Genetic Predisposition in NAFLD and NASH: Impact on Severity of Liver Disease and Response to Treatment

Paola Dongiovanni¹, Quentin M. Anstee²* and Luca Valenti¹*

¹Department of Pathophysiology and Transplantation, section Internal Medicine, Università degli Studi Milano, UO Medicina Interna 1B, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy; ²Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom.

Abstract: Liver fat deposition related to systemic insulin resistance defines non-alcoholic fatty liver disease (NAFLD) which, when associated with oxidative hepatocellular damage, inflammation, and activation of fibrogenesis, i.e. non-alcoholic steatohepatitis (NASH), can progress towards cirrhosis and hepatocellular carcinoma. Due to the epidemic of obesity, NAFLD is now the most frequent liver disease and the leading cause of altered liver enzymes in Western countries. Epidemiological, familial, and twin studies provide evidence for an element of heritability of NAFLD. Genetic modifiers of disease severity and progression have been identified through genome-wide association studies. These include the Patatin-like phospholipase domain-containing 3 (PNPLA3) gene variant I148M as a major determinant of inter-individual and ethnicity-related differences in hepatic fat content independent of insulin resistance and serum lipid concentration. Association studies confirm that the I148M polymorphism is also a strong modifier of NASH and progressive hepatic injury. Furthermore, a few large multicentre case-control studies have demonstrated a role for genetic variants implicated in insulin signalling, oxidative stress, and fibrogenesis in the progression of NAFLD towards fibrosing NASH, and confirm that hepatocellular fat accumulation and insulin resistance are key operative mechanisms closely involved in the progression of liver damage. It is now important to explore the molecular mechanisms underlying these associations between gene variants and progressive liver disease, and to evaluate their impact on the response to available therapies. It is hoped that this knowledge will offer further insights into pathogenesis, suggest novel therapeutic targets, and could help guide physicians towards individualised therapy that improves clinical outcome.

Keywords: Non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polymorphism, genetic predisposition.

1. INTRODUCTION

Liver fat deposition related to systemic insulin resistance (IR) defines non-alcoholic fatty liver disease (NAFLD) [1]. In susceptible individuals this maybe associated with oxidative hepatocellular damage, inflammation, and activation of fibrogenesis, i.e. non-alcoholic steatohepatitis (NASH) [2], potentially progressing towards cirrhosis and hepatocellular carcinoma [3]. Due to the epidemic of obesity and the metabolic syndrome, NAFLD is now the most frequent liver disease (prevalence 20-34%) and the leading cause of altered liver enzymes in Western countries [4, 5]. Although NASH is still an emerging health problem, it is already projected to become the leading cause of end-stage liver disease, liver transplantation and hepatocellular carcinoma within the next 10-20 years.

Epidemiological, familial, and twin studies provide evidence for an element of heritability of hepatic fat content, NAFLD, and non-alcoholic steatohepatitis, which is strongly associated with oxidative hepatocellular damage, inflammation, and activation of fibrogenesis, i.e. non-alcoholic steatohepatitis (NASH) [2], potentially progressing towards cirrhosis and hepatocellular carcinoma [3]. Due to the epidemic of obesity and the metabolic syndrome, NAFLD is now the most frequent liver disease (prevalence 20-34%) and the leading cause of altered liver enzymes in Western countries [4, 5]. Although NASH is still an emerging health problem, it is already projected to become the leading cause of end-stage liver disease, liver transplantation and hepatocellular carcinoma within the next 10-20 years.

Epidemiological, familial, and twin studies provide evidence for an element of heritability of hepatic fat content, NAFLD, and non-alcoholic steatohepatitis, which is strongly associated with oxidative hepatocellular damage, inflammation, and activation of fibrogenesis, i.e. non-alcoholic steatohepatitis (NASH) [2], potentially progressing towards cirrhosis and hepatocellular carcinoma [3]. Due to the epidemic of obesity and the metabolic syndrome, NAFLD is now the most frequent liver disease (prevalence 20-34%) and the leading cause of altered liver enzymes in Western countries [4, 5]. Although NASH is still an emerging health problem, it is already projected to become the leading cause of end-stage liver disease, liver transplantation and hepatocellular carcinoma within the next 10-20 years.

Epidemiological, familial, and twin studies provide evidence for an element of heritability of hepatic fat content, NAFLD, and non-alcoholic steatohepatitis, which is strongly associated with oxidative hepatocellular damage, inflammation, and activation of fibrogenesis, i.e. non-alcoholic steatohepatitis (NASH) [2], potentially progressing towards cirrhosis and hepatocellular carcinoma [3]. Due to the epidemic of obesity and the metabolic syndrome, NAFLD is now the most frequent liver disease (prevalence 20-34%) and the leading cause of altered liver enzymes in Western countries [4, 5]. Although NASH is still an emerging health problem, it is already projected to become the leading cause of end-stage liver disease, liver transplantation and hepatocellular carcinoma within the next 10-20 years.

Epidemiological, familial, and twin studies provide evidence for an element of heritability of hepatic fat content, NAFLD, and non-alcoholic steatohepatitis, which is strongly associated with oxidative hepatocellular damage, inflammation, and activation of fibrogenesis, i.e. non-alcoholic steatohepatitis (NASH) [2], potentially progressing towards cirrhosis and hepatocellular carcinoma [3]. Due to the epidemic of obesity and the metabolic syndrome, NAFLD is now the most frequent liver disease (prevalence 20-34%) and the leading cause of altered liver enzymes in Western countries [4, 5]. Although NASH is still an emerging health problem, it is already projected to become the leading cause of end-stage liver disease, liver transplantation and hepatocellular carcinoma within the next 10-20 years.

Epidemiological, familial, and twin studies provide evidence for an element of heritability of hepatic fat content, NAFLD, and non-alcoholic steatohepatitis, which is strongly associated with oxidative hepatocellular damage, inflammation, and activation of fibrogenesis, i.e. non-alcoholic steatohepatitis (NASH) [2], potentially progressing towards cirrhosis and hepatocellular carcinoma [3]. Due to the epidemic of obesity and the metabolic syndrome, NAFLD is now the most frequent liver disease (prevalence 20-34%) and the leading cause of altered liver enzymes in Western countries [4, 5]. Although NASH is still an emerging health problem, it is already projected to become the leading cause of end-stage liver disease, liver transplantation and hepatocellular carcinoma within the next 10-20 years.

Epidemiological, familial, and twin studies provide evidence for an element of heritability of hepatic fat content, NAFLD, and non-alcoholic steatohepatitis, which is strongly associated with oxidative hepatocellular damage, inflammation, and activation of fibrogenesis, i.e. non-alcoholic steatohepatitis (NASH) [2], potentially progressing towards cirrhosis and hepatocellular carcinoma [3]. Due to the epidemic of obesity and the metabolic syndrome, NAFLD is now the most frequent liver disease (prevalence 20-34%) and the leading cause of altered liver enzymes in Western countries [4, 5]. Although NASH is still an emerging health problem, it is already projected to become the leading cause of end-stage liver disease, liver transplantation and hepatocellular carcinoma within the next 10-20 years.

Epidemiological, familial, and twin studies provide evidence for an element of heritability of hepatic fat content, NAFLD, and non-alcoholic steatohepatitis, which is strongly associated with oxidative hepatocellular damage, inflammation, and activation of fibrogenesis, i.e. non-alcoholic steatohepatitis (NASH) [2], potentially progressing towards cirrhosis and hepatocellular carcinoma [3]. Due to the epidemic of obesity and the metabolic syndrome, NAFLD is now the most frequent liver disease (prevalence 20-34%) and the leading cause of altered liver enzymes in Western countries [4, 5]. Although NASH is still an emerging health problem, it is already projected to become the leading cause of end-stage liver disease, liver transplantation and hepatocellular carcinoma within the next 10-20 years.

2. PATHOPHYSIOLOGY OF NAFLD AND NASH

A short overview of the pathophysiology of NAFLD and NASH is required to introduce the genetic determinants of disease pathogenesis and progression. The acronym NAFLD defines a wide spectrum of liver disease ranging from simple uncomplicated hepatic fat accumulation in the form of triglycerides exceeding 5% of liver mass (steatosis) in the absence of significant alcohol consumption to severe hepatitis characterized by steatosis, lobular inflammation, and hepatocellular damage and apoptosis with activation of fibrogenesis (steatohepatitis, NASH) [3]. Hepatic fat accumulation results from an unbalance between triglycerides acquisition and removal [14], and initially represents a protective mechanism to protect hepatocytes from the toxicity resulting from an increased flux of free fatty acids (FFAs) to the liver [15]. Several lines of evidence support the hypothesis that most of the FFAs accumulated as triglycerides during steatosis derive from increased peripheral lipolysis [16] related to adipose tissue insulin resistance [17], followed by increased lipogenesis induced by hyperinsulinaemia and diet. Indeed, the major risk factor for NAFLD is systemic insulin resistance related to hepatic fat deposition and systemic insulin resistance (IR) defines non-alcoholic fatty liver disease (NAFLD) which, when associated with oxidative hepatocellular damage, inflammation, and activation of fibrogenesis, i.e. non-alcoholic steatohepatitis (NASH), can progress towards cirrhosis and hepatocellular carcinoma. Due to the epidemic of obesity, NAFLD is now the most frequent liver disease and the leading cause of altered liver enzymes in Western countries. Epidemiological, familial, and twin studies provide evidence for an element of heritability of NAFLD. Genetic modifiers of disease severity and progression have been identified through genome-wide association studies. These include the Patatin-like phospholipase domain-containing 3 (PNPLA3) gene variant I148M as a major determinant of inter-individual and ethnicity-related differences in hepatic fat content independent of insulin resistance and serum lipid concentration. Association studies confirm that the I148M polymorphism is also a strong modifier of NASH and progressive hepatic injury. Furthermore, a few large multicentre case-control studies have demonstrated a role for genetic variants implicated in insulin signalling, oxidative stress, and fibrogenesis in the progression of NAFLD towards fibrosing NASH, and confirm that hepatocellular fat accumulation and insulin resistance are key operative mechanisms closely involved in the progression of liver damage. It is now important to explore the molecular mechanisms underlying these associations between gene variants and progressive liver disease, and to evaluate their impact on the response to available therapies. It is hoped that this knowledge will offer further insights into pathogenesis, suggest novel therapeutic targets, and could help guide physicians towards individualised therapy that improves clinical outcome.

Keywords: Non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polymorphism, genetic predisposition.
resistance due to central obesity and the metabolic syndrome [1, 18]. Steatosis per se may then further contribute to hepatic insulin resistance, exacerbating these metabolic disturbances and increase the risk of other extra-hepatic complications including cardiovascular disease [19, 20]. Impaired ability to secrete lipoproteins [21] and changes in fatty-acid oxidation also contribute to hepatic fat accumulation.

Development of NASH has been classically explained by the occurrence of a so-called “second-hit”, leading to the activation of inflammation, in the context of hepatic steatosis [22]. This second insult is likely to actually represent a combination of insults related to direct hepatic lipotoxicity, hepatocellular oxidative stress secondary to free radicals produced during β- and ω- oxidation of FFAs, inflammation triggered by endotoxin engaging TLR-4 receptors in Kupffer cells and hepatocytes due to increased intestinal permeability, bacterial overgrowth and altered intestinal flora [23-25], cytokines release, and endoplasmic reticulum stress. These combine to produce inflammation, cellular damage, and activation of fibrogenesis [26]. A working model of NASH pathogenesis is presented in (Fig. 1), whereas the role of inflammation is presented in greater details in (Fig. 2).

3. EVIDENCE OF HERITABILITY OF NAFLD AND NASH

A possible explanation for the observed inter-individual variability in the susceptibility to NAFLD and progressive NASH is provided by heritability [26]. In addition to evidence of heritability provided by epidemiological, familial, and twin studies [6, 7, 27-29], clinical case series have also shown familial clustering of NAFLD [29]. Although shared environmental risk factors may contribute to development of steatosis, the variation in NAFLD phenotypic expression in persons with similar risk factors implicates a genetic contribution. Recently, a familial aggregation study of fatty liver in overweight children with and without NAFLD showed that liver fat fraction and in particular the condition of fatty liver are strongly heritable traits [6]. In addition, a family study of 157 individuals with familial combined hyperlipidemia (FCHL) showed that ALT levels and the prevalence of fatty liver were increased not only in FCHL probands but also in their relatives, suggesting the presence of a genetic component [30].

Twin studies confirmed that, in subjects without evidence of alcohol abuse or viral hepatitis, alanine transaminases (ALT) levels, mostly reflecting liver fat content, were a heritable trait, with genetic factors explaining almost up to 60% of variability [27].

Fig. (1). Mechanisms involved in the pathogenesis of NASH.

NAFLD is characterized by the hepatic fat accumulation resulting from an unbalance between triglycerides acquisition and removal. Most of free fatty acids (FFAs) that are stored as triglycerides during hepatic steatosis derive from peripheral lipolysis related to adipose tissue insulin resistance, followed by de novo lipogenesis induced by hyperinsulinemia, and diet. In the liver, FFAs can be catabolized through β-oxidation, re-esterification to triglycerides and stored as lipid droplets, or exported as very low density lipoproteins (VLDL). Impaired ability to secrete lipoproteins and decreased β-oxidation due to mitochondrial damage (especially in the presence of NASH) may play a role in hepatic fat accumulation. Long-term injury arising from i) hepatocellular triglycerides storage and lipotoxicity, ii) hepatocellular oxidative stress secondary to free radical produced during β- and omega- oxidation of FFAs, iii) inflammation triggered by endotoxin, iv) cytokines release, v) and endoplasmic reticulum (ER) stress lead in the end to inflammation, perpetuation of cellular damage, and activation of fibrogenesis.
study population among Danish twins identified substantial heritability (35-61%) for levels of biochemical liver indices including ALT, gamma-glutamyl peptidase (GGT), the remaining variation being attributed to environmental factors [31]. Furthermore, GGT levels have also been shown to represent a highly heritable trait (roughly 50%), which had a significant covariance with risk factors for NAFLD determining the metabolic syndrome, such as IR, lipid levels, and diastolic blood pressure [32], and several studies demonstrated a role of genetic factors in the pathogenesis of metabolic alterations typical of hepatic IR and NAFLD [33], although some genetic risk factors for NAFLD are specific for hepatic fat content (see below). The estimates for heritable components of liver fat and liver enzymes associated with steatosis are presented in (Fig. 3). However, it should be underlined that a recent study concluded that ultrasonosonographically detected NAFLD has a low heritability in Hungarian twins [34].

In line with a strong role of genetics in the pathogenesis of NASH, racial and ethnic differences have been reported in the prevalence of NAFLD, NASH and cryptogenic cirrhosis, which is believed to represent an evolution of NASH in the majority of cases [4, 35]. In the US for example, Hispanic subjects are at higher risk than subjects of European descent, whereas African-Americans are protected independently of diabetes and BMI [7]. The results of the Insulin Resistance Atherosclerosis Study (IRAS) Family Study with 1,142 participants of Hispanic or African-American descent was also consistent with a role of heritability in the pathogenesis of NAFLD [36].

4. PNPLA3 I148M IS A MAJOR RISK FACTOR FOR NAFLD AND NASH

A major determinant of the inter-individual and ethnicity-related differences hepatic fat content quantified by magnetic reso-
The coexistence of the rs738409 G risk allele (148M) and an independent environmental stressor such as obesity [58] or chronic alcohol consumption [59], is associated with elevated serum alanine transaminase levels and higher liver damage, suggesting that these stressors appear to uncover the association between 148M and hepatic injury. Indeed, the magnitude of the association between the I148M PNPLA3 variant and liver enzymes was related to abdominal fat mass [60, 61], and to high dietary carbohydrate and sugar consumption [62]. The rs738409 PNPLA3 genotype also influences steatosis development in chronic hepatitis C patients and is independently associated with cirrhosis and other steatosis-related clinical outcomes, such as lack of response to antiviral treatment and possibly hepatocarcinoma [63-66], and with of cirrhosis and hepatocellular carcinoma in patients with alcohol abuse [59, 67-71]. Importantly, it has recently been reported that the I148M PNPLA3 variant is a risk factor for the development of hepatocellular carcinoma in severely obese subjects from Northern Europe [72].

Therefore, current evidence suggests that the PNPLA3 I148M variant is a genetic determinant of liver damage progression associated with steatohepatitis, which may be triggered by a number of factors including obesity, IR, excessive alcohol intake, and chronic hepatitis C (Fig. 4) [53].

5. OTHER GENETIC VARIANTS INFLUENCING NAFLD SUSCEPTIBILITY IDENTIFIED BY GENOMEWIDE SCANS

Besides confirming that rs738409 of PNPLA3 is the major common genetic risk factor of NAFLD, a recent meta-analysis of combined GWAS datasets identified four other SNPs associated with liver fat content and other aspects of the NAFLD phenotype. These were localized in or near the genes neurocan (NCAN, SNP rs2228603), protein phosphatase 1, regulatory (inhibitor) subunit 3B (PPP1R3B, SNP rs4240624), glucokinase regulator (GCKR, SNP rs780094) and lysophospholipase-like 1 (LYPLAL1, SNP rs12137855). NCAN is involved in the regulation of cell adhesion and likely lipoprotein metabolism, and was also associated with histologically validated steatosis in a replication study. GCKR, a regulator of glucose metabolism and LYPLAL1, which exerts a complementary function to the PNPLA3 protein in triglyceride breakdown, were also associated with histologically assessed lobular inflammation and/or fibrosis [73]. The rs780094 GCKR polymorphism is in strong linkage disequilibrium with rs1260326, encoding for the P446L protein variant, which influences the ability of GCKR to inhibit glucokinase in response to fructose-6-phosphate, thereby resulting in a constant increase in hepatic glucokinase activity and glucose uptake by the liver [74]. Unrestricted hepatic glycolysis associated with carriage of the minor 446L allele leads on one hand to lower glucose and insulin levels, but on the other hand to increased levels of malonyl-CoA, which in turn may favor hepatic fat accumulation by serving as a substrate for lipogenesis and by blocking fatty acid oxidation through the inhibition of carnitine-palmitoyl transferase-1 (CPT-1). The combined effects of PNPLA3 I148M and GCKR P446L polymorphisms has been proposed to explain up to one third of variability in liver fat content amongst obese children of European descent [75, 76].

To date one other GWAS has been reported. This identified variants conferring a predisposition to disease progression in a small cohort of patients with histologically proven NAFLD [77]. The study highlighted an association between severity of histological NAFLD activity score and SNP rs2645424 on chromosome 8, in the gene encoding farnesyl diphosphate farnesyl transferase 1 (FDFT1), an enzyme involved in cholesterol biosynthesis. Other associations observed included rs433062 on chromosome 7 with degree of fibrosis, and rs1227756 on chromosome 10 in the COL13A1 gene, rs887304 on chromosome 12 in the EFCA4B gene with lobular inflammation. It was however perhaps surprising that this study did not identify PNPLA3 given that it has been re-
peatedly validated in some many other studies. These findings therefore require validation.

6. GENETIC FACTORS INFLUENCING LIVER DISEASE PROGRESSION IN NAFLD FROM CANDIDATE GENE STUDIES

6.1. Variants Involved in the Regulation of Lipid Metabolism

The hallmark of hepatic steatosis is triglyceride (TG) accumulation within hepatocytes caused by alterations in hepatic lipid metabolism changing the balance between the pathways of uptake, synthesis, degradation and secretion on a background of systemic insulin resistance [78]. Genes that affect hepatic fat storage and mobilization are therefore likely candidates to influence the development and progression of NAFLD as are variants of transcription factors controlling lipid metabolism in the liver and adipose. Peroxisome proliferator-activated receptor-alpha (PPARα) is a member of the nuclear hormone receptor superfamily. A molecular target of long chain fatty acids, eicosanoids and fibrates [79], it is highly expressed in tissues that catabolize fatty acids such as the liver and skeletal muscle. Under condition of increased hepatic fatty acid influx or decreased fatty acid efflux, PPARα activation prevents the accumulation of triglycerides by increasing the rate of fatty acid catabolism. PPARα downregulation is involved in NASH pathogenesis by reducing FFA catabolism [80]. The Val227Ala SNP in the PPARα gene may be implicated in the pathogenesis of NAFLD and it could play a protective role against the development of obesity [81]. It has been hypothesized that the substitution of Valine to Alanine at codon 227 causes a functional change in PPARα and that the Ala227 isoform has higher activity than the Val227 isoform [82]. However, in Italian subjects the Leu162Val PPARα loss-of-function polymorphism did not influence the risk of NAFLD, where it was associated with IR but not histologically assessed disease severity [83], suggesting that the risk related to increased insulin resistance may be balanced by the protective effect of decreased oxidative stress.

Peroxisome proliferator-activated receptor-gamma (PPARγ), the molecular target of glitazones, is highly expressed in adipose tissue and regulates adipocyte differentiation, FFA uptake and storage. Pharmacological activation of PPARγ improves insulin resistance in diabetes and has been reported to decrease liver damage in NAFLD by restoring adipose tissue insulin sensitivity, decreasing FFA flux to the liver [84, 85]. The Pro12Ala loss-of-function SNP in PPARγ2, which is thought to induce a modest impairment of transcriptional activation due to decreased DNA-binding affinity, was associated with a reduction of PPARγ activity in adipose tissue as well as decreased IR and diabetes in Caucasians [86]. The data in liver disease are conflicting however, Rey et al showed a significantly higher risk of developing histological necro-inflammation in alcoholic liver disease patients carrying the 12Ala allele but found that the 12Ala allele was not associated with the progression of liver disease in NAFLD patients [87]. Similarly, the 12Ala allele was not associated with NAFLD susceptibility, liver damage or IR in 212 Italian patients with NAFLD [83]. However an association with carriage of the minor PPARγ haplotypes encompassing the 12Ala allele was reported with increased risk of progressive liver disease in a US cohort of similar size [88].

Another interesting candidate is represented by Lipin1 (LPIN1), a phosphatidate phosphatase that is highly expressed in adipose tissue, is involved in the metabolism of phospholipids and triacylglycerol, and is required for adipogenesis and the normal metabolic
flux between adipose tissue and liver, where it also acts as an inducible transcriptional co-activator to regulate fatty acid metabolism [89]. LPIN1 mRNA expression in the liver and adipose tissue has been positively associated with body mass and IR. LPIN1 SNPs and haplotypes that may confer variability in the protein activity have been associated with several components of the metabolic syndrome, including body mass, insulin levels, resting metabolic rate and to responsiveness to insulin sensitizers [90, 91]. Whereas in a case-control population study of 17538 Danes LPIN1 variants and haplotypes did not influence type 2 diabetes, obesity, or related quantitative metabolic phenotypes [92]. However, in a recent meta-analysis conducted in 8504 subjects the LPIN1 rs13412852 T allele was associated with lower BMI and insulin levels [93], confirming that it possibly represents a protective factor towards metabolic syndrome alterations.

We evaluated whether the LPIN1 rs13412852 C>T polymorphism was associated with NASH and fibrosis in pediatric Italian patients with NAFLD, finding that the TT genotype was under-represented in pediatric but not in adult patients with NAFLD, was associated with less severe dyslipidemia, and that children with this genotype had a trend for a lower prevalence of NASH and significantly less severe liver damage independently of PNPLA3 genotype and other risk factors [94]. Although independent validation of these results is required, these data suggest that LPIN1 genotype may predispose to progressive NASH at early age by influencing lipogenesis and lipid metabolism.

Hepatic uptake of fatty acids (FA), as one of the routes to development of steatosis, is of clinical relevance. Fatty acid transport proteins (FATP) hold a crucial role in mediating FA uptake in different tissues [95-97]. In the liver two different FATP isoforms are predominantly expressed; FATP2 and FATP5 [98]. The FATP5 gene encodes a multifunctional protein which increases the hepatic uptake of FA and activates very long-chain fatty-acids and has bile-CoA ligase activity [99, 100]. Overexpression as well as inhibition of FATP5 in cell culture and experimental animals underlines its function in hepatic fatty acid trafficking [101, 102]. Furthermore, mice lacking FATP5 have defective bile acid conjugation and are protected from obesity [100]. FATP5 silencing reverses diet-induced NAFLD and improves hyperglycemia in mice [101].

Since variations in the promoter region may alter the transcriptional activity [103] we investigated the association of a FATP5 promoter polymorphism with parameters of the fasting and postprandial lipid and glucose metabolism in a cohort study and in subjects with histologically proven NAFLD. A total of 716 male subjects from the Metabolic Intervention Cohort Kiel (MICK) and 103 male subjects with histologically proved non-alcoholic fatty liver disease (NAFLD) were genotyped for the rs56225452 FATP5 polymorphism and phenotyped for features of the metabolic syndrome. In the MICK cohort, ALT levels, postprandial insulin levels and triglyceride concentrations were higher in subjects carrying the rare A-allele than in GG homozygotes. Accordingly, the insulin sensitivity index determined after a mixed meal and standardized glucose load was lower in A-allele carriers. NAFLD cases carrying allele A were presented with also higher ALT activities. In NAFLD subjects the association of BMI with the degree of steatosis and glucose concentration differed across FATP5 promoter polymorphisms [104]. Therefore, the FATP5 promoter polymorphism rs56225452 seems to be associated with higher ALT levels, insulin resistance and dyslipidemia in the general population. The impact of the BMI on the severity of steatosis in NAFLD cases seems to depend partly on the FATP5 polymorphism, but additional studies are needed to define the association with progressive liver damage.

Synthesis of phosphatidylcholine is required for VLDL formation, when it is not available fat droplets accumulate in the cytosol of hepatocytes [105], [106]. This observation underpins the use of choline deficient diets as a well-recognised animal model of NASH [107, 108]. Thus, modifier genes of choline metabolism provide another source of candidates for study. Phosphatidylethanolamine N-Methytransferase (PEMT) catalyzes the de novo synthesis of phosphatidylethanolamine in the liver [109]. Synthesis of new phosphatidylethanolamine molecules is required for VLDL formation and when they are not available fat droplets accumulate in the cytosol of hepatocytes [105], [106]. PEMT knockout mice do not display any PEMT activity in the liver and depend completely on dietary choline intake [110, 111], and when fed a choline-deficient diet develop severe steatosis [112]. Song et al. identified a non-synonymous SNP in the PEMT gene (523 G>A in exon 8), which results in a loss-of-function valine to methionine (V175M) substitution in the encoded protein. A higher occurrence of the low-activity 175M variant was found in 28 subjects of mixed ethnicity with biopsy-proven NAFLD. A lower occurrence of the high-activity 175V allele was associated with also higher ALT activities. In NAFLD cases carrying the 175M variant were associated with increased liver enzymes and increased susceptibility to progressive NASH at early age by influencing lipogenesis and lipid metabolism.

A defect in lipid export as lipoprotein may also contribute to the pathogenesis of steatosis [21]. Microsomal triglyceride transfer protein (MTTP) is necessary for assembly and secretion of VLDL from hepatocytes [117]. It has a key role in lipoprotein assembly by transferring triglycerides, to nascent apolipoproteins B. Abetalipoproteinemia, a rare autosomal recessive disorder caused by mutations in the coding region of the MTTP gene, results in very low total cholesterol, undetectable plasma apoB levels and fat malabsorption, and is characterized by liver steatosis although this seldom progresses to steatohepatitis [118, 119]. A common functional SNP in the MTTP gene promoter (-493G/T) has been described [120], with the G allele associated with decreased MTTP transcription, less export of triglycerides from hepatocytes, and greater intracellular triglyceride accumulation. Namikawa et al. showed that NASH patients had a higher incidence of the G allele and of the G/G genotype compared to controls, even if the number of both patients and controls included in the study was limited [121]. Moreover, the stage of NASH was more advanced in Japanese patients with the G/G genotype than in patients with G/T genotype. A relatively small study from Italy demonstrated that the -493 G/T SNP influences liver disease and postprandial lipid metabolism in NASH. Patients with the G/G genotype were found to have more severe liver disease and a more atherogenic postprandial lipoprotein profile, in spite of similar degrees of adiposity and insulin resistance, adipokine profile and dietary habits [122]. In 40 non-diabetic normo-lipidemic NASH patients compared to 40 healthy controls, the -493 G/T polymorphism modulated beta-cell function, an effect mediated by postprandial HDL-C and oxLDL metabolism [123]. In 271 French patients with type II diabetes the -493 G/T SNP was associated with increased liver enzymes and increased susceptibility to steatohepatitis, however this study provided only indirect evidence of a link between MTTP genotype and NASH, as liver biopsy specimens were not available and the authors adopted raised ALT as a surrogate for NASH [124]. This potential association was not supported by a Brazilian study in 131 patients with biopsy proven disease compared to 141 healthy volunteers: the presence of at least one -493 G allele was only marginally different between NASH and simple steatosis [125] (Tables 1, 2, and 3).
Variants in apolipoproteins influencing serum lipid metabolism might be involved as well. Apolipoprotein E (ApoE) is a plasma protein involved in lipid transport and metabolism [126]. Three alleles, /g2, /g3 and /g4, at the ApoE locus determine three isoforms, ApoE2, ApoE3 and ApoE4, resulting in six ApoE genotypes (E2/2, E3/3, E4/4, E2/3, E2/4, E3/4). These isoforms differ by single amino acid substitution at position 112 and 158 of ApoE, which lead to a different association with lipoproteins and affinity for the LDL receptor [126, 127]. Homozygosity for the /g2 allele is associated with hyperlipidemia, but no significant difference in ApoE genotypes and allele frequencies was observed between NAFLD patients and controls. Nevertheless, comparing non obese patients with controls it was found that the /g2 allele and the /g2/3 genotype were more prevalent in the control group, suggesting that occurrence of this allele and of this genotype may be protective against the development of NAFLD [128]. In line with these data, the ApoE3/3 genotype was associated with an increased risk of NASH in a cohort of Turkish patients, whereas the ApoE3/4 genotype had a protective effect [129]. Interestingly, Price et al. reported that in patients with persistent hepatitis C virus (HCV), which circulates in the plasma associated with VLDL, allowing the virus to enter target cells via lipoprotein receptors [130], the common genetic variations at the ApoE locus influence the outcome of HCV infection, with the /g2 and /g4 alleles favoring viral clearance [131].

Apolipoprotein C3 (ApoC3) is another major constituent of plasma very low density lipoprotein (VLDL), chylomicrons and HDL-C which inhibits lipoprotein lipase and triglyceride clearance [132]. Petersen et al reported that two common ApoC3 T-455C and C-482T promoter SNPs, which hamper the regulation of apolipoprotein C3 expression by insulin signalling via FOXO1 phosphory-

| Gene | SNP | Effect on steatosis | Effect on NASH/fibrosis/inflammation |
|------|-----|---------------------|--------------------------------------|
| PNPLA3, patatine-like phospholipase domain containing 3 [48] | rs738409 rs6006460[8] | ↑ | ↓ | ↑ |
| FDFT1, farnesyl diphosphate farnesyl transferase 1 | rs2645424 | | | ↑ |
| COL13A1, collagen, type XIII, alpha1 | rs1227756 | | | ↑ |
| EFCAB4B, EF-hand calcium binding domain 4B | rs887304 | | | ↑ |
| NCAN, neurocan | rs2228603 | | | ↑ |
| LYPLAL1, lysophospholipase-like 1 | rs12137855 | | | ↑ |
| GCKR, glucokinase regulatory protein | rs780094 | | | ↑ |
| PPP1R3B, protein phosphatase 1, regulatory subunit 3b | rs4240624 | | | ↑ |

Table 1. Genetic variants influencing NAFLD susceptibility identified by genomewide scans (GWAS) [8, 77, 273].
lation [133], predispose to liver fat accumulation in Indian men by altering lipid metabolism and IR [134]. The relationship with altered liver enzymes and liver damage was not assessed. This association was not replicated by a later study which found no association between these two ApoC3 polymorphisms and hepatic triglyceride content, IR or fasting triglycerides levels in a large multi-ethnic US cohort [135]. Neither was this validated in an Italian study of 585 obese subjects [136]. Furthermore, APOC3 genotype was not associated with elevated liver enzymes or with the histological severity of liver damage in Italian and UK patients [137], suggesting that the initial observation may represent a type 1 error.

6.2. Variants Involved in the Pathogenesis of IR

IR is the key factor in NAFLD pathophysiology and is deeply entangled with the progression of liver disease [138], but the causal relationship between IR and fibrogenesis remains unclear. Functional common SNPs of genes included in the insulin signalling pathway influence IR and the susceptibility to type 2 diabetes. A schematic representation of the insulin signalling pathway in the liver is presented in (Fig. 5). Plasma cell antigen-1, also known as ENPP1 is a membrane glycoprotein, which inhibits insulin signalling. The ENPP1 Lys121Gln gain-of-function polymorphism enhances the interaction between the ENPP1 glycoprotein and insulin receptor (INSR), contrasting INSR kinase activity, and is associated with an increased diabetes risk [139]. IRS-1 transduces INSR signalling to downstream kinases regulating glucose and lipid metabolism, cell survival, and proliferation. The loss-of-function Gly972Arg SNP decreases IRS-1 activity and inhibits INSR autophosphorylation and activity [140], increasing the risk of IR and diabetes [141, 142]. We recently demonstrated that the combination of ENPP1 121Gln and IRS-1 972Arg alleles was associated with decreased activation of the insulin signalling pathway in the liver and influenced fibrosis severity in a large multicentre series of NAFLD patients [9]. These data suggest that hepatic IR has a causal role in the progression of liver damage in NASH and therefore, that amelioration of IR may improve the long-term progression of the disease. However, additional independent studies are required to replicate this association and to establish the efficacy of insulin sensitizers in ameliorating the progression of liver fibrosis.

Adiponectin, the major adipokine that has insulin-sensitizing, anti-inflammatory and anti-fibrotic effects [143], and whose decreased levels have been shown to correlate with hepatic fat accumulation [144-146], is another molecule of interest. Increased adipose tissue IR leading to reduced adiponectin levels has been described in patients with severe NASH and hyperglycemia compared to healthy controls independently of body mass [84, 85]. Several papers have reported a significant decrease in the serum levels of adiponectin in NASH patients [147, 148], and the overall evidence support the existence of an inverse relationship between adiponectin levels and the severity of NAFLD [149-151], suggesting a

| Table 3. Genetic risk factors for progressive liver disease in NAFLD evaluated in case-control studies. |
|-----------------------------------------------|
| **Gene**                                      | **SNP** |
| Genetic variants involved in modulation of steatosis |         |
| MTTP, microsomal triglyceride transfer protein [121, 122, 124, 125] | rs1800591 |
| PEMT, phosphatidylethanolamine N-methyltransferase [113, 114] | rs7946 |
| PPARγ, peroxisome proliferative activated receptor gamma [88] | rs1805192 |
| APOE, apolipoprotein E [129] | N/A |
| LPIN1, lipin 1 [94] | rs13412852 |
| Genetic variants involved in glucose metabolism |         |
| ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase1 or PC-1 [9] | rs1044498 |
| IRS1, insulin receptor substrate 1 [9] | rs1801278 |
| ADIPOQ, adiponectin [152] | rs2241766 |
| Genetic variants influencing redox status and stress response |         |
| SOD2, superoxide dismutase 2, mitochondrial [11, 160] | rs4880 |
| UCP3, uncoupling protein 3, mitochondrial [167] | rs1800849 |
| HFE, hemochromatosis [175, 290] | rs1800562 |
| Genetic variants influencing inflammation |         |
| TNFα, tumor necrosis factor alpha [207] | rs361525 |
| IL-6, interleukin [218] | rs1800795 |
| TLR4, toll-like receptor 4 [192] | rs986791 |
| IL28B [65, 230, 232, 237, 238] | rs12979860 |
| Genetic variants involved in HSCs activation and fibrogenesis |         |
| KLF6, kruppel-like factor 6 [275] | rs3750861 |
| TGF-β1, transforming growth factor beta [248, 253] | rs1800471 |

N/A: not available
role for adiponectin dysregulation in the pathogenesis of NASH [149]. Nevertheless, the available evidence on the correlation between adiponectin genetic variants and the progression of NAFLD is still debated. Tokushige et al. reported that two SNPs (+45T>G of exon 2 and +276G>T of intron 2) were associated with the progression of liver fibrosis and insulin resistance in NAFLD Japanese patients [152]. Musso et al. added that the same adiponectin SNPs modulate the acute adiponectin response to dietary fat, and are associated with the presence of NAFLD in the Italian population. Moreover, +45 TT and +276 GT/TT carriers had significantly increased prevalence and severity in NAFLD than in other genotypes [153]. Wang and colleagues observed hypo-adiponectinemia and insulin resistance in Chinese NAFLD patients with metabolic syndrome. However, they concluded that the T45G and G276T SNPs were not important determinants of NAFLD, even if they might influence serum ALT, BMI, insulin resistance, lipid metabolism, and plasma adiponectin concentration [154]. Interestingly, it seems that genetic variation in the hepatocellular receptor of adiponectin (ADIPOR2) may also influence liver fat content in Northern European subjects [155].

6.3. Variants Influencing Redox Status and the Stress Response

Increased FFA flux to the liver on a background of IR plays a key role in the pathogenesis of NASH through hepatocellular oxidative stress, which leads to reactive oxygen species (ROS) production during FFA oxidation.

The mitochondrial enzyme manganese-dependent superoxide dismutase (MnSOD) encoded by the nuclear SOD2 gene, plays an important role in protecting cells from superoxide radicals [156]. A common polymorphism in the SOD2 gene (C47T, rs4880) results in an amino acid substitution (Ala16Val) in the signal sequence targeting the enzyme to the mitochondrial matrix, where it exerts its function [157]. The Ala allele has been associated with more efficient protein import and with a higher enzyme activity [158, 159]. Therefore, the SOD2 rs4880 polymorphism has been investigated as a possible susceptibility factor in NASH and several other diseases related to oxidative stress including hereditary hemochromatosis [160]. In the largest available study in NAFLD, Al-Serri et al. examined a possible association between SOD2 genotype and susceptibility to NASH using two complementary approaches: a family study in which they analyzed trios consisting of children with fibrotic NAFLD and their two parents, and a classical case-control allelic association study in unrelated patients with NAFLD of varying severity. Using both the methodologies, a consistent association between the C47T SNP and fibrosis was demonstrated, providing persuasive genetic evidence that mitochondria-derived oxidative stress is important in the pathogenesis of advanced NAFLD [11]. Consistent with these findings, Namikawa et al. reported that Japanese patients with NASH had a higher incidence of the T/T SOD2 genotype [121].

Further evidence supporting a role for mitochondrial ROS comes from the evaluation of uncoupling protein 3 (UCP3), a mitochondrial transporter that uncouples the oxidative phosphorylation by increasing the proton leak of the inner mitochondrial membrane. Some studies have pointed to a role for UCP3 in the regulation of whole body energy homeostasis [161], diet induced obesity [162] and regulation of lipids substrates [163]. A SNP in the UCP3 promoter (-55CT, rs1800849) has been associated with increased expression of UCP3 mRNA in the skeletal muscle of Pima Indians [164] as well as with body mass [165] and an atherogenic lipid profile in French Caucasians [166]. More relevant, in NAFLD patients, the rs1800849 UCP3 -55CT genotype was associated with insulin resistance, adiponectin levels, the presence of moderate-severe steatosis and NASH [167].
It has been suggested that excess hepatic iron deposition, a frequent feature observed in patients with NAFLD, may contribute to oxidative stress within the liver [22, 168]. The C282Y and H63D mutations of the hemochromatosis (HFE) gene represent a common cause of inherited iron overload in individuals of European ancestry [169]. The mechanism is related to decreased hepcidin release leading to increased iron absorption and parenchymal accumulation [170]. Although initial studies reported that mild iron overload associated with heterozygosity for C282Y HFE mutation may confer susceptibility to NAFLD and cause relative insulin deficiency [171], the available literature suggests that HFE mutations do not predispose to steatosis [172]. It is conceivable that HFE mutations could contribute to oxidative stress via hepatic iron loading among patients with NASH, and increase the susceptibility to fibrosis progression however this is not entirely supported. The relationship between HFE mutations and liver fibrosis is controversial too. Initial reports suggested that increased ferritin levels were markers of histological damage, but that HFE mutations did not contribute to hepatic fibrosis in many patients with NAFLD [173]. Later, Nelson et al. suggested that the presence of the C282Y mutation was a risk factor for development of advanced hepatic fibrosis among US Caucasian patients with NASH [174]. More recently, two large multi-centre studies conducted in Northern Italy and the US again reported that iron deposition was a risk factor for moderate/severe fibrosis in patients with NAFLD, but that HFE genotype determination was not clinically useful in these patients unless evidence of severe parenchymal iron accumulation is obtained [175, 176]. As other genetic factors more reliably predicted hepatic iron accumulation (e.g. the beta-thalassemia trait [169]), it is likely that a wider panel of genetic variants influencing iron metabolism would be required to refine the risk of progressive disease. Independent of the genetic background, iron overload could be an appealing therapeutic target in some patients with NASH but this remains to be realised [168].

Finally, endoplasmic reticulum stress and the activation of the unfolded protein response has been implicated in NASH pathogenesis independently of oxidative stress [177]. Alpha-1-antitrypsin (AAT), is the principal serum protease inhibitor synthesized by the liver. Several variants of this gene have been described, the most common being the PiZ (Glu342Lys) and PiS (Glu264Val) alleles, whose prevalence is about 1% and 4% respectively in Northern Italy and show a decreasing gradient from North to South in Europe [178, 179], and potentially represent genetic modifiers of hepatocellular damage and inflammation. These amino acid substitutions lead to abnormal folding and spontaneous protein polymerization, determining endoplasmic reticulum stress and hepatocellular damage. Heterozygosity for the PiZ and to a lesser extent for the PiS alleles has been associated with cirrhosis and hepatocellular carcinoma (HCC) [180, 181]. In Italian patients with NAFLD, the presence of the PiS and PiZ alleles was associated with hyperferritinaemia and non-parenchymal iron accumulation, likely in response to the activation of the unfolded protein response in the endoplasmic reticulum [182], but on the other hand were not associated with more severe liver disease [183]. However, whether AAT mutations predispose to hepatocellular carcinoma in patients with NAFLD remains to be evaluated.

6.4. Variants Influencing Inflammation

Obesity and NAFLD are associated with the increased production of cytokines by hepatocytes and Kupffer cells in response to bacterial products of intestinal origin [24], leading to hepatic and systemic IR, and contributing to the progression from steatosis to NASH [2].

Toll-like receptor 4 (TLR4) is a transmembrane receptor which signals through adaptor proteins in activating downstream effectors that include nuclear factor kB (NF-kB) [184], mitogen-activated protein kinase, and phosphatidylinositol 3-kinase (PI3K) [185] that control cell survival and apoptosis [186], and plays a critical role in mediating the activation of Kupffer cells in the response to LPS in NAFLD [23, 187]. In the liver TLR4 signalling contributes to hepatic inflammation and injury in NAFLD [23, 188, 189]. The T399I (1196C=T) SNP of TLR4 gene emerged as conferring protection from fibrosis progression [190] along with the highly co-segregated D229G (896A=G) polymorphism. The TLR4 T399I and D229G are two common, highly linked non-synonymous SNPs within the extracellular domain of TLR4 protein, which may affect the strength of interactions with either agonists and/or co-receptors [191]. Guo et al. demonstrated that TLR4 D229G and T399I SNPs that are associated with protection from hepatic fibrosis reduce TLR4-mediated inflammatory and fibrogenic signalling, and lower the apoptotic threshold of activated hepatic stellate cells (HSCs) [192], thus suggesting a critical role of TLR4 signalling in regulating HSCs activation.

Evidence indicating that apoptosis is the major pathway of cell death during NASH make TNFα, a pro-inflammatory cytokine, a good candidate for a role in mediating liver injury given its ability to induce apoptosis in hepatocytes under conditions of oxidative stress and to induce IR. Two polymorphisms in the TNFα promoter region have been studied more extensively: one at position 308 (TNF2 allele) [193] and another at position 238 (TNFA allele) [194]. TNF2 allele is associated to increased constitutive and inducible expression of TNFα [195, 196]. Conflicting data have been reported on the TNFA allele [197], but most investigators believe that TNFA allele is associated to an increased release of this cytokine. Increasing evidence suggests that TNFα is involved in the pathogenesis and progression of liver disease of different aetiology [198-202]. TNFα SNPs have been reported to influence susceptibility to several hepatic diseases including alcoholic steatohepatitis [197] as TNFα appears to be involved in both the early stage of fatty liver disease and also the transition to steatohepatitis and more advanced stages of liver damage [203]. Conflicting data have been reported on the association of TNFα polymorphisms, serum insulin levels, IR index, per cent body fat, and type 2 diabetes mellitus [204-206]. The prevalence of the -238 TNFα polymorphism was reportedly higher in Italian patients with NAFLD than in controls, and TNFα polymorphisms were associated with IR, pancreatic ρ-cell function, and NASH [207]. Pastor et al. found that the TNFA allele is associated with a higher risk to develop liver cirrhosis in a Spanish alcoholic cohort [208]. In contrast, in a prospective cohort of Chinese patients with histology-proven NAFLD, TNFα polymorphisms were not associated with either presence of NAFLD or disease severity [209]. HCV infection is also associated with increased production of TNFα [210]. Our group reported that in patients with HCV chronic hepatitis TNFα genotype modulates the activity of the cytokine pathway, influences insulin sensitivity and the severity of HCV chronic hepatitis, but not liver steatosis [211]. On the contrary, Sanchez-Munoz et al. did not find any difference in insulin resistance, ρ-cell reserve, insulin and leptin levels between HCV patients with or without mutation at the promoter region of the TNFα gene [212]. All in all, results are not consistent across the populations evaluated, and therefore it is likely that the reported associations are explained by the extensive linkage disequilibrium and genetic variability within the HLA-C region including the TNFα locus, determining the associations of different TNFα alleles with other causal variants near the TNFA locus.

Interleukin 6 (IL-6) is another cytokine involved in both inflammation and IR [213]. However, whether specific IL-6 SNPs are associated with IR remains still disputed, given that conflicting results have been reported, probably due to population specific differences in the predisposition to IR and type 2 diabetes. The -174G/C promoter SNP has been reported to have either a protective or a promoting role for the development of type 2 diabetes [214, 215]. Several studies have shown that C allele is associated with IR, diabetes, and metabolic syndrome [215-217]. Carulli et al. found
that the IL-6 -174C variant was significantly more prevalent in NAFLD than in healthy subjects, was associated with increased fasting insulin and HOMA-IR, and was an independent predictor of NAFLD and NASH [218]. However, this study considered a very limited number of NAFLD patients of whom only half had had liver biopsy [218].

6.5. IL28B Genotype, Steatosis, and NASH

Genome-wide association studies have recently identified genetic variations near the IL28B gene, encoding for interferon (IFN)-α3, as a strong predictor of spontaneous and treatment-induced clearance of hepatitis C viral infection [219-224]. Protective variants at the rs12979860 and rs8099917 loci have consistently been associated with faster decline of viral load and an approximately two-fold increase in SVR rate during standard of care treatment, in particular in patients affected by the difficult to cure HCV genotype 1 and 4 [225, 226]. The mechanism by which these genetic variants influence the outcome of HCV infection, i.e. whether they influence IFN-α3 expression by affecting gene transcription or are linked a coding variant (Lys70Arg) of the IFN-α3 protein, is still debated [220, 224], but it seems to result in a different pattern of activation of the innate immune system against HCV infection, as determined by the different basal and IFN-α induced expression of interferon stimulated genes and inflammatory activity [227, 228], possibly influencing viral evolution under the selective pressure of the immune system [229].

Steatosis is frequently observed in patients with chronic hepatitis C (CHC), particularly those with genotype 3 infection, and is associated with fibrosis progression and treatment failure [230, 231]. Tillmann et al. recently reported a negative association between the interleukin 28B (IL28B) rs12979860 CC genotype, predicting sustained virological response [219], and steatosis in genotype-1 CHC [232]. Therefore, through the effect of inflammatory cytokines on lipid metabolism or by favouring a better control of HCV replication [211], IL28B favourable variants protect from the development of steatosis [232], and possibly from the steato-associated fibrosis progression and increased risk of HCC [230, 233]. In addition, the negative association between the CC genotype and steatosis may partly explain the association of steatosis with resistance to peginterferon plus ribavirin therapy in genotype 1 CHC patients. However, the effect of IL28B variants likely on fibrosis progression rate in patients with ongoing HCV was still controversial [231, 234, 235].

As discussed in the previous paragraphs, the PNPLA3 rs738409 polymorphism is a strong determinant of hepatic fat accumulation and steatohepatitis [8, 52], but also influences steatosis and fibrosis progression in CHC [64, 233, 236]. A previous study [236], also reported an association between IL28B rs12980275 genotype and steatosis in CHC non-genotype 3 patients, but the rs12979860 IL28B polymorphism was not tested and the interaction with PNPLA3 genotype was not analyzed in details. The negative association of rs12979860 CC with histologically-determined steatosis was recently confirmed in 567 naïve, consecutive, non-genotype 3 patients from referral centres in Milan and Vienna, without excessive alcohol intake [237]. The association between IL28B genotype and steatosis was independent of acquired risk factors, and of the PNPLA3 GG genotype. Interestingly however, the rs12979860 CC genotype protected form steatosis in patients positive, but not in those negative for the PNPLA3 G variant at risk, suggesting that an interaction occurs between IL28B and PNPLA3 genotypes in the pathogenesis of steatosis in CHC non-genotype-3 patients. In the same cohort of patients, a significant interaction between the rs12979860 IL28B CC and PNPLA3 genotype on liver damage was also observed, as the IL28B CC genotype was associated with advanced fibrosis only in patients negative for the PNPLA3 GG genotype independently of age, BMI, and ALT levels [65]. These data indicate that stratification for PNPLA3 GG genotype unmasked an association between IL28B CC genotype and more severe liver fibrosis, which may be related to increased hepatic inflammation associated with the favourable rs12879860 allele.

Interestingly enough, Petta et al. have recently reported that in 160 consecutive patients with biopsy-proven NAFLD, the IL28B rs12979860 CC genotype was not associated with protection from steatosis in the absence of viral infection, but it was associated with about a four-fold increased risk of moderate-severe lobular inflammation independently of age, gender, triglycerides, hyperuricemia, and steatosis grade, and was significantly associated with severe fibrosis (stage 3-4) at univariate analysis [238]. Intriguingly, the risk of more severe inflammation conferred by the “at risk” CC variant was particularly evident in subjects carrying also the PNPLA3 G allele. Provided that the association between IL28B genotype and hepatic inflammation complicating NAFLD is confirmed in larger series that are urgently awaited, data would suggest that the IL28B CC genotype represent a host factor influencing hepatic inflammation in different liver diseases, and the incorporation together with PNPLA3 in non-invasive scores could be useful to refine the risk of NASH.

6.6. Variants Involved in HSCs Activation and Fibrogenesis

Activated HSCs are the major source of extracellular matrix (ECM) deposition during fibrogenesis [239]. HSCs also release fibrogenic cytokines with autocrine and paracrine effects, including TGF-β1, and over-express tissue inhibitors of metalloproteinase, which promote ECM accumulation by inhibiting matrix degradation.

Kruppel-like factor 6 (KLF6) belongs to the Kruppel-like family of transcription factors that play diverse roles in differentiation, cell growth, apoptosis and angiogenesis [240]. KLF6 was identified as an early gene expressed in activated hepatic stellate cells (HSCs) after liver injury [241, 242], raising the possibility that it may be involved in the process of liver fibrogenesis. Indeed, KLF6 transactivates several genes critical for the development of liver fibrosis, including collagen 1, TGF-β1 and types I and II TGF-β receptors in HSCs [242], [243]. A functional SNP, the IVS1-27G>A SNP (rs3750861) located within the first intron, has been identified in the KLF6 gene [244]. Miele et al. showed that the presence of the KLF6 IVS1-27G>A SNP, which was demonstrated to reduce fibrogenesis in HSCs, was associated with less fibrosis in a UK cohort with biopsy-proven NAFLD patients. This trend was confirmed in an independent group of Italian patients. Moreover, analysis of the combined UK and Italian groups identified the presence of wild-type KLF6 as a predictor of moderate/advanced fibrosis independently of all other risk factors of progressive disease, suggesting that the wild-type KLF6 genotype is a significant susceptibility factor for fibrotic NAFLD, whereas KLF6 IVS1-27G>A protects against the development of fibrosis [12]. The effect of KLF6 genotype on NASH might not be limited to modulation of fibrogenesis, as it also influenced fasting glucose levels. Bechmann et al. observed that KLF6 IVS1-27G wild-type allele was associated with stepwise increase in fasting plasma glucose and insulin and reduced hepatic insulin sensitivity [245], and the effect was at least partially mediated by reduced expression of glucokinase, raising the possibility that the effect of this variant on the progression of liver damage in NASH might entail regulation of glucose and lipid metabolism.

The growth factor TGF-β1 also plays a dominant role in mediating hepatic fibrosis by contributing to the activation of HSCs [246, 247]. Several polymorphic sites have been described within the TGF-β1 gene. One non-synonymous SNP at codon 25 (+915) C/G, encoding an Arg25Pro substitution, modulates TGF-β1 production in vitro and occurs within the peptide signal sequence that is cleaved from the active TGF-β1 protein. Individuals with the Arg/Arg homozygous genotype produce substantially more TGF-β1 protein than individuals with the Arg/Pro genotype. In patients with chronic hepatitis C, those with the high TGF-β1-producing
future, it is likely that the evaluation of the role of these variants in including NAFLD. Given the recent technological developments establish genes involved in telomere maintenance as attractive targets exhaustion of the parenchymal regenerative compartment, and es-on the activation of fibrogenesis without taking into account the recurrence of HCC, and poor outcome after liver transplantation Most importantly however, mutations in TERT and in TERC, having a frequent risk factor for cirrhosis, being observed in 3-8% of [257]. Furthermore, they have recently been demonstrated to repre-sent a frequent risk factor for NASH identified so far, and both liver enzymes and steatosis was soon confirmed in obese children of different ethnicity [55, 56, 271, 272], and in one family study in Italian trios [52], indicating that it exerts its effect early in life. Most importantly, the magnitude of the association between the II48M variant of PNPLA3 and liver enzymes was shown to be related to the size of abdominal fat [60], and to high dietary carbohydrate and sugar consumption specifically during the developmental age [62]. Furthermore, PNPLA3 genotype influenced the histological severity of NASH alterations and fibrosis in obese pediatric patients who underwent biopsy because of persistently altered liver enzymes [54]. Interestingly, the association with fibrosis was stronger than in adults [50], in that, after adjustment for other risk factors such as age, waist circumference, hyperglycemia, and ALT levels, each 148M allele increased the risk of fibrosis by almost two-fold [54]. A more recent GWAS conducted in a larger population was able to identify a wider set of genetic variants influencing steatosis besides II48M of PNPLA3 [273], of whom the rs2854116 SNP of Glucokinase regulator (GCKR), involved in the regulation of the uptake of monosaccharides and lipogenesis was confirmed to predispose to fatty liver and dyslipidemia in obese children and ado-lescents independently of PNPLA3 [75], although the effect on histological progression of liver disease is still unknown, especially in view of the ameliorating effect on insulin resistance. Additional variants in genes implicated in NASH pathogenesis have been shown to influence liver damage and fibrosis progression in candidate gene case-control studies using pediatric patients. These include genetic variants regulating insulin receptor activity, namely the ENPP1 Lys121Gln and the IRS-1 Gly972Arg functional SNPs [9], the SOD2 C477T rs4880 SNP regulating SOD2 mitochondrial import and anti-oxidant activity [274], and the KLF6 IVS1-27G>A SNP regulating alternative splicing isoforms of the tran-scription factor KLF6 involved in the regulation of the regulation of metabolism in hepatocytes and fibrogenesis in hepatic stellate cells [275]. In contrast, variants in the APOC3 regulating VLDL metabo-
lism were not confirmed to influence the susceptibility to steatosis and NASH [276].

Finally, there is growing awareness that the expression of some genetic variants may be age-dependent, i.e. that the phenotype might be more (or less) marked or involve different traits during the developmental age. For example, the common variant (rs13412852) influencing the expression of Lipin-1 (LPIN1) [277], was associated with lipid levels, NASH severity, and hepatic fibrosis in children with NAFLD, whereas it influenced body mass, but not the severity of liver histology, in adults with NAFLD of the same ethnicity [278, 279].

To summarize, genetics has a key role in determining who among the large fraction of the pediatric population with metabolic risk factors will develop progressive liver disease [6]. The I148M variant of PNPLA3 is likely the major genetic determinant of increased hepatic fat content by interacting with body fat and dietary factors [8, 54, 60, 62], but it also influences the susceptibility to NASH and fibrosis, [278]. Additional studies are required to validate these findings at population level and in prospective studies, to evaluate whether PNPLA3 influences the response to therapy (see below), and to define the possible relevance of I148M genotype for the clinical management of patients, and in particular to develop new non-invasive scores that may avoid to perform liver biopsy that is especially problematic in young children. Evaluation of the interaction of PNPLA3 with other genetic variants influencing steatosis and NASH, including GCKR [75], ENPP1 and IRS-1 [9], SOD2 [274], KLIF6 [275], LPIN1 [278], and possibly other SNPs will be instrumental to achieve these goals.

8. INFLUENCE OF GENETICS ON TREATMENT OUTCOME

As the previous sections have highlighted, the genetic basis of susceptibility to NAFLD and progressive NASH is beginning to be elucidated, but very little is known about the effect of genetics on the response to treatment. Much of the uncertainty is obviously related to the lack of effective pharmacological treatment specific for NASH. However, lifestyle changes and in particular sustained weight loss has unequivocally demonstrated to improve histological features of NASH in the majority of patients [280, 281], suggesting that in most cases genetic risk factors are not able to cause NASH in the absence of environmental triggers. A recent systematic review and meta-analysis has shown that exercise interventions per se reduce liver fat despite minimal or no weight loss confirming a role for exercise as a therapeutic target in NAFLD [282].

The minor G allele of PNPLA3 has been suggested to impair triglyceride hydrolysis in vivo studies and several studies have shown that the GG carriers have an increase risk of NASH, for which weight loss is considered the perhaps best treatment [283]. In a recent study Sebastianova et al. evaluated whether weight loss is able to decrease liver fat in homozygous carriers of the G allele (PNPLA3-148MM). They found that 148II and 148MM patients lost similar amounts of body weight in response to a 6-day hypocaloric, low carbohydrate diet. However, liver fat content decreased significantly more in the 148MM group than in the 148II one, although this was in part because of the higher baseline levels. These data suggest that weight is an effective means for reducing liver fat content in subjects with PNPLA3-148MM [284], highlighting that NAFLD is a complex trait exhibiting a strong interaction between genetic and acquired risk factors for NASH, and most importantly confirming that behavioural changes can counteract the effects of the strongest known inherited risk factor for progressive NASH [50]. Pending more definitive studies, these data could provide a rationale to support use of low carbohydrate diet in subjects with NASH that poses the 148MM PNPLA3 genotype. Unfortunately, there are no published data addressing the interaction between other genetic variants and weight loss. We do not know whether the effect of antioxidant therapies such as vitamin E, which may provide some benefit in a sub-set of patients [285], might be influenced by polymorphisms modulating oxidative stress response [11]. Although SNPs in the PPARγ gene influence IR, it is not known whether the effect of insulin sensitizing drugs such as glitazones that target this transcription factor and reduce steatosis in some patients with NASH [285] are influenced by PPARγ genotype.

Limited data are available on possible role of genetic factors in influencing the effect of iron depletion therapy. Indeed, hyperferritinaemia reflecting increased body iron stores is frequently observed in NAFLD due to the association with steatosis and IR, and associated with faster progression of organ damage [168]. Iron depletion by phlebotomy has been reported to decrease both IR and liver enzymes in NAFLD patients more than lifestyle changes alone [286]. A retrospective study has also shown that iron depletion produced a significantly greater improvement in insulin sensitivity than nutritional counselling alone and that iron depletion was effective in reducing HOMA-R in patients with high ferritin concentrations and in carriers of HFE genetic mutations causing hereditary hemochromatosis [287], suggesting that HFE genotyping might be used to select subjects who might benefit most from this approach. However, the study was not designed to test this hypothesis and the outcome was not evaluated histologically, this therefore remains speculative.

9. FUTURE DIRECTIONS

Despite the recent progresses, several key issues remains to be addressed in the next years, including, but not limited to:

1. The mechanism linking the I148M PNPLA3 variant with progressive liver disease, its role in other liver diseases, and the potential clinical utility of its determination for best tailoring the clinical management of the patients with NASH.
2. The validation of the association of other genetic variants associated with liver fat content in GWAS studies (e.g. GCKR) with the progression of liver diseases associated with steatosis.
3. The evaluation of risk factors associated with advanced disease in patients at risk undergoing liver biopsy by a GWAS approach.
4. The evaluation of the role of copy number variants and relatively rare gene variants associated with a high effect on the risk of progressive NASH.
5. The evaluation of the interaction between genetic and acquired risk factors in the pathogenesis of NASH.
6. The development and assessment of the utility diagnostic and prognostic scores incorporating multiple genetic risk factors associated with clinically relevant end-points.
7. The evaluation of the effect of genetic factors on the response to therapy, including specific diets and physical activity programs and drugs.

10. CONCLUSION

Genes play a key role in the development and progression of NAFLD by interacting with environmental factors. To date, PNPLA3 polymorphisms are the best validated susceptibility modifiers for steatosis and progressive hepatic injury. However, several other genetic variants that contribute to steatosis and/or steatohepatitis have been identified through GWAS studies, and risk factors of progressive NASH have been validated in other large multi-centre studies (Tables 1-3). It is now important to explore the molecular mechanisms underlying these associations between gene variants and progressive liver disease, and to evaluate their impact on the response to available therapies. It is hoped that this knowledge will offer further insights into pathogenesis, suggest novel therapeutic targets, and help guide physicians towards individualised therapy that improves clinical outcome.
CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

QMA is the recipient of a Clinical Senior Lectureship Award from the Higher Education Funding Council for England (HEFCE) and is a member of the FLIP research consortium funded by the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement Health-F2-2009-241762.

REFERENCES

[1] Marchesini G, Brizi M, Bianchi G, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes 2001; 50: 1844-50.

[2] Day CP. From fat to inflammation. Gastroenterology 2006; 130: 207-10.

[3] Bugianesi E, Leone N, Vanni E, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002; 123: 134-40.

[4] 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: 1387-95.

[5] Bellentani S, Saccoccio G, Masutti F, et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. Ann Intern Med 2000; 132: 112-7.

[6] Schwimmer JB, Celedon MA, Lavine JE, et al. Heritability of nonalcoholic fatty liver disease. Gastroenterology 2009; 136: 1585-92.

[7] Guerrero R, Vega GL, Grundy SM, Browning JD. Ethnic differences in hepatic steatosis: an insulin resistance paradox? Hepatology 2009; 49: 791-801.

[8] Romeo S, Kozlitina J, Vega GL, Grundy SM, Browning JD. Heritability of nonalcoholic fatty liver disease. J Hepatol 2010; 53: 927-33.

[9] Al-Serri A, Anstee QM, Valenti L, et al. Prevalence of nonalcoholic fatty liver disease and associated variables in the Framingham offspring study. Hepatology 2009; 50: 267-73.

[10] Valenti L, Canavesi E, Galmozzi E, et al. Beta-globin mutations are associated with parenchymal siderosis and fibrosis in patients with non-alcoholic fatty liver disease. J Hepatol 2010; 53: 134-42.

[11] Al-Serri A, Anstee QM, Valenti L, et al. The SOD2 C47T polymorphism influences NAFLD fibrosis severity: evidence from case-control and intraindividual allele association studies. Journal of Hepatology 2012; 56: 54-56.

[12] Miele L, Beale G, Patman G, et al. The Kruppel-like factor 6 genotype in nonalcoholic fatty liver disease. Proc Natl Acad Sci U S A; 107: 134: 1369-75.

[13] Miele L, Valenza V, La Torre G, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology 2009; 49: 1877-87.

[14] Korenblat KM, Fabbrini E, Mohammed BS, Klein S. Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. Gastroenterology 2008; 134: 1369-75.

[15] Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N Engl J Med 2010; 363: 1341-50.

[16] Fabbri E, Mohammed BS, Magkos F, Korenblat KM, Patterson BW, Klein S. Alterations in adipose tissue and hepatic lipid kinet-ics in obese men and women with nonalcoholic fatty liver disease. Gastroenterology 2008; 134: 424-31.

[17] Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroen-terology 1998; 114: 842-5.

[18] Valenti L, Fracanzani AL, Fargin S. The immunopathogenesis of alcoholic and nonalcoholic steatohepatitis: two triggers for one disease? Semin Immunopathol 2009; 31: 359-69.

[19] Valenti L, Valenza V, La Torre G, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology 2009; 49: 1877-87.

[20] Valenti L, Fracanzani AL, Fargin S. The immunopathogenesis of alcoholic and nonalcoholic steatohepatitis: two triggers for one disease? Semin Immunopathol 2009; 31: 359-69.

[21] Miele L, Valenza V, La Torre G, et al. Increased intestinal perme-ability and tight junction alterations in nonalcoholic fatty liver dis-ease. Hepatology 2009; 49: 1877-87.

[22] Valenti L, Valenza V, La Torre G, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology 2009; 49: 1877-87.

[23] Valenti L, Fracanzani AL, Fargin S. The immunopathogenesis of alcoholic and nonalcoholic steatohepatitis: two triggers for one disease? Semin Immunopathol 2009; 31: 359-69.

[24] Miele L, Valenza V, La Torre G, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology 2009; 49: 1877-87.
Miraglia Del Giudice E, Grandone A, Cirillo G, Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain-containing 3 (PNPLA3) with histological severity of non-alcoholic fatty liver disease. Hepatology 2010; 52: 904-903.

Kollerits B, Coassin S, Beckmann ND, et al. Genetic evidence for a role of adiponutrin in the metabolism of apolipoprotein B-containing lipoproteins. Hum Mol Genet 2009; 18: 4669-76.

Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain-containing 3 gene (PNPLA3) on the susceptibility and histological severity of non-alcoholic fatty liver disease. Hepatology 2011; 53: 1833-94.

Valenti L, Al-Serri A, Daly AK, et al. Homozygosity for the patatin-like-phospholipase-3/adiponutrin 1148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology; 51: 1209-17.

Valenti L, Al-Serri A, Daly AK, et al. Homozygosity for the PNPLA3/adiponutrin 1148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2010; 51: 1209-17.

Valenti L, Alisi A, Nobili V. I148M PNPLA3 variant and progressive liver disease: A new paradigm in hepatology. Hepatology 2012; 56: 1883-9.

Valenti L, Alisi A, Galmizzi E, et al. I148M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric non-alcoholic fatty liver disease. Hepatology 2010; 52: 1274-80.

Valenti L, Alisi A, Galmizzi E, et al. I148M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric non-alcoholic fatty liver disease. Hepatology; 52: 1274-80.

Romero 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 (Lond) 2009.

Tian C, Stokowski RP, Kershohenbich D, Ballinger DG, Hinds DA. Variant in PNPLA3 is associated with alcoholic liver disease. Nat Genet 2009; 42: 21-3.

Miraglia Del Giudice E, Grandone A, Cirillo G, et al. The Association of PNPLA3 Variants with Liver Enzymes in Childhood Obesity Is Driven by the Interaction with Abdominal Fat. PLoS One 2011; 6: e27933.

Graff M, North KE, Franceschini N, et al. PNPLA3 gene-by-visceral adipose tissue volume interaction and the pathogenesis of fatty liver disease: The NHLBI Family Heart Study. Int J Obes (Lond) 2012.

Davvis JN, Le KA, Walker RW, et al. Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in PNPLA3 and high dietary carbohydrate and sugar consumption. Am J Clin Nutr 2011; 92: 1522-7.

Valenti L, Rumi M, Galmizzi E, et al. Patatin-like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. Hepatology; 53: 791-9.

Trepo E, Pradat P, Potthoff A, et al. Impact of PNPLA3 (rs738409 C>G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. Hepatology 2011; 54: 60-9.

Valenti L, Aghemo A, Stuttermayer AF, et al. Implications of PNPLA3 polymorphism in chronic hepatitis C patients receiving peginterferon plus ribavirin. Aliment Pharmacol Ther 2012; 35: 1434-42.

Valenti L, Colombo M, Fargion S. Modulation of the effect of PNPLA3 I148M mutation on steatosis and liver damage by alcohol intake in patients with chronic hepatitis C. J Hepatol 2011; 55: 1470-1; author reply 1-2.

Stickel F, Buch S, Lau K, et al. Genetic variation in the PNPLA3 (rs738409 C>G) polymorphism in cirrhosis: relationship with the aetiology of liver disease and hepatocellular carcinoma occurrence. Liver Int 2011; 31: 1137-43.

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: 1307-8.

Nischalke HD, Berger C, Luda C, et al. The PNPLA3 rs738409 148/M genotype is a risk factor for liver cancer in alcoholic cirrhosis but shows no or weak association in hepatitis C cirrhosis. PLoS One 2012; 7: e27087.

Stickel F, Hampe J. Genetic determinants of alcoholic liver disease. Gut 2011; 61: 150-9.

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: 1037-41.

Speliotes EK, Yerges-Armstrong LM, Wu J, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet; 7: e1001324.

Beer NL, Tribble ND, McCulloch LJ, et al. The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. Hum Mol Genet 2009; 18: 4081-3.

Santoro N, Zhang CK, Zhao H, et al. Variant in the glucokinase regulatory protein (GCKR) gene is associated with fatty liver in obese children and adolescents. Hepatology 2011; 55: 781-9.

Valenti L, Alisi A, Nobili V. Unraveling the genetics of fatty liver in obese children: Additive effect of P446L GCKR and 1148M PNPLA3 polymorphisms. Hepatology 2012; 55: 661-3.

Chalasani N, Guo X, Loomba R, et al. Genome-wide association study identifies variants associated with histologic features of non-alcoholic fatty liver disease. Gastroenterology; 139: 1567-76, 76 e1-6.

Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002; 346: 1221-31.

Kim H, Haluzik M, Asghar Z, et al. Peroxisome proliferator-activated receptor-alpha agonist treatment in a transgenic model of type 2 diabetes reverses the lipotoxic state and improves glucose homeostasis. Diabetes 2003; 52: 1770-8.

Stienstra R, Saudale F, Duval C, et al. Kupffer cells promote hepatic steatosis via interleukin-1beta-dependent suppression of peroxisome proliferator-activated receptor alpha activity. Hepatology; 51: 511-22.

Chen S, Li Y, Li S, Yu C. A Val227Ala substitution in the peroxisome proliferator activated receptor alpha (PPAR alpha) gene is associated with non-alcoholic fatty liver disease and decreased waist circumference and waist-to-hip ratio. J Gastroenterol Hepatol 2008; 23: 1415-8.

Yamakawa-Kobayashi K, Ishiguro H, Arinami T, Miyazaki R, Hamaguchi H. A Val227Ala polymorphism in the peroxisome proliferator-activated receptor alpha (PPARalpha) gene is associated with variations in serum lipid levels. J Med Genet 2002; 39: 189-91.

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; 10: 102.

Belfort R, Harrison SA, Brown K, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med 2006; 355: 2297-307.
[85] Gastaldelli A, Harrison SA, Belfort-Aguilar R, et al. Importance of changes in adipose tissue insulin resistance to histological response during thiazolidinedione treatment of patients with nonalcoholic steatohepatitis. Hepatology 2009; 50: 1087-93.

[86] Tonjes A, Scholz M, Loeflfer 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: 2489-97.

[87] Rey JW, Noetel A, Hardt A, et al. Pro12Ala polymorphism of the peroxisome proliferator-activated receptor gamma2 in patients with fatty liver disease. World J Gastroenterol; 16: 5830-7.

[88] Gavrielis S, Marion MC, Konnori K, et al. Genetic Variation in the Peroxisome Proliferator Activated Receptor-Gamma Gene Is Associated with Histologically Advanced NAFLD. Dig Dis Sci 2012; 57: 952-7.

[89] Reue K, Zhang P. The lipin protein family: dual roles in lipid biosynthesis and gene expression. FEBS Lett 2008; 582: 90-6.

[90] Reue K. The lipin family: mutations and metabolism.Curr Opin Lipidol 2009; 20: 165-70.

[91] 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: 209-17.

[92] Burgdorf KS, Sandholt CH, Sparso T, et al. Studies of association between LPIN1 variants and metabolic phenotypes among 17,538 Danes. Eur J Endocrinol; 163: 81-7.

[93] Fawcett KA, Grimesy 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: 2527-33.

[94] Valentl L, Motta BM, Alisi A, et al. LPIN1 rs13412852 Polymorphism and Fatty Acid Transport in Pediatric Non-Alcoholic Fatty Liver Disease. J Pediatr Gastroenterol Nutr

[95] Gimeno RE, Hirsch DJ, Punreddy S, et al. Targeted Deletion of Fatty Acid Transport Protein-4 Results in Early Embryonic Lethality. J Biol Chem 2003; 278: 49512-6.

[96] Motojima K, Passilly P, Peters JM, Gonzalez FJ, Latruffe N. Expression and functional analysis of a human homologue of the bacterial acyl-CoA ligase EC 6.2.1. 2. J Biol Chem 2002; 277: 16961-8.

[97] Hirsch DJ, Stahl A, Lodish HF. A family of fatty acid transporters conserved from mycobacterium to man. Proc Natl Acad Sci U S A 1998; 95: 8625-9.

[98] Steinberg SJ, Mihalik SJ, Kim DG, Cuevas DA, Watkins PA. The human liver-specific homolog of very long-chain acyl-CoA synthetase is cholate:CoA ligase. J Biol Chem 2000; 275: 15609-8.

[99] Hubbard B, Doege H, Punreddy S, et al. Mouse deletion of fatty acid transport protein 5 have defective bile acid conjugation and are protected from obesity. Gastroenterology 2006; 130: 1259-69.

[100] Doege H, Grimm D, Falcon A, et al. Sphingolipids 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: 22186-92.

[101] Doege H, Baillie RA, Ortegon AM, et al. Targeted deletion of FATP5 reveals multiple functions in liver metabolism: alterations in hepatic lipid homeostasis. Gastroenterology 2006; 130: 1245-58.

[102] Chang LW, Nagarajan R, Magee JA, Milbrandt J, Stormo GD. A systematic model to predict transcriptional regulatory mechanisms based on overrepresentation of transcription factor binding profiles. Genome Res 2006; 16: 405-13.

[103] Auerger A, Valenti L, Pfueffer 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: 854-9.

[104] Yao ZM, Vance DE. Head group specificity in the requirement of phosphatidylethanolamine N-methyltransferase from liver. Biochim Biophys Acta 1997; 1348: 18-30.

[105] Zhu X, Song J, Mar MH, Edwards LJ, Zeisel SH. Phosphatidyl ethanolamine N-methyltransferase (PEMT) knockout mice have hepatic steatosis and abnormal hepatic choline metabolism concentrations despite ingesting a recommended dietary intake of choline. Biochem J 2003; 370: 987-93.

[106] Caballero F, Fernandez A, Matias N, et al. Specific contribution of methionine and choline in nutritional nonalcoholic steatohepatitis: impact on mitochondrial S-adenosyl-L-methionine and glutathione. J Biol Chem; 285: 18528-36.

[107] Walkey CJ, Yu L, Agellon LB, Vance DE. Biochemical and evolutionary significance of phospholipid methylation. J Biol Chem 1998; 273: 27043-6.

[108] 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: 1266-71.

[109] 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: 209-17.

[110] Burgdorf KS, Sandholt CH, Sparso T, et al. Studies of association between LPIN1 variants and metabolic phenotypes among 17,538 Danes. Eur J Endocrinol; 163: 81-7.

[111] Fawcett KA, Grimesy 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: 2527-33.

[112] Valentl L, Motta BM, Alisi A, et al. LPIN1 rs13412852 Polymorphism and Fatty Acid Transport in Pediatric Non-Alcoholic Fatty Liver Disease. J Pediatr Gastroenterol Nutr

[113] Gimeno RE, Hirsch DJ, Punreddy S, et al. Targeted Deletion of Fatty Acid Transport Protein-4 Results in Early Embryonic Lethality. J Biol Chem 2003; 278: 49512-6.

[114] Motojima K, Passilly P, Peters JM, Gonzalez FJ, Latruffe N. Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor alpha and gamma activators in a tissue- and inducer-specific manner. J Biol Chem 1998; 273: 16710-4.

[115] Wu Q, Ortegon AM, Tsang B, Doege H, Feingold KR, Stahl A. FATP1 is an insulin-sensitive fatty acid transporter involved in diet-induced obesity. Mol Cell Biol 2006; 26: 3455-67.

[116] 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: 8625-9.

[117] Steinberg SJ, Mihalik SJ, Kim DG, Cuevas DA, Watkins PA. The human liver-specific homolog of very long-chain acyl-CoA synthetase is cholate:CoA ligase. J Biol Chem 2000; 275: 15609-8.

[118] Hubbard B, Doege H, Punreddy S, et al. Mouse deletion of fatty acid transport protein 5 have defective bile acid conjugation and are protected from obesity. Gastroenterology 2006; 130: 1259-69.

[119] Doege H, Grimm D, Falcon A, et al. Sphingolipids 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: 22186-92.

[120] Doege H, Baillie RA, Ortegon AM, et al. Targeted deletion of FATP5 reveals multiple functions in liver metabolism: alterations in hepatic lipid homeostasis. Gastroenterology 2006; 130: 1245-58.

[121] Chang LW, Nagarajan R, Magee JA, Milbrandt J, Stormo GD. A systematic model to predict transcriptional regulatory mechanisms based on overrepresentation of transcription factor binding profiles. Genome Res 2006; 16: 405-13.

[122] Auerger A, Valenti L, Pfueffer 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: 854-9.

[123] Yao ZM, Vance DE. Head group specificity in the requirement of phosphatidylethanolamine N-methyltransferase from liver. Biochim Biophys Acta 1997; 1348: 18-30.

[124] Noga AA, Zhao Y, Vance DE. An unexpected requirement for phosphatidylethanolamine N-methyltransferase in the secretion of very low density lipoproteins. J Biol Chem 2002; 277: 42358-65.

[125] Hebbard L, George J. Animal models of nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol 2011; 8: 35-44.
Genetic Predisposition in NAFLD and NASH

[129] Sazci A, Akipinar G, Aygun C, Ergul E, Senturk O, Halagu S. Association of apolipoprotein E polymorphisms in patients with non-alcoholic steatohepatitis. Dig Dis Sci 2008; 53: 3218-24.

[130] Agnello V, Abel G, Elfahal M, Knight GB, Zhang QX. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. J Gastroenterol Sci U S A 1999; 96: 1276-82.

[131] Price DA, Bassendine MF, Norris SM, et al. Apolipoprotein epsilon 3 allele is associated with persistent hepatitis C virus infection. Gut 2006; 55: 715-8.

[132] Ginsberg HN, Le NA, Goldberg J, et al. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI. Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase in vivo. J Clin Invest 1986; 78: 1287-95.

[133] Altomonte J, Cong L, Harbaran S, et al. Foxo1 mediates insulin action on apoC-III and triglyceride metabolism. J Clin Invest 2004; 114: 1493-503.

[134] Petersen KE, Dufour S, Hariri A, et al. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. N Engl J Med; 362: 1082-9.

[135] Kozlitina J, Boerwinkel E, Cohen JC, Hobbs HH. Dissociation between APOC3 variants, hepatic triglyceride content and insulin resistance. Hepatology; 53: 467-74.

[136] 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 subjects. European Lipids Health Dis; 10: 93.

[137] 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.

[138] Fracanzani AL, Valenti L, Nobili V, Al-Serri A, et al. The association of apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins. J Lipid Res 2009; 50: 976-82.

[139] Kozlitina J, Boerwinkel E, Cohen JC, Hobbs HH. Dissociation between APOC3 variants, hepatic triglyceride content and insulin resistance. Hepatology; 53: 467-74.

[140] Sutton A, Khoury H, Prip-Buus C, Cepanece D, Dengoul F. The Ala16Val genetic polymorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. Pharmacoenet Genomics 2005; 15: 311-9.

[141] Jellema A, Zeegers MP, Feskens EJ, Mensink RP. ENPP1 (PC-1) gene on insulin resistance, obesity, and Type 2 diabetes: a meta-analysis of 27 studies. Diabetologia 2003; 46: 990-5.

[142] Prudente S, Trischitta V. Editorial: The pleiotropic effect of the ENPP1 (PC-1) gene on insulin resistance, obesity, and type 2 diabetes. J Clin Endocrinol Metab 2006; 91: 4767-8.

[143] Kadowaki T, Yamouchi T, Kadowaki T. ApoCIII and adiponectin receptors. Endoher Rev 2005; 26: 439-51.

[144] Bugianesi E, Pagotto U, Manini R, et al. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. J Clin Endocrinol Metab 2005; 90: 3498-504.

[145] Mussio G, Gambino R, Biroti G, et al. Hydropioidpioncinemia predicts the severity of hepatic fibrosis and pancreatic Beta-cell dysfunction in nondiabetic obese patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2005; 100: 2438-46.

[146] Bianchi G, Bugianesi E, Frestyck J, Tarnow L, Flyvbjerg A, Marchesini G. Adiponectin isoforms, insulin resistance and liver histology in non alcoholic fatty liver disease. Dig Liver Dis; 43: 73-7.

[147] Hui JM, Hodge A, Farrell GC, Kench JG, Krietos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? Hepatology 2004; 40: 46-54.

[148] Shimada M, Kawahara H, Ozaki K, et al. Usefulness of a combined evaluation of the serum adiponectin level, HOMA-IR, and serum type IV collagen 7S level to predict the early stage of nonalcoholic steatohepatitis. J Gastroenterol 2007; 102: 1931-8.

[149] Polyzos SA, Toulis KA, Goulis DG, Zavos C, Kountouras J. Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. Metabolism; 60: 313-26.

[150] Kamada Y, Tamura S, Kiso S, et al. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. Gastroenterology 2003; 125: 1796-907.

[151] Matsumoto H, Tamura S, Kamada Y, et al. Adiponectin deficiency exacerbates lipopolysaccharide-D-galactosamine-induced liver injury in mice. World J Gastroenterol 2006; 12: 3352-8.

[152] Tokushige K, Hashimoto E, Noto H, et al. Influence of adiponectin gene polymorphisms in Japanese patients with non-alcoholic fatty liver disease. J Gastroenterol 2009; 44: 976-82.

[153] Musso G, Gambino R, De Michiel F, Durazzo M, Pagano G, Casadero M. Adiponectin gene polymorphisms modulate acute adiponectin response to dietary fat: Possible pathogenetic role in NASH. Hepatology 2008; 47: 1167-77.

[154] Wang ZL, Xia B, Shrestha U, et al. Correlation between adiponectin polymorphisms and non-alcoholic fatty liver disease with or without metabolic syndrome in Chinese population. J Endocrinol Invest 2008; 31: 1086-91.

[155] Kotronen A, Yki-Jarvinen H, Aminoff A, et al. Genetic variation in the ADIPOR2 gene is associated with liver fat content and its surrogate markers in three independent cohorts. Eur J Endocrinol 2008; 160: 593-601.

[156] Macmillan-Crow LA, Cuthirds DL. Invited review: manganese superoxide dismutase in disease. Free Radic Res 2001; 34: 325-36.

[157] Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa-Hattori Y, Shimizu Y, Mizuno Y. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change that influence mitochondrial transport and a study of allelic association in Parkinson's disease. Biochem Biophys Res Commun 1996; 226: 561-9.

[158] Sutton A, Imbert A, Igoudji A, et al. The manganese superoxide dismutase Ala16Val polymorphism modulates both mitochondrial import and mRNA stability. Hepatology 2007; 51: 3748-57.

[159] de Luis DA, Aller R, Izaola O, Sagrado MG, Conde R. Modulation of adipokynines response and weight loss secondary to a hypocaloric diet in obese patients by -55CT polymorphism of UCP3 gene. Horm Metab Res 2008; 40: 214-8.

[160] de Luis DA, Aller R, Izaola O, Gonzalez Sagrado M, Conde R, Perez Carriolion J. Lack of association of -55CT polymorphism of UCP3 gene with fat distribution in obese patients. Ann Nutr Metab 2007; 51: 374-8.

[161] Samec S, Seydoux J, Dullio AG. Role of UCP homologues in skeletal muscles and brown adipose tissue: mediators of thermogenesis or regulators of lipids as fuel substrate? FASEB J 1998; 12: 715-24.

[162] Onbe S, Clement K, Dina C, et al. A genetic variation in the 5′ flanking region of the UCP3 gene is associated with body mass index in humans in interaction with physical activity. Diabetologia 2000; 43: 245-9.

[163] Meirhaeghe A, Amoyel P, Helbecque N, et al. An uncoupling protein 3 gene polymorphism associated with a lower risk of developing Type II diabetes and with atherogenic lipid profile in a French cohort. Diabetologia 2000; 43: 1426-7.

[164] Aller R, de Luis DA, Izaola O, et al. Role of -55CT polymorphism of UCP3 gene on non alcoholic fatty liver disease and insulin resistance in patients with obesity. Nutr Hosp; 25: 572-6.

[165] Dongiovanni P, Fracanzani AL, Fargion S, Valenti L. Iron in fatty liver and in the metabolic syndrome: A promising therapeutic target. J Gastroenterol Hepatol 2011; 26: 1322-33.

[166] Adams PC, Reboussin DM, Barton JC, et al. Hemochromatosis and iron-overload screening in a racially diverse population. N Engl J Med 2005; 352: 1769-78.

[167] Pietrangelo A. Hemochromatosis: an endocrine liver disease. Hepatology 2004; 40: 46-54.

[168] Bruguera M, Rollet J, Garcia-Tabanera J, et al. Evaluation of the serum adiponectin level, HOMA-IR, and serum type IV collagen 7S level to predict the early stage of nonalcoholic steatohepatitis. Dig Dis Sci 2008; 53: 1276-81.
D’Alfonso S, Richardi PM. A polymorphic variation in a putative regulation box of the TNFA promoter region. Immunogenetics 1994; 39: 150-4.

Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. Mol Cell Biol 1993; 13: 6883-8.

Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci U S A 1997; 94: 3195-9.

Grove J, Daly AK, Bassendine MF, Day CP. Association of a tumor necrosis factor promoter polymorphism with susceptibility to alcoholic steatohepatitis. Hepatology 1997; 26: 143-6.

Hohler T, Kruger A, Gerken G, Schneider PM, Meyer zum Buschenfelde KH, Rittner C. Tumor necrosis factor alpha polymorphism at position -238 is associated with chronic active hepatitis C infection. J Med Virol 1998; 54: 173-7.

Jones DE, Watt FE, Grove J, et al. Tumour necrosis factor-alpha promoter polymorphisms in primary biliary cirrhosis. J Hepatol 1999; 30: 232-6.

Cookson S, Constantini PK, Clare M, et al. Frequency and nature of cytokine gene polymorphisms in type 1 autoimmune hepatitis. Hepatology 1999; 30: 851-6.

Bernal W, Donaldson P, Underhill J, Wendon J, Williams R. Tumor necrosis factor-alpha promoter polymorphism and outcome of acetaminophen (paracetamol)-induced acute liver failure. J Hepatol 1998; 29: 53-9.

Fargion S, Valenti L, Dongiovanni P, et al. Tumor necrosis factor alpha promoter polymorphisms influence the phenotype expression of hereditary hemochromatosis. Blood 2001; 97: 3707-12.

Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. N Engl J Med 2000; 343: 1467-76.

Rasmussen SK, Urhammer SA, Jensen JN, Hansen T, Borch-Johnsen K, Pedersen O. The -238 and -308 G-->A polymorphism of the tumor necrosis factor alpha gene promoter are not associated with features of the insulin resistance syndrome or altered birth weight in Danish Caucasians. J Clin Endocrinol Metab 2000; 85: 1731-4.

Fernandez-Real JM, Gutierrez C, Ricart W, et al. The TNF-alpha gene Nco I polymorphism influences the relationship among insulin resistance, percent body fat, and increased serum leptin levels. Diabetes 1997; 46: 1468-72.

Koch M, Reit K, Volk A, et al. The tumor necrosis factor alpha -238 G-->A promoter polymorphism is associated with insulin sensitivity and insulin secretion in young healthy relatives of Type II diabetic patients. Diabetologia 2000; 43: 181-4.

Valenti L, Fracanzani AL, Dongiovanni P, et al. Tumor necrosis factor alpha promoter polymorphisms and insulin resistance in nonalcoholic fatty liver disease. Gastroenterology 2002; 122: 274-80.

Pastor II, Laso FJ, Romero A, Gonzalez-Sarmiento R. -238 G-->A polymorphism of the tumor necrosis factor alpha gene (TNFA) is associated with alcoholic liver cirrhosis in alcoholic Spanish men. Alcohol Clin Exp Res 2005; 29: 1928-31.

Wong VW, Wong GL, Tsang SW, et al. Genetic polymorphisms of adiponectin and tumor necrosis factor-alpha and nonalcoholic fatty liver disease in Chinese people. J Gastroenterol Hepatol 2008; 23: 914-21.

Gochee PA, Jonsson JR, Clouston AD, Pandeya N, Purdie DM, Powell EE. Steatosis in chronic hepatitis C: association with increased messenger RNA expression of collagen I, tumor necrosis factor-alpha and cytochrome P450 2E1. J Gastroenterol Hepatol 2003; 18: 386-92.

Valenti L, Pulixi E, Fracanzani AL, et al. TNFalpha genotype affects TNFalpha release, insulin sensitivity and the severity of liver disease in HCV chronic hepatitis. J Hepatol 2005; 43: 944-50.

Sanchez-Munoz D, Romero-Gomez M, Gonzalez-Escritano MF, et al. Tumor necrosis factor alpha polymorphisms are not involved in the development of steatosis in chronic hepatitis C. Eur J Gastroenterol Hepatol 2004; 16: 761-5.

Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation 2005; 111: 1446-54.
Genetic Predisposition in NAFLD and NASH

Fernandez-Real JM, Broch M, Vendrell J, et al. Interleukin-6 gene polymorphism and insulin sensitivity. Diabetes 2000; 49: 517-20.

Kubaszek A, Phlajamaki J, Punnonen K, Karhapaa P, Vauhkonen I, Laakso M. The C-174G promoter polymorphism of the IL-6 gene affects energy expenditure and insulin sensitivity. Diabetes 2003; 52: 556-61.

Berthier MT, Paradis AM, Tchernof A, et al. The interleukin 6-174G/C polymorphism is associated with indices of obesity in men. J Hum Genet 2003; 48: 14-9.

Wernstedt I, Eriksson AL, Berndtsson A, et al. A common polymorphism in the interleukin-6 gene promoter is associated with overweight. Int J Obes Relat Metab Disord 2004; 28: 1272-9.

Carulli L, Canedi I, Rondinella S, et al. Genetic polymorphisms in non-alcoholic fatty liver disease: interleukin-6-174G/C polymorphism is associated with non-alcoholic steatohepatitis. Dig Liver Dis 2009; 41: 823-8.

Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009; 461: 399-401.

Lange CM, Zeuzem S. IL28B single nucleotide polymorphisms in the treatment of hepatitis C. J Hepatol 2011; 55: 692-701.

Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 2009; 461: 798-801.

Rauch A, Kutalik Z, Descomps P, et al. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 2010; 138: 1338-45, 45 e1-7.

Suppiah V, Moldovan M, Ahlenstiel G, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009; 41: 1100-4.

Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009; 41: 1105-9.

Galmozzi E, Del Menico B, Rametta R, et al. A tetra-primer amplification refractory mutation system polymerase chain reaction for the evaluation of rs12979860 genotype. J Viral Hepatitis 2010; 18: 628-30.

De Nicola S, Aghemo A, Rumi MG, et al. An IL28B polymorphism predicts pegylated interferon plus ribavirin treatment outcome in chronic hepatitis C genotype 4. Hepatology 2011; 53: 348-56.

Honda M, Sakai A, Yamashita T, et al. Hepatic ISG expression is associated with genetic variation in interleukin 28B and the outcome of IFN therapy for chronic hepatitis C. J Gastroenterology 2010; 139: 499-509.

Abe H, Ochi H, Macaek T, et al. A common variation of IL28 affects gamma-GTP levels and inflammation of the liver in chronically infected hepatitis C virus patients. J Hepatol 2010; 53: 439-45.

Valenti L, Puliti E, La Spina S. IL28B, HCV core mutations, and hepatocellular carcinoma: does host genetic make-up shape viral evolution in response to immunity? Hepatology International 2012; 6: 356-9.

Leandro G, Mangia A, Hui J, et al. Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. Gastroenterology 2006; 130: 1363-42.

Marabita F, Aghemo A, De Nicola S, et al. Genetic variation in the Interleukin-28B gene is not associated with fibrosis progression in patients with chronic hepatitis C and known date of infection. Hepatology 2011; 54: 1127-34.

Tillmann HL, Patel K, Muir AJ, et al. Beneficial IL28B genotype associated with lower frequency of hepatic steatosis in patients with chronic hepatitis C J Hepatol 2011.

Valenti L, Rumi M, Galmozzi E, et al. Patatin-Like phospholipase domain-containing 3 1148M polymorphism, steatosis, and liver damage in chronic hepatitis C. Hepatology 2011; 53: 791-9.

Fabris C, Falletti E, Cussigh A, et al. IL28B rs12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. J Hepatol 2011; 54: 716-22.

Bochud PY, Biber S, Kutalik Z, et al. IL28B alleles associated with poor HCV clearance protect against inflammation and fibrosis in patients infected with non-1 HCV genotypes. Hepatology 2011; 55: 368-94.

Cai T, Dufour JF, Muelhaupt B, et al. Viral Genotype-Specific Role of PNPLA3, PPARG, METT and IL28B in Hepatitis C Virus-Associated Steatosis. J Hepatol 2011; 55: 529-35.

Valenti L, Aghemo A, Stattmayer AF. Interaction between IL28B and PNPLA3 genotypes in the pathogenesis of steatosis in chronic hepatitis C non-genotype 3 patients. J Hepatol 2012; 56: 1209-10.

Petta S, Grimaudo S, Camma C, et al. IL28B and PNPLA3 polymorphisms affect histological liver damage in patients with non-alcoholic fatty liver disease. J Hepatol 2012; 56: 1356-62.

Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008; 134: 1655-69.

Black AR, Black JD, Aizikhan-Clifford J. Sp1 and kruppel-like factor family of transcription factors in cell growth regulation and cancer. J Cell Physiol 2001; 188: 143-60.

Lalazar A, Wong L, Yamaski G, Friedman SL. Early genes induced in hepatic stellate cells during wound healing. Gene 1997; 195: 235-43.

Ratziu V, Lalazar A, Wong L, et al. ZBP, a Kruppel-like transcription factor up-regulated in vivo during early hepatic fibrosis. Proc Natl Acad Sci U S A 1998; 95: 9500-5.

Kim Y, Ratziu V, Choi SG, et al. Transcriptional activation of transforming growth factor beta1 and its receptors by the Kruppel-like factor ZBP/core promoter-binding protein and Sp1. Potential mechanisms for autocrine fibrogenesis in response to injury. J Biol Chem 1998; 273: 37580.

Nagia G, Difeo A, Reeves HL, et al. A germline DNA polymorphism enhances alternative splicing of the KL6 tumor suppressor gene and is associated with increased prostate cancer risk. Cancer Res 2005; 65: 1213-22.

Bechmann LP, Gastaldelli A, Vetter D, et al. Glucokinase links Kruppel-like factor 6 to the regulation of hepatic insulin sensitivity in nonalcoholic fatty liver disease. Hepatology 2012; 55: 1083-93.

Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIR groups. Lancet 1997; 349: 825-32.

Baker SJ, Reddy EP. Transducers of life and death: TNF receptor superfamily and associated proteins. Oncogene 1996; 12: 1-9.

Powell EE, Edwards-Smith CJ, Hay JL, et al. Host genetic factors influence disease progression in chronic hepatitis C. Hepatology 2000; 31: 828-33.

Li B, Khanna A, Sharma V, Singh T, Suthanthiran M, August P. TGF-beta1 DNA polymorphisms, protein levels, and blood pressure. Hypertension 1999; 33: 271-5.

Kim S, Ohta K, Hamaguchi A, et al. Angiotensin II type I receptor antagonist inhibits the gene expression of transforming growth factor-beta1 and extracellular matrix in cardiac and vascular tissues of hypertensive rats. J Pharmacol Exp Ther 1995; 273: 509-15.

LeeLK, Meyer TW, Pollock AS, Lovett DH. Endothelial cell injury initiates glomerular sclerosis in the rat remnant kidney. J Clin Invest 1995; 96: 953-64.

Noble NA, Border WA. Angiotensin II in renal fibrosis: should TGF-beta rather than blood pressure be the therapeutic target? Semin Nephrol 1997; 17: 455-66.

Dixon JB, Bhatal PS, Jonsson JR, Dixon AF, Powell EE, O'Brien PE. Pro-fibrotic polymorphisms predictive of advanced liver fibrosis in the severely obese. J Hepatol 2003; 39: 967-71.

Chaiterakar R, Roberts LR. Telomerase mutation: a genetic risk factor for cirrhosis. Hepatology 2011; 53: 1430-2.

Diaz de Leon A, Cronkite JT, Katzenstein AL, et al. Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations. PLoS One 2010; 5: e10680.

Alder JK, Chen JI, Lancaster L, et al. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. Proc Natl Acad Sci U S A 2008; 105: 13051-6.

Calado RT, Regal JA, Kleinerman DE, et al. A spectrum of severe familial liver disorders associate with telomerase mutations. PLoS One 2009; 4: e7926.

Calado RT, Bruando J, Mehta P, et al. Constitutional telomerase mutations are genetic risk factors for cirrhosis. Hepatology 2011; 53: 1600-7.

Hartmann D, Srivastava U, Thaler M, et al. Telomerase gene mutations are associated with cirrhosis formation. Hepatology 2011; 53: 1608-17.
[260] Llovet JM, Chen Y, Wurmbach E, et al. A molecular signature to discriminate dysplastic nodules from early hepatocellular carcinoma in HCV cirrhosis. Gastroenterology 2006; 131: 1758-67.

[261] Barshop NJ, Sirlin CB, Schwimmer JB, Lavine JE. Review article: epidemiology, pathogenesis and potential treatments of paediatric non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2008; 28: 13-24.

[262] Alisi A, Manco M, Panera N, Nobili V. Association between type two diabetes and non-alcoholic fatty liver disease in youth. Ann Hepatol 2009; 8 Suppl 1: S44-50.

[263] Patton HM, Sirlin C, Behling C, Middleton M, Schwimmer JB, Lavine JE. Pediatric nonalcoholic fatty liver disease: a critical appraisal of current data and implications for future research. J Pediatr Gastroenterol Nutr 2006; 43: 413-27.

[264] Papandreou D, Rousso I, Mavromichalis I. Update on non-alcoholic fatty liver disease in children. Clin Nutr 2007; 26: 409-15.

[265] Dunn W, Schwimmer JB. The obesity epidemic and nonalcoholic fatty liver disease in children. Curr Gastroenterol Rep 2008; 10: 67-72.

[266] Manco M, Marcellini M, Devito R, Comparcola D, Sartorelli MR, Nobili V. Metabolic syndrome and liver histology in paediatric non-alcoholic steatohepatitis. Int J Obes (Lond) 2008; 32: 381-7.

[267] Fraser A, Longnecker MP, Lawlor DA. Prevalence of elevated alanine aminotransferase among US adolescents and associated factors: NHANES 1999-2004. Gastroenterology 2007; 133: 1814-20.

[268] Schwimmer JB, Behling C, Newbury R, et al. Histopathology of pediatric nonalcoholic fatty liver disease. Hepatology 2005; 42: 641-9.

[269] Strauss RS, Pollack HA. Epidemic increase in childhood overweight, 1986-1998. JAMA 2001; 286: 2845-8.

[270] Wilfred de Alwis NM, Day CP. Genetics of alcoholic liver disease and nonalcoholic fatty liver disease. Semin Liver Dis 2007; 27: 44-54.

[271] Lin YC, Chang PF, Hu FC, Yang WS, Chang MH, Ni YH. A Common Variant in the PNPLA3 Gene is a Risk Factor for Non-Alcoholic Fatty Liver Disease in Obese Taiwanese Children. J Pediatr 2011; 158: 740-4.

[272] Goran MI, Walker R, Le KA, et al. Effects of PNPLA3 on liver fat and metabolic profile in Hispanic children and adolescents. Diabetes 2010; 59: 3127-30.

[273] Speliotes EK, Yerges-Armstrong LM, Wu J, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet 2011; 7: e1001324.

[274] 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 2011.

[275] 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: 282-91.

[276] 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 1148M genotype in patients with nonalcoholic fatty liver. J Hepatol 2011; 55: 1409-14.

[277] Alisi A, Da Sacco L, Bruscalupi G, et al. Mirnome analysis reveals novel molecular determinants in the pathogenesis of diet-induced nonalcoholic fatty liver disease. Lab Invest 2010; 91: 283-93.

[278] Valenti L, Motta BM, Alisi A, et al. LPIN1 rs13412852 Polymorphism in Pediatric Non-Alcoholic Fatty Liver Disease. J Pediatr Gastroenterol Nutr 2012; 54: 588-93.

[279] Manco M, Alisi A, Real JM, et al. Early interplay of intra-hepatic iron and insulin resistance in children with non-alcoholic fatty liver disease. J Hepatol 2011; 54: 675-53.

[280] Neuschwander-Tetri BA. Lifestyle modification as the primary treatment of NASH. Clin Liver Dis 2009; 13: 649-65.

[281] Promrat K, Kleiner DE, Niemeier HM, et al. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. Hepatology; 51: 121-9.

[282] Keating SE, Hackett DA, George J, Johnson NA. Exercise and non-alcoholic fatty liver disease: a systematic review and meta-analysis. J Hepatol 2012; 57: 157-66.

[283] Musso G, Gambino R, Cassader M, Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. Hepatology; 52: 79-104.

[284] Sevastianova K, Kotronen A, Gambino R, Cassader M, Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. Hepatology; 52: 79-104.

[285] Sanyal AJ, Chalasani N, Kowdley KV, et al. Genetic variation in PNPLA3 (adiponutrin) confers sensitivity to weight loss-induced decrease in liver fat in humans. Am J Clin Nutr; 91: 1082-9.

[286] Petersen KF, Dufour S, Hariri A, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010; 362: 1675-85.

[287] Valenti L, Moscetiello S, Vanni E, et al. Venesection for non-alcoholic fatty liver disease unresponsive to lifestyle counselling—a propensity score-adjusted observational study. QJM 2010; 104: 141-9.

[288] Valenti L, Fracanzani AL, Dongiovanni P, et al. Iron depletion by phlebotomy improves insulin resistance in patients with nonalcoholic fatty liver disease and hyperferritinemia: evidence from a case-control study. Am J Gastroenterol 2007; 102: 1251-8.

[289] Petersen KF, Dufour S, Hariri A, et al. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. N Engl J Med 2010; 362: 1082-9.

[290] Motta BM, Dongiovanni P, Fargion S, Valenti L. IL28B rs12979860 polymorphism influences serum TNFalpha levels in chronic hepatitis C. Dig Liver Dis 2012.

[291] 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: 723-9.