thematic review

*Thematic Review Series: Genetics of Human Lipid Diseases*

# Genetic determinants of hepatic steatosis in man

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**Abstract Hepatic steatosis is one of the most common liver disorders in the general population. The main cause of hepatic steatosis is nonalcoholic fatty liver disease (NAFLD), representing the hepatic component of the metabolic syndrome, which is characterized by type 2 diabetes, obesity, and dyslipidemia. Insulin resistance and excess adiposity are considered to play key roles in the pathogenesis of NAFLD. Although the risk factors for NAFLD are well established, the genetic basis of hepatic steatosis is largely unknown. Here we review recent progress on genomic variants and their association with hepatic steatosis and discuss the potential impact of these genetic studies on clinical practice. Identifying the genetic determinants of hepatic steatosis will lead to a better understanding of the pathogenesis and progression of NAFLD.**—Hooper, A. J., L. A. Adams, and J. R. Burnett. **Genetic determinants of hepatic steatosis in man.** ***J. Lipid Res.*** **2011.** 52: **593–617.**

**Supplementary key words** alcoholic fatty liver disease • cirrhosis • fibrosis • genetic factors • gene variants • nonalcoholic fatty liver disease • nonalcoholic steatohepatitis • single nucleotide polymorphisms • susceptibility

Hepatic steatosis is extremely common in the general population of both Western and Eastern countries, with a prevalence of up to 17% and 30% in China and the United States, respectively (1, 2). The predominant cause of hepatic steatosis is nonalcoholic fatty liver disease (NAFLD), which is associated with pathogenic factors of diabetes and obesity that are approaching epidemic proportions worldwide (3, 4). Excess alcohol consumption may also lead to hepatic steatosis, although it is unclear what quantities of alcohol are required to precipitate hepatic fat accumulation. Despite this, it is thought that alcohol is a relatively uncommon cause of hepatic steatosis (approximately 10% in one series) compared with NAFLD (2). NAFLD may begin in childhood in some individuals, with a prevalence of 10% found in an autopsy study of 2-19 year olds from the United States (5). The prevalence of NAFLD increases with advancing age, with a peak between 40 and 69 years of age (2, 6).

A cut-off of 5.5% hepatic steatosis has been accepted as the threshold to diagnose fatty liver, based on the 95th centile of hepatic fat content in low-risk subjects [normal body mass index (BMI), normal glucose tolerance, lack of excessive alcohol ingestion, and normal liver function tests] (1, 7). Subjects with NAFLD may have a range of histopathological changes (**Fig. 1**). Fatty liver itself is relatively benign and characterized by accumulation of triglyceride-rich lipid droplets within hepatocytes, but without hepatic inflammation or evidence of liver injury. A minor-

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Abbreviations: ABCC2, ATP-binding cassette subfamily C member 2; ADIPOR, adiponectin receptor; AFLP, acute fatty liver of pregnancy; AGTR1, angiotensin II type I receptor; ALT, alanine aminotransferase; apo, apolipoprotein; AST, aspartate aminotransferase; ATGL, adipocyte triglyceride lipase; BMI, body mass index; CESD, cholesterol ester storage disease; CGI-58, comparative gene identification-58; CGL, congenital generalized lipodystrophy; CREBP, carbohydrate response element binding protein; CTLN2, citrullinemia type II; DGAT, acylCoA: diacylglycerol acyltransferase; ENPP1, ectoenzyme nucleotide pyrophosphate phosphodiesterase 1; FHBL, familial hypobetalipoproteinemia; GCLC, glutamate-cysteine ligase; $^{1}$H MRS, proton magnetic resonance spectroscopy; HCV, hepatitis C virus; HOMA, homeostasis model assessment; IRS1, insulin receptor substrate 1; LCAD, long-chain acyl-CoA dehydrogenase deficiency; LCHAD, long-chain 3-hydroxyacyl-CoA dehydrogenase; LIPA, lysosomal acid lipase; LPS, lipopolysaccharide; MCAD, medium-chain acylcoenzyme-A dehydrogenase; MRP2, multidrug resistance protein 2; MTHFR, methylenetetrahydrofolate reductase; MTTP, microsomal triglyceride transfer protein; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NOS2, nitric oxide synthase; OGTT, oral glucose tolerance test; PEMT, phosphatidylethanolamine N-methyltransferase; PNPLA3, patatin-like phospholipase domain containing 3, adiponutrin; PPAR, peroxisomal proliferator-activated receptor; ROS, reactive oxygen species; SLC25A13, solute carrier family 25, member 13; SNP, single nucleotide polymorphism; SOD2, manganese superoxide dismutase; SREBP, sterol regulatory element binding protein; STAT3, signal transducer and activator of transcription 3; TCF7L2, transcription factor 7-like 2; TFP, trifunctional protein; TNF, tumor necrosis factor; UGT1A1, UDP glucuronosyltransferase 1 family, polypeptide A1; VLCAD, very long-chain acyl-CoA dehydrogenase.

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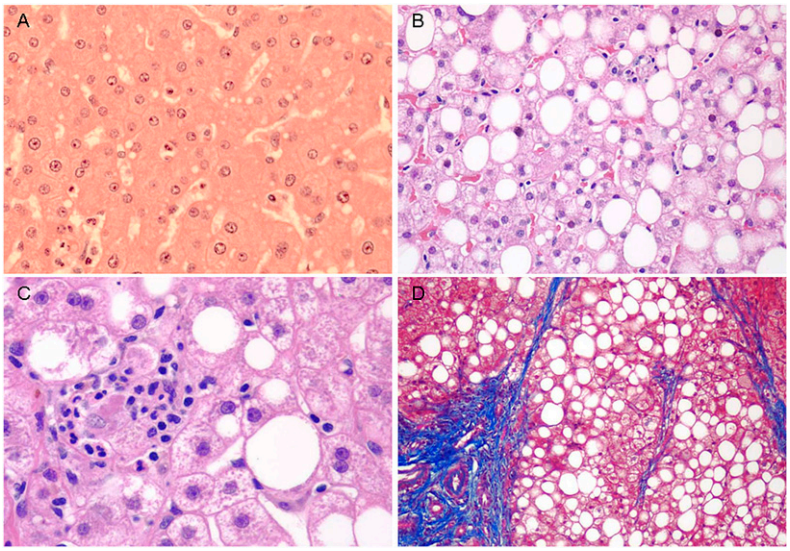


**Fig. 1.** The histopathological progression of NAFLD. A: Normal liver (H&E, 200×). B: Macrovesicular steatosis (H&E, 100×). C: Neutrophils surrounding Mallory bodies (H&E, 400×). D: Cirrhotic nodule with steatosis (MT, 100×). H&E, hematoxylin and eosin; MT, Masson's trichrome; NAFLD, nonalcoholic fatty liver disease.

ity of NAFLD subjects have nonalcoholic steatohepatitis (NASH), which is characterized by inflammation and hepatocyte ballooning on a background of hepatic steatosis (7). These subjects may develop progressive fibrosis and are at risk of developing cirrhosis with complications of liver failure and hepatocellular carcinoma (8). Although the risk of cirrhosis is low, with an estimated 5% incidence over a ten-year period, the high prevalence of NAFLD translates to a significant health burden over the coming decades (8). Exemplifying the increasing health burden related to NAFLD is the proportion of liver transplants performed for NAFLD in the United States, which increased 15-fold over the ten-year period ending in 2007 (9). Thus, a better understanding of the pathogenesis of NAFLD is vital to guide appropriate treatment to minimize future morbidity and mortality.

NAFLD is generally asymptomatic, although it may present with right upper-quadrant pain or discomfort in some individuals, presumably related to capsular distension related to hepatomegaly from significant hepatic steatosis (10). Fatty liver may be diagnosed after investigation of abnormal liver enzymes performed for other reasons or as an incidental finding on radiological imaging. Rarely, NAFLD may present as decompensated cirrhosis with complications of portal hypertension, such as ascites, variceal bleeding, encephalopathy, or hepatocellular carcinoma (11).

## PATHOGENESIS

Insulin resistance and related conditions of diabetes and obesity are closely linked to the development of NAFLD (12). Insulin resistance promotes peripheral adipose lipolysis, thereby increasing FFA flux to the liver, which drives hepatic triglyceride production. Human studies have demonstrated peripheral adipose lipolysis, systemic free fatty acid levels, and de novo hepatic lipogenesis to be upregulated in subjects with NAFLD (13–15). Fatty acid (palmitate) release from peripheral adipose deposits is increased approximately 35% in NAFLD patients compared to age-, gender-, and fat mass-matched controls, and it accounts for approximately 60% of hepatic lipid in subjects with NAFLD (14). De novo lipogenesis accounts for 25% of hepatic fat content in NAFLD subjects compared with 10% in obese hyperinsulinemic subjects and 5% in healthy individuals (16). Dietary lipid is thought to account for 15% of hepatic lipid in NAFLD patients (16).

Animal models have demonstrated that hyperinsulinemia and hyperglycemia promote de novo lipogenesis by upregulating lipogenic transcription factors, such as sterol regulatory element binding protein-1c (SREBP-1c) and carbohydrate response element binding protein (CREBP) (17, 18). Insulin-mediated activation of SREBP-1c increases malonyl-CoA, a key intermediate of fatty acid synthesis, which in vivo animal studies have demonstrated to inhibit carnitine palmitoyltransferase 1, thereby inhibiting long chain fatty acid entry into the mitochondria for β-oxidation and favoring hepatic triglyceride accumulation (18–20). In subjects with NAFLD, upregulation of hepatic levels of SREBP-1c was observed, which correlated with the expression of insulin and insulin receptor substrate 1 (21, 22). The role of CREBP remains to be confirmed in human studies. Interestingly, the addition of insulin to peritoneal dialysis causes a characteristic pattern of subcapsular hepatic steatosis, highlighting its role in the development of fatty liver (23).

Aside from increased lipid import and hepatic lipid synthesis, hepatic lipid export in the form of triglyceride-rich VLDL is inadequate to match the lipid accumulation in humans with NAFLD (14) (**Fig. 2**). In particular, defective incorporation of triglyceride into apolipoprotein (apo) B or reduced apoB synthesis and secretion have been documented in animal models of drug-induced NAFLD and in obese patients with NASH (24, 25).

Despite the presence of hepatic steatosis, only a minority subjects will develop evidence of liver injury and inflammation (i.e., NASH). A number of factors have been implicated in the development of NASH, including oxidative stress, altered adipocytokine concentrations, and lipotoxic FFAs (26). Increased hepatic FFA oxidation can generate

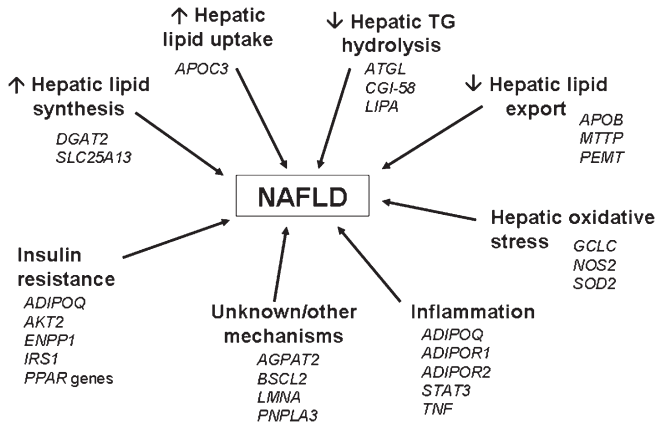


**Fig. 2.** Overview of the metabolic processes influencing the development of NAFLD. Variants within genes involved in these processes may impact the development and/or progression of NAFLD. NAFLD, nonalcoholic fatty liver disease.

oxygen radicals with subsequent lipid peroxidation, cytokine induction, and mitochondrial dysfunction (27, 28). Saturated FFAs, to a greater extent than unsaturated FFAs, induce hepatocyte apoptosis in hepatocyte cell line models (29). Hepatocyte apoptosis is one mechanism of cellular injury in patients with NAFLD and correlates with stage and grade of disease (30, 31).

Increased visceral fat mass is associated with increased levels of pro-inflammatory cytokines, such as tumor necrosis factor (TNF) α and reduced anti-inflammatory and insulin-sensitizing hormones, such as adiponectin, and correlates with severity of liver injury in humans with NASH (31–35). Animal models of fatty liver have demonstrated that adiponectin ameliorates hepatic steatosis and liver injury (36, 37), whereas hepatic adiponectin and expression of its receptor (R2) are downregulated in human subjects with NASH compared to those with simple steatosis (38). In addition, clinical trials in humans of the insulin-sensitizing drug pioglitazone have shown increasing plasma adiponectin levels that correlate with histological improvement in subjects with NASH, suggesting it is an important mediator of hepatic fat accumulation and liver injury (39).

Intestinal-derived bacterial lipopolysaccharide (LPS) has also been implicated in causing progressive liver injury in subjects with fatty liver, with animal models of fatty liver demonstrating increased hepatitis and liver injury after LPS exposure (40, 41). Humans with NASH may be predisposed to LPS-stimulated liver injury related to small bowel bacterial overgrowth and increased intestinal permeability (34). Although many of the above factors have been implicated in leading to progressive liver injury in subjects with fatty liver, understanding the pathogenic mechanisms that lead to end-stage liver disease in a very small proportion of individuals remains to be fully elucidated. Genetic alterations may affect the likelihood of developing liver injury (i.e., NASH) and progressive liver disease in subjects with fatty liver, as well as directly influence the pathogenic pathways of insulin resistance and lipid metabolism, and thus, predisposition to hepatic fat accumulation. This review will focus on genes and their variants - from rare, inherited disorders to common single nucleotide polymorphisms (SNPs) in the general population - that influence the presence and/or severity of hepatic steatosis and liver injury.

## HERITABILITY OF HEPATIC STEATOSIS

NAFLD is considered to be a "complex" disorder with numerous genetic factors contributing to the considerable variability in the natural history of the disease (42). It is now clear that NAFLD is more common in some ethnic populations than others, suggesting heritability and/or environmental factors influence the development of NAFLD. In 2004, Browning et al. reported that the frequency of hepatic steatosis varied significantly with ethnicity among subjects from the multiethnic, population-based Dallas Heart Study (1). The black, white, and Hispanic groups had mean ages of 46, 46, and 41 years, and BMI of 29, 31, and 30 kg/m$^2$, respectively. Hepatic steatosis, defined as hepatic triglyceride content greater than 5.5% as measured by proton magnetic resonance spectroscopy ($^1$H MRS), was found in 45% of Hispanics, 33% of whites, and 24% of blacks. This paralleled differences in intra-abdominal adipose accumulation; African-Americans had less intraperitoneal fat and more lower extremity fat than Hispanics and Caucasians (43). These ethnic differences mirror the findings in NAFLD-related cirrhosis (44). Further examination of this cohort revealed African Americans had higher levels of insulin resistance, defined as homeostasis model assessment (HOMA) score greater than 4.04, but lower triglyceride levels than Caucasians or Hispanics, independent of intra-abdominal adipose and hepatic steatosis, suggesting genetic differences in the relationship between lipid metabolism, insulin resistance, and hepatic steatosis (43).

In view of the disproportionate incidence of NAFLD in Hispanics, Wagenknecht et al. studied risk factors and heritability of NAFLD in the IRAS Family Study cohort, which included 795 Hispanic-American and 347 African-American adults from large families recruited based on family size (45). The mean age of the cohort was 49 years, and 63% were female. Although the prevalence of diabetes was similar in both groups (17%), metabolic syndrome was more common in Hispanics than African Americans (33% versus 19%), as was NAFLD (24% versus 10%). Liver density as determined by computed tomography scans was modestly heritable in both groups, estimated as 0.35 in Hispanics and 0.32 in African Americans (45).

Petersen et al. studied the impact of ethnicity on insulin resistance and hepatic steatosis in young healthy BMI- and age-matched individuals of Eastern Asian, Asian-Indian, black, and Caucasian backgrounds (mean age, 24-26 years) recruited from New Haven, Connecticut (46). An increased prevalence of insulin resistance was seen in Asian-Indian men and was associated with a 2-fold increase in hepatic fat content (and plasma IL-6 concentrations) compared

with Caucasian men. These results also suggest a genetic contribution to NAFLD risk, consistent with the findings from the Dallas Heart Study and the IRAS Family Study (1, 45). Asian-Indian men may be genetically predisposed to develop hepatic steatosis and hepatic insulin resistance at a lower BMI than other ethnic groups due to a tendency to accumulate visceral adiposity, which is more insulin-resistant than subcutaneous adipose tissue (47).

Brouwers et al. used nonparametric quantitative trait locus analysis to identify three loci associated with fatty liver in males, as determined by serum alanine aminotransferase (ALT) activity and ultrasound in 157 members from Dutch familial combined hyperlipidemia kindred (48). Of the familial combined hyperlipidemia probands, 40% had fatty liver, compared with 35% of relatives and 15% of spouses. The heritability of NAFLD was calculated as 20-36%. The three loci in males were located on chromosome 1q42.3 ($P$ = 0.001 for fatty liver), 7p12-21 ($P$ = 0.0002 for log ALT), and 22p13-q11 ($P$ = 0.0007 for fatty liver). The lack of association in females was ascribed to the heterogeneous female population in terms of menopausal status, together with the well-known effects of estrogens on lipid and liver metabolism (49).

Subsequently, Schwimmer et al. performed a familial aggregation study to test the hypothesis that NAFLD is highly heritable. $^{1}$H MRS was used to quantify liver fat in 33 overweight children, with and without biopsy-proven NAFLD, and their relatives. It estimated the heritability of liver fat fraction as 39%, after controlling for age, gender, race, and BMI (50). Of 152 family members, fatty liver (liver fat content ⩾5%) was present in 59% of siblings and 78% of parents of children with NAFLD, and only 17% of siblings and 37% of parents of overweight children without NAFLD. When the heritability of fatty liver was calculated as a dichotomous trait with adjustment for age, gender, race, and BMI, the heritability was much higher (85-100%) (50). However, there are potential problems with the modeling of dichotomous fatty liver traits, suggesting that this finding should be interpreted with caution (51).

## INHERITED DISORDERS ASSOCIATED WITH HEPATIC STEATOSIS

In the majority of NAFLD patients, an inherited inborn error of metabolism is not the cause of hepatic steatosis. However, these extreme "experiments of nature," while rare, can provide valuable insight into the pathogenesis of many disease processes, including NAFLD, and the metabolic pathways involved (**Table 1**). The following examples of disorders inherited in a Mendelian fashion may result in hepatic steatosis by disturbing hepatic triglyceride export (e.g., abetalipoproteinemia, familial hypobetalipoproteinemia), or as a generalized systemic defect in lipid metabolism (e.g., citrullinemia type 2, familial lipodystrophies, neutral lipid storage disease, cholesterol ester storage disease), fatty acid oxidation disorders, or insulin-signaling defects (e.g., postreceptor hepatic insulin resistance). Such defects are associated with other prominent phenotypic manifestations, such as neurological or cardiac deficits, which may dominate the clinical presentation.

### Impaired hepatic triglyceride export

*Abetalipoproteinemia (MTTP).* Abetalipoproteinemia is an autosomal recessive disorder of lipoprotein metabolism characterized by the virtual absence of apoB-containing lipoproteins in blood. This extremely rare (one in one million) condition is associated with deficiencies in fat-soluble vitamins secondary to malabsorption, atypical retinitis pigmentosa, and neuromuscular abnormalities. Patients often present in childhood with failure to thrive (52–54). Hepatic lipid accumulation can be observed, as patients are unable to export triglyceride from the liver (55–57). Cirrhosis, particularly in association with medium-chain triglyceride supplementation, has also been reported (58, 59). Partin et al. suggested that continuous supplementation of medium-chain triglycerides is biochemically analogous to chronic ethanol ingestion, and may explain the possible link among medium-chain triglyceride supplementation, cirrhosis, and alcoholic hyaline

TABLE 1. Summary of inherited disorders associated with fatty liver

| Disorder | Gene | Inheritance | Prevalence |
|---|---|---|---|
| Abetalipoproteinemia | *MTTP* | Recessive | ∼1 in 1,000,000 |
| Familial hypobetalipoproteinemia | *APOB* | Co-dominant | ∼1 in 3,000 (heterozygous)∼1 in 1,000,000 |
| Citrullinemia type II | *SLC25A13* | Recessive | ∼1 in 100,000 to 230,000 (Japan) |
| Familial partial lipodystrophy type 2 | *LMNA* | Dominant | Unknown |
| Familial partial lipodystrophy type 3 | *PPARG* | Dominant | Unknown |
| Congenital generalized lipodystrophy | *AGPAT2, BSCL2* | Recessive | ∼1 in 10,000,000 |
| Neutral lipid storage disorder | *PNPLA2, CGI-58* | Recessive | Unknown |
| Wolman disease | *LIPA* | Recessive | <1 in 300,000 |
| Cholesterol ester storage disease | *LIPA* | Recessive | ∼1 in 40,000 |
| Medium-chain acylcoenzyme-A dehydrogenase deficiency | *ACADM* | Recessive | ∼1 in 20,000 |
| Very long-chain acyl-CoA dehydrogenase deficiency | *ACADVL* | Recessive | ∼1 in 100,000 |
| Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency | *HADHA* | Recessive | ∼1 in 100,000 |

formation observed in liver biopsies of abetalipoproteinemia patients (59).

Although similar in clinical presentation to homozygous familial hypobetalipoproteinemia (FHBL), abetalipoproteinemia is caused by mutations in the 97 kDa microsomal triglyceride transfer protein (MTTP) subunit (60, 61). MTTP plays a critical role of incorporating hepatic triglyceride with apoB, leading to the formation of VLDL that is subsequently the vehicle of lipid export from the liver. The absence of MTTP activity results in undetectable circulating apoB-containing lipoproteins, and after a fat load, chylomicrons do not appear in plasma. Heterozygous carriers of *MTTP* mutations typically have a normal lipid profile and liver function tests.

Abetalipoproteinemia is an extreme form of MTTP inhibition. MTTP has been identified as a molecular target for therapies aimed at reducing plasma LDL cholesterol and apoB, and MTTP inhibitors have potential as lipid-regulating and anti-atherosclerotic agents (62–66). However, MTTP is strongly expressed in the intestine and liver. Gastrointestinal symptoms such as steatorrhea and diarrhea occur related to the effect of MTTP inhibition on chylomicron assembly by enterocytes. Furthermore, current therapies for MTTP inhibition can also increase plasma transaminases and cause hepatic steatosis due to effects on VLDL export (65). Other approaches to target MTTP, including the use of lower doses and tissue-specific inhibition, have been used to circumvent these major side effects (62, 63). Interestingly, MTTP has recently been implicated in immune processes: abetalipoproteinaemia is associated with loss of CD1 function (67). It is possible that the resulting dysfunction of natural killer T cells may contribute to hepatic steatosis.

*Familial hypobetalipoproteinemia (APOB).* Increased serum transaminase concentrations and fatty liver are a common occurrence in FHBL (68–73). This inherited disorder of lipoprotein metabolism is characterized by low plasma levels of apoB and LDL-cholesterol and has an estimated population prevalence of 1 in 3,000 people (54, 74). As FHBL is an autosomal codominant disorder, heterozygotes are usually detected by routine lipid screens. They are typically asymptomatic with mild liver dysfunction, whereas homozygotes have extremely low or undetectable plasma apoB concentrations and a range of clinical symptoms that may present from infancy to adulthood, including deficiency of fat-soluble vitamins and gastrointestinal and neurological dysfunction (53). Fatty liver and one case of liver cirrhosis with hepatocarcinoma have been reported in homozygous FHBL (75, 76). FHBL is caused by mutations in the *APOB* gene, which in most cases, lead to production of a truncated apoB molecule (77). Missense mutations have also been reported that impair apoB folding and secretion (78–80).

Sankatsing et al. reported that both the prevalence (54% versus 29%) and severity of hepatic steatosis were significantly higher in subjects with heterozygous FHBL (n = 41) compared with sex- and BMI-matched controls (81). FHBL subjects were recruited from eight families carrying four different truncating apoB mutations. FHBL heterozygotes were younger (mean age, 41 years) and had a 3- to 5-fold higher liver fat content compared with control subjects (mean age, 46 years) with similar measures of adiposity and insulin resistance (70, 71). Hepatic steatosis was not more severe in those FHBL subjects carrying shorter truncated apoBs (apoB-18 and apoB-29) compared with those carrying longer truncated apoB species ($P = 0.68$). Although lean FHBL subjects and lean controls had similar amounts of liver fat, the regression of liver fat percentage on abdominal fat was steeper for FHBL subjects, indicating that FHBL subjects are more susceptible to developing fatty liver (71). Although FHBL subjects are protected against atherosclerosis, the long-term consequences of fatty liver in FHBL are not known (81).

The hepatic accumulation of lipid in FHBL could be explained by the reduced capacity of short apoB truncations for triglyceride combined with a low rate of production of normal apoB-100 (82), resulting in an accumulation of lipid in the liver. However, there is some degree of compensation: heterozygous and homozygous mouse models of FHBL show a reduction in intestinal triglyceride absorption and suppressed hepatic cholesterol and fatty acid synthesis (83–85). It could be that fatty liver only occurs in FHBL in the presence of additional factors, such as hyperinsulinemia, a high-fat diet, or obesity. Studies have already shown that FHBL subjects appear to be more susceptible to the effects of adiposity and insulin resistance (70, 71). Amaro et al. studied hepatic and muscle insulin sensitivity in lean subjects and groups of obese subjects with normal hepatic triglyceride content, NAFLD, and high hepatic triglyceride content due to FHBL (86). Hepatic and muscle insulin sensitivity was similar in obese heterozygous FHBL subjects and obese subjects with normal hepatic triglyceride content. This finding suggests that the hepatic steatosis seen in FHBL is a marker rather than a cause of metabolic dysfunction (86).

### Enhanced de novo lipogenesis and hepatic fatty acid uptake

*Citrullinemia type II (SLC25A13).* Citrin deficiency is an autosomal recessive disorder caused by mutations in the solute carrier family 25, member 13 (*SLC25A13)* gene on chromosome 7q21.3 that encodes citrin and causes citrullinemia type II (CTLN2) (87). Over 30 *SLC25A13* mutations (mostly deletions, splice site, and nonsense mutations) causing CTLN2 have been described worldwide. Mutations are particularly common in East Asia, where approximately 1 in 70 people are heterozygous carriers (88, 89). CTLN2 is characterized by adult-onset, recurring episodes of hyperammonemia and associated neuropsychiatric symptoms. Onset is sudden usually between the ages of 20 and 25 years, and most patients die within a few years of onset from cerebral edema (90, 91). Fatty liver or NASH is also a common observation in CTLN2 patients (88, 91–93). Komatsu et al. studied 19 Japanese patients with adult-onset citrin deficiency (mean age, 37 years) and found increased ALT activity and fatty liver, including NAFLD and NASH, in 17 of the 19 patients (89%) at the time of

admission (92). Interestingly, these patients were not obese (median BMI, 18.3 kg/m$^2$) and lacked the characteristics typical of the metabolic syndrome.

Citrin is an aspartate/glutamate transporter in mitochondria. The major sites of its expression are the liver, kidneys, and heart. Citrin deficiency limits the activity of the enzyme argininosuccinic acid synthase, which catalyzes the reaction of aspartate and citrulline to form argininosuccinic acid (94). Compensatory changes in the malate-citrin shuttle may favor fatty acid and triglyceride synthesis, in combination with increased fatty acid uptake capacity into hepatocytes, thereby promoting hepatic steatosis (92, 95). Mutations in the *SLC25A13* gene can also cause another condition, neonatal intrahepatic cholestasis; the mechanism is thought to involve primary mitochondrial impairment associated with the delayed maturity of bile acid metabolism (96, 97). Severe hepatic steatosis and fibrosis is a common finding in these infants (98). Symptoms ameliorate by one year of age, but some patients go on to develop CTLN2 later in life (99).

### Familial lipodystrophies

*Familial partial lipodystrophy type 2 (LMNA).* Familial partial lipodystrophy type 2 (FPLD2; Dunnigan-type) is an autosomal dominant condition characterized by progressive and gradual subcutaneous loss of adipose tissue from the extremities, usually commencing at the time of puberty (100). FPLD2 is associated with hypertrophy of type 1 and 2 muscle fibers and hepatomegaly with hepatic steatosis is a common occurrence (100, 101). Patients may also have acanthosis nigricans, and in women, menstrual irregularities, hirsutism, and polycystic ovarian syndrome. Hyperinsulinemia is associated with a cluster of other metabolic abnormalities (including hypertriglyceridemia, low HDL-cholesterol, and increased FFA concentrations), which usually presents before the onset of frank diabetes after the age of 20 years.

FPLD2 results from mutations in the *LMNA* gene encoding nuclear lamin A/C (102). Lamin A and C are intermediate filament proteins that form the nuclear lamina, a meshwork associated the inner nuclear membrane. The mechanism whereby *LMNA* mutations cause dystrophy of adipose cells is not well understood. Specific mutations throughout the *LMNA* gene are associated with other disorders, including muscular dystrophies, Charcot-Marie-Tooth syndrome, and Hutchinson-Gilford progeria syndrome. However, about 90% of mutations causing FPLD2 occur within *LMNA* exon 8, most commonly affecting codons 482 and 486 (103).

Lüdtke et al. studied hepatic steatosis in six FPLD2 families carrying either the *LMNA* R482W or R482Q mutations (104). Hepatic steatosis was found in all 15 subjects with FPLD2 who underwent ultrasound examinations. Nine of these subjects, all R482W carriers, had increased serum transaminase levels, consistent with NASH. Interestingly, none of the six R482Q carriers, all female, had increased transaminases, suggesting a mutation-specific effect on steatohepatitis. Liver biopsy confirmed hepatic steatosis in two subjects. FPLD2 patients had additional metabolic abnormalities; 14 of 18 patients from the six FPLD2 families had abnormal glucose tolerance tests or diabetes, and 16 of 18 patients had Frederickson type IIb, IV, or V hyperlipoproteinemia. Taken together, their findings are consistent with hepatic steatosis being a common feature of FPLD2.

*Familial partial lipodystrophy type 3 (PPARG).* Peroxisomal proliferator-activated receptor γ *(PPARG)* gene mutations cause familial partial lipodystrophy type 3 (FPLD3), characterized by a similar host of metabolic abnormalities as seen in FPLD2 (100). The disorder has a variable age of onset from the second decade to later life. The hepatic steatosis seen in FPLD3 is more severe than FPLD2 (100). A 32-year-old FPLD3 patient heterozygous for the dominant negative mutation PPARG P467L had diabetes and elevated fasting FFA concentrations, and excessive and uncontrolled generation of FFAs directly from triglyceride-rich lipoproteins was observed (105).

PPARG is essential for adipogenesis: adipose-specific knockout of PPARG in mice results in lipodystrophy and, on a high-fat diet, severe insulin resistance (106). Mutations associated with FPLD3 appear to act either via a dominant negative mechanism, where the mutant disrupts the wild-type protein, or haploinsufficiency, where gene expression is reduced 50% due to a nonfunctional allele (100).

*Congenital generalized lipodystrophy (AGPAT2, BSCL2).* The very rare congenital generalized lipodystrophy (CGL) is usually diagnosed soon after birth with metabolic complications similar to those seen in FPLD2. It is also associated with hepatomegaly secondary to hepatic steatosis, which can progress to cirrhosis (100, 107). CGL1 is caused by mutations in the *AGPAT2* gene, which encodes an acylglycerol-3-phosphate transferase that catalyzes esterification of a fatty acid to lysophosphatidic acid, a key intermediate step in the biosynthesis of glycerophospholipids and triacylglycerols (108–110). It is expressed in adipose tissue and is induced during adipogenesis (111, 112). Agpat2 knockout mice develop severe lipodystrophy, insulin resistance, diabetes, and hepatic steatosis (113). Interestingly, in the liver of these mice, the expression of lipogenic genes and rates of de novo fatty acid biosynthesis was increased approximately 4-fold, and monoacylglycerol acyltransferase isoform 1 levels were markedly increased, suggesting that the alternative monoacylglycerol pathway for triglyceride biosynthesis is activated in the absence of AGPAT2 and contributes to hepatic steatosis (113).

CGL2, which has a similar pattern of lipodystrophy to, but of earlier onset than, CGL1 (114) is mainly caused by nonsense mutations in the *BSCL2* gene, encoding an integral membrane protein called "seipin," the function of which is unknown (115). However, seipin is predominantly expressed in brain, testis, and adipose tissue, and it is essential for normal adipogenesis (110, 116). Seipin seems to work upstream or at the level of PPAR-γ (117).

### Reduced hepatic triglyceride hydrolysis

*Neutral lipid storage disorder (PNPLA2, CGI-58).* Mobilization of adipose stores by triglyceride hydrolysis to produce

free fatty acids occurs through a variety of enzymes, including adipocyte triglyceride lipase (ATGL or PNPLA2). ATGL is a 505 amino acid protein that localizes around lipid droplets in adipose and other tissues, including the liver. ATGL null mice develop reduced plasma FFA levels, fat cell hypertrophy, mild obesity, and significant ectopic fat accumulation in the heart, liver, testis, and kidney (118). Interestingly, insulin sensitivity, which is increased in these animals, is hypothesized to be related to the lack of FFA-induced insulin resistance in muscle and increased use of glucose rather than FFA as a substrate for energy production (118). Activation of ATGL relies on the 349 amino acid protein, comparative gene identification-58 (CGI-58). CGI-58 is thought to be normally bound to perilipin, but β-adrenergic stimulation causes disassociation and colocalization with ATGL with subsequent triglyceride lipolysis (119, 120). Inhibition of CGI-58 by antisense oligonucleotides results in hepatic steatosis in adult mice fed a high-fat diet (121). Surprisingly, these animals were resistant to weight gain and demonstrated increased insulin sensitivity, implying an as yet undefined role in protection of diet-induced obesity and insulin resistance.

Mutations in ATGL or CGI-58, which are rare in humans, lead to the development of neutral lipid storage disorder characterized by lipid accumulation in the liver, skeletal, and cardiac muscle inherited in an autosomal recessive manner (118, 120, 122). A total of eight *PNPLA2* mutations have currently been described, all of which result in premature stop codons (122). The truncated ATGL proteins appear to either lack enzymatic activity or retain enzymatic activity but are unable to bind to lipid droplets (123, 124). Sixteen *CGI-58* mutations have been described throughout the seven exons of the gene (120). These appear to result in truncated proteins that lack functional activity. Interestingly, the phenotype of neutral lipid storage disease appears to differ according to which gene is affected, with subjects with ATGL mutations developing significant muscle triglyceride accumulation causing myopathy, whereas those with CGI-58 mutations are effected by ichthyosis (Chanarin-Dorfman syndrome). Subjects with Chanarin-Dorfman syndrome may also be affected by developmental defects, including hearing abnormalities, ataxia, and mental retardation. Although hepatic steatosis has been documented in both types of neutral lipid storage disease, it appears to be more commonly associated with CGI-58 mutations, where it has also been reported to lead to the development of cirrhosis (122).

*Wolman disease and cholesterol ester storage disease.* Wolman disease and cholesterol ester storage disease (CESD) are autosomal recessive conditions resulting from mutations in the lysosomal acid lipase (*LIPA*) gene whose protein product breaks down triglyceride and cholesterol esters that are delivered to lysosomes (125). The two disorders are distinguished by the level of residual activity of the mutant enzyme (126). Wolman disease occurs in the complete absence of lysosomal acid lipase and is uncommon (<1 in 300,000) (127). The disease manifests in the first months of life as massive triglyceride and cholesteryl ester accumulation in the viscera, including the liver and lung. Intestinal engorgement of lipid-filled macrophages leads to malabsorption and cachexia. The mean lifespan is six months. CESD occurs in partial deficiency of lysosomal acid lipase and is milder, presenting later in life with hepatomegaly, combined hypertriglyceridemia and hypercholesterolemia, low HDL cholesterol, and premature atherosclerosis (128, 129). Accumulation of lipid occurs in hepatic stellate cells as well as hepatocytes and may lead to the development of cirrhosis (130). A splice junction mutation in the last base of exon 8 is the most commonly described CESD mutation and results in aberrant splicing and an in-frame deletion of exon 8 (131, 132). Although the mutant protein lacks 24 amino acids and has no detectable activity (133), the mutation allows a small amount (3-5%) of normal splicing to occur, enabling residual acid lipase activity (126).

**Fatty acid oxidation disorders**

Hepatic steatosis is a common feature in patients with fatty acid oxidation disorders, including medium-chain acylcoenzyme-A dehydrogenase (MCAD) deficiency, long-chain acyl-CoA dehydrogenase deficiency (LCAD), very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency and mitochondrial trifunctional protein (TFP) deficiency (134). LCAD knockout mice have hepatic insulin resistance as well as steatosis. Plasma cholesterol levels were 2-fold higher than in wild-type mice, but this was not due to changes in SREBP2, LXRα, or HMG-CoA reductase expression (135). Insulin stimulation resulted in a 4-fold increase in hepatic diacylglycerol content (135). A VLCAD-deficient mouse model revealed that the long-term, medium-chain triglyceride-based diet, which is the treatment for VLCAD deficiency, induces severe hepatic steatosis by stimulating lipogenesis and impairing hepatic lipid metabolism (136).

The LCHAD gene product, mitochondrial TFP, is involved in mitochrondrial β-oxidation of FFAs. Interestingly, pregnant women with heterozygous LCHAD mutations whose fetuses are homozygous and have a deficiency of LCHAD are at risk of acute fatty liver of pregnancy (AFLP) (137). AFLP is a rare (1 in 10,000-15,000 deliveries) but serious condition that occurs in the third trimester of pregnancy, with reported maternal and neonatal mortalities of 2-18% and 7-58%, respectively (138, 139). Patients with AFLP typically present with a 1-2 week history of nausea, vomiting, headache, abdominal pain, and fatigue. Some women develop hypoglycemia, hepatic encephalopathy, and coagulopathy (138, 139). In the presence of the Glu474Gln mutation in the α subunit of the TFP, it is suggested that long-chain 3-hydroxyacyl metabolites produced by the fetus or placenta accumulate in the mother and are highly hepatotoxic (137). Liver histology reveals abundant microvesicular fat, supporting the concept of a mitochrondrial defect in the β oxidation pathway (140). Children with LCHAD and TFP deficiency have a high incidence of obesity (30%) as defined by a BMI ⩾ 95% for age (141).

**Post-receptor hepatic insulin resistance**

Semple et al. examined liver fat content and lipid metabolism in patients with severe insulin resistance due to insulin receptor-signaling defects (142). While four patients with insulin receptor missense mutations (mean age, 31 years and BMI, 22 kg/m$^2$) did not show evidence of hepatic steatosis, two patients (aged 40 and 59 years; BMI, 27 kg/m$^2$) with a dominant-negative protein kinase B (*AKT2*) mutation, R274H (143), had markedly elevated liver fat content, in combination with increased de novo lipogenesis, hypertriglyceridemia, and low HDL-cholesterol concentrations (142). AKT2, which is part of the insulin signaling pathway, is highly expressed in insulin-sensitive tissues and activated in response to growth factors and related stimuli (143). Semple et al. suggest that partial post-receptor hepatic insulin resistance is likely to be present in most forms of human insulin resistance and therefore plays a key role in the development of hepatic steatosis and metabolic dyslipidemia (142). In hyperinsulinemia, whereas the insulin-signaling pathway is downregulated, the transcription factor SREBP-1c is upregulated and mediates enhanced lipogenesis.

## GENETIC POLYMORPHISMS ASSOCIATED WITH HEPATIC STEATOSIS

While it is now established that there are strong environmental and lifestyle influences on the development of hepatic steatosis, there is also emerging evidence of numerous genetic modifiers. Genetic disorders (e.g., FHBL) may be directly associated with the development of hepatic steatosis, or genetic polymorphisms may influence susceptibility in the presence of other pathogenic risk factors. SNPs are single nucleotide substitutions in DNA which may result in the altered expression of a particular gene or altered function of the expressed protein. The increased risk of disease related to a single SNP is generally small, and it is likely that multiple SNPs may influence the phenotypic expression of NAFLD (i.e., a "polygenic" disease). Genetic variants may predispose to hepatic steatosis by influencing lipid trafficking or indirectly via effect on insulin resistance.

Most of the studies discussed below were driven by the selection of a candidate gene, followed by performance of a case-control SNP association study. Candidate genes are selected for examination by their putative or known role in pathogenesis of NAFLD or are based on results of genomic and proteomic studies. However, there are methodological limitations to some of the association studies described, including limited phenotypic characterization and the use of surrogate markers (e.g., ALT) for NAFLD; limited numbers and, therefore, power to detect or refute a true association; potential confounding factors, such as obesity; and the lack of validation studies in independent populations. In addition, studied SNPs may be in linkage disequilibrium with true functional SNPs, and SNPs may interact with each other. Conflicting results may also be related to the different ethnicities of the populations studied.

A genome-wide association study for liver fat has been performed in the multi-ethnic, population-based Dallas Heart Study. A SNP in *PNPLA3,* rs738409 (I148M), was the only variant in the 9,000 studied that was strongly associated with hepatic fat content (144). This association has since been validated in several studies, and despite the plethora of published genetic association studies in NAFLD and NASH, it remains the only robust and convincing association between a single SNP and the presence of hepatic steatosis.

The following sections (summarized in **Table 2**) describe the various gene association studies performed for hepatic steatosis, with emphasis on NAFLD and NASH.

***PNPLA3***

Patatin-like phospholipase domain containing 3, adiponutrin (PNPLA3) is a 481 amino acid protein of the patatin-like phospholipase family (145, 146). While its function is unknown, adiponutrin is a transmembrane protein with both lipolytic and lipogenic activity in vitro, and it is highly expressed in liver and adipocytes (145, 147). Nutritional control of PNPLA3 expression is regulated by a feed-forward loop: SREBP-1c activates PNPLA3 expression and inhibits its degradation through the stimulation of fatty acid synthesis (148). Surprisingly, loss of Pnpla3 in knockout mice had no effect on body weight, body composition, or adipose mass, and it did not cause fatty liver, liver enzyme elevation, or insulin resistance (149).

A genome-wide survey of over 9,000 nonsynonymous SNPs identified rs738409 (I148M) in PNPLA3 as the only variant strongly associated with hepatic fat content in the multi-ethnic, population-based Dallas Heart Study (144) (**Fig. 3**). $^1$H MRS was used to quantify liver fat in 2,111 subjects (1,032 African Americans, 696 European Americans, and 383 Hispanics). The association remained highly significant after adjusting for known risk factors, such as BMI, diabetes, and alcohol intake. In addition, no association was found between PNPLA3 and BMI, markers of insulin sensitivity and dyslipidemia. This is consistent with findings in animal models where increased hepatic fat content is not found with insulin resistance (150), and it provides further support to the notion that multiple mechanisms are responsible for the development of NAFLD.

Interestingly, the highest frequency of I148M was found in Hispanics (0.49), who also have a higher prevalence of NAFLD. The presence of I148M was also associated with higher ALT and aspartate aminotransferase (AST) activities (144). Conversely, African Americans, who have a lower prevalence of NAFLD, carried I148M at a lower frequency (0.17).

On resequencing, 3 of 160 subjects having the highest hepatic fat content were found to carry null mutations of *PNPLA3* (144). In addition, one variant, S453I, was found to be common in African Americans (frequency = 0.104) compared with European Americans (0.003) and Hispanics (0.008), and it was associated with a lower hepatic fat content. Within the African-American group, carriers of the I allele had ~20% lower median hepatic fat content compared with wild-type allele carriers (2.7% versus 3.3%).

TABLE 2. Summary of gene variants associated with the presence and/or severity of NAFLD

| Gene | Protein/Function | Study Size | Major Findings | Reference |
|---|---|---|---|---|
| *PNPLA3* 22q13.31 | Patatin-like phospholipase domain containing 3 (adiponutrin); membrane protein with lipase activity, exact function unknown | n = 2111 | Strong association ($P = 5.9 \times 10^{-10}$) between rs738409 (I148M) and hepatic fat content in subjects from the Dallas Heart Study. | 144 |
| | | n = 103 | I148M associated with the degree of hepatic steatosis (OR 1.50-5.20) and plasma ALT and AST activities. | 158 |
| | | n = 574 | rs738409 (I148M) associated with hepatic steatosis, steatohepatitis. and fibrosis. | 162 |
| *APOC3* 11q23.1-q23.2 | Apolipoprotein C-III; inhibits lipoprotein lipase and triglyceride clearance | n = 95 Replicated in n = 163 | NAFLD present in 38% of variant allele (C-482T and T-455C) carriers and none of the wild-type homozygotes. | 172 |
| *MTTP* 4q24 | Microsomal triglyceride transfer protein; essential for synthesis of triglyceride-rich lipoproteins; binds and lipidates apoB | n = 63 (NASH) n = 150 (control) | −493G allele frequency higher in NASH than control (OR 3.24; 95% CI, 1.65-6.36). Increased hepatic fat content (58.1 ± 22.2% vs. 43.9 ± 8.7%) and more advanced NASH in patients with −493 G/G compared with G/T. | 178 |
| | | n = 131 (NAFLD) n = 141 (control) | No significant difference in MTTP −493 allele frequency between NASH and simple hepatic steatosis or between NAFLD and control. | 244 |
| *DGAT2* 11q13.5 | Diacylglycerol acyltransferase 2; synthesis of intracellular triglycerides | n = 187 | No association of two DGAT SNPs with hepatic steatosis, but after nine months of lifestyle interventions, subjects carrying rs1944438-T showed a smaller decrease in liver fat content. | 187 |
| *PEMT* 17p11.2 | Phosphatidylethanolamine N-methyltransferase; converts phosphatidylethanolamine to phosphatidylcholine in the liver by sequential methylation | n = 28 (NAFLD) n = 59 (control) | Higher frequency of V175M in NAFLD (0.81 vs. 0.61 in control, $P < 0.03$). | 193 |
| | | n = 2349 | No association between V175M and hepatic fat content in Dallas Heart Study participants. | 194 |
| | | n = 107 (NASH) n = 150 (control) | V175M more common in Japanese NASH patients than control (OR 5.79; 95% CI, 2.46-13.60). | 195 |
| | | n = 195 (NAFLD) n = 393 (control) | No association between V175M and hepatic fat content in Koreans. | 196 |
| *ADIPOQ* 3q27 | Adiponectin; adipokine secreted by adipose tissue with effects on glucose and lipid metabolism | n = 70 (NAFLD) n = 70 (control) | Prevalence of haplotype 45TT+276GT/TT was higher in NAFLD than control (64% vs. 28%, $P = 0.0002$). | 199 |
| | | n = 79 (NAFLD) n = 40 (control) | No association of −11391, −11377, +45, or +276 SNPs with NAFLD. | 204 |
| | | n = 165 (NAFLD) n = 160 (control) | No association of +45 or +276 SNPs with NAFLD. | 205 |
| | | n = 119 (NAFLD) n = 115 (control) | Higher prevalence of 45G/G in NAFLD severe fibrosis group (23.4%) compared with mild fibrosis group (2.8%) ($P < 0.01$). | 200 |
| *ADIPOR1* 1q32.1 | Adiponectin receptor-1; mediates effects of adiponectin | n = 85 | Higher liver fat in subjects carrying the −8503 A and −1927 C alleles ($P = 0.02$). | 207 |
| *ADIPOR2* 12p13 | Adiponectin receptor-2; mediates effects of adiponectin | n = 302; validated in n = 619 and n = 3050 | rs767870 associated with hepatic steatosis; mean liver fat content 7.0% in TC carriers vs. 3.0% in CC homozygotes (adjusted $P = 0.02$). | 206 |
| *ENPP1* 6q22-q23 | Ectoenzyme nucleotide pyrophosphate phosphodiesterase 1; involved in ATP hydrolysis and modulates insulin sensitivity | n = 702 (NAFLD) n = 310 (control) | Lys121Gln associated with fibrosis stage >1 (OR 1.55; 95% CI, 1.24-1.97). | 208 |
| *IRS1* 2q36 | Insulin receptor substrate 1; mediates insulin signaling | n = 702 (NAFLD) n = 310 (control) | Gly972Arg associated with fibrosis stage >1 (OR 1.57; 95% CI, 1.12-2.23). | 208 |
| *PPARGC1A* 4p15.1 | Peroxisome proliferator-activated receptor γ, coactivator 1 α; transcriptional coactivator that regulates genes involved in lipid and glucose metabolism | n = 115 (NAFLD) n = 441 (control) | Rs2290602-T associated with hepatic steatosis (OR 2.73; 95% CI, 1.48-5.06) and lower intrahepatic mRNA levels of PPARGC1A. | 221 |
| | | n = 96 (NAFLD) n = 96 (control) | No difference in allele frequency of G482S in NAFLD patients compared with healthy control. | 222 |
| *PPARG* 3p25 | Peroxisome proliferator-activated receptor γ; regulates genes involved in lipid and glucose metabolism | n = 96 (NAFLD) n = 96 (control) | Allele frequency of C161T higher in NAFLD (0.25) compared with control (0.135, $P = 0.004$). | 222 |
| *PPARA* 22q12-q13.1 | Peroxisome proliferator-activated receptor α; regulates genes involved in lipid and glucose metabolism | n = 79 (NAFLD) n = 63 (control) | Allele frequency of Val227Ala was lower in NAFLD (0.03) compared with control (0.11, $P = 0.01$). | 232 |
| *TCF7L2* 10q25.3 | Transcription factor 7-like 2; regulates gene expression in cellular metabolism and growth | n = 78 (NAFLD) n = 156 (control) | Association of rs7903146C/T with hepatic steatosis; 19% of NAFLD patients were homozygous for the C allele compared with 51% of control ($P = 0.0001$). | 237 |

TABLE 2. *Continued.*

| Gene | Protein/Function | Study Size | Major Findings | Reference |
|---|---|---|---|---|
| *GCLC* 6p12 | Glutamate-cysteine ligase, catalytic subunit; rate-limiting enzyme involved in the synthesis of glutathione | n = 131 (NAFLD) n = 141 (control) | −129T allele associated with NASH (OR 12.14; 95% CI, 2.01-73.35). | 244 |
| *NOS2* 17q11.2-q12 | Inducible nitric oxide synthase 2; produces nitric oxide in the liver | n = 115 (NAFLD) n = 435 (control) | Four linked SNPs were associated with protection against NAFLD (OR 0.49; 95% CI, 0.32-0.75). | 246 |
| *SOD2* 6q25.3 | Manganese superoxide dismutase; antioxidant enzyme localizing to mitochondria | n = 63 (NASH) n = 150 (control) | Higher frequency of 1183T/T in NASH patients (0.84) vs. control (0.68, $P = 0.016$). | 178 |
| *STAT3* 17q21 .31 | Signal transducer and activator of transcription 3; transcription factor that regulates genes encoding acute phase proteins | n = 108 (NAFLD) n = 55 (control) | Carriers of two SNPs (rs6503695-T and rs9891119-A) were 2.3- and 2.5-fold more likely to have NAFLD compared with noncarriers. | 252 |
| *TNF* 6p21.3 | Tumor necrosis factor; pro-inflammatory cytokine produced by macrophages | n = 99 (NAFLD) n = 172 (control) | Prevalence of −238 (but not −308) promoter polymorphism higher in NAFLD (31% vs. 15% in control, $P < 0.0001$). | 33 |
| | | n = 102 (NAFLD) n = 100 (control) | No association between promoter polymorphisms and presence of NAFLD; however, −1031C and −863A more common in NASH than simple hepatic steatosis. | 254 |
| | | n = 79 (NAFLD) n = 40 (control) | No association of promoter SNPs with NAFLD. | 204 |
| *MTHFR* 1p36.3 | Methylenetetrahydrofolate reductase; catalyzes conversion of a cosubstrate for homocysteine remethylation | n = 116 (chronic hepatitis C) | Homozygosity for C677T associated with severe hepatic steatosis (OR 20.0; 95% CI, 1.6-89.1). | 255 |
| *HFE* 6p21.3 | Membrane protein that regulates iron absorption by modulating the interaction of transferrin with its receptor | n = 57 (NASH) n = 348 (control) | Presence of either C282Y or H63D higher in NASH (61%) than control (38%) ($P = 0.008$). | 258 |
| | | n = 93 (NASH) n = 206 (control) | 15% NASH were heterozygous for C282Y, compared with 6.3% of healthy blood donors ($P = 0.04$). | 259 |
| | | n = 134 (NAFLD) n = 291 (control) | Prevalence of C282Y higher in NAFLD than control ($P < 0.0001$). | 260 |
| | | n = 52 (NAFLD) | No association of C282Y or H63D with NAFLD. | 261 |
| | | n = 126 (NASH) | Heterozygosity for C282Y associated with advanced fibrosis (44% vs. 21% in other genotypes, $P = 0.05$) among Caucasians with NASH. | 264 |
| *SERPINA1* 14q32.1 | Alpha-1-antitrypsin; serine protease inhibitor | n = 353 (NAFLD) n = 144 (control) | 10.8% of NAFLD patients carried nonMM (non-wild-type) genotypes, compared with 3.5% of control ($P = 0.02$). | 270 |
| | | n = 1405 | Higher prevalence (5.0%) of the Z allele among NAFLD patients with decompensated liver disease, compared with NAFLD patients with less severe liver disease (1.9%, $P = 0.017$). | 271 |
| *UGT1A1* 2q37 | UDP glucuronosyltransferase 1 family, polypeptide A1; hepatic conjugation of bilirubin and other compounds | n = 234 | Variant UGT1A1*6 alleles associated with a lower risk of NAFLD in obese children (OR 0.31; 95% CI, 0.11-0.91). | 273 |
| *ABCC2* 10q24 | ATP-binding cassette, sub-family C, member 2; efflux pump for conjugated compounds | n = 109 (NAFLD) n = 58 (control) | The A allele of both rs17222723A/T and rs8187710G/A had 3-fold lower risk for NAFLD. | 277 |
| *AGTR1* 3q21-q25 | Angiotensin II receptor, type 1; mediates the effects of angiotensin II in the renin-angiotensin system | n = 167 (NAFLD) n = 435 (control) | rs3772622 associated with NAFLD (A allele frequency 0.70 in NAFLD and 0.55 in control, $P = 0.000001$) and fibrosis index ($P = 0.05$). | 286 |

NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SNP, single nucleotide polymorphism.

Overall, the two SNPs in *PNPLA3*, I148M and S453I, accounted for an astonishing 72% of the ethnic differences in hepatic fat content (144).

The findings of Romeo et al. were replicated in a study of 291 Finnish individuals aged 20-75 years, of whom 83 had type 2 diabetes (151). Mean liver fat was 9.0 ± 0.8% in I148 wild-type allele homozygotes; 10.4 ± 1.1% in I/M subjects, and 14.1 ± 1.9% in I148M homozygotes ($P < 0.01$ compared with wild-type homozygotes). In addition, using the euglycemic hyperinsulinemic clamp, the gold standard to assess insulin sensitivity, Kotronen et al. found no association of insulin sensitivity with the I148M SNP (151). This was supported by data from Kantartzis et al., who also found no association between I148M and insulin sensitivity as measured by the oral glucose tolerance test (OGTT) and euglycemic-hyperinsulinemic clamp in 222 subjects (152). More recently, Santoro et al. demonstrated that obese children and adolescents carrying I148M had comparable hepatic glucose production, peripheral glucose disposal, and glycerol turnover to those homozygous for the wild-type allele (153). Interestingly, they also showed that adipocytes from a subcutaneous fat biopsy were

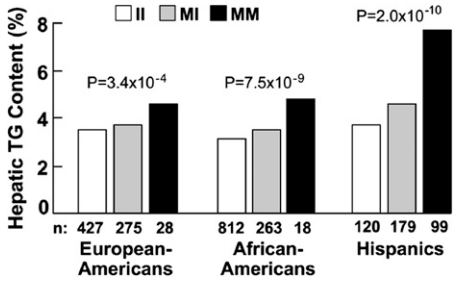


**Fig. 3.** Median hepatic fat content and PNPLA3 I148M genotypes in European-Americans, African-Americans, and Hispanics in the Dallas Heart Study. PNPLA3, patatin-like phospholipase domain containing 3, adiponutrin. Reprinted by permission from Macmillan Publishers Ltd: Nat Genet 2008;40:1461-5, copyright 2008.

smaller in I148M carriers compared with wild-type ($P$ = 0.005). In 218 type 2 diabetic patients, Petit et al. found that while the association of I148M with hepatic fat content remained, liver fat content was closely linked to BMI and visceral fat area in wild-type homozygotes, but not in I148M carriers (154).

A study of three independent cohorts (Bruneck, Italy, population-based; SAPHIR, Austria, healthy working population; and Utah, obesity case-control study) found a strong association of I148M with age- and gender-adjusted ALT and AST activities (155).

A further study by Kollerits et al. found that each I148M allele decreased nonHDL-cholesterol by 2.4 mg/dl and LDL-cholesterol by 1.5 mg/dl, indicating a role in hepatic lipoprotein metabolism of apoB-containing lipoproteins (156). Similar observations were made for rs2072907, an adiponutrin SNP that had previously been associated with obesity (157). However, the two SNPs together only explained less than 1% of total cholesterol concentrations at a population level (156). The mechanism for the effect of adiponutrin variants on circulating lipoprotein concentrations is yet to be elucidated, but it could involve impaired secretion of VLDL from the liver (156).

Sookoian et al. examined the effect of the rs738409 I148M SNP on histologically determined NAFLD severity (158). A total of 103 NAFLD patients (40 with simple hepatic steatosis, 63 with NASH) underwent liver biopsy. An association was observed between rs738409 and the histological spectrum of NAFLD. The rs738409-G allele (frequency 0.34) was associated with the degree of hepatic steatosis (OR 1.50-5.20). The association persisted when adjusted for HOMA score and diabetes, again supporting a mechanism independent of type 2 diabetes mellitus. The proportion of total variation in hepatic fat content attributed to I148M was calculated as 5.3%. A significant association of I148M with plasma ALT and AST activities was observed, after adjusting for age, BMI, HOMA, and even plasma triglyceride concentrations. Rotman et al. studied 894 NAFLD patients, of which 59% had a histological diagnosis of NASH (159). The I148M allele was associated with increased hepatic steatosis, portal and lobular inflammation, and higher fibrosis scores. However, unlike Sookoian et al., the allele frequency between the steatosis-only and steatohepatitis groups did not differ, possibly relating to differences in study design and minor allele frequency (158, 159). Also studied were 223 pediatric patients with NAFLD (64% Hispanic), where carriers of the rs738409-G allele were younger at the time of biopsy, although the association was of borderline-significance (159). An Italian pediatric study found that I148M was strongly associated with both the severity of steatosis and the presence of NASH (160). Of 149 Italian children with biopsy-proven NASH, all I148M homozygotes and 75% of I148M heterozygotes had NASH, compared with 3% of wild-type homozygotes.

In addition, I148M is strongly associated with alcoholic liver disease and clinically evident alcoholic cirrhosis (OR 2.25, $P = 1.7 \times 10^{-10}$) (161). A further study examining the influence of adiponutrin polymorphisms and severity of liver injury in 574 NAFLD patients found the rs738409 genotype to be associated with a 50% increased risk of NASH and significant hepatic fibrosis (162).

The rs738409 I148M SNP is located in the patatin domain of adiponutrin. Prediction algorithms confirm that this variant is likely to affect protein function (158). The I148M SNP is found within a consensus sequence for a Ser-Asp catalytic dyad, which has lipase activity in vitro (146, 163, 164). However, neither overexpression of PNPLA3 in human embryonic kidney cells nor knockdown of PNPLA3 by siRNA changed cellular triglyceride content (165, 166). Structural modeling predicts that substituting methionine for isoleucine at residue 148 would restrict access of the substrate to the catalytic serine at residue 47, due to occlusion by the longer side chain of methionine (167) (**Fig. 4**). Consistent with this prediction, studies in Sf9 cells showed that the I148M substitution abolished triglyceride hydrolysis; the subcellular distribution of the protein was unaffected (167). Overexpression of wild-type and I148M

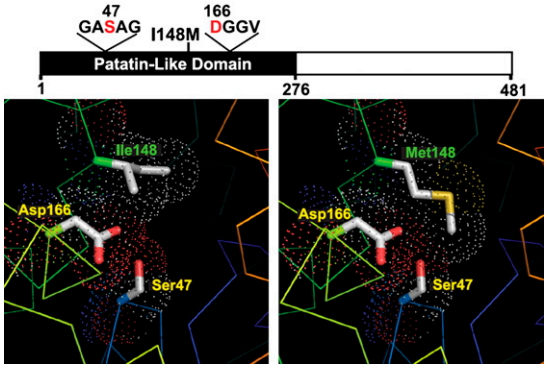


**Fig. 4.** Structural model of wild-type and I148M PNPLA3. The domain structure of PNPLA3, showing the patatin-like domain (black) and locations of the catalytic dyad (Ser47 and Asp166) and the I148M substitution. Structure models of normal (Ile148) and mutant (Met148) PNPLA3 are shown in the left and right panels, respectively. Protein traces are rainbow-colored from N to C terminus (blue to red) with side chains of catalytic dyad residues (positions 47 and 166). The dots indicate a space-filling model corresponding to van der Waals atomic radii. Oxygen and sulfur atoms are colored red and yellow, respectively. PNPLA3, patatin-like phospholipase domain containing 3, adiponutrin. Reprinted by permission from He et al. J Biol Chem 2010;285:6706-15.

PNPLA3 in mice showed that wild-type PNPLA3 failed to lower triglyceride content, whereas I148M actually increased hepatic triglyceride levels. In the human hepatoma cell line HuH-7, I148M promoted the hepatic accumulation of triglycerides and cholesterol esters by inhibiting the hydrolysis of triglycerides (167).

Expression of adiponutrin is stimulated by insulin and glucose (168). There is an inverse relationship between adiponutrin mRNA levels and fasting plasma glucose, and there is a positive correlation between mRNA levels and insulin sensitivity (169). However, there is limited evidence for a relationship between PNPLA3 SNPs and insulin sensitivity. One study showed that carriers of rs738409-C (I148) had decreased insulin secretion in response to an OGTT, whereas I148M carriers were more insulin-resistant at a lower BMI. However, no significant differences were observed in fasting plasma glucose, β-cell function, or HOMA score among allele carrier groups (170). In an obese cohort of Italians, 50% of I148M homozygotes had ALT activities above the reference interval compared with 25% of I148 homozygous wild-type subjects, but without any difference in insulin sensitivity or glucose tolerance (163). A genome-wide association study found that *PNPLA3* had the strongest association with plasma AST and ALT activities (171).

Adiponutrin expression is increased by 50-fold in obese fa/fa Zucker rats (145). In fasting obese individuals, adiponutrin mRNA levels in adipose tissue biopsies were higher than in nonobese subjects, and two SNPs (I148M and rs2072907) were associated with obesity (157). Carriers had lower adipose mRNA levels and an increase in basal lipolysis (157). It is still to be determined whether this association with obesity is a cause or a consequence. Obesity-associated expression of adiponutrin may be secondary to hyperinsulinemia and may actually be a protective physiological response.

In summary, these convincing data show a strong association of a *PNPLA3* genetic variant with liver fat content and liver injury, and they support a role for PNPLA3 in the modulation of hepatic fat content as well as hepatocellular damage.

## GENES AFFECTING LIPID METABOLISM

### *APOC3*

A recent paper by Petersen et al. describes an association of two SNPs in apolipoprotein C3 (*APOC3*) with NAFLD and insulin resistance (172). Ninety-five healthy Asian-Indian men were genotyped for *APOC3* C-482T and T-455C. Allele carriers had a 30% increase in fasting plasma apoC-III concentration and a 60% increase in fasting plasma triglyceride concentration compared with wild-type homozygotes. NAFLD was present in 38% of *APOC3* variant allele carriers and none of the wild-type homozygotes. The association between *APOC3* variants and NAFLD was confirmed in a validation study of 163 healthy non-Asian-Indian men. In addition, after an oral fat load, plasma triglyceride clearance was reduced by almost 50% in variant allele carriers (n = 15) compared with wild-type controls (n = 4). Seven NAFLD subjects with insulin resistance underwent a hypocaloric dietary intervention, losing on average 5.5 kg over a 3-6 month period. A significant reduction in the hepatic triglyceride content was observed (14.0-3.8%), accompanied by marked improvement in insulin sensitivity.

The proposed mechanism by which *APOC3* variants increase the risk of NAFLD is that the increased plasma levels of apoC-III inhibit lipoprotein lipase and triglyceride clearance, leading to fasting and postprandial hypertriglyceridemia due to an increase in chylomicron remnants (172). The chylomicron remnants are preferentially taken up by the liver, resulting in NAFLD and hepatic insulin resistance.

### *MTTP*

MTTP is essential for synthesis of triglyceride-rich lipoproteins; it is a chaperone that binds and lipidates nascent apoB (173). The hepatic expression level of MTTP mRNA is significantly lower in NASH compared with NAFLD patients (174). Interestingly, MTTP inhibition has been implicated in hepatitis C virus (HCV)-related hepatic steatosis (175). Moreover, HCV-associated hypobetalipoproteinemia has been shown to be negatively correlated with hepatic steatosis and HCV viral load (176). Taken together, these studies would be consistent with the concept that, by inhibiting VLDL secretion, HCV core protein might induce liver steatosis and hypobetalipoproteinemia.

A functional promoter polymorphism in MTTP, −493G>T, is associated with increased MTTP transcription in vitro and lower levels of LDL-cholesterol in healthy subjects, whereas the G allele may be associated with hepatic steatosis (177, 178). However, in chronic HCV infection, the T allele is associated with decreased MTTP expression, which may occur either through a direct binding of some HCV proteins at the −493 site or through an upregulation of MTTP-suppressor(s) by HCV (179). There is also an association between −493T and hepatic steatosis in both HCV genotype 3- and nongenotype 3-infected patients (180, 181). Increased lipid droplet accumulation in hepatocytes may provide a safe environment for HCV latency (179).

A recent study has also associated the *MTTP* G allele with β-cell dysfunction in nondiabetic normolipidemic NASH patients (182), providing a link between the *MTTP* SNP and diabetes incidence. *MTTP* GG carriers also have a more atherogenic postprandial lipid profile (as determined by the magnitude of triglyceride, FFA, and LDL-conjugated diene response and fall in HDL-cholesterol and apoA-I) than other genotypes, which is independent of adipokines and insulin resistance (183).

### *DGAT2*

DGAT2, an isoform of the enzyme acylCoA: diacylglycerol acyltransferase, catalyses the final stage of triglyceride synthesis in the liver (184). Overexpression of DGAT2 in mice led to a 2.4-fold increase in hepatic triglyceride content, but it had no effect on production of VLDL triglyceride or apoB (185). In addition, mice on a

high-fat diet that overexpress DGAT develop fatty liver but not glucose or insulin intolerance (150), showing that hepatic steatosis can occur independently of insulin resistance. Interestingly, antisense therapy reducing DGAT improves hepatic steatosis but not insulin sensitivity (186).

Kantartzis et al. studied *DGAT2* SNPs in 187 Caucasians at risk of, but without, type 2 diabetes. Risk factors included a family history of type 2 diabetes, BMI > 27 kg/m$^2$, and impaired glucose tolerance or gestational diabetes (187). Two SNPs (rs10899116 and rs1944438) that, due to high linkage disequilibrium, represented all the commonly occurring SNPs (minor allele frequency > 0.05) in the general population were genotyped. Although there was no association of DGAT SNPs with hepatic steatosis, after nine months of lifestyle interventions that were aimed to reduce body weight by ⩾5%, subjects carrying minor T allele of rs1944438 showed a smaller decrease in liver fat (TT, −17 ± 10%; TC, −24 ± 5%). compared with homozygous C carriers (−39 ± 7%). Changes in total body fat, visceral fat, and insulin sensitivity did not vary between genotypes. These observations are consistent with studies in mice, which show a predominant effect of DGAT on hepatic steatosis (150). There was also no association of DGAT2 variants with early-onset obesity in children and adolescents (188).

### *PEMT*

Phosphatidylethanolamine N-methyltransferase (PEMT) catalyzes the synthesis of ∼30% of phosphatidylcholine formed in the liver (189), which is required for hepatic secretion of triglyceride-rich VLDL (190, 191). Dietary choline deficiency can cause the development of fatty liver (192).

A small study of reported that the frequency of the *PEMT* variant V175M was higher in 28 biopsy-confirmed NAFLD patients (0.81) compared with control subjects with normal hepatic triglyceride content (0.61) (193). In addition, in vitro transfection experiments in McArdle-RH7777 cells showed that V175M had diminished activity compared with wild-type PEMT (193). However, no association was observed between *PEMT* V175M and liver fat in the Dallas Heart Study participants (2,349 total; grouped as black, white, or Hispanic) who underwent $^1$H-MRS, even after adjusting for BMI, which called into question the validity of the smaller study (194). In Japanese subjects, V175M occurred more frequently in NASH patients (n = 107, frequency 0.121) than in healthy controls (n = 150, frequency 0.023), and it was associated with a lower BMI among NASH patients (195). A subsequent study in Koreans found no association between PEMT V175M and fatty liver (196). It is possible that conflicting findings seen in the association studies could relate to the different frequencies of the *PEMT* variant among different populations, and also to interactions with other genes, such as *MTHFR*. The need for diet- or PEMT-synthesized choline is reduced by the availability of methyl groups via methyltetrahydrofolate (197).

## GENES AFFECTING INSULIN RESISTANCE / SENSITIVITY

### Adiponectin and adiponectin receptor

Adiponectin is a 244 amino acid protein that modulates a number of metabolic processes, including inflammation, glucose regulation, and fatty acid catabolism (198). This hormone is secreted from adipose tissue into the circulation, and it is the most abundant and adipose-specific adipokine. The plasma concentrations of adiponectin are decreased in type 2 diabetics compared with nondiabetics, whereas weight reduction and insulin-sensitizing agents increase the circulating levels (39). Adiponectin concentrations correlate with hepatic steatosis grade and severity of NAFLD; levels are lower in NASH compared with NAFLD (198). This observation opens the possibilities of adiponectin being a noninvasive predictor of NASH and a potential therapeutic agent in NAFLD.

Musso et al. assessed the prevalence of adiponectin gene (*ADIPOQ*) SNPs 45G>T and 276G>T in 70 nonobese, nondiabetic, normolipidemic Italian NAFLD patients (of which 30 had biopsy-proven NASH) and 70 matched healthy controls (199). The genotypes 45TT and 276GT/TT were more prevalent in NAFLD than controls (64% versus 28%, $P = 0.0002$). The NASH patients and 30 controls underwent a standardized oral fat challenge. Both genotypes (45TT and 276GT/TT) were associated with a lower postprandial adiponectin increase in NASH patients compared with NASH patients carrying other genotypes. In addition, NASH patients with 45TT and 276GT/TT genotypes had higher postprandial triglyceride, VLDL, and FFA responses. These results need confirmation in NAFLD patients without NASH.

In a subsequent Japanese study of 119 histologically proven NAFLD (54 females) and 115 age- and gender-matched healthy controls (57 females), the only association found with the adiponectin 276 SNP was among females, where a higher allele frequency of 276G in NAFLD was observed (0.78 versus 0.65 in controls, $P = 0.03$) (200). More 45 and 276G homozygotes were seen among patients with severe fibrosis (23.4%) than among patients with mild fibrosis (2.8%). The conflicting findings of Musso et al. and Tokushige et al. could relate to ethnic differences; in several Asian studies, homozygosity for the 276G allele is associated with low adiponectin, diabetes, and coronary artery disease (201–203).

A study in Chinese patients (79 with NAFLD, including 18 with fibrosis; and 40 healthy controls) did not show an association between *ADIPOQ* polymorphisms and NAFLD or fibrosis (204). A larger Chinese study of 165 NAFLD patients, 83 NAFLD patients with metabolic syndrome, and 160 healthy controls also failed to find any association of the 45 and 276 adiponectin SNPs with the presence of NAFLD (205).

Adiponectin receptor 1 (ADIPOR1) is expressed predominantly in skeletal muscle, whereas adiponectin receptor 2 (ADIPOR2) is found predominantly in the liver. Hepatitic ADIPOR2 expression is reduced in human subjects

with NASH compared with simple steatosis, suggesting it may play a role in the progression of NAFLD (38). A candidate gene approach study of 302 Finns who had hepatic steatosis determined by $^{1}$H MRS found a SNP in ADIPOR2 (rs767870) but not any SNPs in ADIPOR1 associated with hepatic steatosis (206). The TT allele was associated with increased hepatic fat content after controlling for age, gender, and BMI, and it was subsequently associated with biochemical surrogates of NAFLD (serum γ glutamyltransferase activity and triglyceride concentrations in men) in two independent validation cohorts. The first validation cohort comprised 619 50-year-old Swedish men randomly selected from a population registry; the second validation cohort comprised 3,050 adults taking part in a population-based study from the Botnia region of Western Finland (Prevalence, Prediction, and Prevention of Diabetes Study). In contrast, in a smaller German study of 85 nondiabetics, no association between MRS-detected hepatic steatosis and ADIPOR2 was detected; however, a trend ($P = 0.056$) toward an association with ADIPOR1 was found after controlling for age, gender, and percentage of body fat (207). The association between ADIPOR1 SNP and hepatic steatosis became significant after weight loss through dietary intervention, suggesting it may play a role in predicting treatment responses. These findings need to be interpreted with caution due to the small sample size; further confirmatory studies in larger groups are required to validate these conflicting findings.

### *ENPP1* and *IRS1*

A recent study by Dongiovanni et al. examined two SNPs known to affect insulin receptor activity in ectoenzyme nucleotide pyrophosphate phosphodiesterase 1 (*ENPP1*) and insulin receptor substrate 1 (*IRS1*) genes in 702 biopsy-proven NAFLD patients in Italy and the UK and 310 Italian controls (208). The SNPs had previously been associated with increased risk of type 2 diabetes (209, 210). Dongiovanni et al. showed that the *ENPP1* Lys121Gln and *IRS1* Gly972Arg SNPs were independently associated with fibrosis stage >1 (OR 1.55; 95% CI, 1.24-1.97; and OR 1.57; 95% CI, 1.12-2.23, respectively). Genotype frequencies were not significantly different between NAFLD patients and controls for *ENPP1*. A borderline significant decrease in *IRS1* frequency was observed in NAFLD. In NAFLD patients, *ENPP1* 121Gln was associated with higher BMI and waist circumference and lower HDL-cholesterol concentrations, whereas *IRS1* 972 Arg was associated with lower triglyceride levels and a trend toward a higher prevalence of type 2 diabetes. Both polymorphisms were associated with a 70% reduction in AKT activation status, reflecting reduced insulin-signaling activity (208).

### *PPARGC1A*, *PPARG*, and *PPARA*

PPARγC1α is involved in insulin resistance, mitochondrial biogenesis, and oxidative phosphorylation (211, 212). *PPARγC1α* interacts with peroxisome proliferator activated receptors (PPARs), transcription factors that regulate series of genes involved in glucose and lipid metabolism (213). Levels of PPARγC1α are tightly regulated, increased by glucagon during fasting, and inhibited by insulin (214, 215). Chronically and mildly reduced hepatic expression of *PPARγC1α* in mice causes hepatic insulin resistance (216). Mice with absence of *PPARγC1α* expression in liver exhibit fasting-induced hepatic steatosis (217), and mouse models of diabetes (liver insulin receptor knockout, ob/ob and streptozotocin-administered) have increased *PPARγC1α* expression levels (218). PPARα plays a key role in utilizing lipid in the liver for energy by stimulating fatty acid oxidation. Thus, genetic knockout animal models develop hepatic steatosis (219). PPARγ is expressed predominantly in adipocytes and is involved in adipocyte differentiation and insulin sensitivity. Thiazolidinediones are PPARγ ligands that have demonstrated promise as therapeutic agents for the treatment of NAFLD (220).

A Japanese study in 115 biopsy-proven NAFLD patients (65 with NASH and 50 with simple hepatic steatosis) showed a significant association of the PPAR-γ coactivator 1α gene (*PPARGC1A*) rs2290602-T, an intron 7 SNP, with the development of NAFLD [OR 2.73 (1.48-5.06)] (221). Although the numbers were small, the intrahepatic mRNA levels of *PPARγC1α* were lower in subjects homozygous for the T allele (TT) compared with wild-type GG or heterozygous GT subjects.

A Chinese study of 96 NAFLD patients found no significant difference in allele frequencies of another *PPARGC1A* SNP, Gly482Ser, compared with 96 matched healthy controls (222). This study also found that the *PPARG* C161T allele frequency was higher (0.25) compared with healthy controls (0.135) and that plasma adiponectin concentrations were lower in NAFLD patients with CT/TT genotypes compared with NAFLD CC homozygotes (3.0 ± 0.6 versus 4.3 ± 0.9 mg/l, $P = 0.02$) (222). Other studies have implicated Gly482Ser in obesity, hypertension, and diabetes (223–228). Gly482Ser is associated with reduced *PPARGC1A* gene expression and gives an 11% increase in type 2 diabetes risk per copy (229, 230). However, the SNP does not lie in or alter known *PPARγC1α* transcription factor binding sites (223). Gly482Ser has also been associated with FFA levels in obese subjects. FFAs have been shown to downregulate *PPARγC1α* in skeletal muscle (231). Carriers of Ser482 show blunted clearance of glucose load (231).

Chen et al. genotyped 79 NAFLD patients and 63 healthy controls for PPARα Val227Ala, and they found that the allele frequency of Val227Ala was lower in NAFLD (0.03) than controls (0.11) (232). However, note that the above human studies were limited in sample size; thus, appropriately powered studies examining the effects of these gene variants on hepatic steatosis in a variety of cohorts are needed.

### *TCF7L2*

Transcription factor 7-like 2 (TCF7L2) is a receptor for β-catenin. It regulates the expression of a multitude of genes involved in cellular metabolism and growth. Previous studies have linked TCF7L2 variation with impaired insulin secretion and risk of diabetes, possibly mediated by reduced secretion of incretin and altered β-cell glucose

response (233–235). TCF7L2 also regulates adipokine secretion and triglyceride metabolism through effects on PPAR-γ, CCAAT/enhancer-binding protein-α, and lipoprotein lipase. TCF7L2 SNPs are associated with serum triglyceride concentrations in familial hyperlipidemia (236).

Musso et al. found an association of a variant in the *TCF7L2* gene, rs7903146C/T, with hepatic steatosis in 78 nondiabetic, normolipidemic NAFLD patients (including 34 patients with a histological diagnosis of NASH) compared with 156 age-, gender-, and BMI-matched controls (237). A total of 19% of NAFLD patients were homozygous for the C allele compared with 51% of controls ($P = 0.0001$). In this study, derived measures of pancreatic β-cell function, incretin effect, and hepatic insulin resistance based on oral glucose tolerance testing were more severe in CC homozygotes compared with TT/CT subjects in both controls and NASH patients. In addition, plasma levels of a hepatocyte apoptotic marker (cytokeratin-18) were increased in NASH patients with the CC genotype compared with the CT/TT genotype.

## GENES AFFECTING OXIDATIVE STRESS

Oxidative stress is prominent in humans with NASH, and it appears to be a key factor in the development of liver injury in patients with NAFLD, leading to necroinflammatory activity and fibrosis (238, 239). Furthermore, oxidative stress has been implicated in cell line and animal model studies to exacerbate insulin resistance by disruption of insulin signaling, which may enhance the development of hepatic steatosis (240, 241). Increased oxidative stress levels in subjects with NAFLD may be related to production of reactive oxygen species (ROS), fatty acid oxidation, mitochondrial dysfunction, or the presence of hepatic iron (28, 242).

### *GCLC*

Glutamate-cysteine ligase (GCLC) is the first and rate-limiting enzyme in the synthesis of glutathione, the major antioxidant in the liver. Liver-specific deletion of GCLC in mice rapidly leads to hepatic steatosis and progressive severe parenchymal damage (243). In the association study of Oliveira et al. (as described above in the "MTTP" section), the *GCLC*-129T allele was found to be independently associated with NASH (OR 12.1; 95% CI, 2.1-73.35) (244). The T allele lies in the promoter region of *GCLC* and gives rise to a 50-60% decrease in GCLC gene expression compared with C (245). It is hypothesized that carriers of one or more T alleles form less intracellular glutathione in response to hepatic ROS generation and have increased susceptibility to ROS-induced hepatocellular injury (244).

### *NOS2*

Yoneda et al., who studied associations of *PPARγC1α*, also examined the influence of SNPs in the inducible nitric oxide synthase (*NOS2*) gene on their NAFLD cohort (246). Ten common SNPs were genotyped. Four of these were found to be in complete linkage disequilibrium (rs2297510, rs2297511, rs2797512, and rs1060822) and were significantly associated with the presence of NAFLD, with the major alleles being protective (OR 0.49; 95% CI, 0.32-0.75). Minor allele rs1060822-T was also associated with a higher fibrosis index in TT homozygotes than in TC or TT subjects. Plasma hyaluronic acid and type IV collagen 7S domain levels were higher in patients with the TT genotype. There was a trend toward higher intrahepatic mRNA expression of iNOS in TC (n = 9) compared with wild-type CC (n = 4) carriers, although this failed to reach significance. iNOS is expressed as part of the inflammatory response, and in the presence of superoxide radicals, it forms peroxynitrite, which can cause endoplasmic reticulum stress and cell death (247).

### *SOD2*

Manganese superoxide dismutase (SOD2) is a nuclear-encoded antioxidant enzyme that localizes to the mitochondria and protects the mitochondrial components from superoxide created as a by-product of respiration. A single study by Namikawa et al. found a higher frequency of *SOD2* genotype 1183-TT in 63 biopsy-proven NASH patients (frequency 0.841) compared with 150 healthy controls (0.680) (178). The minor C allele results in a valine-to-alanine change that may alter the helical structure of the mitochondrial targeting sequence, enhancing transport of manganese SOD to the mitochondria (248). The increased oxidative stress could lead to greater risk of the development of NASH. The 1183 polymorphism does not influence susceptibility to alcohol-induced liver fibrosis or alcohol-induced oxidative stress (248).

## GENES AFFECTING IMMUNE REGULATION

### *STAT3*

Signal transducer and activator of transcription 3 (STAT3) is an acute-phase transcription factor. After hepatic necrosis, it activates pathways associated with liver regeneration and acute inflammation (249). STAT3 is also implicated in nutrient metabolism and the development of metabolic syndrome. Transgenic mice with hepatic deficiency of STAT3 develop insulin resistance and disturbed glucose homeostasis, whereas the constitutive liver-specific expression of STAT3 in diabetic mice reduces blood glucose and plasma insulin concentrations and downregulates gluconeogenic gene expression (250). STAT3 is activated by IL-6; in vivo treatment with IL-6 or overexpression of STAT3 ameliorates fatty liver in several mouse models of NAFLD (250, 251).

Sookoian et al. compared the frequency of three *STAT3* gene tagSNPs (rs2293152) representing 24 polymorphic sites in 108 NAFLD patients and 55 controls (252). Carriers of rs6503695-T and rs9891119-A alleles were, respectively, 2.3- and 2.5-fold more likely to have NAFLD compared with noncarriers ($P = 0.011$ and 0.005, respectively). However, no association was observed between

SNP allele frequencies and the necro-inflammatory grade or overall fibrosis score.

### *TNF*

TNF-α is a key pro-inflammatory hepatic cytokine produced by macrophages. It plays a role in the development of liver injury, including the pathogenesis of NASH (253). TNF mRNA levels are increased in the liver and peripheral adipose tissue of NASH subjects, as are circulating levels of TNF-α (35, 253).

In one of the earliest NAFLD gene association studies, *TNF* promoter polymorphisms at −238 and −308 were genotyped in 99 NAFLD patients and 172 controls in Italy (33). The prevalence of the −238 allele, but not the −308 allele, was higher in NAFLD (31% versus 15% in controls). Tokushige et al. subsequently studied *TNF* promoter polymorphisms (−1031, −863, −857, −308, and −238) in Japanese patients with simple steatosis (n = 36) and NASH (n = 66) compared with 100 healthy controls (254). No association was seen between *TNF* SNPs and the presence of NAFLD. However, −1031C and −863A were more common in patients with NASH than in patients with simple steatosis, suggesting a role for *TNF* polymorphisms in the progression of NAFLD. Levels of serum soluble TNF receptor (sTNFR)-2 were significantly higher in NASH patients than in patients with simple steatosis or control subjects. No significant association of *TNF* promoter polymorphisms −863, −308, or −238 was seen in Chinese NAFLD patients (n = 79) (204).

## OTHER GENES

### *MTHFR*

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (a cosubstrate for homocysteine remethylation to methionine). Adinolfi et al. studied the relationship among the *MTHFR* gene C677T polymorphism, homocysteinemia, and hepatic steatosis in 116 patients with chronic hepatitis C (255). The T allele was associated with elevated levels of circulating homocysteine and a greater prevalence of hepatic steatosis (79% in TT or CT versus 41% in CC, $P = 0.01$). The mechanism is thought to involve reduced MTHFR enzyme activity for the T allele, leading to increased homocysteine levels, endoplasmic reticulum stress, and increased uptake of cholesterol and triglycerides, leading to hepatic steatosis (256). In cultured human hepatocytes, homocysteine-induced endoplasmic reticulum stress activates SREBPs and is associated with increased expression of genes responsible for cholesterol/triglyceride biosynthesis and uptake (256). Consistent with these in vitro findings, mice with diet-induced hyperhomocysteinemia show accumulation of cholesterol and triglycerides in the liver (256).

### *HFE*

There have been conflicting reports whether carrying *HFE* mutations associated with hemochromatosis (H63D or C282Y) will increase a person's risk of developing hepatic steatosis. George et al. found that 31% of NASH patients were heterozygous or homozygous for C282Y and concluded that the C282Y is associated with hepatic damage in these patients, due to its association with Perls' stain grade and liver iron content (257). Although Bonkovsky et al. found a higher prevalence of heterozygosity for C282Y or H63D in 22 of 36 NASH patients (61%) compared with controls (38%, $P = 0.008$), the prevalence of C282Y alone was not higher in NASH (258). However, it was observed that there was more hepatic fibrosis in NASH C282Y carriers compared with NASH without C282Y (258). In a cohort of 93 NASH patients of predominantly northern European ancestry, 15% were heterozygous for C282Y, compared with 6.3% of healthy blood donors ($P = 0.04$); the frequency of H63D was not significantly different between patients and controls (259).

In 134 consecutive Italian NAFLD patients without a previous diagnosis of diabetes, the prevalence of C282Y was higher (18%, $P < 0.0001$) compared with population controls, and C282Y carriers developed NAFLD despite lower BMI and triglyceride levels (260). However, several other Italian studies have shown no association of *HFE* mutations with NAFLD. Loria et al. studied consecutive NAFLD patients referred on the basis of ultrasonographic evidence of "bright liver" and found that the prevalence of C282Y and H63D was not different from that in the healthy Italian population, although only 52 patients were genotyped (261). In a study of 272 Italians with NASH with BMI < 26 kg/m$^2$, the frequency of H63D or C282Y was not increased, compared with frequencies in 430 healthy controls (262). Of interest, C282Y heterozygosity was rare, composing only 0.81% of NASH and 0.79% of controls. Another negative finding was reported in a large study of 587 NAFLD patients (C282Y heterozygosity in 5.8% of patients versus 4.4% of 184 controls), which also found no association between *HFE* mutations and severity of hepatic fibrosis (263). In contrast, a North American multicentre cohort showed that C282Y heterozygotes (14.3% of patients) were more likely to have fibrosis or cirrhosis (44% versus 21% in other genotypes, $P = 0.05$) among 126 liver-biopsied Caucasian NASH patients (264). The C282Y variant is absent or rare in Asian populations, and H63D does not seem to be associated with hepatic steatosis in NAFLD patients (265, 266).

In nonobese, nonalcoholic patients with abnormal liver function tests without hemochromatosis, high serum ferritin concentration is a risk factor for hepatic steatosis (267). Bugianesi et al. noted that 21% of 263 prospectively enrolled NAFLD patients had hyperferritinemia (268). However, the C282Y and H63D mutations were not associated with iron overload, and their prevalence was similar to the general population, indicating that the increased ferritin levels are markers of liver damage and not necessarily iron overload.

### *SERPINA1*

α1-Antitrypsin is a protease inhibitor. Homozygous or compound heterozygous carriers of null (*PI**Null) or

defective (*PI**Z) alleles exhibit α1-antitrypsin deficiency, which puts patients at high risk of developing respiratory problems and cirrhosis at an early age (269). Because of the additional risk for liver disease, patients with α1-antitrypsin deficiency are often excluded from NAFLD cohort studies.

A significant finding among 353 NAFLD patients was that 10.8% carried α1-antitrypsin *SERPINA1* (*PI*) nonMM (non-wild-type) genotypes, compared with 3.5% of controls (270). Although these *PI* mutations were associated with hyperferritinemia and sinusoidal iron accumulation, they were not associated with more severe liver damage in NAFLD (270). Regev et al. showed that, in patients with liver disease, there was a disproportionately higher prevalence of *PI**Z among NAFLD patients with decompensated liver disease (5.0%) compared with NAFLD patients with less severe liver disease (1.9%) (271).

### *UGT1A1*

Bilirubin is formed from the breakdown of heme and is conjugated in the liver by UDP glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1). The common promoter polymorphism UGT1A1*28 is associated with reduced UGT1A1 activity, elevated bilirubin concentrations, and Gilbert's syndrome, whereas the UGT1A1*6 allele is within the coding region (G71R) and is associated with reduced UGT1A1 activity (272).

A recent study by Lin et al. found that variants in the *UGT1A1* gene were associated with the risk of pediatric NAFLD (273). Of 234 obese children aged between 6 and 13 years, 12% had NAFLD. *UGT1A1*6* variant alleles were found to be protective, with an estimated adjusted OR of 0.31 (95% CI, 0.11-0.91), whereas *UGT1A1*28* was not associated with NAFLD. This protective effect could be attributed to the intrahepatocytic bilirubin acting as an antioxidant in counteracting oxidative stress.

### *ABCC2*

ATP-binding cassette subfamily C member 2 (ABCC2, also known as multidrug resistance protein 2, MRP2) is an efflux pump located on the apical membrane of hepatocytes for elimination of conjugated endogenous and xenobiotic compounds into bile (274). Genetic variation in *ABCC2* may affect toxin metabolism by impairing clearance or by causing accumulation of bile acids in the liver (275). Hepatic expression of ABCC2 is reduced in obese Zucker rats, which exhibit impaired bile secretion and early cholestatic changes that occur before the development of fatty liver (276).

The association between the *ABCC2* gene and hepatic steatosis was studied in 109 consecutive NAFLD (mean age, 56 years; BMI, 36.3 kg/m$^2$; n = 80 females) and 58 healthy controls (mean age, 46 years; BMI, 25.6 kg/m$^2$; n = 40 females) in Argentina (277). Four SNPs representing 46 polymorphic sites were studied, plus an extra two linked SNPs that had been correlated with hepatic ABCC2 expression in another study (278). These two SNPs, rs17222723A/T (V1188E) and rs8187710G/A (C1515Y), showed significantly different frequencies between NAFLD and controls (OR 2.80, 1.11-7.04), and they were associated with NAFLD disease severity ($P$ = 0.003 and $P$ = 0.015, respectively) (277). The A alleles conferred protection such that carriers had almost 3-fold lower risk for NAFLD (277). The A alleles also showed a 1.5-fold increase in hepatic ABCC2 expression ($P$ = 0.006) (278).

### *AGTR1*

The angiotensin II type I receptor (AGTR1) mediates the effects of angiotensin II in the renin-angiotensin system and is a candidate gene for NAFLD (279). AGTR1 blockers inhibit fibrosis and control progression of NASH (280–282), and AGTR1 is expressed in activated hepatic stellate cells (283). Angiotensin II via production of transforming growth factor β 1 enhances liver fibrosis (284). The SNPs studied are intronic, but they may affect transcriptional activity or possibly be in linkage disequilibrium with functional coding variants. It is also possible the *AGTR1* SNPs may affect response to treatment with blockers; there is a relationship between rs5182 and therapeutic response to the angiotensin II receptor antagonist losartan in patients with cirrhosis and portal hypertension (285).

Yoneda et al. studied 167 biopsy-diagnosed NAFLD patients (106 with NASH, 61 with simple hepatic steatosis) and 435 controls (286). Twelve SNPs in the *AGTR1* gene were genotyped. One SNP, rs3772622 was associated with fatty liver (OR 1.95, 1.49-2.55) and fibrosis index. A particular haplotype block of SNPs, ATATG (rs3772633, rs2776736, rs3772630, rs3772627, and rs3772622) was protective against NAFLD, whereas the haplotype GCGCA increased susceptibility to NAFLD. Frequencies of rs3772633-G, rs3772627-C, and rs3772622-A were significantly higher in NASH than controls, but a difference in frequency between NASH and simple hepatic steatosis could not be seen, possibly relating to insufficient sample numbers.

## CONCLUSION

Hepatic steatosis affects a large proportion of the world's population. Environmental and lifestyle influences play a significant role in the development and progression of NAFLD, the main cause of hepatic steatosis. Genetic factors may also be important in determining the susceptibility to NAFLD and its progression to cirrhosis. Several inherited disorders of lipid metabolism are associated with hepatic steatosis. In addition, polymorphisms in genes affecting lipid metabolism, oxidative stress, insulin resistance, and immune regulation have been identified as predisposing factors to the development of hepatic steatosis and the development of progressive liver injury. Unfortunately, none of these association studies is conclusive. Most are limited by their inadequate study design, small sample sizes, low statistical power, and lack of validation in different ethnic populations. Moreover, only modest differences in gene frequencies between patients and controls have been reported. The independent association of PNPLA3 polymorphisms with the occurrence of fatty liver

and severity of liver injury offers an avenue of investigation into novel pathogenic mechanisms. Future genetic data may be able to assist by predicting risk of NAFLD and the risk of progression to cirrhosis, particularly in the presence of known risk factors, such as alcohol, caloric excess, and liver injury.

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