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The Rise in the Prevalence of Nonalcoholic Fatty Liver Disease and Hepatocellular Carcinoma

Zaki A. Sherif

Abstract

Nonalcoholic fatty liver disease (NAFLD) affects a third of the world's population and its rapid rise parallels the increase in hepatocellular carcinoma (HCC). NAFLD replacing hepatitis C virus (HCV) infection as a leading indicator for liver transplantation (LT) in the United States. NAFLD is a spectrum of disease ranging from simple steatosis (NAFL) to nonalcoholic steatohepatitis (NASH), which can progress to advanced fibrosis (AF) and cirrhosis, culminating in HCC. The main clinical concern of public health administrators is that many patients who are unaware of NAFLD remain undiagnosed and risk developing end-stage liver disease (ESLD). Clinicians overly rely on surrogate liver enzymes to identify patients with NAFLD, allowing for substantial liver disease to go unnoticed and untreated. Furthermore, according to epidemiological studies, in patients diagnosed with NAFLD, ethnicity plays a role in complications and treatment response, and ethnic correlations with NAFLD are thoroughly underreported. Although liver biopsy is the gold standard method for appropriately diagnosing and staging NAFLD, most patients can be effectively diagnosed non-invasively with imaging modalities and integrated tests that are routinely available in the clinic today. This chapter discusses the current global rise in the rates of NAFLD and HCC; the current key findings incidences and the recommended diagnostic approaches and in therapeutic methods.

Keywords: obesity, cirrhosis, hepatocellular carcinoma (HCC), insulin resistance (IR), liver transplantation (LT), metabolic syndrome (MetS), nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH)

1. Introduction

The liver is a 1.5 kg reddish-brown biochemical processing plant of immense responsibilities that include protein synthesis, xenobiotic or drug metabolism, blood detoxification, and the release of bile acids for digestion. In short, the liver plays a key role in the hemostasis of the body by regulating the levels of sugar, protein, and fat that circulate in the blood. However, obesity, which must be carefully defined according to ethnic-specific BMI cut-off points, may alter normal liver physiology and lead to liver disease [1]. Obesity is at the intersection of the chronic liver disease pathway that includes diabetes, metabolic syndrome (MetS), nonalcoholic fatty liver disease (NAFLD), and hepatocellular carcinoma (HCC). The complex association between obesity and liver function involving NAFLD, HCC, histopathology, and genetic factors is the subject of several collaborative research investigations [2–7].

Over the past few decades, dramatic changes in lifestyle behaviors and health priorities have contributed to a significant rise in noncommunicable diseases such as obesity and NAFLD. Obesity is highly prevalent in the United States of America, estimated to represent between 30 and 38% of adults with a body mass index (BMI) greater than 30 kg/m² [8, 9]. Obesity is also a risk factor for metabolic syndrome (MetS), which increases hepatic triglyceride (TGs) depositions. NAFLD is the most common cause of impaired liver function in Western countries, affecting over one quarter of the population [10, 11]. Obesity is driving the rise of NAFLD and nonalcoholic steatohepatitis (NASH), the culmination of the fatty liver disease spectrum that is manifested by ballooning, scarring, cirrhosis, and finally liver failure and HCC [12]. It is estimated that globally the prevalence of NAFLD in the general population is 24–30% [13, 14]. Accounting for errors in accuracy that may exist in indirect measurement methodologies, in the United States, the prevalence of NAFLD in adults has risen from 18% in 1988–1991, to 29% in 1999–2000, to 31% in 2011–2012 [15]. However, the prevalence of NAFLD in the United States diagnosed by ultrasonography alone was estimated to be 24.13% (95% CI 19.73–29.15%) [16].

1.1 NAFLD definition

Nonalcoholic fatty liver disease (NAFLD) is a broad-spectrum disease ranging from fat infiltration of hepatocytes with no symptoms (simple steatosis aka nonalcoholic fatty liver, NAFL) to excess intrahepatic macroglobular and macrovesicular fat accumulation (5–10% by weight of liver) with aggravated inflammation (steatohepatitis aka nonalcoholic steatohepatitis, NASH) in the presence of little ethanol (typically <30 g per day for men and <20 g per day for women) or no alcohol consumption in the last 12 months [12, 17]. It should be noted, however, there is now convincing evidence demonstrating that even “safe” levels of alcohol consumption are associated with adverse health outcomes [17–20] suggesting that future studies should include only nondrinker individuals in the “NAFLD definition” [21]. Therefore, for NAFLD classification, the patient must show evidence of hepatic fat accumulation in the absence of declared chronic alcohol consumption, or drug use that can induce steatosis, or hereditary disorders. This NAFLD designation excludes both macrovesicular and microvesicular steatosis encompassing certain drugs, toxins, viral hepatitis B (HBV), hepatitis C (HCV) or human immunodeficiency virus (HIV) infections, celiac disease, α -1 antitrypsin deficiency, hepatobiliary infectious diseases, hepatolenticular degeneration, hepatic malignancies, genetic hemochromatosis, Wilson’s disease, lipodystrophy, abetalipoproteinemia, Reye’s syndrome, HELLP syndrome, or decompensated cirrhosis, which may contribute to secondary causes of steatosis and elevated liver enzymes [22–24]. Additional medications targeted for exclusion are estrogen, sodium valproate, nonsteroidal anti-inflammatory drugs (NSAIDs), calcium antagonists, perhexiline-maleate, and antiretroviral drugs [25–27]. Appropriate medical history must also be taken to exclude the uncommon causes of fatty liver secondary to treatment with drugs such as amiodarone, diltiazem, steroids, synthetic estrogens, tamoxifen, and highly active antiretroviral therapy; refeeding syndrome and total parenteral nutrition; severe weight loss after jejunioileal or gastric bypass; lipodystrophy; and other rare disorders [28]. There are also strong opinions for the exclusion of “whole-body system diseases” such as inflammatory bowel syndrome, hypothyroidism, and lipoatrophy [25] from the “secondary fatty liver diseases” category because they may also induce liver steatosis.

NAFLD can be distinguished from alcoholic steatohepatitis (ASH) by the absence of alcohol consumption and on histological markers such as sclerosing hyaline necrosis, hepatocyte ballooning, portal granulocytic inflammation, lobular

inflammation, satellitosis, perisinusoidal fibrosis, Mallory-Denk bodies, and acute cholestasis among others [29–31]. However, it is important to note that NAFLD can also coexist with other liver diseases including HCV, hemochromatosis, and alcoholic liver disease, which can accelerate progression to end-stage liver disease (ESLD) [32].

1.2 The natural history of NAFLD

The pathophysiology of NAFLD and its variants is still incompletely understood thereby limiting the availability of effective diagnostic and therapeutic intervention. The ongoing persistence of obesity and the accompanying high rates of diabetes will increase the prevalence of NAFLD [33]. In many cases, the natural cause of the disease is the development of cirrhosis and ESLD as the population ages. Increased mortality rates have been reported in studies that compared NAFLD patients with a normal reference population [34–36]. The primary cause of death for NAFLD patients is cardiovascular disease followed by nonliver cancer, whereas the third leading cause of mortality is liver-related complications including cirrhosis [33]. The exact prevalence of fatty liver condition is not known, but population studies from the United States and China estimate that 28–30% of the general population has simple steatosis that carries a relatively benign prognosis and is measured using magnetic resonance spectroscopy (the most accurate imaging modality) and that 8% of the population has elevated alanine transaminase (ALT) [37, 38]. A follow-up of population-based studies examining the natural history of NAFLD patients in Minnesota revealed that 3.1% of the patients developed cirrhosis-related complications including ascites (2%), jaundice (2%), encephalopathy (2%), variceal bleeding (1%), and HCC (0.5%) [34]. Approximately 10–30% of those with steatosis develop NASH, and the development of NASH cirrhosis is associated with a poor long-term prognosis for 2.6% of them who will be at a risk of developing HCC [39–41]. Ten years following diagnosis, 45% will decompensate and the mortality rate for subjects with Child-Pugh A disease will be 20% [42]. Furthermore, besides having an increased liver-related mortality rate compared to the general population, patients with NASH also have an increased risk of cardiovascular death (15.5 vs. 7.5%, $p = 0.04$) [35]. Generally, NAFLD is a slowly progressing disease, which does not culminate in ESLD in most patients. Identifying those who will develop a complete liver failure is a difficult proposition [43]. NAFLD data are limited on predictors of clinical progression to NASH and beyond. Due to the compounding effect of obesity, prospective longitudinal studies are needed to help in the prediction of outcomes for individual patients. On the other hand, patients with NASH have a worse prognosis and attempts should be made to include them in clinical trials of novel treatments for this condition. The sequence of steps in liver disease commencing with steatosis and eventually culminating in HCC (i.e., ESLD) is presented in **Figure 1** [44].

1.3 NAFLD diagnosis and staging

The general classification of NAFLD as stated above and accepted by the American Association for the Study of Liver Diseases (AASLD) is a hepatic fat accumulation exceeding 5–10% by weight of the liver [45]. Accordingly, NAFLD diagnosis in the liver is based on: (i) the presence of simple steatosis, as determined by histological or imaging procedure; (ii) a total weekly consumption of less than 140 g of ethanol for men and less than 70 g for women in the last 12 months; and (iii) the absence of competing etiologies for simple liver steatosis and the absence of coexisting causes for chronic liver disease [46]. An appropriate diagnosis of

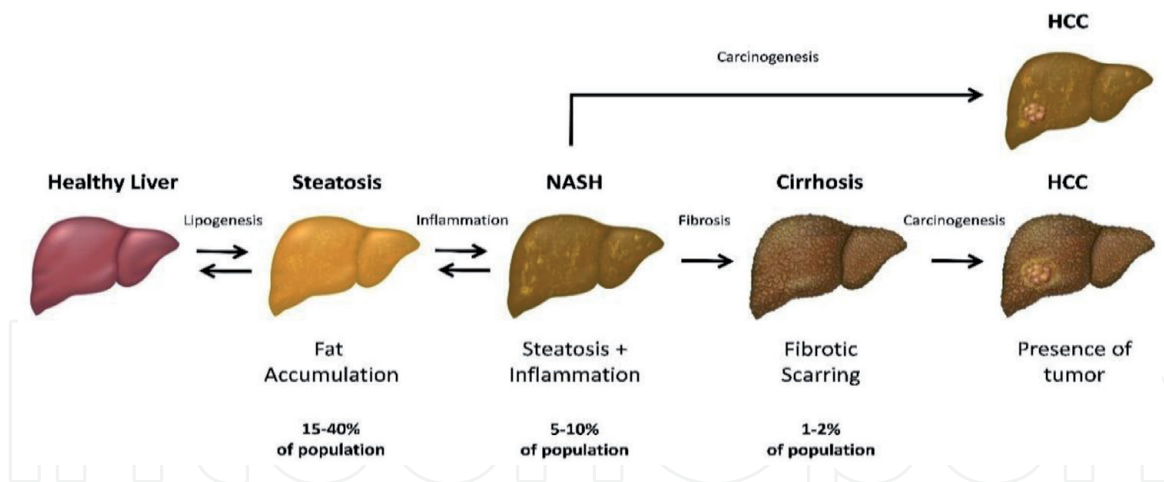


Figure 1.

The progression and stages of NAFLD (adapted from Baranova et al., [44]). Steatosis is the initial NAFLD stage and is characterized by excessive accumulation of fat in hepatocytes. Subsequent inflammatory conditions accelerate the progression to NASH followed by liver cirrhosis, which may lead to HCC. Both steatosis and NASH can reverse to NAFLD.

NAFLD, which is multifaceted, requires that there is evidence of hepatic steatosis upon imaging and histology or both and that other causes of liver disease including steatosis have been excluded [23].

The increasing prevalence of obesity in the past few decades has led to a surge in NAFLD, which manifests liver cells as bloated with droplets of fat. It has been reported that 70% of centrally obese patients with diabetes and hypertension (HTN) harbor steatohepatitis on liver biopsy [47]. Imaging has enabled the observation of central obesity in 70–80% of these subjects and in 50–80% of patients with type 2 diabetes mellitus (T2DM). NAFLD is typically asymptomatic; therefore, diagnosis usually follows the subsidiary finding of abnormal liver enzymes or steatosis on imaging. Early diagnosis of NAFLD requires skilled and informed practitioners to halt fibrosis progression to more advanced stages. Liver needle puncture biopsy, although invasive, is the gold standard. Less-invasive methods of image detection tools may not provide consistent information due to the subjective interpretations of the data by radiologists [48]. But imaging tools such as abdominal ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI) are beginning to meet this need. Ultrasound or sonography is very effective in diagnosing steatosis where greater than 33% of hepatocytes are steatotic but can be unreliable with lesser degrees of steatosis [49]. The other imaging modalities such as CT or MRI can also detect hepatic steatosis even though they are not used in the evaluation of steatosis. Currently, the combination of MRI and proton magnetic resonance spectroscopy (MRI/¹H-MRS) is the most accurate noninvasive measuring tool of steatosis [50, 51]. ¹H-MRS, which defines NAFLD as hepatic fat accumulation (steatosis) >5% of total weight of the liver, is the most reliable quantitative tool. However, due to its prohibitive cost, it is not widely available. Ultrasonography, on the other hand, is the instrument of choice for most of the clinics due to its low cost and wide availability even though it is still relatively limited in the detection of inflammation, a more important and higher risk concern than steatosis for fibrosis, cirrhosis, and HCC [52, 53].

Controlled attenuation parameter (CAP), which is a novel ultrasound-based technique that assesses liver stiffness and steatosis simultaneously by employing transient elastography (TE) [54]. This CAP technique has been shown to accurately detect steatosis although its diagnostic threshold has not yet been determined. Obesity and diabetes are the main risk factors for NAFLD [55]. It has been reported that the presence of T2DM significantly increases the prevalence

of NAFLD regardless of the diagnostic tool [56]. For example, using controlled attenuation parameter (CAP), the prevalence of NAFLD is estimated at 75% in T2DM population and 40% in the general population, whereas it is 65% and about 37% respectively when measured by ¹H-MRS. The prevalence rate goes down when assessed by liver ultrasound, computed tomography, and plasma ALT in that order [56].

In contrast, most global population studies base their NAFLD characterization on less sensitive and less specific surrogate markers of the disease including elevated liver-associated enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT >40 IU/L in males; >31 IU/L in females) [57, 58]. Furthermore, serum ALT levels are within the range currently considered “normal” in a sizeable proportion of NAFLD subjects [59]. Typically, depending on the reference values from different laboratories, the broad range for normal AST is reported between 10 and 40 IU/L and ALT between 7 and 56 IU/L. This is because ALT usually falls (and AST may rise) as fibrosis progresses to cirrhosis. Mild elevations, which are generally asymptomatic, are considered to be 2–3 times higher than the normal range, and drastic elevations are 5 times higher than the upper limit of normal, which varies according to gender [60]. Moreover, the very selective measurement of ALT level based on race or ethnicity underscores the lack of effective surrogate markers for NAFLD/NASH in the absence of biopsy [61]. Therefore, an innovative approach is needed to use metabolic risk factors to identify subjects with NAFLD/NASH rather than relying on liver enzyme abnormalities.

There is an active research that is underway to discover serum biomarkers for NASH since it is associated with increased apoptosis and therefore blood markers of apoptosis may be instrumental in distinguishing NASH from simple steatosis [62]. Apoptosis activates caspases that cleave various substrates such as cytokeratin-18 (CK-18), a key intermediate filament protein in hepatocytes, that can be detected with an ELISA test using an M30 antibody to identify patients with NASH [63, 64]. However, liver biopsy provides a superior assessment of hepatic steatosis, hepatocellular injury, inflammation, and fibrosis as well as its ability to demonstrate the presence of hepatocyte ballooning and degeneration in association with steatosis as the key histological feature that distinguishes NASH from simple steatosis. Notwithstanding its limitations such as inherent variability in histologic assessment of NAFLD stage and activity, its invasiveness, its high possibility of complications related to liver damage, its proneness to sampling error generated by the operators, and its limitations in accessibility and reproducibility, liver biopsy is still the standard criterion for the most accurate diagnosis of NAFLD and NASH. Also, because only 7–30% of NAFLD patients in the world population had an indication of biopsy for accurate measurement, Younosis et al., re-evaluated and reported the global prevalence of NASH to be between 1.5 and 6.45% and the North American rate at an average of 8.69% (between 7.2 and 14.63%) [4, 65]. Regarding obesity, reports show that NASH can be verified by histological examination in about 47% of all NAFLD cases among obese individuals [66].

Liver fibrosis is the inordinate accretion of extracellular matrix proteins that include collagen in most types of liver disease including NAFLD. Fibrosis stage is a crucial histological variable to predict mortality. There are well-known independent predictors of fibrosis, which is a subway to chronic liver disease state. Some of these risk factors are age (>45–50), BMI (>28–30 kg/m²), insulin resistance (IR), diabetes, and HTN [67]. Staging hepatic fibrosis is essential in all patients with NAFLD to identify individuals with advanced fibrosis (AF) who may later develop liver-related complications such as hepatocellular dysfunction and portal hypertension

(PHTN). A noninvasive and an indirect assessment, which is performed in all liver disease patients including children, may include blood tests such as liver function tests (low albumin), complete blood count (thrombocytopenia and neutropenia), and coagulation profile (prolonged prothrombin time) [68]. Among the diagnostic tools used to measure the prevalence of AF in the setting of T2DM versus the general population, vibration-controlled transient elastography shows the highest prevalence rate followed by NAFLD fibrosis score and FibroTest in that order. It should be noted that the prevalence of T2DM significantly increases the prevalence of AF in similar ways to NAFLD [56].

The most widespread clinically implemented histological grading and staging system is the ‘NAFLD activity score’ (NAS) [6] (see **Table 1**). More recently, the SAF score encompassing an assessment of steatosis (S), activity (A), and fibrosis (F) has been used to produce more accurate measurements of NASH [5]. These recent developments underscore the fact that NAFLD patients can be diagnosed and staged effectively using noninvasive strategies even though liver biopsy can still be applied for individuals with dubious diagnostic tests or if noninvasive staging is unspecified [69]. However, there is no widely available simple blood test or imaging modality that can differentiate simple steatosis from NASH.

In summary, early diagnosis of NAFLD is essential to halting the progression of the disease. Biopsy is intrusive and therefore cannot be routinely applied. Ultrasound (sonography) and magnetic resonance imaging tools have become alternative noninvasive detection tools that can be routinely employed in clinical practice. The NAFLD activity score is important as part of the diagnosis procedure. But the fibrosis score is just as important. **Table 2** shows the fibrosis score currently used to stage the degree of fibrosis in the liver. There are a few noninvasive fibrosis imaging tests on the market such as Fibroscan that offers a liver stiffness measurement (LSM) using pulsed-echo ultrasound as a surrogate marker of fibrosis [70] and acoustic radiation force impulse (ARFI), which uses conventional B-mode ultrasonography to produce an ultrasonic pulse and measure the response of the liver tissue as shear wave velocity [71]. The Centers for Disease Control (CDC) and Prevention projects that diabetes mellitus is likely to impact the fibrosis progression rates, given the close link between diabetes and fibrosis in those with NAFLD [72, 73].

Some commercial biomarker tests include enhanced liver fibrosis (ELF), a panel of markers of matrix turnover as tissue inhibitor of matrix metalloproteinase 1 (TIMP1), hyaluronic acid and PIIINP [74] and FibroTest (FT), a panel of markers of fibrosis widely used in France.

Grade	Steatosis (%)	S score	Lobular (L) inflammation	L score	Hepatocyte ballooning (B)	B score
0	<5	0	No foci	0	None	0
1	5–33	1	<2 foci per 200 × field	1	Few cells	1
2	34–66	2	2–4 foci per 200 × field	2	Many cells	2
3	>66	3	>4 foci per 200 × field	3	N/A	N/A

NASH activity grade = total score: S + L + B range (0–8). Score of ≥5 is equivalent to NASH; score of 3 or 4 is borderline NASH; score of ≤2 denotes non-NASH NAFLD. The NAFLD activity score is based on three pathologic features: Steatosis, hepatocyte ballooning degeneration, and lobular inflammation. Higher scoring denotes severity of NASH: >5 = NASH; <5 = No NASH; 3–4 = borderline; none (0); few (1); many (2).

Table 1.
NAFLD activity score (NAS).

Liver injury	Fibrosis score (0–4) [*]	Fibrosis stage
None	0	0
Mild (delicate fibrosis)/zone 3 presinusoidal fibrosis	<5% (0), 5–33% = (1)	1a
Moderate (dense fibrosis)/zone 3 presinusoidal fibrosis	>33–66% (2), >66% = (3)	1b
Periportal/portal fibrosis	0 (0), <2 (1), 2–4 (2), > (3)	1c
Portal and periportal fibrosis/presinusoidal fibrosis	None (0)	2
Bridging fibrosis	Few (1)	3
Cirrhosis	Many (2)	4

^{*}Fibrosis score of F1-F4 is generally considered NASH [4].

Table 2.
 NAFLD fibrosis score (NFS) and stage.

1.4 The metabolic syndrome (MetS)

Recognizing patients with the metabolic syndrome (MetS) is key to identifying patients at risk of NAFLD. MetS is a group of risk factors that raises risk of heart disease, diabetes, stroke, etc. [75] and is diagnosed when any three of the following five clinical risk factors are present [76]: impaired fasting serum glucose, low levels of serum HDL cholesterol, elevated serum triglycerides (i.e., hypertriglyceridemia), central obesity or larger than cut-off waist circumference (varies according to gender and ethnicity), and high blood pressure (HTN) (see **Table 3**).

Insulin resistance is also a major risk factor for the development of steatosis. Once considered benign, NAFL (or simple steatosis), which is defined as the presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of the hepatocytes, is now believed to be a serious risk factor for progression to liver disease, cardiovascular disease, and mortality [37, 77]. This is because an excess of abdominal fat is most tightly associated with the metabolic risk factors [78, 79]. The duration of obesity and the presence of MetS in an individual patient are closely tied to the risk of developing NASH-related cirrhosis and HCC [80]. Some of the characteristics of MetS are present in most NAFLD individuals, with 65–71% being obese, 57–68% having deranged lipid profiles, 36–70% suffering from HTN, and 12–37% having impaired fasting glucose tolerance [81]. Approximately a third of patients with NAFLD have the full metabolic syndrome and >90% have at least one feature [47]. There is a consensus that considers NAFLD as a hepatic manifestation of the MetS [82, 83]. On the other hand, clinical signs of the disease are manifested in 70–75% of T2DM patients and up to 95% of obese patients [84]. Thus, the development of the MetS, which is an important

Features	Terms of condition
Blood glucose (sugar)	Fasting, ≥ 100 mg/dL
Blood HDL (“good”) cholesterol	$\text{♂} < 40$ mg/dL; $\text{♀} < 50$ mg/dL
Blood triglycerides (TGs)	Fasting, ≥ 150 mg/dL
Waist circumference	$\text{♂} > 40$ ”; $\text{♀} > 35$ ”
Blood pressure (HTN)	$\geq 130/85$ mm Hg

^{*}The MetS is present with any three of the features shown in the table.
 ♂ = male; ♀ = female; HDL = high-density lipoprotein; ” = inches.

Table 3.
 Features of the metabolic syndrome.*

predictor of NASH in NAFLD patients, poses a sweeping and unfavorable prognosis [85]. IR is a key mediator that links NAFLD and MetS, which is a constellation of anthropometric and metabolic abnormalities (see **Table 3** above).

According to the latest data from NHANES (National Health and Nutrition Examination Survey) study conducted between 2011 and 2012, the prevalence of MetS has increased to 35% in American adults [86]. MetS is a risk factor for diabetes and cardiovascular diseases. It induces an abnormal production of hormones such as leptin, adiponectin, and cytokines such as TNF (tumor necrosis factor)-alpha that regulate inflammatory responses and cause disequilibrium between the pro-inflammatory and anti-inflammatory state of the organ [86]. These are mutually antagonistic: the pro-inflammatory factors such as TNF-alpha promote pro-apoptotic processes, recruit white blood cells, and promote insulin resistance. On the other hand, adiponectin acting as an anti-inflammatory factor inhibits fatty acid uptake, stimulates fatty acid oxidation and lipid export, and enhances insulin sensitivity. Both an increase in pro-inflammatory factors and a decrease in anti-inflammatory factors cause a cytokine imbalance that would lead to steatosis (NAFL) followed by necroinflammation (NASH) and IR. There is a supporting evidence that a high TNF to adiponectin ratio promotes fatty liver and steatohepatitis in animal [87] and human [88] studies. The importance of MetS including IR is that it predicts the occurrence of diabetes and cardiovascular diseases, which can further promote the development and progression of arteriosclerosis and HTN leading to significant morbidity and mortality [89]. Also, NAFLD and obesity are risk factors for the progression to fibrosis among HCV-infected patients [90–93]. Furthermore, elevated levels of ferritin are common in NAFLD patients and typically reflect active IR or underlying inflammatory activity [68, 81, 94]. Therefore, because of many different correlates and etiological factors and an assortment of assessment tools associated with MetS, there are some unresolved uncertainties in the current estimates of the global and the United States prevalence of NAFLD.

1.5 The genetics of NAFLD

Genetic disorders of lipid metabolism can cause hepatic fat deposition. However, they are far less common than excess body weight and features of MetS as risk factors for NAFLD and NASH. Several genes have been associated with NAFLD. These include *NCAN*, which may have a protective effect for Hispanics but increases risk of steatosis for non-Hispanic blacks; *LYPLAL1*, *GCKR*, as well as *PPP1R3B*, which may confer increased risk for hepatic steatosis but the data of distinctive serum lipid profiles in all these genes are sparse [61, 95–97]. *GCKR* is reported to be closely associated with NAFLD in Chinese [98]. Another gene, Patatin-like phospholipase domain-containing 3 (*PNPLA3* or adiponutrin), has emerged as the genetic factor predisposing Hispanics more at risk for fatty liver disease [99]. This adiponutrin gene is a single variant considered responsible for increased hepatic TG levels, fibrosis, and inflammation, observed among ethnic groups [100, 101]. Homozygote patients have a twofold rise in hepatic fat content than heterozygotes, and Hispanic populations exhibit the highest frequency of this polymorphism (49%) compared to 23% in European-Americans (EAs) and 17% in African-Americans (AAs) [101]. It also shows more allelic frequency with Hispanics than other ethnic groups. Romeo and colleagues [102, 103] along with Singal and colleagues published papers in 2008 in which they reported that *PNPLA3* is strongly associated with hepatic steatosis and elevated ALT and also recently showed that *PNPLA3* is associated with NASH, fibrosis progression, and hepatocellular cancer as well [102].

A genetic marker, *TM6SF2*, discovered in an exome-wide association study of liver fat content, has also shed some light on its association with hepatic steatosis. It

is involved in the loss of function mutation in very low-density lipoprotein (VLDL) secretion, and its association with NASH and advanced fibrosis has been recently validated even though its precise function has not been delineated [102]. However, its mutation is associated with elevated ALT, hepatic steatosis, and lower level of alkaline phosphatase, LDL, and TGs. This gene is most prevalent in European ancestry and less in Hispanics and AAs [104].

There is a reported 52% heritability rate of NAFLD, but evidence pertaining to specific genetic mutations is scant according to multivariable models used after adjusting for sex, age, and ethnicity [13]. Although the mechanism is not well understood, genetic mutations in hemochromatosis (HFE) gene, which is responsible for iron uptake and transferrin plasma concentration, may also be associated with NAFLD development [105, 106]. Several other factors have been indicated in the development and outcomes of NAFLD including epigenetic alterations [107, 108], maternal perinatal nutrition [109–111], and gut microbiota [107, 112–114]. A recent study also reports a novel pathway in which hepatic vitamin D receptor (VDR) expression is increased in patients with simple steatosis (nonalcoholic fatty liver without inflammation), and the activated VDR upregulates angiopoietin-like protein 8 (ANGPTL8) expression, thus contributing to triglyceride accumulation in human hepatocytes [115]. At any rate, studies have reported that fibrosis-initiated fatty liver disease progresses over many years, thus providing a potential window for intervention by examining disease-progression/modifying factors in NAFLD [116–118]. It is important to note that increased BMI and insulin resistance have been associated with a more rapid progression to fibrosis [35, 119].

1.6 The epidemiology and prevalence of NAFLD

In most epidemiological studies, the prevalence of NAFLD in the general population is determined by imaging or other indirect methods. Accordingly, the epidemiology and demographic characteristics of NAFLD vary worldwide [12, 16]. In epidemiological studies, the pathophysiological aspects, the natural history, and the determinants of NAFLD are important parameters for the diagnosis and evaluation of therapeutic interventions. This section will provide global perspectives on the prevalence of NAFLD (and later HCC) with emphasis on the United States and the possible reasons for the rapid rise.

There are wide-ranging estimates of NAFLD prevalence in the general population of the United States. An estimated 17–51% of adults have NAFLD [23, 120, 121]. Analysis of liver ultrasound data collected between 1988 and 1994 from the NHANES III reported that 19% of adults have NAFLD [122], whereas a meta-analysis of studies from 2006 to 2014 estimated a NAFLD prevalence of 24% (20–29%) in the general population [65]. The prevalence of NASH is difficult to estimate as biopsy is the necessary tool for screening, but it is cost-prohibitive and impractical for a population study.

Globally, NAFLD is a growing cause of chronic liver disease and NASH is replacing HCV infection as the primary reason for LT [13, 123, 124]. The broad category of NAFLD can manifest as NAFL or NASH. Fibrosis precedes cirrhosis and is therefore used as a prognosticator of the clinical risk of progression to cirrhosis and long-term liver-related adverse outcomes and mortality [34]. Recent evidence has shown that NAFLD and NASH can progress to HCC even in the absence of cirrhosis [125–127]. In most epidemiological studies including the NHANES data set, the assumptions about NASH in the NAFLD population are based on a post-hoc application of liver enzymes (i.e., AST and ALT) and clinical measurements. In the same vein, the fibrosis stages in population-based studies reflect best estimates derived

from clinical aids (e.g., fibrosis-4, ALT to platelet ratio index, and NAFLD fibrosis scores) [128, 129]. The current prevalence rates for NAFLD, NASH, and HCC based on definitive clinical manifestations are shown in **Table 4**.

Certain risk factors such as advanced age, obesity, ethnicity, and T2DM increase the incidence and prevalence of NAFLD and NASH and have been consistently identified as salient risk factors for fibrotic progression to cirrhosis [130] (see **Table 5**).

The current global estimate is that 24–30% of the world’s population is affected by NAFLD [65] and that includes between 80 and 100 million Americans (<http://www.mayoclinic.com>), making it the primary etiology for liver disease in the United States; see **Figure 2**.

The increasing incidence of obesity, diabetes, and metabolic syndrome in the United States and Europe may soon catapult NAFLD/NASH to become the most common cause of HCC in developed countries. In the United States, among the more than 26 million people with diabetes, the prevalence of biopsy-proven NAFLD and NASH is as high as 74 and 11%, respectively [138, 139].

1.7 The rise in burden of NAFLD/NASH

The global rise of NAFLD has exasperated the looming healthcare burden of disease. It may be difficult to accurately forecast the current and future burden of a disease that is rapidly progressing. However, there are modeling techniques and approaches that incorporate real-world surveillance data for NAFLD and NASH incidences, which are growing causes of cirrhosis and HCC. As with many models, the utility of the model is linked to the validity of the inputs into the model. One of these modeling approaches is based on the premise that public awareness and

Status	Definition	Prevalence	Prognosis
NAFLD	Spectrum of fatty liver disease with <140 g for men and < 70 g for women per week of alcohol consumption	Estimated at 24–30% of global population [13, 14, 16] and at least 31% of US population (7)	—
NAFL	>5% simple hepatic steatosis by weight of liver without evidence of hepatocellular injury (i.e., hepatocyte ballooning)	>80% of NAFLD patients	Low probability of progression to cirrhosis
NASH	>5% hepatic steatosis by weight of liver with inflammation and hepatocellular injury with or without fibrosis Confirmed histologically	Estimated at up to 21–59% of patients with NAFLD Estimated at 1.5–6.45% of US general US population [40, 96, 122]	11% progress to cirrhosis over 15 years
NASH cirrhosis	Presence of cirrhosis with current or past histologic evidence of steatosis	10–30% of patients with NASH	About 31% have liver decompensation over 8 years; about 7% develop HCC over 6.5 years
NASH HCC	Hepatocellular carcinoma induced by NASH	Estimated at annual rate of 2.6–12.8% [*]	Progresses to end-stage liver disease

^{*}The prevalence of NASH-HCC is not firmly established. Data in the table are the annual incidence rate of developing HCC in patients with NASH-related cirrhosis.

Table 4.
Prevalence of NAFLD and its more progressive forms, NASH and HCC.

Risk factor	Description	References
Age	Risk increases with age	[4, 40]
Gender	More common in men Higher risk of advanced fibrosis in women	[4, 96, 131, 132]
Genetics	Patatin-like phospholipase domain-containing 3 gene	[133]
MetS**	70–90% of patients have NAFLD MetS is an independent predictor of fibrosis	[85, 95]
Ethnicity	Elevated risk in Hispanics Lower risk in blacks	[61]
Diet	Elevated levels of cholesterol and saturated fats High fructose intake, low carbohydrates	[89, 134–136]
OSA***	Increased risk of hepatic fibrosis	[137]

*NAFLD/NASH = nonalcoholic fatty liver disease/nonalcoholic steatohepatitis.
 **MetS = metabolic syndrome.
 ***OSA = obstructive sleep apnea.

Table 5.
 NAFLD/NASH* risk factors.

government health policies will be able to eventually level off national obesity incidences and prevalence, which in return will level off NAFLD [4]. The interpretation of the output of this and other models attempting to analyze the burden of NAFLD is constrained by the lack of accurate diagnosis of steatohepatitis with simple epidemiologic tools. Nevertheless, the proportion of individuals with NASH in the NAFLD population will probably continue to rise through the next 15 years based on the rising prevalence of diabetes mellitus [4].

Analyzing the cost and burden of disease with respect to NASH has several potential implications. First, it helps introduce strategies and treatment regimen that will stem its exponential rise in incidence and mortality rates; it will reduce the growing contribution of NASH to LT, which is expensive; and due to an oversupply of decompensated cirrhosis, matching organ availability is rare, and insurance companies have exclusive policy of qualifying subjects with NASH-induced cirrhosis based on whether they have associated co-morbidities.

The epidemiology and demographic characteristics of NAFLD vary worldwide. The rise in NAFLD and NASH will balloon the number of patients with decompensated cirrhosis and pose a major emotional and financial burden on subjects and

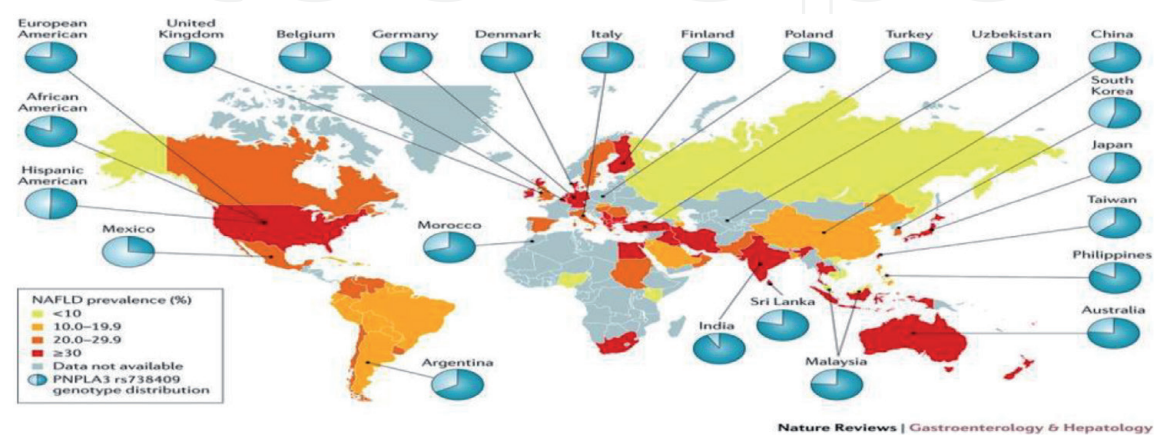


Figure 2.
 Global picture of estimated prevalence of NAFLD and distribution of PNPLA3 genotypes adopted from Zobair Younossi [16]. PNPLA3 is presented as minor allele frequency in some areas (light blue section of the pie chart).

their caregivers, thus adding to the overall cost of health care. Furthermore, the main etiologic factor adding to the burden of HCC is NAFLD [4]. In select NASH-related HCC patients, liver resection and transplantation provide potentially curative therapeutic options; however, these procedures place a significant burden to health-care resources and utilization [140]. Currently, NASH-related HCC has replaced HCV-related HCC as the fastest growing indication for LT in HCC candidates.

2. Hepatocellular carcinoma (HCC)

Liver cancer, which has limited therapeutic choices, has the second highest mortality rate in the world [141]. HCC, which can lead to complications such as portal vein thrombosis (PVT), accounts for the majority of primary liver malignancies and is one of the leading causes of death in patients with advanced fibrosis or cirrhosis [141–144]. HCC can be caused by chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), alcohol abuse, as well as obesity and diabetes-induced MetS. NAFLD often occurs in the setting of metabolic disorders such as obesity and T2DM. These same metabolic conditions are also risk factors for NAFLD-associated HCC, which can materialize in individuals even in the absence of advanced fibrosis or cirrhosis. NASH-HCC appears to be phenotypically different from HCC arising from other chronic liver diseases (**Table 6**). By all accounts, the formation and progression of HCC are multistep processes. Therefore, the specific and detailed molecular events that underlie HCC development remain only partially understood [143].

2.1 The epidemiology and prevalence of HCC

Primary liver cancer in 2012 was identified as the second most common cause of cancer-related death in the world. In the United States, HCC is the most common histological subtype of liver cancer that accounts for 70–85% of primary liver malignancies [145, 146]. It is also the most rapidly rising cause of cancer and

Hepatitis B infection (HBV)
Hepatitis C infection (HCV)
Hepatitis D infection (HDV)
Alcohol (ethanol)
Nonalcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis (NASH)
Obesity/diabetes
Hereditary hemochromatosis
Aflatoxin B
Tyrosinemia
Glycogen storage disease type 1a
Oral contraceptives
Smoking
Cirrhosis*

*Diagnosis of cirrhosis is based on the presence of the ICD-9 codes for cirrhosis or complications of cirrhosis (gastroesophageal varices, encephalopathy, and nonmalignant ascites) recorded at least twice in any inpatient or outpatient encounter.

Table 6.
Common risk factors for hepatocellular carcinoma.

cancer-related deaths with an incidence that has more than tripled over the last two decades. This high mortality reflects a poor prognosis and a poorer therapeutic intervention [147]. Compared to HCC caused by alcoholic liver disease and viral hepatitis, there is a lack of strong epidemiological data associated with the incidence and prevalence of HCC precipitating from NAFLD [140, 148]. While the prevalence of NAFLD is thought to be highest among Hispanics and Caucasians, the ethnic distribution among NAFLD-/NASH-related HCC patients has yet to be defined. Male patients are overrepresented in NASH-related HCC; however, gender has not been proven to be a statistical risk factor in NASH progression to HCC [7]. The rising incidence of NAFLD/NASH in the setting of obesity has led to a drastic growth in NASH-related HCC incidence [149]. Although NAFLD can present with HCC in the absence of NASH or cirrhosis, the cumulative annual incidence rate for developing HCC in patients with NASH-related cirrhosis is approximately 2.4–12.8% [125]. This suggests or utmost underlies that cirrhosis may be the main cause of HCC despite new emerging data suggesting that NAFLD may be an independent risk factor for HCC, even in the absence of cirrhosis [126, 150, 151].

There was also a twofold increase in the incidence of HCC in the United States over the past two decades, and it is projected to double over the next two decades. Compared to HCC in alcoholic liver disease and viral hepatitis, there is a lack of strong epidemiological data regarding the incidence and prevalence of HCC in NAFLD [148]. It is projected that in just 12 more years, HCC at its current pace of growth in the United States will outstrip breast and colorectal cancers as the third leading cause of cancer-related death. This is because the prevalence of HCC is expected to increase by 149% from 10,000 to 24,900 during 2015–2030, while the incidence of HCC cases is expected to increase from 5160 to 12,240 in 2030, an increase of 137% [4]. This alarming incidence is attributed to several different genetic and epigenetic alterations that are under investigation [4].

Modeling the epidemic of HCC suggests that in 2015, 3280 incident HCC cases were estimated to have progressed from compensated cirrhosis (64% of total), with the remaining 1880 incident cases occurring among \leq F3 (fibrosis score-3) cases [4]. By 2030, 8790 incident HCC cases are predicted to occur among compensated cirrhotic cases or 72% of the annual incidence, reflecting aging and disease progression [4].

The true prevalence of NASH and NASH-related HCC is probably underestimated. This is because in 6.9–29% of HCC cases, the underlying etiology is unknown, further questioning the designation that the liver disease is secondary to cryptogenic cirrhosis [148]. Traits of NASH are more frequently observed in HCC patients with cryptogenic cirrhosis than in age- and sex-matched HCC patients of well-defined viral or alcoholic etiology [152]. In the past several years, myriad studies have tried to determine the variability of relationships between NAFLD/NASH, cryptogenic cirrhosis, and HCC. In a recent meta-analysis, White et al. [125] estimated that 60% of HCC cases ascribed to NAFLD/NASH had cirrhosis either prior to diagnosis or at the time of diagnosis. This same analysis showed that NASH-associated cirrhosis consistently manifested an increased HCC risk. Furthermore, the study also revealed that when compared to those with chronic HCV, the risk of developing HCC is lower in patients with cirrhosis due to NAFLD/NASH (HCV, 19.7% vs. NAFLD/NASH, 26.9%) [125]. Although the prevalence of NAFLD-/NASH-related HCC is not well delineated, the growing incidence of obesity and diabetes suggests the impact of NAFLD-/NASH-related HCC will continue to grow.

2.2 The genetics of HCC

Genomic analyses promise to improve tumor characterization for the optimization of precision or personalized medicine for patients with HCC. Recent

developments and molecular techniques have significantly improved our understanding of the pathogenesis of HCC and its complex genetic landscape [153–156]. The integration of several profiling data from various sources may provide additional insight into the molecular mechanisms of HCC [153]. The first large-scale multiplatform analysis of HCC conducted as part of The Cancer Genome Atlas (TCGA) network included valuation of somatic mutations by whole exome sequencing and DNA copy number analyses in 363 patients whose tissue and tumor specimens were obtained [157]. This high-throughput analysis also included further investigation of DNA methylation, mRNA expression, microRNA (miRNA) expression, and proteomic expression in 196 patients. To decipher the molecular landscape of HCC and extract biological insights for therapeutic targets and prognostic implications, analyses were made by integrating multiple data platforms with the available clinical data for HCC [157]. Mutational and DNA sequencing analyses identified an array of genes altered either by downregulation or by mutation. Among the significantly mutated genes were *EEF1A1*, *SMARCA4*, *LZTR1*, and *SF3B1* [157]. Those genes downregulated by hypermethylation including *ALB*, *APOB*, and *CPS1* may cause metabolic reprogramming in HCC. The analysis of integrated molecular platform also yielded the identification of a subtype linked to poorer prognosis in three HCC cohorts. This large-scale multiplatform, high-throughput analysis enabled the design of a p53 target gene expression signature correlating with poor survival. This TCGA network analysis produced potential therapeutic targets including WNT signaling, *IDH1*, *MET*, *VEGFA*, *MCL1*, *MDM4*, *TERT*, and immune checkpoint proteins PD-1, PD-L1, and CTLA-4 [157]. This is significant because effective inhibitors already exist for these targets, which alter hepatocyte energy balance [157].

In exome sequencing analysis of over 200 liver tumors, investigators identified mutational signatures that are associated with specific risk factors such as alcohol and tobacco consumption and exposure to aflatoxin B₁ [158]. As a result, they found that 161 putative driver genes were associated with 11 recurrently altered pathways involving *CTNNB1* (alcohol), *TP53* (hepatitis B virus, HBV), and *AXIN1* [158]. Further analysis of tumor stage progression identified *TERT* as an early event, whereas *FGF3*, *FGF4*, *FGF19*, or *CCND1* amplification and *TP53* and *CDKN2A* alterations were prominent in aggressive tumors. The involvement of these many altered genes and pathways in the development and/or progression of HCC leads to the extensive landscape and multifaceted nature of this lethal cancer. **Figure 3** shows the salient signaling pathways associated with HCC.

In another recent study, gene expression and DNA methylation profiles were screened to identify potential genetic biomarkers of HCC. The findings from this study suggest potential HCC biomarker roles for certain genes such as *DTL*, *DUSP1*, *NFKBIA*, and *SOCS2* [160]. Similar to TCGA Research Network analyses mentioned above [157], these investigators also suggest that the tumor protein ‘p53 signaling’ and ‘metabolic’ pathways may serve important roles in the pathogenesis of HCC [160]. Other polymorphic variants serving as potential risk factors for HCC in high-risk patients infected with HBV/HCV have also been reported [161]. As for prognostic biomarkers, recent RNA sequencing data from the Cancer Genome Atlas (TCGA) reveal that among the 12 tissue types studied, the liver had the largest number of tissue-enriched genes, which are associated with the prognosis of patients with HCC and represent distinct physiological patterns [162]. A further study of the characteristics of liver-enriched genes showed that hypermethylation might be partially responsible for the downregulation of these genes, most of which were metabolism-related genes associated with pathological stage and dedifferentiation in patients with HCC. The authors suggest that hypermethylation might be a mechanism

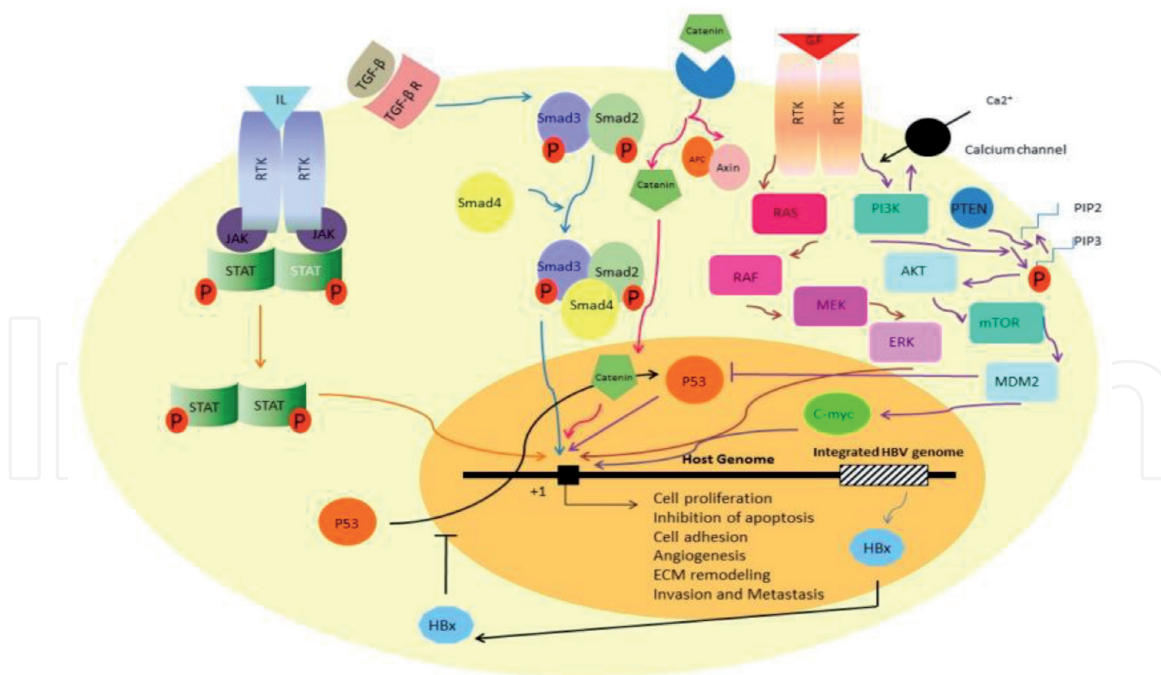


Figure 3.
 The most reported signaling pathways in HCC. Adapted from Birgani et al. [159].

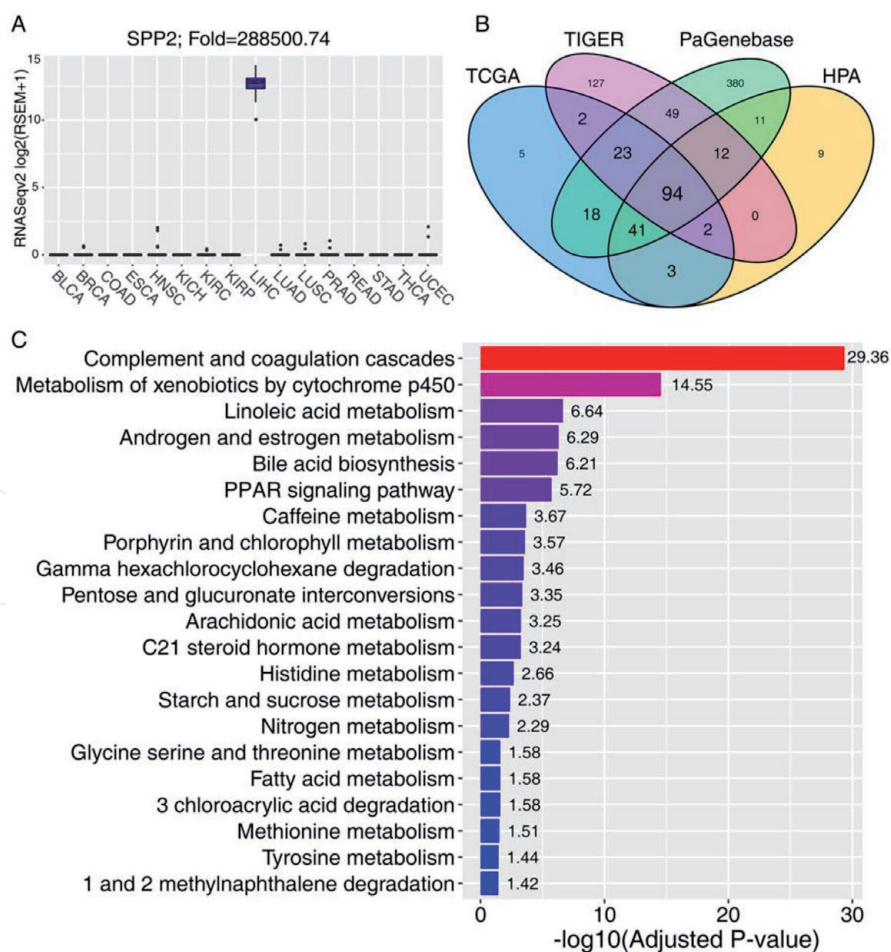


Figure 4.
 Validation of liver-enriched genes and KEGG analysis. (A) an example of liver-enriched genes. SPP2 was exclusively expressed in the corresponding nontumor tissues of HCC. (B) Four-set Venn diagram showing the overlap of the liver-enriched genes derived from the TCGA and three other databases, including HPA, PaGeneBase, and TiGER. (C) Significantly enriched KEGG pathways of 188 liver-enriched genes. $-\log_{10}$ (adjusted p-value) was annotated on each bar of the KEGG pathway. Adapted from Binghua et al. [162].

underlying the downregulation of these liver-enriched genes. When they overlapped the tissue-enriched and prognostic genes across cancer types, they found that, in HCC, 55% (84/188) of the liver-enriched genes were prognostic (see **Figure 4**).

Circulating regulatory nucleic acids like miRNA profiles can also reflect the pathogenic changes occurring in organs including the liver. Changes in miR-21, miR-122, and miR-223 were correlated with the histological status of the human liver and were specific for liver injury [163]. These miRNA levels were significantly higher in the serum of chronic hepatitis (i.e., HBV and HCV) and HCC patients compared to healthy controls [44]. Yet, the biological heterogeneity of HCC makes it difficult to clarify the key mechanisms of cancer initiation and progression, and thereby develop and implement effective therapies [164].

3. The projection of NAFLD and HCC

A recent Markov model was used to predict incidence of NAFLD and to forecast NAFLD disease progression in the United States. The model was based on historical and projected changes in adult prevalence of obesity and T2DM as well as national surveillance data for incidence of NAFLD-related HCC [4]. The report forecasts that prevalent NAFLD cases will increase to 21% (100.9 million) by 2030, while prevalent NASH cases will increase 63% from 16.5 million to 27.00 million cases [4]. Overall NAFLD prevalence among the adult population (aged ≥ 15 years) is projected at 33.5% in 2030, and the median age of the NAFLD population will increase from 50 (estimated at 2015 level) to 55 years between 2015 and 2030 [4]. In 2015, approximately 20% of NAFLD cases were classified as NASH and are expected to increase to 27% by 2030, a reflection of both disease progression and an aging population. The estimated prevalence of NASH in adults living in the United States is 3–5% [6, 23, 121, 165] and is projected to increase by 63% from 16.5 million in 2015 to 27.00 million cases in 2030 [4]. This prevalence of NASH was calculated based on published estimates and modeling of fibrosis progression. It was assumed that up to 5% of NAFLD cases without NASH could be NASH regressors, with most NASH regressors still in F0 stage [4]. Similarly, the incidence of decompensated cirrhosis will surge by 168% to 105,430 cases in 2030, while incidence of HCC will increase by 137% to 12,240 cases. Liver deaths are estimated to increase 178% to 78,300 deaths in 2030. During 2015–2030, there are projected to be nearly 800,000 excess liver deaths. The aging population, the continuing high rates of adult obesity, and T2DM will propel NAFLD-related liver disease and mortality in the United States. Immediate strategies are required to curtail new NAFLD cases and mitigate disease burden.

Currently, NAFLD is estimated to affect more than 80 million and up to 90 million Americans, making it the most common etiology for liver disease in the United States [16, 65]. In the United Kingdom, NAFLD has now become the most common cause of abnormal liver function tests (LFTs) [166]. Although NAFLD has emerged as a serious disease in affluent Western economies, its global prevalence encompasses the Middle East (32%), South America (31%), Asia (27%), the United States (24%), Europe (23%), and Africa (14%) [167]. Because of the increasing incidence of obesity and diabetes around the world, NAFLD has become a global public health concern. The prevalence of NAFLD varies according to age, sex, and the methodology used to measure the condition in each geographical location [61]. Currently, NAFLD is the most prevalent liver disease observed in patients with obesity, diabetes, and metabolic syndrome (MetS), all of which can confer insulin resistance (IR) and are known risk factors for the development of HCC, a growing indicator of LT [45, 69]. While HCV infection has been the most common indication for liver transplants to date, NASH is surpassing it as obesity reaches historic

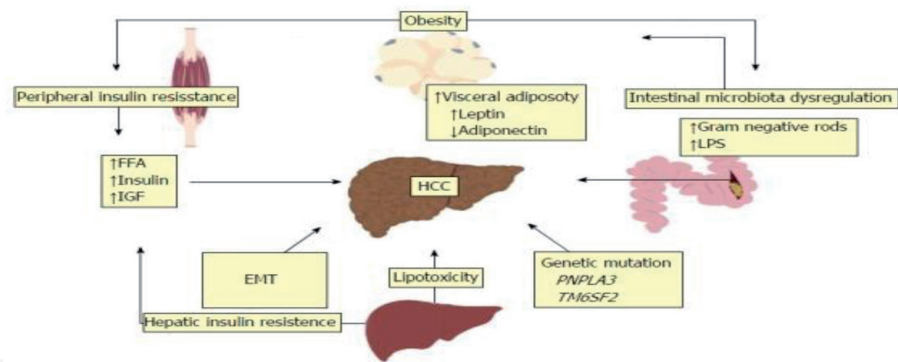


Figure 5. Risk factors and proposed mechanisms for NAFLD- and NASH-related HCC, which is multifactorial. Proposed pathogenic mechanisms include obesity, peripheral and hepatic IR from T2DM, increased hepatic lipid storage and lipotoxicity, genetic mutations, and intestinal microbiota dysregulation. HCC, hepatocellular carcinoma; EMT, epithelial to mesenchymal transition; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; FFA, free fatty acid; IGF, insulin-like growth factor; LPS, lipopolysaccharide; PNPLA3, patatin-like phospholipase domain-containing 3; TM6SF2, transmembrane 6 superfamily member 2. Adapted from Cholankeril [140].

highs and new direct-acting antiviral (DAA) drugs are essentially curing hepatitis C [168]. Furthermore, with the continued decline in the prevalence of HCV infection, the proportion of NASH-HCC is anticipated to increase exponentially due to the growing epidemic of obesity and diabetes [140]. Currently, NASH-related HCC is the fastest growing indication for LT in HCC candidates [140]. NAFLD and NASH are a growing cause of cirrhosis and HCC.

Globally, Asia is leading the rise in NAFLD followed by the United States. Although our understanding of NAFLD is steadily evolving, it is not an isolated disease. It is commonly associated with the leading metabolic comorbidities such as obesity, MetS, T2DM, and dyslipidemia. The potential progression of NAFLD subtypes is from fibrosis to advanced fibrosis, ESLD, and HCC (**Figure 1**). As the incidence of obesity and concurrently diabetes and MetS continues to surge in Europe and the United States, NAFLD/NASH may become the most common cause of HCC in developed countries in the foreseeable future [169–171].

A 2002–2012 retrospective cohort study among adult patients revealed a four-fold increase in patients undergoing LT for NASH-related HCC in contrast to only twofold increase in number of patients undergoing transplantation for HCV-related HCC. In the United States, about 6000–7000 liver transplants are performed annually, and the rapid increase in the percentage (44.9%) of obese individuals during a 14-year period (2000–2014) is expected to escalate to 55% the number of NASH patients awaiting LT by 2030 [172]. The increased morbidity and mortality, healthcare costs, and declining health-related quality of life associated with NAFLD require more in-depth analysis. **Figure 5** depicts the proposed mechanisms that ties NAFLD/NASH and HCC.

4. Ethnic and gender differences in NAFLD and HCC

Although still not fully resolved, the prevalence of NAFLD in the United States can vary by ethnicity. Even in this context, there are several factors that could explain the reported ethnic disparities. These include access to health care, genetic factors, environmental factors, affliction with chronic diseases, and the presence of chronic diseases such as the MetS [61, 65, 173]. In this context, the prevalence of NAFLD is reported to be highest in Hispanic-Americans, followed by Americans of European descent and then African-Americans [40, 61, 65, 122, 173]. Several

studies have shown a relative sparsity of NAFLD cases among individuals of African descent living in or coming from Africa or the Caribbean region. Although the prevalence of metabolic disease and obesity is high in Afro-Caribbean ethnic groups compared to Caucasian and Hispanic groups, the frequency of NAFLD/NASH is reported to be low [61, 174]. This discrepancy might be due to an actual low number rate or biases that include low-recognition and low-referral rates in these ethnic minorities [175], as Afro-Caribbean patients are categorically less likely to be referred to other tertiary hospitals [176].

There are also ethnic differences in the incidence of HCC in the United States (see Sherif et al. for a comprehensive review) [61]. Compared to European-Americans (EAs), the incidence of HCC is higher in African-Americans (AAs) and is associated with more advanced tumor stage at diagnosis and lower survival rates overall. Assessment of changes in the levels of metabolites of samples stratified by race was made using gas chromatography-mass spectrometry in selected ion monitoring mode to identify ethnically diverse biomarkers in HCC between EA and AAs [177]. Race-specific metabolites including alpha tocopherol for AA and EA combined, glycine for EA, and valine for AA exhibited better sensitivity and specificity than the standard serological marker for HCC, alpha-fetoprotein (AFP) that is widely used for the diagnosis of HCC [177–180]. It is hypothesized that there is a variation in HCC-associated epigenetic modifications between AAs and EAs. Thus, the identification of aberrant DNA methylation and differentially modulated miRNAs can be used to better understand the mechanisms of disparities in HCC between races. Also, identifying epigenetic markers for HCC in a specific population will enhance personalized medicine that targets specific therapeutic approaches [181, 182]. This also demands the gathering together of a highly interdisciplinary team of experts to investigate changes in both DNA methylation and miRNA expression patterns between tumor, cirrhotic, and normal liver tissues from AA and EA participants. Identifying molecular cancer gene drivers and mutations may 1 day become critical for precision oncology.

Most epidemiological studies document prevalence of individual diseases in selected tertiary hospital populations [183]. This widespread practice, particularly when imaging and liver enzyme tests are involved and when the patients may be asymptomatic in the early stage of diagnosis, leads to underestimation and underdiagnosis of NAFLD. This is especially true for minority populations in whom the natural development and progression of NAFLD and NASH are understudied and underreported as reflected by the paucity of data in the literature. Furthermore, the predictive value of the MetS may not reflect the true state of NAFLD in AAs since the criteria for the syndrome were developed for non-Hispanic whites [184] thereby influencing underdiagnosis or misdiagnosis of NAFLD and NASH in Hispanics and non-Hispanic blacks (NHB). There is also a strong relationship between insulin resistance and hypertriglyceridemia, one of the crucial components of MetS. However, NHB often have normal triglycerides (TG) level [185], which is used as a diagnostic criterion of the MetS leading to underdiagnosis of the MetS in NHB [186]. This suggests that lowering the threshold for TG level in AAs will lead to grasping the true cases of NAFLD. Moreover, the racial differences in NAFLD and NASH may be a function of the differences in TGs or the differences in the distribution of adiposity (e.g., subcutaneous vs. visceral) since AAs have relatively less VAT and lower TGs than Hispanics [119, 173, 175]. In addition, AAs may be more resistant to both the accretion of TG in the abdominal visceral compartment (adipose tissue and liver) and hypertriglyceridemia associated with IR [119].

Epidemiologic studies establish the foundational framework for the control and prevention of diseases. In the case of NAFLD and NASH, it should be done by first tracking the prevalence of the disease, characterizing its natural history, and

identifying both its social and health determinants along ethnic lines. This type of study is critical for the proper diagnosis and early intervention of NAFLD especially in minority populations [61].

Genome-wide association studies have revealed several genetic variants that are associated with NAFLD and NASH. Yet, these variants either represent only a limited amount of variation in hepatic steatosis among ethnic groups or may just be markers representing a larger body of genetic variations.

There is an urgent need to gain a better understanding of the underlying biological mechanisms responsible for why some people with NAFLD are more prone to developing HCC, and the causes for disparities in NAFLD-related HCC. There is also an urgent need for a less invasive method than biopsy and for a more sensitive biomarker than ALT for large-scale NAFLD screening. The lack of high-throughput studies employing proteomics or metabolomics for the discovery of novel and reliable diagnostic biomarkers for NAFLD also hampers our understanding of the pathophysiology of the disease among the disparate ethnicities [12, 177].

One recent area of exploration is the involvement of DNA methylation and miRNA regulation. Epigenetic alterations are potentially reversible, and this possibility will facilitate the development of biomarkers and therapeutics in the prevailing disparities between AA and EA patients in HCC initiation and development. The identification and functional validation of race-specific methylation hotspots and miRNAs can be used to understand the mechanisms of disparities in HCC. This can be done by first identifying DNA methylation sites and miRNAs with statistically significant changes between HCC cases and cirrhotic or normal controls in a race-specific manner. Then, network-based methods and hierarchical integrative models can be used to integrate epigenomic data with transcriptomic, proteomic, glycoproteomic, and metabolomic data acquired from the same cirrhotic and HCC participants to select methylation hotspots and miRNAs relevant for understanding the mechanisms of disparities in HCC [177]. The selected candidates can then be validated by independent methods using frozen and formalin-fixed, paraffin-embedded (FFPE) liver tissues collected from patients with HCC and liver cirrhosis. Finally, functional validation of race-specific epigenetic modifications discovered in this type of high-throughput study can be performed through *in vitro* experiments using established cell lines derived from racially diverse populations. These cell cultures may present unique opportunities for targeted functional validation of epigenetic modifications and the downstream consequences.

In addition to exploring the external environment and how it influences HCC disease status, it is also necessary to explore the intestinal environment of different ethnicities. Experimental data from the obesity epidemic have revealed that the composition and products of the gut microbiome, which is altered with obesity and/or a high fat diet, are carcinogenic to the liver [187, 188]. Studies suggest that there are ethnic differences in microbial composition in a cirrhotic population at elevated risk for HCC as a result of metabolites, which can differentiate cirrhotic with HCC from those without HCC. Therefore, a case-control study can be designed to examine the contributions of race/ethnicity, fecal microbiome, fecal metabolome, and host factors (e.g., specific dietary factors and markers of body and liver fat composition) to NAFLD-related HCC. All in all, a multiethnic study of NAFLD and HCC that encompasses all racial/ethnic groups is needed to lay the groundwork for the elucidation of factors that account for health disparities across these populations. The prevalence of NAFLD is reported to be highest among Hispanics and Caucasians as mentioned above. However, NASH was the leading cause of waitlist LT registration in 2016 among Asian, Hispanic, and non-Hispanic white females, whereas HCV is still the leading cause in AA females [189].

As for gender differences in NAFLD or NASH, there are uncertainties including the role of IR in the influence of gender on NAFLD. Ruhl et al. reported that NAFLD is about 2.7 times more prevalent in men than in women [190]. One reasonable explanation for this reported gender difference in NAFLD is due to the higher waist-to-hip circumference (WHR) ratio in men [96]. Pan et al. further state that WHR is associated with visceral adipose tissue (VAT), which is correlated with both peripheral and hepatic IR. Similarly, in the Dallas Heart Study, European-American (EA) men had an approximately twofold higher prevalence of hepatic steatosis than EA women. This gender disparity has been blamed on alcohol use, sex hormones or lifestyle behaviors, and no differences in body weight or insulin sensitivity [96].

The ethnic distribution among NAFLD-/NASH-related HCC patients has yet to be defined [191]. If the increase in the number of ethnic groups waitlisted for LT from 2004 to 2016 is a good indicator of the rise in NASH-HCC, then it could be inferred from a recent retrospective study that Asian females had an 854% change in NASH waitlist registration, while Asian males had a 552% change [189]. The increase in African-American waitlist population was much less compared to the other ethnic groups. In contrast, the Hispanic females had a 3010% change in the rate of waitlist registration for NASH with HCC, while non-Hispanic white females had a 1992% change [189].

NASH-related HCC patients are primarily male even though gender is not a proven statistical risk factor in the progression of NASH to HCC. However, NASH is currently the second leading cause for LT waitlist in females, whereas in men, alcoholic liver disease (ALD) continues to be the leading cause [189]. Although old data of 698 patients from biopsy-proven NASH show that NASH patients are more likely to be female than male possibly reflecting a higher disease burden rate in women [192], it is likely that both gender and racial ethnic differences in NAFLD and NASH are attributed to interaction among genetic, environmental, and lifestyle behaviors.

5. Medical therapy for NAFLD and HCC

The biological heterogeneities of NAFLD and HCC create predicaments in deciphering the key mechanisms of development and progression from NAFLD to ESLD. Although progress is being made in understanding the molecular underpinnings of chronic liver disease and its various offshoots, there are still formidable challenges in providing effective treatment regimens. Aside from a few prophylactic agents that have shown promise in the prevention and treatment of steatohepatitis and fibrosis, there is no treatment consensus due to scarcity of data [140]. Wholesome lifestyle and behavioral changes that include regular physical activity, low caloric intake, and weight loss are the main bulwarks against NAFLD, which may progress to HCC with or without cirrhosis. However, the extent to which these modifications are effective to prevent the development of HCC is unclear. There is currently no effective chemoprevention to decrease the incidence of HCC except using nucleoside analogs to reduce viral replication for those infected with HBV [193] and direct-acting antivirals (DAAs) for those infected with HCV [194], the latter demonstrating very high cure rates but also raising concerns about the recurrence or development of HCC after the achievement of a sustained virological response [195]. Obeticholic acid (OCA), a selective agonist of the Farnesoid X receptors, was touted to be a promising pharmacological drug for the management of NAFLD. However, its low efficacy and specificity have dampened enthusiasm for its practical use. Also, the drug pioglitazone has no long-term impact on NASH. This entails a pressing need to develop more effective and safe agents for NAFLD and HCC. Several other experimental studies suggest a direct role for vitamin D in

modulating liver fibrosis and inflammation by enhancing hepatic response to insulin via binding to vitamin D receptor on liver cells [196–198]. Vitamin E and carotenoids are also shown to decrease plasma levels of patients with NASH [199], whereas dietary antioxidants such as vitamin C and coenzyme Q12, trace minerals such as selenium, anticholesterol medications such as statins, antidiabetic drugs such as metformin, and methyl radical donors such as S-adenosylmethionine have all been touted as potential prophylactic agents [169, 200–202].

6. Key findings, future trends, and unmet needs

Hepatic steatosis is associated with many other morbidities. Therefore, dissecting the myriad causative agents including genetic, hormonal, or environmental factors underlying the pathogenicity of simple hepatic steatosis must be a priority to avoid the maze of complications that may arise during the development of NAFLD and its progression to HCC. The key findings are:

- Global prevalence of NAFLD is at 24% but is rising to greater than 30%; highest rates to lowest rates are found in South America, Middle East, Asia, United States, and Europe.
- The large volume of patients sets NAFLD apart from other liver diseases; thus, clinical care must focus on discerning highest risk of progressive liver disease.
- Overweight in childhood and adolescence is associated with the risk of NAFLD later in life and increases liver-related morbidity and/or mortality.
- NAFLD patients have an elevated risk of liver-related morbidity/mortality and metabolic comorbidities, which place a strain on healthcare systems.
- NAFLD warrants that primary-care physicians, specialists, and health policy-makers stress prevention of excessive weight gain during childhood.
- Bariatric surgery may be an alternative option to committed weight loss.
- Older age, being male, and HA are independent risk factors for NAFLD/NASH.
- NAFLD is linked with higher BMI, higher HTN, and lower physical activity.
- MetS as currently defined is not a good predictor of NAFLD in non-Hispanic blacks (NHB); because in contrast to others, TG level is normal in this group.
- Proton magnetic resonance spectroscopy is currently the best proven alternative tool to biopsy for accurate diagnosis of NAFLD.
- Treatment options require more robust studies on etiology of NAFLD.
- There is no proven medical therapy for NASH.
- Most effective therapeutic strategies include lifestyle changes including diet, exercise, modifying metabolic risk factors, early screening, and intervention.
- Certain genes may be associated with disparities in lipid metabolism.

- Alternative noninvasive markers of NASH may now be available even though there are no proven biomarkers for various stages of the NAFLD spectrum.
- Discovery of new biomolecules during clinical trials and metabolomics studies is crucial for understanding NAFLD/NASH initiation and progression.
- Patients with NASH have a worse prognosis and must be included in clinical trials of new treatments.
- The biological heterogeneity of HCC makes it difficult to assess the key mechanisms of cancer development and thus implement effective therapies.
- Certain genes have been identified to be associated with progression to HCC.

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Conflicts of interest

The author has no conflict of interest.

Abbreviations

AA	African-American
AF	advanced fibrosis
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMI	body mass index
CDC	Centers for Disease Control and Prevention
DAA	direct-acting antiviral
EA	European-American
ESLD	end-stage liver disease
HA	Hispanic-American
HCC	hepatocellular carcinoma
HDL	high-density lipoprotein
HTN	hypertension
IR	insulin resistance
LT	liver transplantation
MetS	metabolic syndrome
NAFL	nonalcoholic fatty liver
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NFS	NAFLD fibrosis score
NAS	NAFLD activity score
NHANES	National Health and Nutrition Examination Survey
NFS	NAFLD fibrosis score
T2DM	type 2 diabetes mellitus
TG	triglyceride
TCGA	the cancer genome atlas

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Author details

Zaki A. Sherif

Department of Biochemistry and Molecular Biology, Howard University College of Medicine, Washington, DC, USA

*Address all correspondence to: zaki.sherif@howard.edu

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References

- [1] Lear SA, Humphries KH, Kohli S, Birmingham CL. The use of BMI and waist circumference as surrogates of body fat differs by ethnicity. *Obesity*. 2007;**15**:2817-2824
- [2] Dongiovanni P, Anstee QM, Valenti L. Genetic predisposition in NAFLD and NASH: Impact on severity of liver disease and response to treatment. *Current Pharmaceutical Design*. 2013;**19**:5219-5238
- [3] Marengo A, Rosso C, Bugianesi E. Liver cancer: Connections with obesity, fatty liver, and cirrhosis. *Annual Review of Medicine*. 2016;**67**:103-117
- [4] Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology*. 2018;**67**:123-133
- [5] Bedossa P, Poitou C, Veyrie N, et al. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. *Hepatology*. 2012;**56**:1751-1759
- [6] Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*. 2005;**41**:1313-1321
- [7] Ballestri S, Nascimbeni F, Baldelli E, Marrazzo A, Romagnoli D, Lonardo A. NAFLD as a sexual dimorphic disease: Role of gender and reproductive status in the development and progression of nonalcoholic fatty liver disease and inherent cardiovascular risk. *Advances in Therapy*. 2017;**34**:1291-1326
- [8] Ogden CL, Carroll MD, Lawman HG, et al. Trends in obesity prevalence among children and adolescents in the United States, 1988-1994 through 2013-2014. *Journal of the American Medical Association*. 2016;**315**:2292-2299
- [9] Flegal KM, Kruszon-Moran D, Carroll MD, Fryar CD, Ogden CL. Trends in obesity among adults in the United States, 2005 to 2014. *Journal of the American Medical Association*. 2016;**315**:2284-2291
- [10] Malik SM, Ahmad J. Outcomes of liver transplantation in patients with cirrhosis due to nonalcoholic steatohepatitis versus patients with cirrhosis due to alcoholic liver disease. *Liver Transplantation*. 2010;**16**:533
- [11] Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: From steatosis to cirrhosis. *Hepatology*. 2006;**43**:S99-S112
- [12] Benedict M, Zhang X. Non-alcoholic fatty liver disease: An expanded review. *World Journal of Hepatology*. 2017;**9**:715-732
- [13] Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nature Reviews Gastroenterology & Hepatology*. 2013;**10**:686-690
- [14] Sayiner M, Koenig A, Henry L, Younossi ZM. Epidemiology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in the United States and the rest of the world. *Clinics in Liver Disease*. 2016;**20**:205-214
- [15] Ruhl CE, Everhart JE. Fatty liver indices in the multiethnic United States National Health and Nutrition Examination Survey. *Alimentary Pharmacology & Therapeutics*. 2015;**41**:65-76
- [16] Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: Trends, predictions, risk factors and prevention. *Nature Reviews Gastroenterology & Hepatology*. 2018;**15**:11-20
- [17] GBD 2016 Alcohol Collaborators. Alcohol use and burden for 195 countries and territories, 1990-2016:

A systematic analysis for the global burden of disease study 2016. *Lancet*. 2018;**392**:1015-1035

[18] Hajifathalian K, Torabi Sagvand B, McCullough AJ. Effect of alcohol consumption on survival in nonalcoholic fatty liver disease: A National Prospective Cohort Study. *Hepatology*. 2018. DOI: 10.1002/hep.30226

[19] Younossi ZM, Stepanova M, Ong J, et al. Effects of alcohol consumption and metabolic syndrome on mortality in patients with non-alcoholic and alcohol-related fatty liver disease. *Clinical Gastroenterology and Hepatology*. 2018;**17**:1625-1633

[20] Wood AM, Kaptoge S, Butterworth AS, et al. Risk thresholds for alcohol consumption: Combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. *Lancet*. 2018;**391**:1513-1523

[21] Mantovani A. Time to revise the definition of NAFLD: A purist vision. *Digestive and Liver Disease*. 2019;**51**:457-458

[22] Gao X, Fan JG, Study Group of Liver and Metabolism, Chinese Society of Endocrinology. Diagnosis and management of non-alcoholic fatty liver disease and related metabolic disorders: Consensus statement from the Study Group of Liver and Metabolism, Chinese Society of Endocrinology. *Journal of Diabetes*. 2013;**5**:406-415

[23] Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology*. 2012;**142**:1592-1609

[24] Cobbold JF, Anstee QM, Thomas HC. Investigating mildly abnormal

serum aminotransferase values. *British Medical Journal*. 2010;**341**:c4039

[25] Farrell GC, Chitturi S, Lau GK, Sollano JD. Asia-Pacific working party on NAFLD. Guidelines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pacific region: Executive summary. *Journal of Gastroenterology and Hepatology*. 2007;**22**:775-777

[26] Pais R, Rusu E, Zilisteanu D, et al. Prevalence of steatosis and insulin resistance in patients with chronic hepatitis B compared with chronic hepatitis C and non-alcoholic fatty liver disease. *European Journal of Internal Medicine*. 2015;**26**:30-36

[27] Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Digestive Diseases*. 2010;**28**:155-161

[28] Sattar N, Forrest E, Preiss D. Non-alcoholic fatty liver disease. *British Medical Journal*. 2014;**349**:g4596

[29] Sakhuja P. Pathology of alcoholic liver disease, can it be differentiated from nonalcoholic steatohepatitis? *World Journal of Gastroenterology*. 2014;**20**:16474-16479

[30] Neuman MG, French SW, French BA, et al. Alcoholic and non-alcoholic steatohepatitis. *Experimental and Molecular Pathology*. 2014;**97**:492-510

[31] Dumitrascu DL, Neuman MG. Non-alcoholic fatty liver disease: An update on diagnosis. *Clujul Medical*. 2018;**91**:147-150

[32] Powell EE, Jonsson JR, Clouston AD. Steatosis: Co-factor in other liver diseases. *Hepatology*. 2005;**42**:5-13

[33] Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver fibrosis, but no other histologic features, is associated

with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology*. 2015;**149**:389-397.e10

[34] Adams LA, Lymp JF, St Sauver J, et al. The natural history of nonalcoholic fatty liver disease: A population-based cohort study. *Gastroenterology*. 2005;**129**:113-121

[35] Ekstedt M, Franzen LE, Mathiesen UL, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology*. 2006;**44**:865-873

[36] Ekstedt M, Hagstrom H, Nasr P, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology*. 2015;**61**:1547-1554

[37] Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: A spectrum of clinical and pathological severity. *Gastroenterology*. 1999;**116**:1413-1419

[38] Teli MR, James OF, Burt AD, Bennett MK, Day CP. The natural history of nonalcoholic fatty liver: A follow-up study. *Hepatology*. 1995;**22**:1714-1719

[39] Dyson JK, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: A practical approach to diagnosis and staging. *Frontline Gastroenterology*. 2014;**5**:211-218

[40] Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity. *Hepatology*. 2004;**40**:1387-1395

[41] Wong VW, Chu WC, Wong GL, et al. Prevalence of non-alcoholic fatty liver disease and advanced fibrosis in Hong Kong Chinese: A population study using proton-magnetic resonance spectroscopy and transient elastography. *Gut*. 2012;**61**:409-415

[42] Sanyal AJ, Banas C, Sargeant C, et al. Similarities and differences in outcomes of cirrhosis due to nonalcoholic steatohepatitis and hepatitis C. *Hepatology*. 2006;**43**:682-689

[43] Lindvig KP, Teisner AS, Kjeldsen J, et al. Allocation of patients with liver cirrhosis and organ failure to intensive care: Systematic review and a proposal for clinical practice. *World Journal of Gastroenterology*. 2015;**21**:8964-8973

[44] Turchinovich A, Baranova A, Drapkina O, Tonevitsky A. Cell-free circulating nucleic acids as early biomarkers for NAFLD and NAFLD-associated disorders. *Frontiers in Physiology*. 2018;**9**:1256

[45] Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: Summary of an AASLD single topic conference. *Hepatology*. 2003;**37**:1202-1219

[46] American Gastroenterological Association. American Gastroenterological Association medical position statement: Nonalcoholic fatty liver disease. *Gastroenterology*. 2002;**123**:1702-1704

[47] Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: Predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology*. 2001;**121**:91-100

[48] Strauss S, Gavish E, Gottlieb P, Katsnelson L. Interobserver and intraobserver variability in the sonographic assessment of fatty liver. *AJR. American Journal of Roentgenology*. 2007;**189**:W320-W323

[49] Saadeh S, Younossi ZM, Remer EM, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology*. 2002;**123**:745-750

- [50] Schwenzer NF, Springer F, Schraml C, Stefan N, Machann J, Schick F. Non-invasive assessment and quantification of liver steatosis by ultrasound, computed tomography and magnetic resonance. *Journal of Hepatology*. 2009;**51**:433-445
- [51] McPherson S, Jonsson JR, Cowin GJ, et al. Magnetic resonance imaging and spectroscopy accurately estimate the severity of steatosis provided the stage of fibrosis is considered. *Journal of Hepatology*. 2009;**51**:389-397
- [52] Li Q, Dhyani M, Grajo JR, Sirlin C, Samir AE. Current status of imaging in nonalcoholic fatty liver disease. *World Journal of Hepatology*. 2018;**10**:530-542
- [53] Lee SS, Park SH. Radiologic evaluation of nonalcoholic fatty liver disease. *World Journal of Gastroenterology*. 2014;**20**:7392-7402
- [54] Wang Y, Fan Q, Wang T, Wen J, Wang H, Zhang T. Controlled attenuation parameter for assessment of hepatic steatosis grades: A diagnostic meta-analysis. *International Journal of Clinical and Experimental Medicine*. 2015;**8**:17654-17663
- [55] Streba LA, Vere CC, Rogoveanu I, Streba CT. Nonalcoholic fatty liver disease, metabolic risk factors, and hepatocellular carcinoma: An open question. *World Journal of Gastroenterology*. 2015;**21**:4103-4110
- [56] Bril F, Cusi K. Management of nonalcoholic fatty liver disease in patients with type 2 diabetes: A call to action. *Diabetes Care*. 2017;**40**:419-430
- [57] Mofrad P, Contos MJ, Haque M, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. *Hepatology*. 2003;**37**:1286-1292
- [58] Yano E, Tagawa K, Yamaoka K, Mori M. Test validity of periodic liver function tests in a population of Japanese male bank employees. *Journal of Clinical Epidemiology*. 2001;**54**:945-951
- [59] Martin-Rodriguez JL, Gonzalez-Cantero J, Gonzalez-Cantero A, Arrebola JP, Gonzalez-Calvin JL. Diagnostic accuracy of serum alanine aminotransferase as biomarker for nonalcoholic fatty liver disease and insulin resistance in healthy subjects, using 3T MR spectroscopy. *Medicine*. 2017;**96**:e6770
- [60] Oh RC, Hustead TR, Ali SM, Pantsari MW. Mildly elevated liver transaminase levels: Causes and evaluation. *American Family Physician*. 2017;**96**:709-715
- [61] Sherif ZA, Saeed A, Ghavimi S, et al. Global epidemiology of nonalcoholic fatty liver disease and perspectives on US minority populations. *Digestive Diseases and Sciences*. 2016;**61**:1214-1225
- [62] Feldstein AE, Canbay A, Angulo P, et al. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. *Gastroenterology*. 2003;**125**:437-443
- [63] Bantel H, Ruck P, Gregor M, Schulze-Osthoff K. Detection of elevated caspase activation and early apoptosis in liver diseases. *European Journal of Cell Biology*. 2001;**80**:230-239
- [64] Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: A multicenter validation study. *Hepatology*. 2009;**50**:1072-1078
- [65] Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of

prevalence, incidence, and outcomes. *Hepatology*. 2016;**64**:73-84

[66] Merriman RB, Ferrell LD, Patti MG, et al. Correlation of paired liver biopsies in morbidly obese patients with suspected nonalcoholic fatty liver disease. *Hepatology*. 2006;**44**:874-880

[67] Bhatt HB, Smith RJ. Fatty liver disease in diabetes mellitus. *Hepatobiliary Surgery and Nutrition*. 2015;**4**:101-108

[68] Pinto RB, Schneider AC, da Silveira TR. Cirrhosis in children and adolescents: An overview. *World Journal of Hepatology*. 2015;**7**:392-405

[69] Anstee QM, McPherson S, Day CP. How big a problem is non-alcoholic fatty liver disease? *British Medical Journal*. 2011;**343**:d3897

[70] Sandrin L, Fourquet B, Hasquenoph JM, et al. Transient elastography: A new noninvasive method for assessment of hepatic fibrosis. *Ultrasound in Medicine & Biology*. 2003;**29**:1705-1713

[71] Dahl JJ, Pinton GF, Palmeri ML, Agrawal V, Nightingale KR, Trahey GE. A parallel tracking method for acoustic radiation force impulse imaging. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*. 2007;**54**:301-312

[72] Gregg EW, Boyle JP, Thompson TJ, Barker LE, Albright AL, Williamson DF. Modeling the impact of prevention policies on future diabetes prevalence in the United States: 2010-2030. *Population Health Metrics*. 2013;**11**:18

[73] Koehler EM, Plompen EP, Schouten JN, et al. Presence of diabetes mellitus and steatosis is associated with liver stiffness in a general population: The Rotterdam study. *Hepatology*. 2016;**63**:138-147

[74] Rosenberg WM, Voelker M, Thiel R, et al. Serum markers detect the presence

of liver fibrosis: A cohort study. *Gastroenterology*. 2004;**127**:1704-1713

[75] Rotman Y, Koh C, Zmuda JM, Kleiner DE, Liang TJ, NASH CRN. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. *Hepatology*. 2010;**52**:894-903

[76] Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nature Reviews Gastroenterology & Hepatology*. 2013;**10**:330-344

[77] Demir M, Lang S, Steffen HM. Nonalcoholic fatty liver disease: Current status and future directions. *Journal of Digestive Diseases*. 2015;**16**:541-557

[78] Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. *The Journal of Clinical Endocrinology and Metabolism*. 2004;**89**:2595-2600

[79] Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: An American Heart Association/National Heart, Lung, and Blood Institute scientific statement: Executive summary. *Critical Pathways in Cardiology*. 2005;**4**:198-203

[80] Hassan MM, Curley SA, Li D, et al. Association of diabetes duration and diabetes treatment with the risk of hepatocellular carcinoma. *Cancer*. 2010;**116**:1938-1946

[81] American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2009;**32** (Suppl 1):S62-S67

[82] Ballestri S, Zona S, Targher G, et al. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from

a systematic review and meta-analysis. *Journal of Gastroenterology and Hepatology*. 2016;**31**:936-944

[83] Ahmed MH, Abu EO, Byrne CD. Non-alcoholic fatty liver disease (NAFLD): New challenge for general practitioners and important burden for health authorities? *Primary Care Diabetes*. 2010;**4**:129-137

[84] Portillo-Sanchez P, Bril F, Maximos M, et al. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. *The Journal of Clinical Endocrinology and Metabolism*. 2015;**100**:2231-2238

[85] Adams LA, Waters OR, Knudman MW, Elliott RR, Olynyk JK. NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: An eleven-year follow-up study. *The American Journal of Gastroenterology*. 2009;**104**:861-867

[86] McCullough AJ. Epidemiology of the metabolic syndrome in the USA. *Journal of Digestive Diseases*. 2011;**12**:333-340

[87] Ruan H, Lodish HF. Insulin resistance in adipose tissue: Direct and indirect effects of tumor necrosis factor- α . *Cytokine & Growth Factor Reviews*. 2003;**14**:447-455

[88] Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF- α function. *Nature*. 1997;**389**:610-614

[89] Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology*. 1999;**30**:1356-1362

[90] Friedenberg F, Pungpapong S, Zaeri N, Braitman LE. The impact of diabetes

and obesity on liver histology in patients with hepatitis C. *Diabetes, Obesity & Metabolism*. 2003;**5**:150-155

[91] Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology*. 2001;**33**:1358-1364

[92] Pekow JR, Bhan AK, Zheng H, Chung RT. Hepatic steatosis is associated with increased frequency of hepatocellular carcinoma in patients with hepatitis C-related cirrhosis. *Cancer*. 2007;**109**:2490-2496

[93] Leandro G, Mangia A, Hui J, et al. Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: A meta-analysis of individual patient data. *Gastroenterology*. 2006;**130**:1636-1642

[94] Afsari A, Lee E, Shokrani B, et al. Clinical and pathological risk factors associated with liver fibrosis and steatosis in African-Americans with chronic hepatitis C. *Digestive Diseases and Sciences*. 2017;**62**:2159-2165

[95] Bambha K, Belt P, Abraham M, et al. Ethnicity and nonalcoholic fatty liver disease. *Hepatology*. 2012;**55**:769-780

[96] Pan JJ, Fallon MB. Gender and racial differences in nonalcoholic fatty liver disease. *World Journal of Hepatology*. 2014;**6**:274-283

[97] Sliz E, Sebert S, Wurtz P, et al. NAFLD risk alleles in PNPLA3, TM6SF2, GCKR and LYPLAL1 show divergent metabolic effects. *Human Molecular Genetics*. 2018;**27**:2214-2223

[98] Yang Z, Wen J, Tao X, et al. Genetic variation in the GCKR gene is associated with non-alcoholic fatty liver disease in Chinese people. *Molecular Biology Reports*. 2011;**38**:1145-1150

- [99] Bruschi FV, Tardelli M, Claudel T, Trauner M. PNPLA3 expression and its impact on the liver: Current perspectives. *Hepatic Medicine : Evidence And Research*. 2017;**9**:55-66
- [100] Palmer ND, Musani SK, Yerges-Armstrong LM, et al. Characterization of European ancestry nonalcoholic fatty liver disease-associated variants in individuals of African and Hispanic descent. *Hepatology*. 2013;**58**:966-975
- [101] Romeo S, Huang-Doran I, Baroni MG, Kotronen A. Unravelling the pathogenesis of fatty liver disease: Patatin-like phospholipase domain-containing 3 protein. *Current Opinion in Lipidology*. 2010;**21**:247-252
- [102] Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nature Genetics*. 2008;**40**:1461-1465
- [103] Singal AG, Manjunath H, Yopp AC, et al. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: A meta-analysis. *The American Journal of Gastroenterology*. 2014;**109**:325-334
- [104] Kozlitina J, Smagris E, Stender S, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nature Genetics*. 2014;**46**:352-356
- [105] Britton LJ, Subramaniam VN, Crawford DH. Iron and non-alcoholic fatty liver disease. *World Journal of Gastroenterology*. 2016;**22**:8112-8122
- [106] Miyake T, Kumagi T, Furukawa S, et al. Non-alcoholic fatty liver disease: Factors associated with its presence and onset. *Journal of Gastroenterology and Hepatology*. 2013;**28**(Suppl 4):71-78
- [107] Zhao F. Dysregulated epigenetic modifications in the pathogenesis of NAFLD-HCC. *Advances in Experimental Medicine and Biology*. 2018;**1061**:79-93
- [108] de Mello VD, Matte A, Perfilyev A, et al. Human liver epigenetic alterations in non-alcoholic steatohepatitis are related to insulin action. *Epigenetics*. 2017;**12**:287-295
- [109] Cordero P, Campion J, Milagro FI, Martinez JA. Transcriptomic and epigenetic changes in early liver steatosis associated to obesity: Effect of dietary methyl donor supplementation. *Molecular Genetics and Metabolism*. 2013;**110**:388-395
- [110] Mouralidarane A, Soeda J, Sugden D, et al. Maternal obesity programs offspring non-alcoholic fatty liver disease through disruption of 24-h rhythms in mice. *International Journal of Obesity*. 2015;**39**:1339-1348
- [111] Oben JA, Mouralidarane A, Samuelsson AM, et al. Maternal obesity during pregnancy and lactation programs the development of offspring non-alcoholic fatty liver disease in mice. *Journal of Hepatology*. 2010;**52**:913-920
- [112] Aron-Wisnewsky J, Gaborit B, Dutour A, Clement K. Gut microbiota and non-alcoholic fatty liver disease: New insights. *Clinical Microbiology and Infection*. 2013;**19**:338-348
- [113] Henao-Mejia J, Elinav E, Jin C, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature*. 2012;**482**:179-185
- [114] Gerhard GS, Malenica I, Llaci L, et al. Differentially methylated loci in NAFLD cirrhosis are associated with key signaling pathways. *Clinical Epigenetics*. 2018;**10**:93
- [115] Garcia-Monzon C, Petrov PD, Rey E, et al. Angiopoietin-like protein 8 is

- a novel vitamin D receptor target gene involved in non-alcoholic fatty liver pathogenesis. *The American Journal of Pathology*. 2018;**188**:2800-2810
- [116] Blond E, Disse E, Cuerq C, et al. EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease in severely obese people: Do they lead to over-referral? *Diabetologia*. 2017;**60**:1218-1222
- [117] Nasr P, Ignatova S, Kechagias S, Ekstedt M. Natural history of nonalcoholic fatty liver disease: A prospective follow-up study with serial biopsies. *Hepatology Communications*. 2017;**2**:199-210
- [118] Lindenmeyer CC, McCullough AJ. The natural history of nonalcoholic fatty liver disease-an evolving view. *Clinics in Liver Disease*. 2018;**22**:11-21
- [119] Guerrero R, Vega GL, Grundy SM, Browning JD. Ethnic differences in hepatic steatosis: An insulin resistance paradox? *Hepatology*. 2009;**49**:791-801
- [120] Angulo P. Nonalcoholic fatty liver disease. *The New England Journal of Medicine*. 2002;**346**:1221-1231
- [121] Rinella ME. Nonalcoholic fatty liver disease: A systematic review. *Journal of the American Medical Association*. 2015;**313**:2263-2273
- [122] Lazo M, Hernaez R, Eberhardt MS, et al. Prevalence of nonalcoholic fatty liver disease in the United States: The third National Health and Nutrition Examination Survey, 1988-1994. *American Journal of Epidemiology*. 2013;**178**:38-45
- [123] Younossi ZM, Otgonsuren M, Henry L, et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology*. 2015;**62**:1723-1730
- [124] Pais R, Barritt AS 4th, Calmus Y, et al. NAFLD and liver transplantation: Current burden and expected challenges. *Journal of Hepatology*. 2016;**65**:1245-1257
- [125] White DL, Kanwal F, El-Serag HB. Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. *Clinical Gastroenterology and Hepatology*. 2012;**10**:1342-1359.e2
- [126] Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: A weighty connection. *Hepatology*. 2010;**51**:1820-1832
- [127] Kawada N, Imanaka K, Kawaguchi T, et al. Hepatocellular carcinoma arising from non-cirrhotic nonalcoholic steatohepatitis. *Journal of Gastroenterology*. 2009;**44**:1190-1194
- [128] Castera L, Friedrich-Rust M, Loomba R. Non-invasive assessment of liver disease in patients with NAFLD. *Gastroenterology*. 2019;**156**:1264-1281
- [129] Papastergiou V, Tsochatzis E, Burroughs AK. Non-invasive assessment of liver fibrosis. *Annals of Gastroenterology*. 2012;**25**:218-231
- [130] Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: A noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology*. 2007;**45**:846-854
- [131] Matsushita N, Hassanein MT, Martinez-Clemente M, et al. Gender difference in NASH susceptibility: Roles of hepatocyte Ikkbeta and Sult1e1. *PLoS One*. 2017;**12**:e0181052. DOI: 10.1371/journal.pone.0181052
- [132] Machado MV, Cortez-Pinto H. Non-invasive diagnosis of non-alcoholic fatty liver disease. A critical

appraisal. *Journal of Hepatology*. 2013;**58**:1007-1019

[133] Sookoian S, Castano GO, Burgueno AL, Gianotti TF, Rosselli MS, Pirola CJ. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. *Journal of Lipid Research*. 2009;**50**:2111-2116

[134] Ouyang X, Cirillo P, Sautin Y, et al. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *Journal of Hepatology*. 2008;**48**:993-999

[135] Musso G, Gambino R, De Michieli F, et al. Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. *Hepatology*. 2003;**37**:909-916

[136] Cortez-Pinto H, Jesus L, Barros H, Lopes C, Moura MC, Camilo ME. How different is the dietary pattern in non-alcoholic steatohepatitis patients? *Clinical Nutrition*. 2006;**25**:816-823

[137] Musso G, Cassader M, Olivetti C, Rosina F, Carbone G, Gambino R. Association of obstructive sleep apnoea with the presence and severity of non-alcoholic fatty liver disease. A systematic review and meta-analysis. *Obesity Reviews*. 2013;**14**:417-431

[138] Byrne CD, Olufadi R, Bruce KD, Cagampang FR, Ahmed MH. Metabolic disturbances in non-alcoholic fatty liver disease. *Clinical Science*. 2009;**116**:539-564

[139] Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: A prospective study. *Gastroenterology*. 2011;**140**:124-131

[140] Cholanckeril G, Patel R, Khurana S, Satapathy SK. Hepatocellular carcinoma

in non-alcoholic steatohepatitis: Current knowledge and implications for management. *World Journal of Hepatology*. 2017;**9**:533-543

[141] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA: A Cancer Journal for Clinicians*. 2015;**65**:87-108

[142] El-Serag HB. Hepatocellular carcinoma. *The New England Journal of Medicine*. 2011;**365**:1118-1127

[143] Zucman-Rossi J, Villanueva A, Nault JC, Llovet JM. Genetic landscape and biomarkers of hepatocellular carcinoma. *Gastroenterology*. 2015;**149**:1226-1239.e4

[144] Llovet JM, Zucman-Rossi J, Pikarsky E, et al. Hepatocellular carcinoma. *Nature Reviews Disease Primers*. 2016;**2**:16018

[145] McGlynn KA, London WT. The global epidemiology of hepatocellular carcinoma: Present and future. *Clinics in Liver Disease*. 2011;**15**:223-243, vii-x

[146] Pittman ME, Brunt EM. Anatomic pathology of hepatocellular carcinoma: Histopathology using classic and new diagnostic tools. *Clinics in Liver Disease*. 2015;**19**:239-259

[147] Rastogi A. Changing role of histopathology in the diagnosis and management of hepatocellular carcinoma. *World Journal of Gastroenterology*. 2018;**24**:4000-4013

[148] Margini C, Dufour JF. The story of HCC in NAFLD: From epidemiology, across pathogenesis, to prevention and treatment. *Liver International*. 2016;**36**:317-324

[149] Michelotti GA, Machado MV, Diehl AM. NAFLD, NASH and liver cancer. *Nature Reviews Gastroenterology & Hepatology*. 2013;**10**:656-665

- [150] Baffy G, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: An emerging menace. *Journal of Hepatology*. 2012;**56**:1384-1391
- [151] Marrero JA, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, Lok AS. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. *Hepatology*. 2002;**36**:1349-1354
- [152] Bugianesi E, Leone N, Vanni E, et al. Expanding the natural history of nonalcoholic steatohepatitis: From cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology*. 2002;**123**:134-140
- [153] Singh AK, Kumar R, Pandey AK. Hepatocellular carcinoma: Causes, mechanism of progression and biomarkers. *Current Chemical Genomics and Translational Medicine*. 2018;**12**:9-26
- [154] Jayachandran M. An updated portrait of pathogenesis, molecular markers and signaling pathways of hepatocellular carcinoma. *Current Pharmaceutical Design*. 2017;**23**:2356-2365
- [155] Liu Y, Yang Z, Du F, et al. Molecular mechanisms of pathogenesis in hepatocellular carcinoma revealed by RNA-sequencing. *Molecular Medicine Reports*. 2017;**16**:6674-6682
- [156] Chen J, Qian Z, Li F, Li J, Lu Y. Integrative analysis of microarray data to reveal regulation patterns in the pathogenesis of hepatocellular carcinoma. *Gut and Liver*. 2017;**11**:112-120
- [157] Cancer Genome Atlas Research Network. Electronic address: wheeler@bcm.edu, Cancer Genome Atlas Research Network. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell*. 2017;**169**:1327-1341.e23
- [158] Huang MN, Yu W, Teoh WW, et al. Genome-scale mutational signatures of aflatoxin in cells, mice, and human tumors. *Genome Research*. 2017;**27**:1475-1486
- [159] Tahmasebi Birgani M, Carloni V. Tumor microenvironment, a paradigm in hepatocellular carcinoma progression and therapy. *International Journal of Molecular Sciences*. 2017;**18**:pii.E405. DOI: 10.3390/ijms18020405
- [160] Meng C, Shen X, Jiang W. Potential biomarkers of HCC based on gene expression and DNA methylation profiles. *Oncology Letters*. 2018;**16**:3183-3192
- [161] De Mattia E, Cecchin E, Polesel J, et al. Genetic biomarkers for hepatocellular cancer risk in a caucasian population. *World Journal of Gastroenterology*. 2017;**23**:6674-6684
- [162] Li B, Xu T, Liu C, et al. Liver-enriched genes are associated with the prognosis of patients with hepatocellular carcinoma. *Scientific Reports*. 2018;**8**:11197
- [163] Xu J, Wu C, Che X, et al. Circulating microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. *Molecular Carcinogenesis*. 2011;**50**:136-142
- [164] Wong CM, Tsang FH, Ng IO. Non-coding RNAs in hepatocellular carcinoma: Molecular functions and pathological implications. *Nature Reviews Gastroenterology & Hepatology*. 2018;**15**:137-151
- [165] Rinella ME. Will the increased prevalence of nonalcoholic steatohepatitis (NASH) in the age of better hepatitis C virus therapy make NASH the deadlier disease? *Hepatology*. 2011;**54**:1118-1120
- [166] Armstrong MJ, Houlihan DD, Bentham L, et al. Presence and severity

of non-alcoholic fatty liver disease in a large prospective primary care cohort. *Journal of Hepatology*. 2012;**56**:234-240

[167] Ofosu A, Ramai D, Reddy M. Non-alcoholic fatty liver disease: Controlling an emerging epidemic, challenges, and future directions. *Annals of Gastroenterology*. 2018;**31**:288-295

[168] Goldberg D, Ditah IC, Saeian K, et al. Changes in the prevalence of hepatitis C virus infection, nonalcoholic steatohepatitis, and alcoholic liver disease among patients with cirrhosis or liver failure on the waitlist for liver transplantation. *Gastroenterology*. 2017;**152**:1090-1099.e1

[169] Nouredin M, Rinella ME. Nonalcoholic fatty liver disease, diabetes, obesity, and hepatocellular carcinoma. *Clinics in Liver Disease*. 2015;**19**:361-379

[170] Wong RJ, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology*. 2014;**59**:2188-2195

[171] Portillo Sanchez P, Bril F, Maximos M, et al. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. *The Journal of Clinical Endocrinology and Metabolism*. 2014;**100**:2231-2238. DOI: 10.1210/jc.2014-2739

[172] Parikh ND, Marrero WJ, Wang J, et al. Projected increase in obesity and non-alcoholic-steatohepatitis-related liver transplantation waitlist additions in the United States. *Hepatology*. 2017. DOI: 10.1002/hep.29473

[173] Schneider AL, Lazo M, Selvin E, Clark JM. Racial differences in nonalcoholic fatty liver disease in the U.S. population. *Obesity*. 2014;**22**:292-299

[174] Weston SR, Leyden W, Murphy R, et al. Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. *Hepatology*. 2005;**41**:372-379

[175] Caldwell SH, Harris DM, Patrie JT, Hespeneide EE. Is NASH underdiagnosed among African Americans? *The American Journal of Gastroenterology*. 2002;**97**:1496-1500

[176] Morgan C, Mallett R, Hutchinson G, et al. Pathways to care and ethnicity. 2: Source of referral and help-seeking. Report from the AESOP study. *The British Journal of Psychiatry*. 2005;**186**:290-296

[177] Di Poto C, He S, Varghese RS, et al. Identification of race-associated metabolite biomarkers for hepatocellular carcinoma in patients with liver cirrhosis and hepatitis C virus infection. *PLoS One*. 2018;**13**:e0192748

[178] Banales JM, Inarrairaegui M, Arbelaiz A, et al. Serum metabolites as diagnostic biomarkers for cholangiocarcinoma, hepatocellular carcinoma, and primary sclerosing cholangitis. *Hepatology*. 2018. Available from: <https://doi.org/10.1002/hep.30319>

[179] Khattab M, Fouad M, Ahmed E. Role of biomarkers in the prediction and diagnosis of hepatocellular carcinoma. *World Journal of Hepatology*. 2015;**7**:2474-2481

[180] Ferrarini A, Di Poto C, He S, et al. Metabolomic analysis of liver tissues for characterization of hepatocellular carcinoma. *Journal of Proteome Research*. 2019. DOI: 10.1021/acs.jproteome.9b00185

[181] Ferrin G, Aguilar-Melero P, Rodriguez-Peralvarez M, Montero-Alvarez JL, de la Mata M. Biomarkers for hepatocellular carcinoma: Diagnostic and therapeutic utility. *Hepatic Medicine: Evidence And Research*. 2015;**7**:1-10

- [182] Kelly TK, De Carvalho DD, Jones PA. Epigenetic modifications as therapeutic targets. *Nature Biotechnology*. 2010;**28**:1069-1078
- [183] Vernon G, Baranova A, Younossi ZM. Systematic review: The epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Alimentary Pharmacology & Therapeutics*. 2011;**34**:274-285
- [184] Sumner AE, Cowie CC. Ethnic differences in the ability of triglyceride levels to identify insulin resistance. *Atherosclerosis*. 2008;**196**:696-703
- [185] Sumner AE, Vega GL, Genovese DJ, Finley KB, Bergman RN, Boston RC. Normal triglyceride levels despite insulin resistance in African Americans: Role of lipoprotein lipase. *Metabolism*. 2005;**54**:902-909
- [186] Giannini E, Testa R. The metabolic syndrome: All criteria are equal, but some criteria are more equal than others. *Archives of Internal Medicine*. 2003;**163**:2787-2788. Author reply 2788
- [187] Grohmann M, Wiede F, Dodd GT, et al. Obesity drives STAT-1-dependent NASH and STAT-3-dependent HCC. *Cell*. 2018;**175**:1289-1306.e20
- [188] Nakagawa H, Hayata Y, Kawamura S, Yamada T, Fujiwara N, Koike K. Lipid metabolic reprogramming in hepatocellular carcinoma. *Cancers*. 2018;**10**:447. DOI: 10.3390/cancers10110447
- [189] Nouredin M, Vipani A, Bresee C, et al. NASH leading cause of liver transplant in women: Updated analysis of indications for liver transplant and ethnic and gender variances. *The American Journal of Gastroenterology*. 2018;**113**:1649-1659
- [190] Ruhl CE, Everhart JE. Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. *Gastroenterology*. 2003;**124**:71-79
- [191] Fleischman MW, Budoff M, Zeb I, Li D, Foster T. NAFLD prevalence differs among hispanic subgroups: The multi-ethnic study of atherosclerosis. *World Journal of Gastroenterology*. 2014;**20**:4987-4993
- [192] Neuschwander-Tetri BA, Clark JM, Bass NM, et al. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. *Hepatology*. 2010;**52**:913-924
- [193] Hosaka T, Suzuki F, Kobayashi M, et al. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. *Hepatology*. 2013;**58**:98-107
- [194] Moon AM, Green PK, Berry K, Ioannou GN. Transformation of hepatitis C antiviral treatment in a national healthcare system following the introduction of direct antiviral agents. *Alimentary Pharmacology & Therapeutics*. 2017;**45**:1201-1212
- [195] Tampaki M, Savvanis S, Koskinas J. Impact of direct-acting antiviral agents on the development of hepatocellular carcinoma: Evidence and pathophysiological issues. *Annals of Gastroenterology*. 2018;**31**:670-679
- [196] Barchetta I, Carotti S, Labbadia G, et al. Liver vitamin D receptor, CYP2R1, and CYP27A1 expression: Relationship with liver histology and vitamin D3 levels in patients with nonalcoholic steatohepatitis or hepatitis C virus. *Hepatology*. 2012;**56**:2180-2187
- [197] Eliades M, Spyrou E. Vitamin D: A new player in non-alcoholic fatty liver disease? *World Journal of Gastroenterology*. 2015;**21**:1718-1727
- [198] Beilfuss A, Sowa JP, Sydor S, et al. Vitamin D counteracts fibrogenic

TGF-beta signalling in human hepatic stellate cells both receptor-dependently and independently. *Gut*. 2015;**64**:791-799

[199] Erhardt A, Stahl W, Sies H, Lirussi F, Donner A, Haussinger D. Plasma levels of vitamin E and carotenoids are decreased in patients with nonalcoholic steatohepatitis (NASH). *European Journal of Medical Research*. 2011;**16**:76-78

[200] Montella M, Crispo A, Giudice A. HCC, diet and metabolic factors: Diet and HCC. *Hepatitis Monthly*. 2011;**11**:159-162

[201] Bhat M, Sonenberg N, Gores GJ. The mTOR pathway in hepatic malignancies. *Hepatology*. 2013;**58**:810-818

[202] Dongiovanni P, Petta S, Mannisto V, et al. Statin use and non-alcoholic steatohepatitis in at risk individuals. *Journal of Hepatology*. 2015;**63**:705-712