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SYSTEMATIC REVIEWS

Genetic factors that affect nonalcoholic fatty liver disease: A systematic clinical review

Tyler J Severson, Siddesh Besur, Herbert L Bonkovsky

Tyler J Severson, Herbert L Bonkovsky, Department of Gastroenterology and Hepatology, Wake Forest University NC Baptist Medical Center, Winston-Salem, NC 27157, United States

Siddesh Besur, Abdominal Transplant Program, Aurora St Luke's Medical Center, Milwaukee, WI 53215, United States

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Correspondence to: Herbert L Bonkovsky, MD, Professor, Department of Gastroenterology and Hepatology, Wake Forest University NC Baptist Medical Center, Nutrition Bldg, E-112, 1 Medical Center Blvd, Winston-Salem, NC 27157, United States. hbonkovs@wakehealth.edu
Telephone: +1-336-7137341
Fax: +1-336-7137322

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Abstract

AIM: To investigate roles of genetic polymorphisms in non-alcoholic fatty liver disease (NAFLD) onset, severity, and outcome through systematic literature review.

METHODS: The authors conducted both systematic and specific searches of PubMed through December 2015 with special emphasis on more recent data (from 2012 onward) while still drawing from more historical data for background. We identified several specific genetic polymorphisms that have been most researched and, at this time, appear to have the greatest clinical significance on NAFLD and similar hepatic diseases. These were further investigated to assess their specific effects on disease onset and progression and the mechanisms by which these effects occur.

RESULTS: We focus particularly on genetic polymorphisms of the following genes: *PNPLA3*, particularly the p. I148M variant, *TM6SF2*, particularly the p. E167K variant, and on variants in *FTO*, *LIPA*, *IFNλ4*, and iron metabolism, specifically focusing on *HFE,* and *HMOX-1*. We discuss the effect of these genetic variations and their resultant protein variants on the onset of fatty liver disease and its severity, including the effect on likelihood of progression to cirrhosis and hepatocellular carcinoma. While our principal focus is on NAFLD, we also discuss briefly effects of some of the variants on development and severity of other hepatic diseases, including hepatitis C and alcoholic liver disease. These results are briefly discussed in terms of clinical application and future potential for personalized medicine.

CONCLUSION: Polymorphisms and genetic factors of several genes contribute to NAFLD and its end results. These genes hold keys to future improvements in diagnosis and management.

Key words: Genetic polymorphisms; Non-alcoholic fatty

liver disease; Non-alcoholic steatohepatitis; PNPLA3; TM6SF2; FTO; Cirrhosis; Iron metabolism

Core tip: Non-alcoholic fatty liver disease (NAFLD) is reaching epidemic proportions not only in the United States but worldwide. Its end results can include non-alcoholic steatohepatitis, cirrhosis, liver failure, and hepatocellular carcinoma. Studies since 2008 have demonstrated and continue to uncover noteworthy genetic factors that influence NAFLD and its onset, severity, and ultimate outcome. Awareness of these genetic elements yields improved understanding of the pathology of the disease and will likely be key to individualizing effective patient therapy in the near future.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome[1-4]. Its already-high worldwide prevalence continues to grow[2,5,6]. Its pathogenesis is related to environmental, dietary, and host factors; chiefly to increasing obesity and sedentary lifestyles[7-9]. Evidence also points to genetic factors playing important roles in modulating the occurrence, severity, and long-term prognosis of NAFLD. It is important for practicing gastroenterologists to be aware of major genetic factors that influence NAFLD and its progression because they hold the key to better understanding of pathogenesis and new and better treatments. In this review, we describe and highlight the most important of these genetic influences. Table 1 summarizes the genes and variants discussed in our review.

MATERIALS AND METHODS

Search strategy and study selection

We conducted a search in PubMed to identify relevant articles published from January 1, 2012 to December 2015. The search terms *(NASH OR NAFLD) AND genetic** yielded 1,481 published references. Filtering for human studies yielded 853; also filtering for English yielded 819. Another search approach using the MeSH terms "Fatty Liver/genetics"[Mesh] for the same time frame yielded 801 published references. Filtering for human studies yielded 539; also filtering for English yielded 511.

The two searches were combined with duplicates eliminated, leaving 997 references, which were sorted by the authors according to subject relevance, leaving 111. After careful evaluation, the 45 recent references which proved most important and relevant are cited in this article.

Searches for major background and findings/publications prior to January 2012 yielded 44 additional citations. Further, each author performed independent searches based on specific keywords and search terms that did not completely intersect with the overall search. This yielded an additional 29 sources cited. Finally, 35 additional citations were incorporated with adaptation of Table 2.

RESULTS

PNPLA3

Function of PNPLA3: Patatin-like phospholipase domain-containing protein 3 (PNPLA3, also called adiponutrin) is a 481-amino acid protein expressed to greatest degree in hepatocytes[10]. It functions as both a triglyceride hydrolase (suggesting catabolic lipase activity) and acetyl-CoA-independent transacylase (suggesting anabolic lipogenic activity)[11-13].

The most commonly studied variant of PNPLA3 is rs738409, altering wild-type cytosine to guanine at nt444 (c.444C>G), which changes isoleucine to methionine at residue 148 (p. I148M). This SNP is associated with increased hepatocellular triglyceride accumulation (up to two-fold greater than wild type[14,15]) and the development of NAFLD[16].

I148M increases hepatocellular lipid retention by altering enzymatic hydrolysis of emulsified triglycerides. The long side chain of the methionine substitution at p.148 restricts substrate access to the enzyme's catalytic site[17,18] despite the functional catalytic dyad (Figures 1 and 2). The defunct I148M protein accumulates on hepatic lipid droplets, preventing other lipolytic elements from accessing the coated droplet and rendering it metabolically inaccessible[19].

I148M subjects have lower hepatic VLDL secretion than wild-type homozygotes with the same degree of steatosis. *In vitro* correlation showed a lower degree of apoB-containing lipoprotein secretion from I148M cells[20].

The I148M variant leads to lower levels of circulating adiponectin[21], associated with susceptibility to NAFLD[22]. Adiponectin has anti-inflammatory effects[23]; reduced levels may allow inflammation leading to progression from NAFLD into NASH[24]. Adiponectin may also inhibit activation of pro-fibrotic hepatic stellate cells[25].

PNPLA3 I148M as ethnic NAFLD risk factor: Many of the studies previously cited were conducted on patients of Caucasian descent. The presence of rs738409 G, however, has been shown to be strongly associated with susceptibility to NAFLD and degree of

Table 1 Genes and variants emphasized in this review

Gene name	Genetic variant	Coding DNA change	Amino acid change	Putative effect of variant
PNPLA3	rs738409	444C>G	I148M	Increased hepatocyte triglyceride content
	rs6006460	1531G>T	S453I	Lower-than-average hepatic triglyceride accumulation
TM6SF2	rs58542926	499A>G	E167K	Elevated AST/ALT, increased hepatic triglyceride levels, decreased serum cholesterol
	rs10401969	613+80A>G	Intron	Lower hepatic *TM6SF2* mRNA levels correlate with larger hepatocellular lipid droplets
LIPA	rs116928232	894G>A	E8SJM	Cholesterol ester storage disease often resulting in fibrosis→cirrhosis
IFNλ4	rs12979860	151-152G>A	Intron	Increased degree of hepatic inflammation and fibrosis
HFE	rs1800562	845G>A	C282Y	Increased hepatic iron uptake, associated with greater NAFLD risk/severity
	rs1799945	187C>G	H63D	Increased hepatic iron uptake, associated with greater NAFLD risk/severity
HMOX1	rs2071746	-413A>T	Affects promoter	Higher HMOX1 activity correlated with less frequent and less severe NAFLD
FTO	rs1421085	46-43098T>C	Affects repressor	Adipocytic phenotype shift from beige (energy-dissipating) to white (energy-storing)
GNPAT	rs11558492	1556A>G	D519G	Worsened iron overload in patients with HFE

Table 2 Variation in frequency of the common PNPLA3 polymorphism in different regions and among different ethnic groups

Descent/ethnicity	Alleles C[1]	Alleles G[2]	Genotypes C|C	Genotypes C|G	Genotypes G|G	Allele count		Genotype count		
						C allele	G allele	C|C	C|G	G|G
All (n = 2504)	73.8%	26.2%	56.9%	33.8%	9.3%	3695	1313	1424	847	233
African (n = 661)	88.2%	11.8%	78.8%	18.8%	2.4%	1166	156	521	124	16
Latin American (n = 347)	51.6%	48.4%	27.7%	47.8%	24.5%	358	336	96	166	85
Asian (n = 504)	65.0%	35.0%	44.0%	41.9%	14.1%	655	353	222	211	71
European (n = 503)	77.4%	22.6%	60.2%	34.4%	5.4%	779	227	303	173	27
Southern Asian (n = 489)	75.4%	24.6%	57.7%	35.4%	7.0%	737	241	282	173	34

[1]Allele C: Wild type; [2]Allele G: Variant rs738409, codes I148M protein.

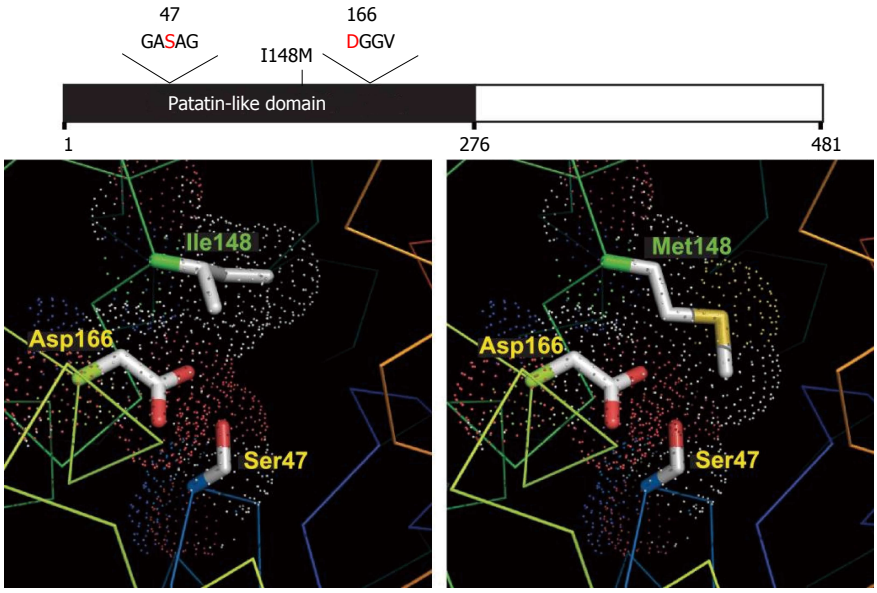

Figure 1 Structural models of wild type and mutant PNPLA3. Structural models of normal (Ile148) and mutant (Met148, associated with increased hepatic triglyceride content) PNPLA3 are shown in the left and right panels, respectively. This change effectively blocks substrate access to the catalytic dyad seen at Ser47 and Asp166. Adapted from He *et al*[8] used under Creative Commons-BY licensing.

steatosis across many ethnic groups. Several studies indicate that the rs738409 GG genotype is associated with development and progression of NAFLD in Asian cohorts, including Chinese[26,27], Japanese[28], Korean[29], and Indian[30,31] populations.

The 1000 Genomes consortium has found significant ethnic variability in the prevalence of rs738409 (Table 3)[32]. The Latin American cohort is particularly noteworthy. Persons of Hispanic descent have been found to have higher prevalence of hepatic

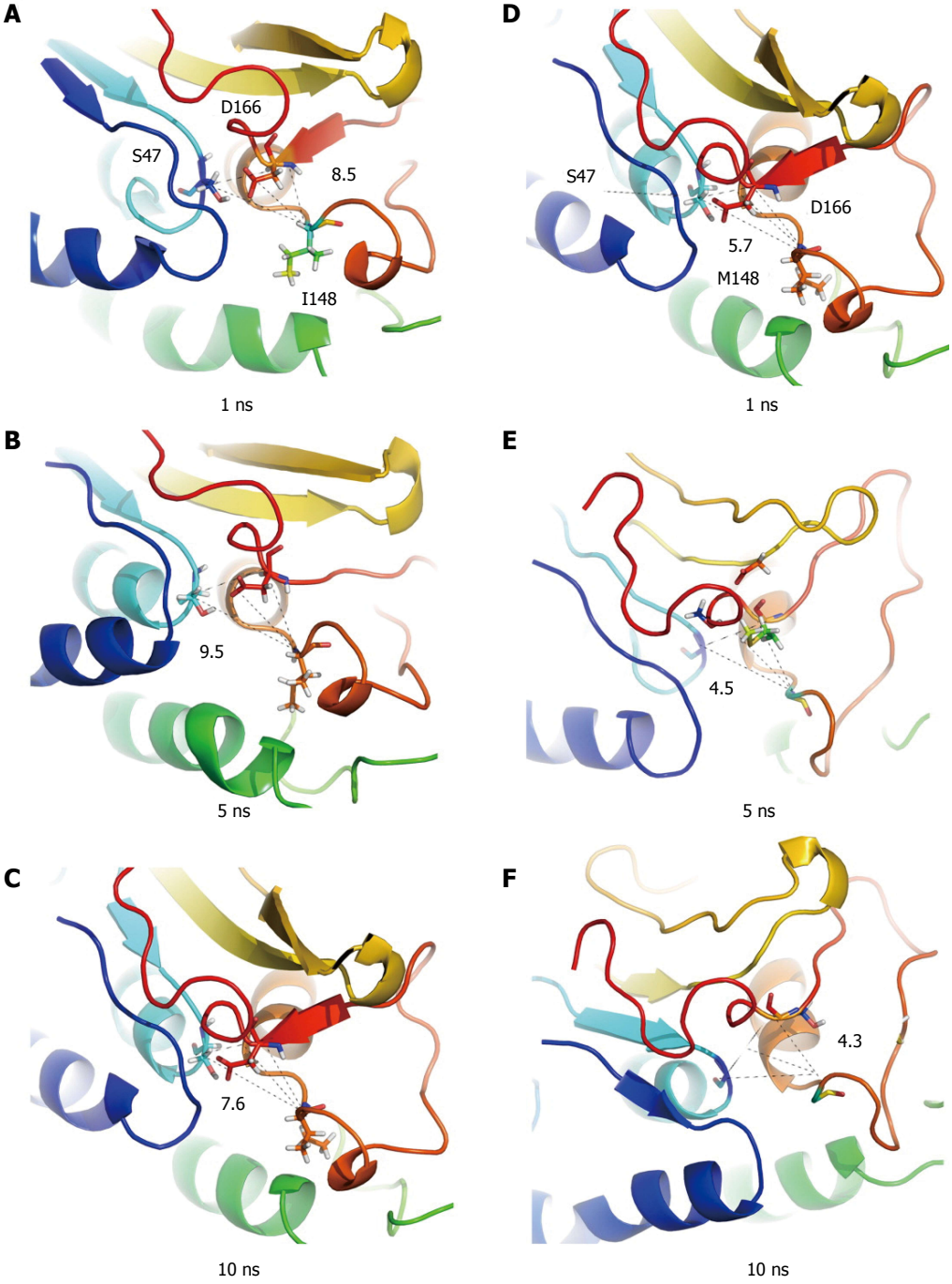

Figure 2 Structural snapshots of wild type and mutant PNPLA3 in substrate-free systems. Subplots A-C present conformations of the wild type protein at 1, 5, 10 ns, respectively, while D-F present the I148M mutant. From Xin *et al*[18] with permission of the copyright holder.

steatosis (45%) than both white (33%) and black (24%) subjects[33]. A study of cryptogenic cirrhosis (most often caused by NASH) showed that, despite similar prevalence of diabetes between patients of Hispanic and African heritages, the prevalence of cryptogenic cirrhosis in Hispanics is 3.1-fold higher than among Caucasian subjects, and the prevalence among persons of African origin was 3.9-fold lower than among Caucasians[34]. In Hispanic populations, variation in PNPLA3 was found to affect not only the degree of liver fat content[35] but also serum aminotransferase elevations[36].

Specifically among Hispanic patients, Mexican Americans studied by 1000 Genomes were found to have 34.4% of GG GG genotypes and 42.2% CG genotypes. It is unsurprising, then, that subjects of Mexican descent were recently found to have higher prevalence of NAFLD than any other group of Hispanic descent[37].

For African Americans, the rs738409 mutation has been found to contribute to the risk of increased hepatic steatosis[38]. However, another mutation of PNPLA3 found in the African American population (rs6006460, c.1531G>T, encoding p.S453I) showed

Table 3 Summary of genetic modifiers of nonalcoholic fatty liver disease

Gene	Protein	Study details and comments
Glucose metabolism and insulin resistance		
ENPP1; IRS1	Ectonucleotide pyrophosphatase/ phosphodiesterase family member 1; insulin receptor substrate 1	Functional variants promote insulin resistance by impairing insulin receptor signaling[114,115]. Carriage of nonsynonymous SNPs in *ENPP1* (rs1044498, encoding Lys121Gln) and *IRS1* (rs1801278, encoding Gln972Arg) reduced AKT activation, promoted insulin resistance, and showed independent association with greater fibrosis[116]
GCKR	Glucokinase regulatory protein	*GCKR* SNP rs780094 has been associated with hepatic TG accumulation[117] and greater NAFLD fibrosis[118]
PPARG	Peroxisome proliferator-activated receptor γ	A loss-of-function SNP (rs1805192, encoding Pro12Ala) impairs transcriptional activation and affects insulin sensitivity[119]
SLC2A1	Solute carrier family 2, facilitated glucose transporter member 1	Variants in SCLA1 are associated with NAFLD independent of insulin resistance or T2DM[120] Downregulation of SLC2A1 *in vitro* promoted lipid accumulation and increased oxidative stress, potentially linking the key pathogenic features of NAFLD: oxidative injury and increased lipid storage
Steatosis: Hepatic lipid import or synthesis		
FTO	Fat mass and obesity-associated protein	SNP rs1421085 (c.46-43098T>C) disrupts a conserved motif, which leads to de-repression of a potent preadipocyte enhancer and to a shift in phenotype from energy-dissipating beige adipocytes to energy-storing white adipocytes, with reduction in mitochondrial thermogenesis[70]
LPIN1	Phosphatidate phosphatase LPIN1	Required for adipogenesis and the normal metabolic flux between adipose tissue and liver; also acts to regulate fatty acid metabolism[121,122] Variants have been associated with multiple components of the metabolic syndrome[121,123]
SLC27A5	Very long chain acyl-CoA synthetase	Silencing *Slc27a5* reverses diet-induced NAFLD and improves hyperglycemia in mice[124] Carriage of the *SLC27A5* rs56225452 polymorphism has been associated with higher ALT and greater postprandial insulin and triglyceride levels[124] In patients with histologically proven NAFLD, the effect of BMI on degree of steatosis differed with *SLC27A5* genotype[125]
Steatosis: Hepatic lipid export or oxidation in steatosis		
APOE	Apolipoprotein E	Plasma protein involved in lipid transport and metabolism[126]. Three alleles (ε2, ε3, and ε4) determine three isoforms (ApoE2, ApoE3, and ApoE4) resulting in six ApoE genotypes (E2/2, E3/3, E4/4, E2/3, E2/4, E3/4). Overall homozygosity for the ε2 allele in one study was associated with dyslipidemia, but not NAFLD[127] In a subgroup of non-obese individuals, the ε2 allele and the E2/3 genotype were more prevalent in controls, suggesting it might be protective[127]. Consistent with this result, the E3/3 genotype was associated with NASH in a Turkish cohort, whereas E3/4 was protective[128]
LEPR	Human leptin receptor	SNP rs1805096 (c.3057G>A) may contribute to the onset of NAFLD via regulation of lipid metabolism[129]. Combination of either of LEPR SNPs rs1137100 or rs1137101 with PNPLA3 rs738409 exacerbates risk of developing NAFLD more than either of the variants on its own[130]
NR1I2	Nuclear receptor subfamily 1 group I member 2 (also known as pregnane X receptor)	NR1I2 encodes a transcription factor that regulates hepatic detoxification and acts through CD36 (fatty-acid translocase) and various lipogenic enzymes to control lipid metabolism[131] *Nr1i2*-deficient mice develop steatosis[131] Two SNPs (rs7643645 and rs2461823) were associated with NAFLD and were also a predictor of disease severity[132]
PNPLA3	Patatin-like phospholipase domain-containing 3	The nonsynonymous c.444C>G nucleotide transversion mutation SNP (rs738409, encoding p.I148M) has been consistently associated with steatosis, steatohepatitis, and hepatic fibrosis. Function remains incompletely understood[39,42]
PPARα	Peroxisome proliferator-activated receptor α	PPAR-α is a molecular sensor for long chain fatty acids, eicosanoids, and fibrates[133]; activated by increased hepatocyte fatty-acid load, it limits TAG accumulation by increasing fatty acid oxidation Carriage of a non-synonymous SNP (rs1800234, encoding p. V227A) increases activity, and was associated with NAFLD despite reduced BMI[134,135] A loss-of-function polymorphism (rs1800206, encoding p. L162V) was not associated with NAFLD[136]
TM6SF2	Transmembrane 6 super family 2	The *TM6SF2* rs58542926 minor allele is associated with greater steatosis, steatohepatitis, and NAFLD fibrosis. The major allele is associated with dyslipidemia and greater CVD risk[61,66,68,69]
Steatohepatitis: Oxidative stress		
ABCC2	ATP-binding cassette, subfamily C (CFTR/MRP), member 2	Association studies support a role for *ABCC2* (also known as *MRP2*), which facilitates terminal excretion and detoxification of endogenous and xenobiotic organic anions, including lipid peroxidation products[137]

GCLC; GCLM	Glutamate-cysteine ligase catalytic unit; glutamate-cysteine ligase regulatory unit	Glutamate-cysteine ligase is the rate-controlling step in glutathione synthesis; absence of the *Gclc* gene causes steatosis and liver failure in mice[138] A study of 131 patients with NFLD reported the *GCLC* promoter region polymorphism (c. c-129t, rs17883901) was associated with steatohepatitis compared with simple steatosis[139]
HFE	Hereditary hemochromatosis protein	Hepatic iron accumulation promotes oxidative stress. Two studies, examining 177 patients, reported carriage of an HFE polymorphism (rs1800562) that was associated with more severe steatohepatitis and advanced fibrosis[95,140] However, three other studies have not shown increased carriage of either the C282Y or H63D (rs1799945) mutations[105-107]. Meta-analysis have also provided conflicting results[108,109]
SOD2	Superoxide dismutase [Mn], mitochondrial	Carriage of the nonsynonymous SNP rs4880 has been associated with advanced hepatic fibrosis in NAFLD in both Japanese[141] and European[142] cohorts
Endotoxin response		
CD14	Monocyte differentiation antigen CD14	A lipopolysaccharide receptor expressed on monocytes, macrophages, and neutrophils that enhances TLR4 endotoxin signaling. An association with promoter-region polymorphism rs2569190 increasing CD14 expression has been reported[143]
TLR4	Toll-like receptor 4	Study of a spontaneous *Tlr4* null mutation in C3H/J mice has established the contribution of TLR4/endotoxin to NAFLD pathogenesis in the laboratory[144] *TLR4* polymorphisms rs4986791 and rs4986790 influence hepatitis-C-related fibrosis[145,146], but no association with NAFLD and *TLR4* variants has been found
Cytokines		
IFNλ4	Interferon lambda 4	The intronic rs12979860 SNP in IFNλ4 is a strong predictor of fibrosis in an etiology-independent manner, including a cohort of 488 NAFLD cases. Those with rs12979860 cc had greater hepatic inflammation and fibrosis[85]
TNF	Tumor necrosis factor	A promoter polymorphism (c.238G>A) has been associated with NASH[147,148] suggesting a primary role in the transition from steatosis to steatohepatitis. A separate study found that two other promoter region polymorphisms (rs1799964 and rs1800630) were more common in NAFLD than a control population[148]
Fibrosis		
AGTR1	Type-1 angiotensin Ⅱ receptor	Studies link SNP rs3772622 with grade of steatohepatitis and stage of fibrosis; the most recent study also suggests an interaction with PNPLA3 genotype[149,150]
KLF6	Kruppel-like factor 6	SNP rs3750861 has been associated with milder NAFLD-related hepatic fibrosis in three separate European cohorts[151]
MERTK	Myeloid epithelial reproductive tyrosine kinase	Homozygosity for common non-coding rs4374383 G>A polymorphism associated with less fibrosis in hepatitis C and NAFLD. Mechanism suggested is modulation of HSC activation[152]

Adapted from Anstee and Day[153] with permission of copyright holder.

association with lower-than-average hepatic fat content[39]. This gene has a minor allele frequency of 10.4% in African American patients, but only 0.3% in Caucasians and 0.8% in Hispanics.

Effect on severity of disease

Fibrosis: Fibrosis is increased in I148M subjects[40,41], possibly resulting from increased inflammation due to increased hepatic steatosis. It has been suggested that there may also be a directly pathogenic mechanism to the variant[42], perhaps *via* fibrogenesis, as I148M is associated with increased fibrosis independent of its effect on hepatocyte lipid content[43].

Cirrhosis: Presence of c.444C>G (both homozygous GG and heterozygous CG) was associated with significantly increased risk of cirrhosis when compared to wild type[44], regardless of etiology.

Hepatocellular carcinoma: Occurrence of HCC is more common in homozygous I148M patients than in wild type patients (OR = 1.76)[45-47]. I148M patients have more than double the risk for Hepatocellular carcinoma (HCC) (adjusted OR = 2.26) for each variant allele[48]. It is unknown if the mutation is directly oncogenic.

Role in progression of other hepatic diseases: Although beyond the scope of this review, it is worthy of mention that I148M is positively correlated with increased susceptibility, progression, and/or severity in alcoholic liver disease[49-51], chronic hepatitis C[52-55], chronic hepatitis B[56,57], hemochromatosis[58], and Wilson disease[59]. So broad is the effect of I148M on hepatic disease, it has been elsewhere suggested as the defining criterion of so-called PNPLA3-associated steatohepatitis, or "PASH"[60].

Transmembrane 6 superfamily 2

Transmembrane 6 Superfamily 2 (TM6SF2), also known as KIAA 1926, is a protein of unknown function with 377 amino acids and molecular mass of 42.6 kDa. The chromosomal location of the *TM6SF2* gene in humans is 19p13.11. It has broad tissue and organ expression with highest relative levels of expression in the small intestine and liver[61-63].

TM6SF2 as NAFLD risk

One variant in *TM6SF2* (rs10401969, c.613+80A>G) is associated with reduced hepatic mRNA levels of

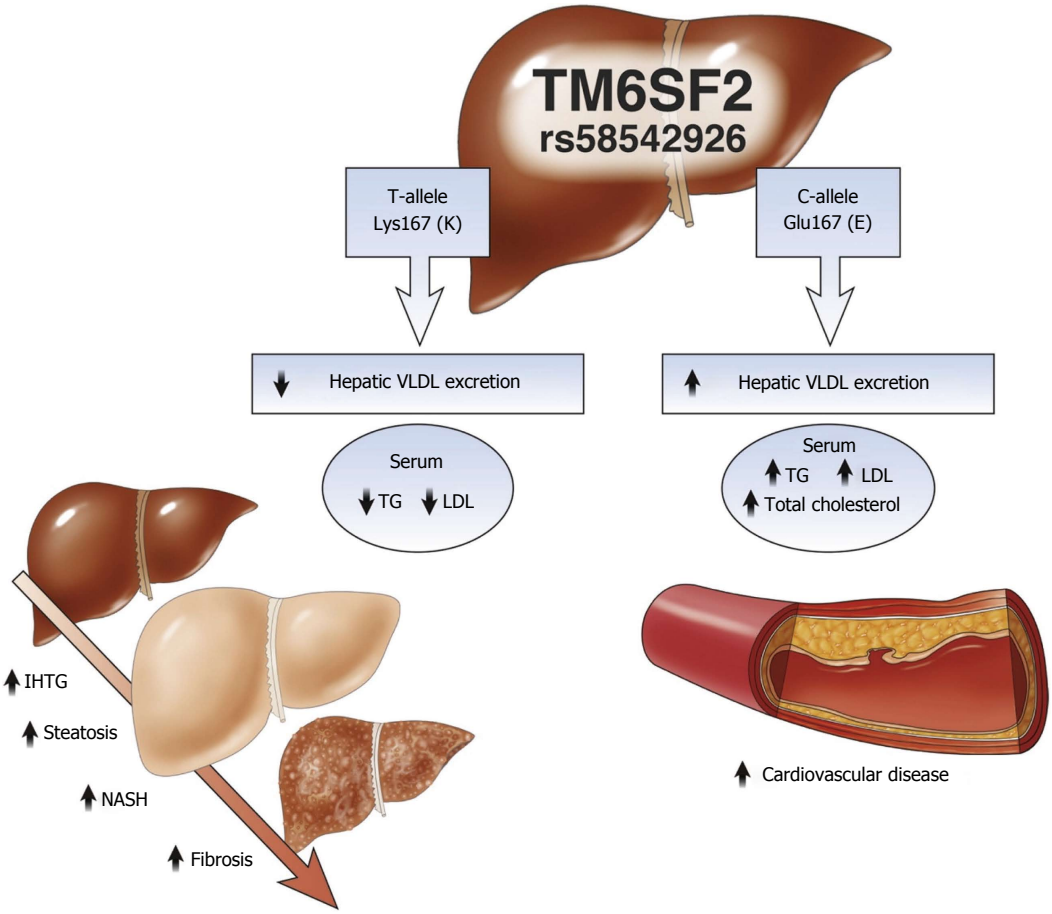

Figure 3 Effects of TM6SF2 genetic variations. TM6SF2 plays a role in VLDL export from liver to serum which results in increased serum lipids and myocardial infarction, and decreased risk of liver steatosis. From Kahali *et al*[67], used by permission of the copyright holder. Chol: Cholesterol; LDL: Low-density lipoprotein cholesterol; IHTG: Intrahepatic triglyceride; NASH: Nonalcoholic steatohepatitis; TG: Triglyceride; VLDL: Very low-density lipoprotein.

TM6SF2[64]. Decreased levels correlated with altered expression of multiple genes involved in triglyceride synthesis (*ACSS2, DGAT1, DGAT2*, and *PNPLA3*) and with increased size and number of hepatocytic lipid droplets, but with no effect on cell damage and proliferation.

Murine hepatocyte-specific silencing of *Tm6sf2* resulted in decreased levels of plasma triglycerides, LDL, HDL, and triglyceride content of VLDL with a threefold increase in hepatic triglyceride levels. Overexpression of the gene, on the other hand, was associated with a reduction in the number and size of the hepatocytic lipid droplets.

Another *TM6SF2* SNP (rs58542926, c.499G>A), changes glutamic acid to lysine amino acid at protein residue 167 (p. E167K). Presence of this variant was positively associated with associated with elevations in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT)[61] and with the development of NASH compared to wild type patients[65]. It was also associated with lower levels of plasma triglyceride and cholesterol. These were concomitant with increases in the hepatic triglyceride levels[66]. Impaired TM6SF2, then, increases the likelihood of NAFLD development while decreasing the likelihood of hypertriglyceridemia and vascular diseases associated with cardiovascular disease, making variation in TM6SF2 a two-edged sword (Figure 3)[67,68].

Effect of TM6SF2 on disease

TM6SF2 rs58542926 variant was strongly associated with NAFLD, advanced fibrosis, and cirrhosis[69], independent of age, body mass index (BMI), type 2 diabetes mellitus and *PNPLA3* rs738409 genotype. It remains unclear if the minor allele is associated with increased risk of HCC.

Fat mass and obesity-related gene

The fat mass and obesity-associated gene (*FTO*) encodes a nuclear protein of 506 amino acids with molecular mass 58.3 kDa that functions as a Fe^{2+}-containing and requiring oxygenase that repairs alkyl DNA and RNA by carrying out oxidative demethylations, especially of N(6) methyladenosine residues on RNA, the most prevalent internal modification of mRNA in higher eukaryotes.

A recent landmark study[70] showed that the single nucleotide variant rs1421085 (c.46-43098T>C) of the *FTO* gene disrupts a conserved motif that is essential for expression of the repressor AT-rich interactive

domain 5B (ARID5B), which, in turn, leads to de-repression of a potent preadipocyte enhancer and to doubling of Iroquois homeobox 3 and 5 (IRX3 and IRX 5) expression during early adipocyte differentiation. This shifts the adipocyte phenotype from energy-dissipating beige to energy-storing white, with a five-fold reduction in mitochondrial thermogenesis.

Down-regulation of *Irx3* in murine adipose tissue reduced body weight and increased heat production without changes in appetite or exercise. Repair of the ARID5B motif of rs1421085 in primary adipocytes from a patient with the C [risk] allele activated gene expression profiles of brown fat and increased thermogenesis seven-fold.

Thus, this single gain-of-function variant in a non-coding region of *FTO* plays a dominant role in BMI set point and possibly in NAFLD and NASH as well. It can be hoped that pharmacologic or other approaches, such as gene editing to restore activity of ARID5B and/or to down-regulate IRX3 and IRX5, will prove to have pronounced anti-obesity and anti-NAFLD/NASH effects.

LIPA gene (lipase A, lysosomal acid, cholesterol esterase)

The lysosomal acid lipase A gene (*LIPA*) is located on human chromosome 10q23.31[71,72]. *LIPA* produces and regulates lysosomal acid lipase (LIPA), also known as cholesterol ester hydrolase. LIPA contains 399 amino acids and has molecular mass of 45.4 kDa. It catalyzes lysosomal hydrolysis of cholesteryl esters and triglycerides, which plays a pivotal role in the intracellular regulation of the endogenous cholesterol synthesis, uptake of low density lipoproteins (LDL) and cholesterol esterification[73,74].

At least six splice variants of *LIPA* have been described. Some mutations lead to reduced or absent production of the LIPA enzyme, yielding increased cholesterol ester storage in the lysosomes. Defective *LIPA* gene inherited as autosomal recessive disorder is clinically known as Wolman's disease (fatal in infancy)[75,76] and cholesterol ester storage disease (CESD, presenting later in life with dyslipidemia[77,78], premature atherosclerosis[79], and cirrhosis[80]). The majority of mutations (42%) are due to deletions/insertions; the remainder are splice-site and missense mutations[81]. The most common mutation is a splice-site at the exon 8, E8SJM (rs116928232, c.894G>A).

Fibrosis leading to cirrhosis and its complications is seen in two-thirds of patients with LIPA deficiency[82]. Of LIPA enzyme deficiency patients[80], 64% had fibrosis and/or cirrhosis, with cirrhosis present in 29% of patients. *LIPA* mutations have not been associated with increased risk of HCC.

Interferon λ 4 gene

IFN Interferon λ 4 gene (IFNλ4)4 codes for a cytokine product thought to trigger antiviral responses, especially to HCV, by activating the JAK-STAT pathway and up-regulating selected interferon-responsive genes. The gene is widely expressed in nearly all tissues. SNPs rs12979860 and rs8099917 are located within intron 1 region of the *IFNλ4* gene on chromosome 19q13.2. These polymorphisms control the inflammatory and immune response pathways[83,84] which form the basis for the interferon-based treatment of HCV.

A recent study involving 4,172 patients with liver disease (chronic HCV, chronic HBV, and NAFLD) found that patients with rs12979860 have greater hepatic inflammation and fibrosis[85]. The exact mechanism for this is unclear. It is thought that NAFLD leads to higher basal interferon stimulated genes, leading to immune activation and cell death.

Genes and proteins of iron and heme metabolism

Hepatic iron toxicity is chiefly related to the role of iron in catalyzing oxidation reactions with formation of the highly reactive and toxic hydroxyl free radical[86]. Insertion of iron into protoporphyrin forms heme, which is also highly reactive and capable of increasing oxidative stress[87]. Thus, genetic variations in genes and proteins involved in iron and heme metabolism may influence NAFLD/NASH, as well as other liver diseases[74-80].

Heavy iron overload, such as occurs in hemochromatosis, is known to lead to hepatic fibrosis, cirrhosis and HCC[88]. Modest amounts of hepatic iron - which of themselves would not produce toxicity - can enhance or synergize hepatotoxicity in chronic viral hepatitis and/or alcoholic and NAFLD[89-92]. Increased levels of serum ferritin are associated with higher severity and stage of fibrosis in NAFLD[93] and with all-cause mortality and with morbidity and mortality[94].

The major (C282Y) and minor (H63D) mutations of *HFE* are risk factors for NAFLD and for more severe disease[95-97]. The most important additional modulating factors are chronic HCV infection and heavy alcohol use. However, other genetic factors, such as genetic variation in one or more of the many other genes involved in iron metabolism (*e.g., BMP2*, *FPN*, *FTL*, *HAMP*, *HJV* and others[98,99]) also play a role. Recently, a genetic variation in *GNPAT* (rs11558492, c. 1556A>G, exon 11; chromosome 1q42; p.D519G) was reported to be significantly associated with more severe iron overload in male subjects homozygous for C282Y, the major mutation of *HFE*[100]. The mechanism for the effect is suggested to relate to an effect of deficient GNPAT to down-regulate hepcidin production.

The above observations led to the idea that iron reduction accomplished by therapeutic phlebotomies might be of benefit in the metabolic syndrome, diabetes mellitus, and NAFLD. Several studies have shown that phlebotomies to near iron-depletion (serum ferritin levels about 25 ng/mL), but short of anemia, lead to improvements in insulin sensitivity and glucose tolerance[101,102], and that chronically sustained iron

reduction leads to lower serum ALT/AST, less necro-inflammation, and less fibrosis[103]. A recently published exception is that of Adams *et al*[104], although the trend of data in this paper also favored the iron reduction cohort. Lesser effectiveness in this work may relate to the shorter duration of study (6 *vs* 18-36 mo); longer duration of iron reduction therapy is probably important for study endpoints such as progression of hepatic fibrosis/cirrhosis and development of HCC.

Other studies did not show increased frequency of carriage of *HFE* mutations in patients with NAFLD than in controls[105-107]. Meta-analyses have also yielded divergent results[108,109]. Thus, the role of *HFE* mutations in modulating NAFLD is not entirely settled. Some of the reason for this may be that other genetic, dietary, and environmental influences, in addition to *HFE*, materially affect iron loading in the liver and probably also in other tissues.

Heme oxygenases (HMOX1, HMOX2) are key cyto-protective enzymes, protecting the liver and other organs from oxidative stress caused by excess heme, potentially a stronger pro-oxidant than iron[87]. HMOX1 is especially important, as it is highly inducible by heme, heavy metals, oxidative stress, and other forms of chemical or physical stress.

Levels of expression of the *HMOX1* gene are also under genetic control in two major ways: the variable length of GT repeats in the promoter and a functional SNP at position -413 upstream of the transcription starting point (c.-413A>T; rs2071746). Shorter GTn repeats [18-22 nts] and -413A are associated with higher levels of *HMOX1* gene expression and higher HMOX1 activities. Higher levels of expression of HMOX1 have been correlated with less frequent and less severe NAFLD/NASH in rodents and humans[110-112].

Results on balance indicate that even modest increases in iron or heme are potentially hepatotoxic, especially in the presence of chronic hepatitis C or the metabolic syndrome. Until more effective treatments become available for NAFLD, iron reduction remains a safe and reasonable therapeutic modality, recent suggestions to the contrary notwithstanding[113].

COMMENTS

Background

The worldwide prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing rapidly, related to multiple causes, some better understood than others. Delving into the genetic underpinnings of NAFLD development and severity is helping us not only to understand better genetic risk factors of NAFLD, but also, by assessing the effects of the genes and proteins involved, we learn more about the pathogenesis and management of the disease itself. The primary aim of this review is to discuss the known major genetic factors that influence NAFLD and to improve awareness and understanding of these factors among physicians and other healthcare providers.

Research frontiers

The prevalence of NAFLD is likely to continue to increase with the worldwide expansion of "Western diets" and sedentary lifestyles *pari passu* with trends toward more frequent and more severe obesity. The number of genetic polymorphisms that predispose a patient to NAFLD or worsen an affected patient's prognosis, however, is also likely to continue expanding. Indeed, we continue to identify additional genetic factors and associations with NAFLD and the metabolic syndrome. It is likely that this will, at some point in the near future, allow us to predict, warn about, and ideally prevent disease before it occurs.

Innovations and breakthroughs

As the author develop a better understanding of the genetic underpinnings of fatty liver disease and its progression, they will likely gain insight not only into the origins and physiological basis of this problem, but also into how they can better combat it. They foresee in the near future development and validation of a panel of genetic tests that will identify subjects at higher or lower risk of development and progression of NAFLD and that will identify subjects for therapies targeted specifically to specific patient genotypes. Regardless of favorable or adverse genetic factors, however, for the foreseeable future the author will need to continue counseling all their patients about the benefits of exercise and sensible diets, consumed in moderation.

Applications

There have already been therapeutic trials of iron reduction for therapy of NAFLD/NASH; such therapy is likely to be more necessary and effective in subjects with mutations in *HFE, GNPAT*, and other genes that tend to increase hepatic iron levels. Genetic testing for the variants discussed above and others yet to be discovered may ultimately be used to assess individual risk of hepatic disease and may direct early detection and prophylactic treatment in patients at risk. Similarly, although less studied thus far, genetic variations in *PNPLA3* and/or *TM6SF2* may be expected to influence efficacy of other therapies and allow for greater individualization of therapy. It will be of increasing value and importance going forward to know and to take into account host genotypes in both observational and interventional studies in NAFLD/NASH.

Terminology

Non-alcoholic fatty liver disease, or NAFLD, is the most common form of chronic liver disease in the United States and continues to increase in prevalence around the world. It is caused by increased intrahepatic accumulation of fatty deposition in the liver (steatosis) and can progress from a largely benign condition to inflammatory hepatitis (NASH), to cirrhosis and beyond. NAFLD is now usually diagnosed based upon history, physical examination, and hepatic imaging. Diagnosis of NASH requires liver biopsy; staging of severity of fibrosis is being done with increasing frequency by assessment of hepatic stiffness by elastography, although liver biopsy remains the gold standard.

Peer-review

This article is informative and presented in a systematic way. Well written and will be of use to the readership.

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