWF19L1 gene cluster (9 SNPs in two haplotype blocks)
Kozlitina et al, 2014 ⁴³	¹ H-MRS measured steatosis	USA (mixed ethnicity)	2,735	247,870 exonic SNPs	PNPLA3 (rs738409), TM6SF2 (rs58542926)
DiStefano et al, 2015 ⁴⁴	Liver histology	USA (mixed ethnicity)	2,300	–	PNPLA3 and SUGP1 (neighboring gene to TM6SF2)

Source: Modified from Anstee QM, Day CP. The genetics of NAFLD. Nat Rev Gastroenterol Hepatol 2013;10(11):645–655.¹⁴
Abbreviations: ALT, alanine transaminase; CT, computed tomography; ¹H-MRS, proton magnetic resonance spectroscopy; SNPs, single nucleotide polymorphisms.

Fig. 1 Linkage disequilibrium patterns in the chromosome 19 (19p13.11) region flanking *TM6SF2*. Visualization of the 19p13.11 region flanking the rs58542926 *TM6SF2* SNP (red diamond) based on 1000 genome data analyzed in SNAP.¹⁸⁹ Strong linkage disequilibrium (LD) with a cluster of variants up to ~400 kb distant is demonstrated. Neighboring genes associated with relevant phenotypes in other studies (including *NCAN*, *SUGP1* (*SF4*), *CILP2*, *PBX4*, and *GMIP*) are likely to have been due to the LD effect across the 19p13.11 region.

identifying new genetic modifiers as the radiologically based studies.

Chalasani et al reported the first histologically based GWAS in 236 female NAFLD patients.³⁸ This study did not report associations with individual components of the histological NAFLD activity score⁵¹ such as steatosis; ballooning degeneration or portal inflammation. Higher overall NAFLD activity scores were associated with a variant in the farnesyl diphosphate farnesyl transferase 1 (*FDFT1*, rs2645424), an enzyme with a role in cholesterol biosynthesis in a multivariate analysis adjusting for age, diabetes, HbA1c level, body mass index [BMI], and waist:hip ratio. Additional loci were associated with fibrosis (rs343062) and lobular inflammation (including rs1227756 in the *COL13A1* gene). *PNPLA3* was not associated with any aspect of the NAFLD phenotype in this study and the *FDFT1* association was not replicated in a subsequent study using a cohort of 340 NAFLD.⁵² Although the associations detected in this study are potentially interesting, they require further independent validation in larger patient cohorts.

Two Japanese histologically based studies have been published.^{41,53} The first, by Kawaguchi et al,⁴¹ examined 484,751 SNPs in a cohort of 529 patients with biopsy-proven NAFLD characterized according to the Matteoni classification⁵⁴ and 932 population controls. The second study by Kitamoto et al⁵³ initially examined 261,540 SNPs in a cohort of 392 NAFLD patients and 934 population controls and then validated their findings in a cohort of 172 patients and 1,012 controls. Both studies confirmed the association between NAFLD and SNPs flanking the *PNPLA3* gene in this ethnically distinct population, but identified no novel signals. Another study in 2,300

obese individuals again provided strong evidence of an association with *PNPLA3* and identified a locus neighboring *TM6SF2*, both linked to a steatosis phenotype.⁴⁴

Clinical Chemistry-Based GWASs

It is accepted that liver biochemistry correlates poorly with the presence of NAFLD or disease severity^{55,56} so that biochemical screening consistently reports a lower disease prevalence (3–12%) than imaging or histology-based studies.⁵⁷ Accepting these limitations, two GWASs have identified genes that influence plasma levels of alanine transaminase (ALT).^{58,59} The first of these studies found that *PNPLA3* I148M was associated with raised serum ALT levels in populations of European and Indian-Asian descent.⁵⁸ A region spanning three genes on chromosome 10, *APN1-ERLIN1-CHUK* (rs11597390, rs11591741, rs11597086) that was also highlighted in this study⁵⁸ has since been associated with radiological steatosis and ALT levels in a GWAS-correlated meta-analysis, which flagged variants in *ERLIN1-CHUK-CWF19L1*.⁴² The second of the two studies identified 42 loci at genome-wide significance levels ($p < 1 \times 10^{-8}$) in a cohort of 61,089 individuals.⁵⁹ Four loci were associated with elevations in ALT: rs6834314 near *HSD17B13* and *MAPK10*; rs2954021 near *TRIB1* (a gene that has previously been associated with variations in plasma cholesterol and triglyceride levels^{48,60,61}); rs10883437 near *CPN1*; and *PNPLA3* rs738409.⁵⁹

The Chromosome 22 Locus: *PNPLA3*

Across all GWASs it is noteworthy that only two genes (*PNPLA3* and *TM6SF2*) have been identified as potential

Fig. 2 Commonality between the largest genome-wide association studies (GWASs) in nonalcoholic fatty liver disease (NAFLD). There is surprisingly little commonality in genes identified in GWASs examining aspects of the NAFLD phenotype. Currently, only two genes (*PNPLA3* and *TM6SF2*) have been identified in more than one study.

modifiers in more than one study (►Fig. 2). Following its association with steatosis in the first reported NAFLD GWAS, there has remained considerable interest in the role of the *PNPLA3* gene on chromosome 22 as a modifier of NAFLD pathogenesis. This association has now been independently replicated in numerous candidate-gene studies examining both adult^{62–66} and pediatric^{66–69} NAFLD cohorts across differing ethnicities, as has the association with raised ALT/aspartate aminotransferase (AST) levels.⁷⁰ There is also evidence from histologically based studies that the rs738409 (I148M) variant is associated with severity of steatohepatitis and greater fibrosis.^{62,64,66} It has also been associated with greater response to dietary or lifestyle modification and so may be a marker of both greater risk and also greater benefit from intervention.^{71,72}

The rise in the burden of NAFLD coincides with a marked increase in the incidence of HCC in many countries,^{73–76} and so it is timely to also consider whether genetic modifiers such as *PNPLA3* may also increase the risk of NAFLD-associated HCC. The I148M variant has been associated with increased HCC risk in a mixed-etiology cohort,⁷⁷ the morbidly obese,⁷⁸ alcohol-related liver disease,^{79–82} and more variably, in chronic viral hepatitis.^{79,80,82–84} This association is supported by a meta-analysis⁸⁵ but is best demonstrated by a recent European study, where in a multivariate analysis adjusted for age, gender, diabetes, BMI, and presence of cirrhosis, carriage of each copy of the rs738409 minor (G) allele conferred an additive risk for HCC (adjusted odds ratio [OR] = 2.26, 95% confidence interval [CI] = 1.23–4.14, $p = 0.0082$), with GG homozygotes exhibiting a fivefold (adjusted OR = 5.05, 95% CI = 1.47–17.29, $p = 0.01$) increased risk over CC homozygotes.⁸⁶ In this study, comparison of genotype frequencies between the NAFLD-HCC cohort and an unselected United Kingdom (UK) general population sample (the MRC/Wellcome Trust UK 1958 Birth Cohort) identified a 12-fold increased risk of HCC (OR = 12.19, 95% CI = 6.89–21.58, $p < 0.0001$) for rs738409 minor (G) allele homozygotes relative to C-allele homozygotes.⁸⁶ These findings have led some to suggest that *PNPLA3*-associated HCC approaches monogenic inheritance.⁸⁷ We would caution against a move toward considering “*PNPLA3*-associated NAFLD,” or by extension “*PNPLA3*-associated HCC,” as distinct, monogenic conditions,

but based on a reanalysis of the data from Liu et al⁸⁶ to assess sensitivity/specificity agree that there may be value in the *PNPLA3* genotyping to select out those individuals least likely to develop HCC and therefore least likely to benefit from surveillance given the very high negative predictive value.⁸⁸ Further studies to determine the utility and health-economic merits of a multifactorial risk stratification that incorporates *PNPLA3* rs738409 genotype along with other recognized risk factors for HCC are warranted.

The Challenge of Determining the Mechanistic Effects of *PNPLA3* Variants

The *PNPLA3* gene encodes a 481 amino acid protein that is structurally related to the principal TG hydrolase in adipose tissue, adipose triglyceride lipase (*ATGL/PNPLA2*).^{46,89} *PNPLA3* differs from classical lipases in possessing a catalytic dyad (S47/D166) rather than the more usual triad.⁹⁰ Although the site of the I148M variant does not lie within this highly conserved catalytic site, the I148M amino acid substitution affects the hydrophobic substrate-binding groove and so may prevent substrate access to the catalytic site.⁹¹

It is accepted that the modifier effect of *PNPLA3* on the degree of hepatic steatosis is not due to a direct change in insulin sensitivity when assessed either by hyperinsulinemic, euglycemic clamp^{63,65} or plasma insulin response to oral glucose tolerance testing.⁹² Furthermore, it does not alter the overall severity of the metabolic syndrome or confer a greater BMI, degree of dyslipidemia or prevalence of overt T2DM.⁹³ Current evidence suggests that the effects are more subtle, and that carriage of the *PNPLA3* variation sensitizes the liver to metabolic stress due to nutritional calorific excess and adiposity, which is consistent with the concept of NASH as a complex disease trait. Thus, carriage of the I148M variant is associated with a greater degree of steatosis for any given degree of insulin resistance or adiposity. This concept is supported by the fact that I148M carriage is more strongly associated with raised AST/ALT in the presence of obesity.⁹²

Despite the strong genetic evidence, the physiological role of *PNPLA3* and how this is perturbed by carriage of the I148M polymorphism is still incompletely understood. The structural changes affecting the catalytic site described above are consistent with the results of studies examining recombinant adiponutrin expressed in HUH-7 cells and the in vitro biochemical actions of purified *PNPLA3*.^{91,94} These demonstrate that *PNPLA3* hydrolyses acylglycerols with maximal hydrolytic activity observed against the three major glycerolipids, triacylglycerol (TG), diacylglycerol, and monoacylglycerol, with a strong preference for oleic acid as the acyl moiety; and that the I148M polymorphism is associated with substantially reduced enzymatic activity without reducing substrate affinity.^{91,94} Stable isotope tracer studies in 55 overweight/obese men and in vitro studies using rat McA-RH7777 cells that secrete ApoB-containing very-low-density lipoprotein- (VLDL-) like particles demonstrate that carriage of the I148M variant reduces VLDL secretion, an effect that may be indicative of a failure to mobilize TG from intracellular lipid droplets due to loss of TG hydrolase activity.⁹⁵ However, this is contentious as other groups have proposed that

PNPLA3 may also possess lysophosphatidic acid acetyltransferase (LPAAT) activity that is enhanced by I148M, increasing TG synthesis.^{96,97}

Interspecies differences in gene expression pattern have complicated in vivo mechanistic studies. PNPLA3 is expressed primarily in liver and adipose tissue, where it partitions to membranes and lipid droplets.⁹¹ However, hepatic expression is highest in man,^{98,99} whereas adipose tissue expression is highest in mice.^{100,101} Moreover, the two species only share approximately 68% PNPLA3 homology. Hepatic PNPLA3 expression is increased after feeding, reduced by fasting,^{46,101} and is also raised in obesity.^{63,101} Insulin controls postprandial PNPLA3 expression through LXR/RXR and the transcription factor SREBP-1c.⁹⁸ Significantly, additional posttranslational control influenced by the constituents of the fatty acid milieu has also been described.⁹⁸ The presence of specific saturated (palmitate, C16:0), monounsaturated (oleate, C18:1) and polyunsaturated fatty acids (linoleic acid, C18:2) increased adiponutrin expression although very long chain fatty acids (e.g., arachidonic acid, C20:4 and eicosapentaenoic acid, C20:5) were not found to effect expression.⁹⁸

Reconciling the apparently conflicting findings of the in vivo experiments with the in vitro studies has proved challenging (► Table 2). Although the initial in vitro data was most consistent with a loss-of-function effect of the I148M polymorphism, deletion of *Pnpla3* did not provoke hepatic TG accumulation, even when mice were fed a high-sucrose diet.^{102,103} Adenoviral mediated overexpression of wild-type human PNPLA3 was also unable to provoke steatosis; however, overexpression of the PNPLA3 I148M variant did cause steatosis.⁹¹ More recently, studies have shown that neither hepatic nor adipose tissue overexpression of wild-type PNPLA3 promotes hepatic TG accumulation. Hepatic overexpression of the I148M variant form did, however, cause TG accumulation.¹⁰⁴ Importantly, three distinct metabolic effects were observed: increased synthesis of fatty acids and TG, impaired TG hydrolysis, and relative depletion of TG long chain polyunsaturated fatty acids.¹⁰⁴ Taken together, these data support the view that PNPLA3 acts to remodel TG in lipid droplets as they accumulate in the fed-state and that I148M affects multiple facets of this process. Furthermore, the apparent mixed enzymatic actions, coupled with the transcriptional regulation of PNPLA3 by feeding and the specific fatty acid profile suggests that the action of PNPLA3 may vary somewhat according to tissue and metabolic milieu. It is noteworthy that in this study hepatic overexpression of the I148M variant led to increased hepatic TG, but no associated changes in TNF α , α -smooth muscle actin or collagen type 1 α mRNA expression were seen, providing no evidence that either inflammatory or fibrotic processes were active.¹⁰⁴

In an attempt to address the concerns that overexpression of human PNPLA3 I148M in mice may not have physiological fidelity, a recent study by Smagris et al used homologous recombination to introduce the *Pnpla3* I148M variant (or a complete loss of function mutation that distorted the catalytic dyad, S47A) in mice while preserving normal physiological regulation of gene expression.¹⁰⁵ Consistent with the concept that the I148M variant sensitizes the liver to metabolic stress

due to nutritional calorific excess and adiposity, a two to threefold increase in hepatic TG accumulation was seen when mice were fed high sucrose diet (but not standard chow), although these effects were not associated with an increase in ALT or circulating lipid levels.¹⁰⁵ Interestingly, *Pnpla3* I148M was not associated with any changes in hepatic *Srebp-1c* expression or the expression profiles of its target genes, and once again no changes in TNF α , α -smooth muscle actin, or collagen type 1 α mRNA expression were observed. A novel finding in this study was that hepatic TG accumulation in the knockin mice was accompanied by a 40-fold increase in either catalytically inactive PNPLA3 variant (I148M or S47A) on the surface of hepatic lipid droplets. This study is of significance as it reconciles the apparent disparity between the in vitro data suggesting a predominant loss-of-function effect of I148M^{91,94,95} and the in vivo data where *Pnpla3* knockout mice did not develop a NAFLD phenotype.^{102,103} The I148M variant confers a dominant-negative effect and so for the variant to drive NAFLD pathogenesis the presence of catalytically inactive PNPLA3 protein is necessary, not simply a complete absence of PNPLA3 activity.¹⁰⁵

While representing a substantial advance in our understanding of disease pathogenesis, the work by Li et al¹⁰⁴ and Smagris et al¹⁰⁵ underline the fact that further research is required to clarify how PNPLA3 drives not-only hepatic lipid accumulation, but also NAFLD progression to steatohepatitis, fibrosis, and hepatocellular carcinoma, where no clear mechanisms have been identified as yet. Addressing this point, only one study has offered any evidence of mechanistic link between PNPLA3 activity and hepatic fibrosis.¹⁰⁶ In that study, Pirazzi et al demonstrated that PNPLA3 is highly expressed in human hepatic stellate cells (HSCs) that purified wild-type PNPLA3 hydrolyzes retinyl palmitate into retinol and palmitic acid and that this enzymatic activity is markedly reduced by the I148M variant.¹⁰⁶ These findings suggest a potential link between HSCs, retinoid metabolism, and PNPLA3 in determining the susceptibility to hepatic fibrosis although no evidence that the observed effects altered HSC activation was provided and so further study is needed before this mechanism can be established as relevant.

The Chromosome 19 Locus: TM6SF2

The controversy regarding the causative gene within the 19p13.11 locus that was initially ascribed to NCAN has now largely been resolved. Kozlitina et al demonstrated that a non-synonymous genetic variant within TM6SF2 (rs58542926 c.449 C > T, p.Glu167Lys) was associated with ¹H-MRS quantified HTGC.⁴³ This SNP lies within 50 kb of the NCAN gene variant (rs2228603) that had previously been associated with HTGC.^{39,47} Although both SNPs are in strong linkage disequilibrium ($D' = 0.926$, $r^2 = 0.798$), conditioning on the TM6SF2 variant abrogated the effect of the NCAN variant while the reverse did not occur, establishing that TM6SF2 rs58542926 is more strongly associated with the HTGC phenotype.⁴³ In vitro and in vivo functional studies have provided further support for this conclusion, but did not provide evidence demonstrating whether the effect of TM6SF2 was limited to hepatic steatosis or

Table 2 Summary of studies examining the physiological role of PNPLA3 and the I148M variant

Study	In vivo/In vitro	Experimental conditions	Findings
Chen et al, 2010 ¹⁰²	In vivo	Global targeted PNPLA3 knockout mouse.	Loss of PNPLA3 (knockout) does not increase hepatic TAG accumulation across a range of dietary and genetic backgrounds.
He et al, 2010 ⁹¹	Both	In vivo: adenoviral overexpression of wild type and I148M in C57BL/6J mice. In vitro: (1) recombinant PNPLA3 purified from Sf9 cells; (2) overexpression of wild type and I148M in HuH-7 cells.	PNPLA3 has hydrolase activity. I148M a loss of function mutation, reducing TAG and DAG hydrolysis.
Basantani et al, 2011 ¹⁰³	In vivo	Global targeted PNPLA3 knockout mouse.	Loss of PNPLA3 (knockout) does not increase hepatic TAG accumulation. Argues against I148M being a loss-of-function mutation.
Huang et al, 2011 ⁹⁴	In vitro	Purified human wild type and I148M	PNPLA3 has hydrolase activity. I148M a loss of function mutation, reducing TAG hydrolysis.
Kumari et al, 2012 ⁹⁷	Both	In vivo: Wild type and PNPLA3 knockout mice fed chow or high sucrose diets. In vitro: (1) enzymatic activity of purified murine and human wild type and I148M protein; (2) overexpression of wild type and I148M PNPLA3 in HepG2, CHO, and Cos-7 cells.	Loss of PNPLA3 (knockout) does not increase hepatic TAG accumulation. PNPLA3 has lysophosphatidic acid acetyltransferase activity (LPAAT). I148M a gain of function mutation, increasing TAG synthesis.
Perttola et al, 2012 ¹³²	In vitro	Immortalized human hepatocytes and HuH-7 cells	PNPLA3 I148M increases hepatic TAG accumulation by reducing hydrolysis.
Pirazzi et al, 2012 ⁹⁵	Both	In vivo: Tracer studies in patients of known PNPLA3 genotype In vitro: Targeted overexpression of I148M in McA-RH 7777 cells.	Carriage (or overexpression) of I148M associated with reduced VLDL (ApoB) secretion and thus TAG accumulation. Proposed mechanism: reduced TAG hydrolysis leading to impaired lipidation on VLDL and so reduced TAG efflux.
Li et al, 2012 ¹⁰⁴	In vivo	Targeted overexpression of wild type and I148M PNPLA3 in mouse adipose tissue and hepatocytes.	No effect of wild-type PNPLA3 overexpression in liver or adipose tissue No effect of I148M overexpression in adipose tissue Hepatocyte I148M overexpression caused hepatic TAG accumulation due to increased FFA and TAG synthesis, impaired TAG hydrolysis, and relative depletion of TAG long-chain polyunsaturated fatty acids.

(Continued)

Table 2 (Continued)

Study	In vivo/In vitro	Experimental conditions	Findings
Pirazzi et al, 2014 ¹⁰⁶	In vitro	Analysis of effects of PNPLA3 on retinol metabolism in HSCs	PNPLA3 is highly expressed in human HSCs. PNPLA3 hydrolyzes retinyl palmitate into retinol and palmitic acid. This enzymatic activity is markedly reduced by the I148M variant.
Smagris et al, 2015 ¹⁰⁵	In vivo	Knockin of I148M variant in mouse <i>Pnpla3</i> gene	No effect of I148M carriage on normal chow, increased steatosis on high sucrose diet. Variant I148M PNPLA3 is a loss of function variant that coats lipid droplets.

Source: Modified from Anstee QM, Day CP. The genetics of NAFLD. Nat Rev Gastroenterol Hepatol 2013;10(11):645–655.¹⁴
Abbreviations: FFA, free fatty acid; HSCs, hepatic stellate cells; LD, linkage disequilibrium; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; TAG, triacylglycerol; VLDL, very low density lipoprotein.

whether it had broader clinical relevance, modifying the risk of steatohepatitis or fibrosis as has been shown for *PNPLA3*.⁴³ Candidate gene studies addressed the effect of the *TM6SF2* rs58542926 variant on presence of steatohepatitis and severity of NAFLD-associated hepatic fibrosis/cirrhosis. The first such study, in a cohort of > 1,000 histologically characterized European Caucasian patients demonstrated that carriage of each copy of the E167K variant was associated with a near twofold increased risk of advanced fibrosis (OR = 1.88 [1.41–2.5] per copy of the minor allele carried), independent of confounding factors including age, diabetes, obesity, or *PNPLA3* genotype.⁵⁰ This association has since been independently validated in another large European cohort.¹⁰⁷ Two studies, including one from China, have failed to replicate the association,^{108,109} although an association with NAFLD has been demonstrated in another Chinese population.¹¹⁰ These two negative results may in part be due to the generally low minor allele frequency of *TM6SF2* rs58542926 and interethnic variations in its carriage. A minor allele frequency of 0.07 in Europeans, 0.04 in Hispanics, and 0.02 in African Americans has been reported,¹¹¹ meaning that the effect of the NAFLD promoting *TM6SF2* allele will be more apparent in individuals of European ancestry than Hispanic or African ancestry. Inadequate statistical power to detect an effect on histological markers of disease progression coupled with a cohort exhibiting predominantly mild disease were contributory flaws in the small South American study.¹⁰⁹ This study contained only 226 NAFLD cases with histologically characterized disease of which 96 had simple steatosis and the remaining 130 exhibited only minimally active disease with little fibrosis (mean fibrosis stage of 1.4 out of 4). Conclusions drawn from this low quality study are questionable and are likely to represent type 2 error. Thus, the modifier role exerted by *TM6SF2* rs58542926 (E167K) at the 19p13.11 locus has been robustly demonstrated across several large independent cohorts for NAFLD phenotypes ranging from steatosis to advanced fibrosis/cirrhosis.^{39,43,50,107,110,112}

The Mechanistic Effects of *TM6SF2* Variants

Little is known of the precise protein structure or functional role of the *TM6SF2* gene product beyond that it is a multipass membrane protein.¹¹³ Based on the analysis of coexpressed gene profiles in the Mouse Genome Informatics (MGI) database, *TM6SF2* is predicted to function as a lipid transporter and may interact with proteins involved in intestinal absorption.¹¹⁴ The *TM6SF2* rs58542926 (E167K) missense mutation maps to an exposed nontransmembrane domain. Its effects appear to extend beyond liver disease alone. Using confocal microscopy, investigators observed localization of GFP-tagged *TM6SF2* to the endoplasmic reticulum and ER-Golgi intermediate compartments (ERGIC) in two human hepatoma cell lines.¹¹² In a series of elegant in vitro experiments they also found that knockdown of *TM6SF2* reduced secretion of triglyceride-rich lipoproteins and APOB. This led to increased cellular triglyceride accumulation, which at the sub-cellular level, manifested as a marked increase in lipid droplet number and average size. Conversely, overexpression of *TM6SF2* caused a decrease in the number and average size of lipid droplets.¹¹²

A GWAS seeking modifiers of serum lipid levels and cardiovascular disease risk published around the same time as the studies linking *TM6SF2* E167K with NAFLD supports these findings.¹¹⁵ Holmen et al provided evidence that carriage of the more common *TM6SF2* rs58542926 major allele was associated with dyslipidemia (raised serum LDL cholesterol and triglyceride) and increased myocardial infarction/cardiovascular disease risk while minor allele carriage was protective.¹¹⁵ This finding was also consistent with several earlier reports regarding the 19p13.11 locus that had variously implicated the nearby genes *NCAN*, *PBX4*, and *GMIP* with dyslipidemia, associations that may probably now be ascribed to *TM6SF2*.^{39,48,49} The association of *TM6SF2* with dyslipidemia and atherosclerosis risk in humans was validated by another study examining two separate groups (a NAFLD patient cohort and an obese population cohort). Patients with NAFLD that carried the E167K variant encoding the minor allele were found to have a lower risk of developing carotid plaques (OR = 0.49, 95% CI = 0.25–0.94) while E167K carriers among a group of 1,819 obese individuals had significantly lower serum lipid levels and a lower incidence of cardiovascular events (hazard ratio = 0.61; 95% CI = 0.39–0.95).¹⁰⁷ As demonstrated in a study examining 300 Finnish subjects, the effects of *TM6SF2* on HTGC and circulating lipid profiles are mediated without inducing greater hepatic insulin resistance.¹¹⁶ Indeed, the effects of insulin on glucose production and lipolysis were significantly higher in carriers of the *TM6SF2* E167K variant than in wild-type subjects.¹¹⁶ The most recent GWAS, addressing the impact of low-frequency and rare variants on lipid levels, examined 9.6 million genetic variants achieved through 1,000 Genomes Project imputation in 62,166 samples.¹¹⁴ Here, two variants within *TM6SF2* were identified that together explained the entire 19p13.11 regional association with

lipid levels (TC, LDL-C, and TG). These variants were rs58542926 (E167K) and a second, less common, missense mutation located in the fifth transmembrane domain (rs1874290064, L156P).¹¹⁴

Further support for the parallel effects of *TM6SF2* on NAFLD pathogenesis and hepatic lipid handling is provided by a series of in vivo studies observing the effects of altered gene expression in mice. Overexpression of human wild-type *TM6SF2* in liver resulted in higher total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, triglycerides (TGs), and lower high-density lipoprotein (HDL) cholesterol, whereas knockdown of mouse *Tm6sf2* in liver resulted in decreased serum TC.¹¹⁵ Knock down of mouse *Tm6sf2* in vivo also resulted in increased hepatic lipid accumulation and a reduction in serum TG, LDL, and VLDL secretion.⁴³ Taken together, these in vivo^{43,115} and in vitro^{43,112,115} functional studies suggest that *TM6SF2* controls hepatic lipid efflux, with its deletion or mutation resulting in a reduction in lipoprotein secretion (VLDL, TG, and APOB) coincident with increased hepatocellular lipid droplet size and TG accumulation. Because the E167K variant results in lower TC in humans and expression levels of the E167K protein is lower than the wild-type form, these data further indicate that the E167K variant confers a loss of function to the protein that may be both qualitative and quantitative.^{43,115}

***TM6SF2* and Cardiovascular Disease: The “Catch-22” Paradigm**

Based on available evidence, we recently described the “*TM6SF2* Catch-22” paradigm.¹¹⁷ Here, on a background of insulin resistance and metabolic stress, *TM6SF2* acts as a determinant of metabolic syndrome-related end-organ damage and thus clinical outcome: protecting the liver at the

Fig. 3 The “*TM6SF2* Catch-22.” On a background of insulin resistance and metabolic stress, *TM6SF2* acts as a determinant of metabolic syndrome related end-organ damage and thus clinical outcome: Protecting the liver at the expense of increased risk of atherosclerosis and cardiovascular disease or vice versa. LDL, low-density lipoprotein; T2DM, type 2 diabetes mellitus; TG, triglyceride; VLDL, very low-density lipoprotein. (Modified from Kahali B, Liu YL, Daly AK, Day CP, Anstee QM, Speliotes EK. *TM6SF2*: catch-22 in the fight against nonalcoholic fatty liver disease and cardiovascular disease? *Gastroenterology* 2015;148(4):679–684¹¹⁷ with permission.)

Table 3 Summary of genetic modifiers of nonalcoholic fatty liver disease

Gene	Protein	Study details and comments
Glucose metabolism and insulin resistance		
ENPP1; IRS1	Ectonucleotide pyrophosphatase/phosphodiesterase family member 1; Insulin receptor substrate 1	Functional variants in <i>ENPP1/PC-1</i> and <i>IRS1</i> impair insulin receptor signaling and promote insulin resistance. ^{133,134} In 702 biopsy-proven NAFLD cases, carriage of nonsynonymous SNPs in <i>ENPP1</i> (rs1044498, encoding Lys121Gln) and <i>IRS1</i> (rs1801278, encoding Gln972Arg) reduced AKT activation, promoted insulin resistance, and were independently associated with greater fibrosis. ¹³⁵
GCKR	Glucokinase regulatory protein	<i>GCKR</i> SNP rs780094 is in strong LD with a functional nonsynonymous SNP (rs1260326, encoding Pro446Leu) and has been associated with hepatic TG accumulation in several studies. ^{39,59} It is also associated with greater NAFLD fibrosis. ¹³⁶
SLC2A1	Solute carrier family 2, facilitated glucose transporter member 1	A study examining 3,072 SNPs across 92 candidate genes identified variants in <i>SLC2A1</i> associated with NAFLD, independent of insulin resistance or T2DM. ¹³⁷ Downregulation of <i>SLC2A1</i> in vitro promoted lipid accumulation and increased oxidative stress, potentially linking the key pathogenic features of NAFLD: oxidative injury and increased lipid storage.
TCF7L2	Transcription factor 7-like 2	A role for <i>TCF7L2</i> , which has a key role in Wnt signaling and has been implicated in T2DM, has been reported in NAFLD. ¹³⁸
PPARG	Peroxisome proliferator-activated receptor γ	A loss-of-function SNP (rs1805192, encoding Pro12Ala) impairs transcriptional activation and affects insulin sensitivity. ¹³⁹ Carriage of haplotypes including the Pro12Ala allele was associated with progressive NAFLD, ¹⁴⁰ but two studies found no association. ^{141,142}
Steatosis		
Hepatic lipid import or synthesis		
SLC27A5	Very long chain acyl-CoA synthetase	Two principal isoforms of FATPs are expressed in the liver, <i>SLC27A2</i> (also known as <i>FATP2</i>) and <i>SLC27A5</i> (also known as <i>FATP5</i>). ¹⁴³ Silencing <i>SLC27a5</i> reverses diet-induced NAFLD and improves hyperglycemia in mice. ¹⁴⁴ Carriage of the <i>SLC27A5</i> rs56225452 promoter region polymorphism has been associated with higher ALT levels, and greater postprandial insulin and triglyceride levels. ¹⁴⁴ In patients with histologically proven NAFLD, the effect of BMI on degree of steatosis differed with <i>SLC27A5</i> genotype. ¹⁴⁵
FADS1	Fatty acid desaturase 1	Alleles associated with decreased hepatic expression of <i>FADS1</i> were associated with greater HTGC. ¹²⁸
LPIN1	Phosphatidate phosphatase LPIN1	LPIN1 is required for adipogenesis and the normal metabolic flux between adipose tissue and liver, where it also acts as an inducible transcriptional

Table 3 (Continued)

Gene	Protein	Study details and comments
		coactivator to regulate fatty acid metabolism. ^{146,147} Variants have been associated with multiple components of the metabolic syndrome. ^{146,148} Although a large case-control study found no association with T2DM, obesity, or related traits in 17,538 individuals, ¹⁴⁹ a meta-analysis in 8,504 individuals found that the <i>LPIN1</i> rs13412852 [T] allele was associated with lower BMI and insulin levels. ¹⁵⁰ This same polymorphism was underrepresented in pediatric (but not adult) NAFLD with a suggestion of less severe liver damage. ¹⁵¹
Hepatic lipid export or oxidation in steatosis		
<i>PNPLA3</i>	Patatin-like phospholipase domain-containing 3	The nonsynonymous 617C > G nucleotide transversion mutation SNP (rs738409, encoding Ile148Met) has been consistently associated with steatosis, steatohepatitis, and hepatic fibrosis; however, function remains incompletely understood. ^{26,62}
<i>TM6SF2</i>	Transmembrane 6 super family 2	The <i>TM6SF2</i> rs58542926 minor allele is associated with greater steatosis, steatohepatitis, and NAFLD fibrosis. The major allele is associated with dyslipidemia and greater CVD risk. ^{43,50,107,115}
<i>NR1H2</i>	Nuclear receptor subfamily 1 group I member 2 (also known as pregnane X receptor)	<i>NR1H2</i> encodes a transcription factor that regulates hepatic detoxification ¹⁵² and acts through CD36 (fatty-acid translocase) and various lipogenic enzymes to control lipid metabolism. ¹⁵³ <i>Nr1h2</i> -deficient mice develop steatosis. ¹⁵³ Two SNPs (rs7643645 and rs2461823) were associated with NAFLD and were also a predictor of disease severity. ¹⁵⁴
<i>PPARα</i>	Peroxisome proliferator-activated receptor α	<i>PPAR-α</i> is a molecular sensor for long chain fatty acids, eicosanoids, and fibrates ¹⁵⁵ ; activated by increased hepatocyte fatty-acid load, it limits TAG accumulation by increasing fatty acid oxidation Carriage of a nonsynonymous SNP (rs1800234, encoding Val27Ala) increases activity, and was associated with NAFLD despite reduced BMI. ^{156,157} A loss-of-function polymorphism (rs1800206, encoding Leu162Val) was not associated with NAFLD. ¹⁴²
<i>PEMT</i>	Phosphatidylethanolamine N-methyltransferase	Two studies have reported an association between NAFLD and a nonsynonymous <i>PEMT</i> exon 8 590G > A transversion (rs7946, encoding Val175Met). ^{126,127}
<i>MTTP</i>	Microsome triglyceride transfer protein large subunit	<i>MTTP</i> mediates hepatic synthesis and secretion of VLDL. Abetalipoproteinemia (OMIM#200100) results from a loss-of-function frameshift mutation in <i>MTTP</i> ; however, whereas this mutation causes severe hepatic TAG accumulation, steatohepatitis and fibrosis are infrequent. ¹⁵⁸ A promoter region transversion (−493G > T; rs1800591), predisposed to steatosis and NASH ^{124,159} in a small cohort, but a larger study in 131 patients found no association. ¹⁶⁰

(Continued)

Table 3 (Continued)

Gene	Protein	Study details and comments
APOC3	Apolipoprotein CIII	Two promoter region SNPs -455T > C; rs2854116 and -482C > T; rs2854117 that increased steatosis were reported in small ($n = 95$ and 163) cohorts of Asian-Indian and non-Asian ethnicity. ¹⁶¹ To date, studies together examining > 4,000 individuals have been unable to replicate these findings. ¹⁶²⁻¹⁶⁵
APOE	Apolipoprotein E	ApoE is a plasma protein involved in lipid transport and metabolism. ¹⁶⁶ Three alleles ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$) determine three isoforms (ApoE2, ApoE3, and ApoE4) resulting in six ApoE genotypes (E2/2, E3/3, E4/4, E2/3, E2/4, E3/4). Overall homozygosity for the $\epsilon 2$ allele in one study was associated with dyslipidemia, but not NAFLD. ¹⁶⁷ In a subgroup of nonobese individuals, the $\epsilon 2$ allele and the E2/3 genotype were more prevalent in controls, suggesting this allele might be protective. ¹⁶⁷ Consistent with this result, the ApoE3/3 genotype was associated with NASH in a Turkish cohort, whereas ApoE3/4 was protective. ¹⁶⁸
Steatohepatitis		
Oxidative stress		
HFE	Hereditary hemochromatosis protein	Hepatic iron accumulation promotes oxidative stress. Two studies, examining 177 patients, reported carriage of an HFE polymorphism (rs1800562, encoding Cys282Tyr) that was associated with more severe steatohepatitis and advanced fibrosis. ^{169,170} However, three other studies ¹⁷¹⁻¹⁷³ have not shown increased carriage of either the Cys282Tyr or His63Asp (rs1799945) mutations. Meta-analyses have also provided conflicting results, with the latest finding no evidence of an effect. ^{174,175}
GCLC; GCLM	Glutamate-cysteine ligase catalytic unit; glutamate-cysteine ligase regulatory unit	Glutamate-cysteine ligase (γ -glutamyl cysteine synthetase) is the rate-limiting step in glutathione synthesis; absence of Gclc causes steatosis and liver failure in mice. ¹⁷⁶ A study of 131 patients with NAFLD found the GCLC promoter region polymorphism (-129C > T, rs17883901) was associated with steatohepatitis compared with simple steatosis. ¹⁶⁰
ABCC2	ATP-binding cassette, subfamily C (CFTR/MRP), member 2	Association studies support a role for ABCC2 (also known as MRP2), which facilitates terminal excretion and detoxification of endogenous and xenobiotic organic anions, including lipid peroxidation products. ¹⁷⁷
SOD2	Superoxide dismutase [Mn], mitochondrial	Carriage of the nonsynonymous SNP (rs4880, encoding Ala16Val) has been associated with advanced hepatic fibrosis in NAFLD in both Japanese ¹²⁴ and European ¹²⁵ cohorts.

Table 3 (Continued)

Gene	Protein	Study details and comments
Endotoxin response		
TLR4	Toll-like receptor 4	Study of a spontaneous <i>Tlr4</i> null mutation in C3H/J mice has established the contribution of TLR4/endotoxin to NAFLD pathogenesis in the laboratory. ¹⁷⁸ <i>TLR4</i> polymorphisms (rs4986791 and rs4986790) influence hepatitis-C related fibrosis, ^{179,180} but no association with NAFLD and either <i>TLR4</i> or <i>NOD2</i> (bacterial cell wall peptidoglycan receptor) variants has been found.
CD14	Monocyte differentiation antigen CD14	CD14 is a lipopolysaccharide receptor expressed on monocytes, macrophages, and neutrophils that enhances TLR4 endotoxin signaling. An association with a promoter-region polymorphism (–159C > T, rs2569190) that increases <i>CD14</i> expression has been reported. ^{181,182}
Cytokines		
TNF	Tumour necrosis factor	A <i>TNF</i> (–238G > A, rs361525) promoter polymorphism has been associated with NASH, ^{183,184} suggesting a primary role in the transition from steatosis to steatohepatitis; a separate study, found that two other promoter region polymorphisms (–1031T > C, rs1799964 and –863C > A, rs1800630) were more common in NASH than steatosis, but were no more common in NAFLD than a control population. ¹⁸⁴
IL6	Interleukin 6	An <i>IL6</i> promoter region polymorphism (–174G > C, rs1800795) has been associated with NASH. ¹⁸⁵
IFNL4	Interferon lambda 4	The intronic rs12979860 SNP in <i>IFNL4</i> is a strong predictor of fibrosis in an etiology-independent manner, including a cohort of 488 NAFLD cases. Those with rs12979860 CC had greater hepatic inflammation and fibrosis. ¹⁸⁶
Fibrosis		
AGTR1	Type-1 angiotensin II receptor	Two studies have linked the <i>AGTR1</i> rs3772622 SNP with grade of steatohepatitis and stage of fibrosis, with the most recent study also suggesting an interaction with <i>PNPLA3</i> genotype. ^{187,188}
<i>KLF6</i>	Kruppel-like factor 6	The <i>KLF6</i> –IVS1–27G > A (rs3750861) SNP has been associated with milder NAFLD-related hepatic fibrosis in three separate European cohorts. ¹³⁰

Source: Modified from Anstee QM, Day CP. The genetics of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013;10(11):645–655.¹⁴

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; FATP, fatty acid transport protein; HTDC, hepatic triglyceride content; LD, linkage disequilibrium; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SNP, single nucleotide polymorphism; TG, triglyceride; T2DM, type 2 diabetes mellitus; VLDL, very low density lipoprotein.

expense of increased risk of atherosclerosis and cardiovascular disease or vice versa (►Fig. 3).¹¹⁷ It is widely accepted that NAFLD is associated with cardiovascular disease, indeed the majority of patients with NAFLD will ultimately die a cardiovascular rather than a liver-related death.^{3,10,57} However, these conditions can be dissociated: Individuals carrying the minor (T) allele of *TM6SF2* rs58542926 (167K) appear prone to developing NAFLD with advanced fibrosis and so are more likely to experience liver-related rather than cardiovascular morbidity and mortality.^{43,50,107} Conversely, carriage of the C-allele is associated with dyslipidemia and cardiovascular disease.^{107,115} As yet, it remains unclear if the effects of *TM6SF2* variants are sufficient to eclipse the many environmental and other genetic factors that determine disease outcome at an individual level, and so merit inclusion of genotype testing within a stratified medicine approach. Nevertheless, further study of this and other NAFLD-associated human genetic variants will greatly inform our understanding of the pathophysiology and interrelationship between NAFLD and its associated metabolic traits.

Other Genetic Modifiers in NAFLD

The current understanding of disease pathogenesis has been achieved through both clinical research and the translational study of specific animal models.¹² It is generally accepted that the initiating events in NAFLD are dependent on the development of obesity and insulin resistance.³ Together, these produce an increased free fatty acid (FFA) flux within the liver which in turn places hepatocytes under considerable metabolic load and promotes hepatocyte lipotoxicity, increased oxidative stress secondary to free radicals produced during β - and ω -FFA oxidation, and endoplasmic reticulum stress. Hepatocellular TAG accumulation (steatosis) is a histologically apparent epiphenomenon reflecting these metabolic changes and is best considered an early adaptive response through which potentially lipotoxic FFAs are partitioned into relatively stable intracellular TAG stores.¹¹⁸ Ultimately, these insults combine with the additive effects of endotoxin/TLR4 induced Kupffer cell cytokine release and immune mediated hepatocellular injury to induce cellular damage and activate cell death pathways, marking the transition to steatohepatitis.^{119–121} If these processes persist stellate cell activation, collagen deposition and hepatic fibrosis occur.¹²²

Potential genetic modifiers therefore fall into four broad categories: (1) those that influence glucose metabolism and insulin sensitivity; (2) those that perturb the handling of fatty acids and the accumulation of TAG in the liver; (3) those that determine progression to NASH (for example, modifiers of oxidative stress, endotoxin response or cytokine and adipokine activity); and (4) those that might influence hepatic fibrogenesis.¹³ Drawing on the results of the GWAS discussed earlier and the numerous candidate gene studies that have been reported, ►Table 3 summarizes the genes that have been associated with aspects of the NAFLD phenotype according to this schema. These are discussed in depth in our previous reviews of the field.^{13,14} It should be remembered, however, that only a minority of the

genes that have been associated with NAFLD either in GWAS or through candidate-gene analysis have been independently validated and can be considered of proven importance. Indeed, if only those genes that have been robustly validated in large independent studies or through the use of transmission disequilibrium testing¹²³ are considered, the list only includes patatin-like phospholipase domain-containing 3 (*PNPLA3*),^{26,62–66} transmembrane 6 superfamily member 2 (*TM6SF2*),^{43,50,107} mitochondrial superoxide dismutase 2 (*SOD2*),^{124,125} phosphatidylethanolamine N-methyltransferase (*PEMT*),^{126,127} fatty acid desaturase 1 (*FADS1*)¹²⁸ and Kruppel-like factor-6 (*KLF6*).^{129,130}

Conclusions

Nonalcoholic fatty liver disease is a complex disease in which subtle interpatient genetic variations and environmental factors interact to determine disease phenotype and progression. The relative importance of these factors will vary between populations depending on background modifier genes, lifestyle choices/challenges, and other factors such as the intestinal microbiome. Beyond identifying associations, it is important to understand the mechanisms through which these variations exert effects and to translate these findings into clinical utility where possible.

Abbreviations

¹ H-MRS	proton magnetic resonance spectroscopy
ALT	alanine transaminase
AST	aspartate aminotransferase
ATGL	adipose triglyceride lipase
BMI	body mass index
<i>FDFT1</i>	farnesyl diphosphate farnesyl transferase 1
GCKR	glucokinase regulator
GWASs	genome-wide association studies
HCC	hepatocellular carcinoma
HTGC	hepatic triglyceride content
LYPLAL	lysophospholipase-like
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
<i>PNPLA3</i>	patatin-like phospholipase domain containing 3
SNPs	single nucleotide polymorphisms
T2DM	type 2 diabetes mellitus
TAG	triacylglycerol
TG	triglyceride
<i>TM6SF2</i>	transmembrane 6 superfamily member 2
VLDL	very low-density lipoprotein

Acknowledgments

QMA is the recipient of a Clinical Senior Lectureship Award from the Higher Education Funding Council for England (HEFCE). QMA and CPD are members of the EPoS (Elucidating Pathways of Steatohepatitis) consortium funded by the Horizon 2020 Framework Program of the European Union under Grant Agreement 634413.

References

- Anstee QM, McPherson S, Day CP. How big a problem is non-alcoholic fatty liver disease? *BMJ* 2011;343:d3897
- Sanyal AJ; American Gastroenterological Association. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology* 2002;123(5):1705–1725
- Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol* 2013;10(6):330–344
- Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010;53(2):372–384
- Das K, Das K, Mukherjee PS, et al. Nonobese population in a developing country has a high prevalence of nonalcoholic fatty liver and significant liver disease. *Hepatology* 2010;51(5):1593–1602
- Charlton MR, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology* 2011;141(4):1249–1253
- Satapathy MBBS, Sanyal AJ. Epidemiology and natural history of nonalcoholic fatty liver Disease. *Semin Liver Dis* 2015;35(3):221–235
- Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol* 2015;13(4):643–54. e1, 9, quiz e39–e40
- McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosis-steatohepatitis using paired biopsies: implications for prognosis and clinical management. *J Hepatol* 2015;62(5):1148–1155
- Angulo P. The natural history of NAFLD. In: Farrell G, McCulloch AJ, Day C, eds. *Non-Alcoholic Fatty Liver Disease: A Practical Guide*. Hoboken, NJ: Wiley Blackwell Press; 2013:37–45
- Day CP. Pathogenesis of steatohepatitis. *Best Pract Res Clin Gastroenterol* 2002;16(5):663–678
- Anstee QM, Goldin RD. Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. *Int J Exp Pathol* 2006;87(1):1–16
- Anstee QM, Daly AK, Day CP. Genetics of alcoholic and nonalcoholic fatty liver disease. *Semin Liver Dis* 2011;31(2):128–146
- Anstee QM, Day CP. The genetics of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013;10(11):645–655
- Hirschhorn JN, Gajdos ZK. Genome-wide association studies: results from the first few years and potential implications for clinical medicine. *Annu Rev Med* 2011;62:11–24
- Altshuler D, Daly MJ, Lander ES. Genetic mapping in human disease. *Science* 2008;322(5903):881–888
- Willner IR, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. *Am J Gastroenterol* 2001;96(10):2957–2961
- Struben VM, Hespeneide EE, Caldwell SH. Nonalcoholic steatohepatitis and cryptogenic cirrhosis within kindreds. *Am J Med* 2000;108(1):9–13
- Schwimmer JB, Celedon MA, Lavine JE, et al. Heritability of nonalcoholic fatty liver disease. *Gastroenterology* 2009;136(5):1585–1592
- Makkonen J, Pietiläinen KH, Rissanen A, Kaprio J, Yki-Järvinen H. Genetic factors contribute to variation in serum alanine aminotransferase activity independent of obesity and alcohol: a study in monozygotic and dizygotic twins. *J Hepatol* 2009;50(5):1035–1042
- Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004;40(6):1387–1395
- Browning JD, Kumar KS, Saboorian MH, Thiele DL. Ethnic differences in the prevalence of cryptogenic cirrhosis. *Am J Gastroenterol* 2004;99(2):292–298
- Guerrero R, Vega GL, Grundy SM, Browning JD. Ethnic differences in hepatic steatosis: an insulin resistance paradox? *Hepatology* 2009;49(3):791–801
- Bambha K, Belt P, Abraham M, et al; Nonalcoholic Steatohepatitis Clinical Research Network Research Group. Ethnicity and nonalcoholic fatty liver disease. *Hepatology* 2012;55(3):769–780
- Szczepaniak LS, Nurenberg P, Leonard D, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005;288(2):E462–E468
- Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008;40(12):1461–1465
- Reich DE, Lander ES. On the allelic spectrum of human disease. *Trends Genet* 2001;17(9):502–510
- Wang WY, Barratt BJ, Clayton DG, Todd JA. Genome-wide association studies: theoretical and practical concerns. *Nat Rev Genet* 2005;6(2):109–118
- Pritchard JK. Are rare variants responsible for susceptibility to complex diseases? *Am J Hum Genet* 2001;69(1):124–137
- Hindorf LA, Sethupathy P, Junkins HA, et al. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc Natl Acad Sci U S A* 2009;106(23):9362–9367
- Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 2003;33(2):177–182
- Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. *Nature* 2009;461(7265):747–753
- Cardon LR, Bell JL. Association study designs for complex diseases. *Nat Rev Genet* 2001;2(2):91–99
- Hirschhorn JN. Genomewide association studies—illuminating biologic pathways. *N Engl J Med* 2009;360(17):1699–1701
- Manolio TA, Brooks LD, Collins FS. A HapMap harvest of insights into the genetics of common disease. *J Clin Invest* 2008;118(5):1590–1605
- Hardy J, Singleton A. Genomewide association studies and human disease. *N Engl J Med* 2009;360(17):1759–1768
- Hirschhorn JN, Daly MJ. Genome-wide association studies for common diseases and complex traits. *Nat Rev Genet* 2005;6(2):95–108
- Chalasani N, Guo X, Loomba R, et al; Nonalcoholic Steatohepatitis Clinical Research Network. Genome-wide association study identifies variants associated with histologic features of nonalcoholic fatty liver disease. *Gastroenterology* 2010;139(5):1567–1576. e1–e6
- Speliotes EK, Yerges-Armstrong LM, Wu J, et al; NASH CRN; GIANT Consortium; MAGIC Investigators; GOLD Consortium. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet* 2011;7(3):e1001324
- Anstee QM, Darlay R, Leathart JB, et al. Genome-wide association analysis confirms importance of PNPLA3 and identifies novel variants associated with histologically progressive steatohepatitis in NAFLD. *Hepatology* 2012;56:265A–266A
- Kawaguchi T, Sumida Y, Umemura A, et al; Japan Study Group of Nonalcoholic Fatty Liver Disease; Takeshi Okanoue. Genetic polymorphisms of the human PNPLA3 gene are strongly associated with severity of non-alcoholic fatty liver disease in Japanese. *PLoS ONE* 2012;7(6):e38322

- 42 Feitosa MF, Wojczynski MK, North KE, et al. The ERLIN1-CHUK-CWF19L1 gene cluster influences liver fat deposition and hepatic inflammation in the NHLBI Family Heart Study. *Atherosclerosis* 2013;228(1):175–180
- 43 Kozlitina J, Smagris E, Stender S, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014;46(4):352–356
- 44 DiStefano JK, Kingsley C, Craig Wood G, et al. Genome-wide analysis of hepatic lipid content in extreme obesity. *Acta Diabetol* 2015;52(2):373–382
- 45 Victor RG, Haley RW, Willett DL, et al; Dallas Heart Study Investigators. The Dallas Heart Study: a population-based probability sample for the multidisciplinary study of ethnic differences in cardiovascular health. *Am J Cardiol* 2004;93(12):1473–1480
- 46 Romeo S, Huang-Doran I, Baroni MG, Kotronen A. Unravelling the pathogenesis of fatty liver disease: patatin-like phospholipase domain-containing 3 protein. *Curr Opin Lipidol* 2010;21(3):247–252
- 47 Gorden A, Yang R, Yerges-Armstrong LM, et al; GOLD Consortium. Genetic variation at NCAN locus is associated with inflammation and fibrosis in non-alcoholic fatty liver disease in morbid obesity. *Hum Hered* 2013;75(1):34–43
- 48 Kathiresan S, Melander O, Guiducci C, et al. Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. *Nat Genet* 2008;40(2):189–197
- 49 Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* 2010;466(7307):707–713
- 50 Liu YL, Reeves HL, Burt AD, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun* 2014;5:4309
- 51 Kleiner DE, Brunt EM, Van Natta M, et al; Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;41(6):1313–1321
- 52 Ballestri S, Day CP, Daly AK. Polymorphism in the farnesyl diphosphate farnesyl transferase 1 gene and nonalcoholic fatty liver disease severity. *Gastroenterology* 2011;140(5):1694–1695
- 53 Kitamoto T, Kitamoto A, Yoneda M, et al. Genome-wide scan revealed that polymorphisms in the PNPLA3, SAMM50, and PARVB genes are associated with development and progression of nonalcoholic fatty liver disease in Japan. *Hum Genet* 2013;132(7):783–792
- 54 Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999;116(6):1413–1419
- 55 Mofrad P, Contos MJ, Haque M, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. *Hepatology* 2003;37(6):1286–1292
- 56 Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011;34(3):274–285
- 57 Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011;43(8):617–649
- 58 Yuan X, Waterworth D, Perry JR, et al. Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. *Am J Hum Genet* 2008;83(4):520–528
- 59 Chambers JC, Zhang W, Sehmi J, et al; Alcohol Genome-wide Association (AlcGen) Consortium; Diabetes Genetics Replication and Meta-analyses (DIAGRAM+) Study; Genetic Investigation of Anthropometric Traits (GIANT) Consortium; Global Lipids Genetics Consortium; Genetics of Liver Disease (GOLD) Consortium; International Consortium for Blood Pressure (ICBP-GWAS); Meta-analyses of Glucose and Insulin-Related Traits Consortium (MAGIC). Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. *Nat Genet* 2011;43(11):1131–1138
- 60 Aulchenko YS, Ripatti S, Lindqvist I, et al; ENGAGE Consortium. Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. *Nat Genet* 2009;41(1):47–55
- 61 Willer CJ, Sanna S, Jackson AU, et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. *Nat Genet* 2008;40(2):161–169
- 62 Valenti L, Al-Serri A, Daly AK, et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2010;51(4):1209–1217
- 63 Kotronen A, Johansson LE, Johansson LM, et al. A common variant in PNPLA3, which encodes adiponutrin, is associated with liver fat content in humans. *Diabetologia* 2009;52(6):1056–1060
- 64 Sookoian S, Castaño GO, Burgueño AL, Gianotti TF, Rosselli MS, Pirola CJ. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. *J Lipid Res* 2009;50(10):2111–2116
- 65 Kantartzis K, Peter A, Machicao F, et al. Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. *Diabetes* 2009;58(11):2616–2623
- 66 Rotman Y, Koh C, Zmuda JM, Kleiner DE, Liang TJ; NASH CRN. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. *Hepatology* 2010;52(3):894–903
- 67 Romeo S, Sentinelli F, Cambuli VM, et al. The 148M allele of the PNPLA3 gene is associated with indices of liver damage early in life. *J Hepatol* 2010;53(2):335–338
- 68 Valenti L, Alisi A, Galmuzzi E, et al. I148M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric nonalcoholic fatty liver disease. *Hepatology* 2010;52(4):1274–1280
- 69 Santoro N, Kursawe R, D'Adamo E, et al. A common variant in the patatin-like phospholipase 3 gene (PNPLA3) is associated with fatty liver disease in obese children and adolescents. *Hepatology* 2010;52(4):1281–1290
- 70 Kollerits B, Coassin S, Kiechl S, et al. A common variant in the adiponutrin gene influences liver enzyme values. *J Med Genet* 2010;47(2):116–119
- 71 Sevastianova K, Kotronen A, Gastaldelli A, et al. Genetic variation in PNPLA3 (adiponutrin) confers sensitivity to weight loss-induced decrease in liver fat in humans. *Am J Clin Nutr* 2011;94(1):104–111
- 72 Shen J, Wong GL, Chan HL, et al. PNPLA3 gene polymorphism and response to lifestyle modification in patients with nonalcoholic fatty liver disease. *J Gastroenterol Hepatol* 2015;30(1):139–146
- 73 El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007;132(7):2557–2576
- 74 Nordenstedt H, White DL, El-Serag HB. The changing pattern of epidemiology in hepatocellular carcinoma. *Dig Liver Dis* 2010;42 (Suppl 3):S206–S214
- 75 [Anonymous] European Association For The Study Of The Liver; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012;56(4):908–943

- 76 Dyson J, Jaques B, Chattopadhyay D, et al. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *J Hepatol* 2014;60(1):110–117
- 77 Hassan MM, Kaseb A, Etzel CJ, et al. Genetic variation in the PNPLA3 gene and hepatocellular carcinoma in USA: risk and prognosis prediction. *Mol Carcinog* 2013;52(Suppl 1): E139–E147
- 78 Burza MA, Pirazzi C, Maglio C, et al. PNPLA3 I148M (rs738409) genetic variant is associated with hepatocellular carcinoma in obese individuals. *Dig Liver Dis* 2012;44(12):1037–1041
- 79 Guyot E, Sutton A, Rufat P, et al. PNPLA3 rs738409, hepatocellular carcinoma occurrence and risk model prediction in patients with cirrhosis. *J Hepatol* 2013;58(2):312–318
- 80 Nischalke HD, Berger C, Luda C, et al. The PNPLA3 rs738409 148M/M genotype is a risk factor for liver cancer in alcoholic cirrhosis but shows no or weak association in hepatitis C cirrhosis. *PLoS ONE* 2011;6(11):e27087
- 81 Trepo E, Guyot E, Ganne-Carrie N, et al. PNPLA3 (rs738409 C>G) is a common risk variant associated with hepatocellular carcinoma in alcoholic cirrhosis. *Hepatology* 2012;55(4):1307–1308
- 82 Trepo E, Nahon P, Bontempi G, et al. Association between the PNPLA3 (rs738409 C>G) variant and hepatocellular carcinoma: evidence from a meta-analysis of individual participant data. *Hepatology* 2014;59(6):2170–2177
- 83 Valenti L, Rumi M, Galmozzi E, et al. Patatin-like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. *Hepatology* 2011;53(3): 791–799
- 84 Falletti E, Fabris C, Cmet S, et al. PNPLA3 rs738409C/G polymorphism in cirrhosis: relationship with the aetiology of liver disease and hepatocellular carcinoma occurrence. *Liver Int* 2011;31(8): 1137–1143
- 85 Singal AG, Manjunath H, Yopp AC, et al. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *Am J Gastroenterol* 2014;109(3):325–334
- 86 Liu YL, Patman GL, Leathart JB, et al. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol* 2014;61(1):75–81
- 87 Krawczyk M, Stokes CS, Romeo S, Lammert F. HCC and liver disease risks in homozygous PNPLA3 p.I148M carriers approach monogenic inheritance. *J Hepatol* 2015;62(4):980–981
- 88 Anstee QM, Liu YL, Day CP, Reeves HL. Reply to: HCC and liver disease risk in homozygous PNPLA3 p.I148M carriers approach monogenic inheritance. *J Hepatol* 2015;62(4):982–983
- 89 Zimmermann R, Strauss JG, Haemmerle G, et al. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science* 2004;306(5700):1383–1386
- 90 Rydel TJ, Williams JM, Krieger E, et al. The crystal structure, mutagenesis, and activity studies reveal that patatin is a lipid acyl hydrolase with a Ser-Asp catalytic dyad. *Biochemistry* 2003; 42(22):6696–6708
- 91 He S, McPhaul C, Li JZ, et al. A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J Biol Chem* 2010;285(9):6706–6715
- 92 Romeo S, Sentinelli F, Dash S, et al. Morbid obesity exposes the association between PNPLA3 I148M (rs738409) and indices of hepatic injury in individuals of European descent. *Int J Obes* 2010;34(1):190–194
- 93 Speliotes EK, Butler JL, Palmer CD, Voight BF, Hirschhorn JN; GIANT Consortium; MIGen Consortium; NASH CRN. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. *Hepatology* 2010;52(3):904–912
- 94 Huang Y, Cohen JC, Hobbs HH. Expression and characterization of a PNPLA3 protein isoform (I148M) associated with nonalcoholic fatty liver disease. *J Biol Chem* 2011;286(43):37085–37093
- 95 Pirazzi C, Adiels M, Burza MA, et al. Patatin-like phospholipase domain-containing 3 (PNPLA3) I148M (rs738409) affects hepatic VLDL secretion in humans and in vitro. *J Hepatol* 2012;57(6): 1276–1282
- 96 Jenkins CM, Mancuso DJ, Yan W, Sims HF, Gibson B, Gross RW. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. *J Biol Chem* 2004;279(47):48968–48975
- 97 Kumari M, Schoiswohl G, Chitruju C, et al. Adiponutrin functions as a nutritionally regulated lysophosphatidic acid acyltransferase. *Cell Metab* 2012;15(5):691–702
- 98 Huang Y, He S, Li JZ, et al. A feed-forward loop amplifies nutritional regulation of PNPLA3. *Proc Natl Acad Sci U S A* 2010;107(17):7892–7897
- 99 Wilson PA, Gardner SD, Lambie NM, Commans SA, Crowther DJ. Characterization of the human patatin-like phospholipase family. *J Lipid Res* 2006;47(9):1940–1949
- 100 Hoekstra M, Li Z, Kruijt JK, Van Eck M, Van Berkel TJ, Kuiper J. The expression level of non-alcoholic fatty liver disease-related gene PNPLA3 in hepatocytes is highly influenced by hepatic lipid status. *J Hepatol* 2010;52(2):244–251
- 101 Lake AC, Sun Y, Li JL, et al. Expression, regulation, and triglyceride hydrolase activity of Adiponutrin family members. *J Lipid Res* 2005;46(11):2477–2487
- 102 Chen W, Chang B, Li L, Chan L. Patatin-like phospholipase domain-containing 3/adiponutrin deficiency in mice is not associated with fatty liver disease. *Hepatology* 2010;52(3): 1134–1142
- 103 Basantani MK, Sitnick MT, Cai L, et al. Pnpla3/Adiponutrin deficiency in mice does not contribute to fatty liver disease or metabolic syndrome. *J Lipid Res* 2011;52(2):318–329
- 104 Li JZ, Huang Y, Karaman R, et al. Chronic overexpression of PNPLA3I148M in mouse liver causes hepatic steatosis. *J Clin Invest* 2012;122(11):4130–4144
- 105 Smagris E, BasuRay S, Li J, et al. Pnpla3I148M knockin mice accumulate PNPLA3 on lipid droplets and develop hepatic steatosis. *Hepatology* 2015;61(1):108–118
- 106 Pirazzi C, Valenti L, Motta BM, et al. PNPLA3 has retinyl-palmitate lipase activity in human hepatic stellate cells. *Hum Mol Genet* 2014;23(15):4077–4085
- 107 Dongiovanni P, Petta S, Maglio C, et al. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. *Hepatology* 2015;61(2): 506–514
- 108 Wong VW, Wong GL, Tse CH, Chan HL. Prevalence of the TM6SF2 variant and non-alcoholic fatty liver disease in Chinese. *J Hepatol* 2014;61(3):708–709
- 109 Sookoian S, Castaño GO, Scian R, et al. Genetic variation in transmembrane 6 superfamily member 2 and the risk of nonalcoholic fatty liver disease and histological disease severity. *Hepatology* 2015;61(2):515–525
- 110 Wang X, Liu Z, Peng Z, Liu W. The TM6SF2 rs58542926 T allele is significantly associated with non-alcoholic fatty liver disease in Chinese. *J Hepatol* 2015;62(6):1438–1439
- 111 Palmer ND, Musani SK, Yerges-Armstrong LM, et al. Characterization of European ancestry nonalcoholic fatty liver disease-associated variants in individuals of African and Hispanic descent. *Hepatology* 2013;58(3):966–975
- 112 Mahdessian H, Taxiarchis A, Popov S, et al. TM6SF2 is a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content. *Proc Natl Acad Sci U S A* 2014; 111(24):8913–8918
- 113 Carim-Todd L, Escarceller M, Estivill X, Sumoy L. Cloning of the novel gene TM6SF1 reveals conservation of clusters of paralogous genes between human chromosomes 15q24—>q26 and 19p13.3 —>p12. *Cytogenet Cell Genet* 2000;90(3–4):255–260

- 114 Surakka I, Horikoshi M, Mägi R, et al; ENGAGE Consortium. The impact of low-frequency and rare variants on lipid levels. *Nat Genet* 2015;47(6):589–597
- 115 Holmen OL, Zhang H, Fan Y, et al. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. *Nat Genet* 2014;46(4):345–351
- 116 Zhou Y, Llauroadó G, Orešič M, Hyötyläinen T, Orho-Melander M, Yki-Järvinen H. Circulating triacylglycerol signatures and insulin sensitivity in NAFLD associated with the E167K variant in TM6SF2. *J Hepatol* 2015;62(3):657–663
- 117 Kahali B, Liu YL, Daly AK, Day CP, Anstee QM, Speliotes EK. TM6SF2: catch-22 in the fight against nonalcoholic fatty liver disease and cardiovascular disease? *Gastroenterology* 2015;148(4):679–684
- 118 Yamaguchi K, Yang L, McCall S, et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology* 2007;45(6):1366–1374
- 119 Malhi H, Gores GJ, Lemasters JJ. Apoptosis and necrosis in the liver: a tale of two deaths? *Hepatology* 2006;43(2, Suppl 1):S31–S44
- 120 Anstee QM, Concas D, Kudo H, et al. Impact of pan-caspase inhibition in animal models of established steatosis and non-alcoholic steatohepatitis. *J Hepatol* 2010;53(3):542–550
- 121 Farrell GC, Larter CZ, Hou JY, et al. Apoptosis in experimental NASH is associated with p53 activation and TRAIL receptor expression. *J Gastroenterol Hepatol* 2009;24(3):443–452
- 122 Iredale JP. Models of liver fibrosis: exploring the dynamic nature of inflammation and repair in a solid organ. *J Clin Invest* 2007;117(3):539–548
- 123 Spielman RS, Ewens WJ. The TDT and other family-based tests for linkage disequilibrium and association. *Am J Hum Genet* 1996;59(5):983–989
- 124 Namikawa C, Shu-Ping Z, Vyselaar JR, et al. Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in non-alcoholic steatohepatitis. *J Hepatol* 2004;40(5):781–786
- 125 Al-Serri A, Anstee QM, Valenti L, et al. The SOD2 C47T polymorphism influences NAFLD fibrosis severity: evidence from case-control and intra-familial allele association studies. *J Hepatol* 2012;56(2):448–454
- 126 Dong H, Wang J, Li C, et al. The phosphatidylethanolamine N-methyltransferase gene V175M single nucleotide polymorphism confers the susceptibility to NASH in Japanese population. *J Hepatol* 2007;46(5):915–920
- 127 Song J, da Costa KA, Fischer LM, et al. Polymorphism of the PEMT gene and susceptibility to nonalcoholic fatty liver disease (NAFLD). *FASEB J* 2005;19(10):1266–1271
- 128 Wang L, Athinarayanan S, Jiang G, Chalasani N, Zhang M, Liu W. Fatty acid desaturase 1 gene polymorphisms control human hepatic lipid composition. *Hepatology* 2015;61(1):119–128
- 129 Narla G, Difeo A, Reeves HL, et al. A germline DNA polymorphism enhances alternative splicing of the KLF6 tumor suppressor gene and is associated with increased prostate cancer risk. *Cancer Res* 2005;65(4):1213–1222
- 130 Miele L, Beale G, Patman G, et al. The Kruppel-like factor 6 genotype is associated with fibrosis in nonalcoholic fatty liver disease. *Gastroenterology* 2008;135(1):282–291.e1
- 131 Anstee QM, Darlay R, Leathart J, et al. A candidate-gene approach to validation of genetic modifier associations using a large cohort with histologically characterised non-alcoholic fatty liver disease. *J Hepatol* 2013;58:S46
- 132 Perttälä J, Huaman-Samanez C, Caron S, et al. PNPLA3 is regulated by glucose in human hepatocytes, and its I148M mutant slows down triglyceride hydrolysis. *Am J Physiol Endocrinol Metab* 2012;302(9):E1063–E1069
- 133 Grarup N, Urhammer SA, Ek J, et al. Studies of the relationship between the ENPP1 K121Q polymorphism and type 2 diabetes, insulin resistance and obesity in 7,333 Danish white subjects. *Diabetologia* 2006;49(9):2097–2104
- 134 McGettrick AJ, Feener EP, Kahn CR. Human insulin receptor substrate-1 (IRS-1) polymorphism G972R causes IRS-1 to associate with the insulin receptor and inhibit receptor autophosphorylation. *J Biol Chem* 2005;280(8):6441–6446
- 135 Dongiovanni P, Valenti L, Rametta R, et al. Genetic variants regulating insulin receptor signalling are associated with the severity of liver damage in patients with non-alcoholic fatty liver disease. *Gut* 2010;59(2):267–273
- 136 Petta S, Miele L, Bugianesi E, et al. Glucokinase regulatory protein gene polymorphism affects liver fibrosis in non-alcoholic fatty liver disease. *PLoS ONE* 2014;9(2):e87523
- 137 Vazquez-Chantada M, Gonzalez-Lahera A, Martinez-Arranz I, et al. Solute carrier family 2 member 1 is involved in the development of nonalcoholic fatty liver disease. *Hepatology* 2013;57(2):505–514
- 138 Musso G, Gambino R, Pacini G, Pagano G, Durazzo M, Cassader M. Transcription factor 7-like 2 polymorphism modulates glucose and lipid homeostasis, adipokine profile, and hepatocyte apoptosis in NASH. *Hepatology* 2009;49(2):426–435
- 139 Tönjes A, Scholz M, Loeffler M, Stumvoll M. Association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor gamma with pre-diabetic phenotypes: meta-analysis of 57 studies on nondiabetic individuals. *Diabetes Care* 2006;29(11):2489–2497
- 140 Gawrieh S, Marion MC, Komorowski R, et al. Genetic variation in the peroxisome proliferator activated receptor-gamma gene is associated with histologically advanced NAFLD. *Dig Dis Sci* 2012;57(4):952–957
- 141 Rey JW, Noetel A, Hardt A, et al. Pro12Ala polymorphism of the peroxisome proliferator-activated receptor γ 2 in patients with fatty liver diseases. *World J Gastroenterol* 2010;16(46):5830–5837
- 142 Dongiovanni P, Rametta R, Fracanzani AL, et al. Lack of association between peroxisome proliferator-activated receptors alpha and gamma2 polymorphisms and progressive liver damage in patients with non-alcoholic fatty liver disease: a case control study. *BMC Gastroenterol* 2010;10:102
- 143 Hirsch D, Stahl A, Lodish HF. A family of fatty acid transporters conserved from mycobacterium to man. *Proc Natl Acad Sci U S A* 1998;95(15):8625–8629
- 144 Doege H, Grimm D, Falcon A, et al. Silencing of hepatic fatty acid transporter protein 5 in vivo reverses diet-induced non-alcoholic fatty liver disease and improves hyperglycemia. *J Biol Chem* 2008;283(32):22186–22192
- 145 Auinger A, Valenti L, Pfeuffer M, et al. A promoter polymorphism in the liver-specific fatty acid transport protein 5 is associated with features of the metabolic syndrome and steatosis. *Horm Metab Res* 2010;42(12):854–859
- 146 Reue K, Zhang P. The lipin protein family: dual roles in lipid biosynthesis and gene expression. *FEBS Lett* 2008;582(1):90–96
- 147 Reue K. The lipin family: mutations and metabolism. *Curr Opin Lipidol* 2009;20(3):165–170
- 148 Wiedmann S, Fischer M, Koehler M, et al. Genetic variants within the LPIN1 gene, encoding lipin, are influencing phenotypes of the metabolic syndrome in humans. *Diabetes* 2008;57(1):209–217
- 149 Burgdorf KS, Sandholt CH, Sparsø T, et al. Studies of association between LPIN1 variants and common metabolic phenotypes among 17,538 Danes. *Eur J Endocrinol* 2010;163(1):81–87
- 150 Fawcett KA, Grimsey N, Loos RJ, et al. Evaluating the role of LPIN1 variation in insulin resistance, body weight, and human lipodystrophy in U.K. Populations. *Diabetes* 2008;57(9):2527–2533

- 151 Valenti L, Motta BM, Alisi A, et al. LPIN1 rs13412852 polymorphism in pediatric nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 2012;54(5):588–593
- 152 Zhang B, Xie W, Krasowski MD. PXR: a xenobiotic receptor of diverse function implicated in pharmacogenetics. *Pharmacogenomics* 2008;9(11):1695–1709
- 153 Zhou J, Zhai Y, Mu Y, et al. A novel pregnane X receptor-mediated and sterol regulatory element-binding protein-independent lipogenic pathway. *J Biol Chem* 2006;281(21):15013–15020
- 154 Sookoian S, Castaño GO, Burgueño AL, Gianotti TF, Rosselli MS, Pirola CJ. The nuclear receptor PXR gene variants are associated with liver injury in nonalcoholic fatty liver disease. *Pharmacogenet Genomics* 2010;20(1):1–8
- 155 Kim H, Haluzik M, Asghar Z, et al. Peroxisome proliferator-activated receptor- α agonist treatment in a transgenic model of type 2 diabetes reverses the lipotoxic state and improves glucose homeostasis. *Diabetes* 2003;52(7):1770–1778
- 156 Chen S, Li Y, Li S, Yu C. A Val227Ala substitution in the peroxisome proliferator activated receptor α (PPAR α) gene associated with non-alcoholic fatty liver disease and decreased waist circumference and waist-to-hip ratio. *J Gastroenterol Hepatol* 2008;23(9):1415–1418
- 157 Yamakawa-Kobayashi K, Ishiguro H, Arinami T, Miyazaki R, Hamaguchi H. A Val227Ala polymorphism in the peroxisome proliferator activated receptor α (PPAR α) gene is associated with variations in serum lipid levels. *J Med Genet* 2002;39(3):189–191
- 158 Lonardo A, Lombardini S, Scaglioni F, et al. Hepatic steatosis and insulin resistance: does etiology make a difference? *J Hepatol* 2006;44(1):190–196
- 159 Bernard S, Touzet S, Personne I, et al. Association between microsomal triglyceride transfer protein gene polymorphism and the biological features of liver steatosis in patients with type II diabetes. *Diabetologia* 2000;43(8):995–999
- 160 Oliveira CP, Stefano JT, Cavaleiro AM, et al. Association of polymorphisms of glutamate-cysteine ligase and microsomal triglyceride transfer protein genes in non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2010;25(2):357–361
- 161 Petersen KF, Dufour S, Hariri A, et al. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. *N Engl J Med* 2010;362(12):1082–1089
- 162 Kozlitina J, Boerwinkle E, Cohen JC, Hobbs HH. Dissociation between APOC3 variants, hepatic triglyceride content and insulin resistance. *Hepatology* 2011;53(2):467–474
- 163 Sentinelli F, Romeo S, Maglio C, et al. Lack of effect of apolipoprotein C3 polymorphisms on indices of liver steatosis, lipid profile and insulin resistance in obese Southern Europeans. *Lipids Health Dis* 2011;10:93
- 164 Valenti L, Nobili V, Al-Serri A, et al. The APOC3 T-455C and C-482T promoter region polymorphisms are not associated with the severity of liver damage independently of PNPLA3 I148M genotype in patients with nonalcoholic fatty liver. *J Hepatol* 2011;55(6):1409–1414
- 165 Verrijken A, Beckers S, Francque S, et al. A gene variant of PNPLA3, but not of APOC3, is associated with histological parameters of NAFLD in an obese population. *Obesity (Silver Spring)* 2013;21(10):2138–2145
- 166 Utermann G, Hees M, Steinmetz A. Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinemia in man. *Nature* 1977;269(5629):604–607
- 167 Demirag MD, Onen HI, Karaoguz MY, et al. Apolipoprotein E gene polymorphism in nonalcoholic fatty liver disease. *Dig Dis Sci* 2007;52(12):3399–3403
- 168 Sazci A, Akpinar G, Aygun C, Ergul E, Senturk O, Hulagu S. Association of apolipoprotein E polymorphisms in patients with non-alcoholic steatohepatitis. *Dig Dis Sci* 2008;53(12):3218–3224
- 169 George DK, Goldwurm S, MacDonald GA, et al. Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. *Gastroenterology* 1998;114(2):311–318
- 170 Nelson JE, Bhattacharya R, Lindor KD, et al. HFE C282Y mutations are associated with advanced hepatic fibrosis in Caucasians with nonalcoholic steatohepatitis. *Hepatology* 2007;46(3):723–729
- 171 Bugianesi E, Manzini P, D'Antico S, et al. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology* 2004;39(1):179–187
- 172 Valenti L, Fracanzani AL, Bugianesi E, et al. HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology* 2010;138(3):905–912
- 173 Raszeja-Wyszomirska J, Kurzawski G, Lawniczak M, Miezynska-Kurtycz J, Lubinski J. Nonalcoholic fatty liver disease and HFE gene mutations: a Polish study. *World J Gastroenterol* 2010;16(20):2531–2536
- 174 Ellervik C, Birgens H, Tybjaerg-Hansen A, Nordestgaard BG. Hemochromatosis genotypes and risk of 31 disease endpoints: meta-analyses including 66,000 cases and 226,000 controls. *Hepatology* 2007;46(4):1071–1080
- 175 Hernaez R, Yeung E, Clark JM, Kowdley KV, Brancati FL, Kao WH. Hemochromatosis gene and nonalcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol* 2011;55(5):1079–1085
- 176 Buch S, Schafmayer C, Völzke H, et al. A genome-wide association scan identifies the hepatic cholesterol transporter ABCG8 as a susceptibility factor for human gallstone disease. *Nat Genet* 2007;39(8):995–999
- 177 Sookoian S, Castaño G, Gianotti TF, Gemma C, Pirola CJ. Polymorphisms of MRP2 (ABCC2) are associated with susceptibility to nonalcoholic fatty liver disease. *J Nutr Biochem* 2009;20(10):765–770
- 178 Spruss A, Kanuri G, Wagnerberger S, Haub S, Bischoff SC, Berghelm I. Toll-like receptor 4 is involved in the development of fructose-induced hepatic steatosis in mice. *Hepatology* 2009;50(4):1094–1104
- 179 Guo J, Loke J, Zheng F, et al. Functional linkage of cirrhosis-predictive single nucleotide polymorphisms of Toll-like receptor 4 to hepatic stellate cell responses. *Hepatology* 2009;49(3):960–968
- 180 Huang H, Shiffman ML, Friedman S, et al. A 7 gene signature identifies the risk of developing cirrhosis in patients with chronic hepatitis C. *Hepatology* 2007;46(2):297–306
- 181 Day CP, Leathart J, McTernan P, et al. Genetic evidence for a role of gut flora in the pathogenesis of NASH in humans. *Hepatology* 2006;44(Suppl 1):261A
- 182 Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A Polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol* 1999;20(5):976–983
- 183 Valenti L, Fracanzani AL, Dongiovanni P, et al. Tumor necrosis factor α promoter polymorphisms and insulin resistance in nonalcoholic fatty liver disease. *Gastroenterology* 2002;122(2):274–280
- 184 Tokushige K, Takakura M, Tsuchiya-Matsushita N, Tanai M, Hashimoto E, Shiratori K. Influence of TNF gene polymorphisms in Japanese patients with NASH and simple steatosis. *J Hepatol* 2007;46(6):1104–1110
- 185 Carulli L, Canedi I, Rondinella S, et al. Genetic polymorphisms in non-alcoholic fatty liver disease: interleukin-6-174C/G polymorphism is associated with non-alcoholic steatohepatitis. *Dig Liver Dis* 2009;41(11):823–828

- 186 Eslam M, Hashem AM, Leung R, et al; International Hepatitis C Genetics Consortium (IHCGC). Interferon- λ rs12979860 genotype and liver fibrosis in viral and non-viral chronic liver disease. *Nat Commun* 2015;6:6422
- 187 Yoneda M, Hotta K, Nozaki Y, et al. Association between angiotensin II type 1 receptor polymorphisms and the occurrence of nonalcoholic fatty liver disease. *Liver Int* 2009;29(7):1078–1085
- 188 Zain SM, Mohamed Z, Mahadeva S, et al. Susceptibility and gene interaction study of the angiotensin II type 1 receptor (AGTR1) gene polymorphisms with non-alcoholic fatty liver disease in a multi-ethnic population. *PLoS ONE* 2013;8(3):e58538
- 189 Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics* 2008;24(24):2938–2939