Title: Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention

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Author contributions

The authors contributed equally to all aspects of this article.

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The authors declare no competing interests for this manuscript.

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Review criteria

PubMed was searched for articles published between 1990 and 2017 using the keywords “non-alcoholic fatty liver disease”, “steatohepatitis” or “fatty liver” combined with “prevalence”, “incidence”, “natural history”, “gene”, “lifestyle”, “lean”, or “children”. Articles published in languages other than English were excluded from the analysis.

Key points:

- Non Alcoholic Fatty Liver Disease (NAFLD) has gained clinical recognition as one of the most important causes of liver disease worldwide both in adults and children as a consequence of the pandemic spread of obesity, though some patients are lean.
- The global prevalence of NAFLD in the general population is currently estimated to be 24%, but the highest rates are reported from South America and the Middle East, followed by Asia, United States and Europe, whereas NAFLD is less common in Africa.
- What clearly sets NAFLD apart from other common liver diseases is the sheer volume of patients. In this context, the major focus of clinical care is discerning NAFLD subjects at highest risk for progressive liver disease
- Being overweight in childhood/late adolescence is associated with an increased risk of liver disease due to NAFLD later in life. As a consequence, the threshold of liver-related morbidity and/or mortality is reached at a younger age.
- NAFLD subjects have a high risk of liver-related morbidity/mortality along with metabolic comorbidities and may place a growing strain on health systems from their need for management.
- NAFLD is a complex disease, affected by inter-related environmental factors and genetic predisposition which varies in different regions of the world. Knowledge of the exact contribution of each of the genetic or environmental components in promoting the burden of NAFLD should be a priority in the research agenda.
- While waiting for effective therapies, NAFLD warrants the attention of primary care physicians, specialists and health policy makers, starting from prevention of excessive weight gain during childhood.

Abstract

NAFLD is one of the most important causes of liver disease worldwide and is likely to emerge as the leading cause of end-stage liver disease (ESLD) in the coming decades, with the disease affecting both adults and children. The epidemiology and demographic characteristics of NAFLD vary worldwide, usually parallel to the prevalence of obesity, but a significant proportion of patients are lean. The large number of NAFLD patients with potential for progressive liver disease creates challenges for screening, as the diagnosis of NASH necessitates invasive liver biopsy. Furthermore, NAFLD subjects have a high frequency of metabolic comorbidities and may place a growing strain on health systems from their need for management. While waiting for effective therapies, this liver disease warrants the attention of primary care physicians, specialists and health policy makers.

Introduction

During the last century, dramatic modifications in lifestyle have radically changed the health priorities in most areas of the world. The new epidemic in chronic liver disease is related to the burden of Nonalcoholic fatty liver disease (NAFLD), paralleling the worldwide increase of obesity. The global prevalence of NAFLD is currently estimated to be 24%.[1] Community surveys utilizing ultrasonography or proton NMR spectroscopy have assessed the prevalence of NAFLD across geographic locales (Figure 1), while studies based on elevated liver enzymes systematically underestimated the true prevalence. NAFLD is highly prevalent in all continents, but the highest rates are reported from South America (31%) and the Middle East (32%), followed by Asia (27%), United States (24%) and Europe (23%), whereas NAFLD is less common in Africa (14%).[1]

NAFLD, particularly its histological phenotype nonalcoholic steatohepatitis (NASH), can potentially progress to advanced liver disease, cirrhosis and hepatocellular carcinoma (HCC).[2] The prevalence of NAFLD is constantly increasing (15% in 2005 to 25% in 2010) and similarly the rate of NASH is almost doubled in the most recent studies compared to the oldest ones (59.1% versus 33%).[1] NASH is now considered the second most common indication for liver transplant in the United States after chronic hepatitis C and is still growing.[2] This review will provide evidence of the global burden of NAFLD and its clinical and economic implications, which should be considered by health policies to secure a better future for coming generations.

Prevalence of NAFLD in the United States of America

Over the recent decades, there has been extensive research to accurately determine the prevalence of NAFLD in the United States.[3-10] These data have recently been summarized in a meta-analysis reporting the worldwide prevalence of NAFLD.[1] In most of these studies, the prevalence of NAFLD in the general population was determined by imaging or other indirect methods. In this context, the prevalence of NAFLD in the United States diagnosed by ultrasound was estimated to be 24.13% [95% CI: 19.73-29.15]. On the other hand, the prevalence of NAFLD as determined by any other non-invasive methods (blood tests, ICD 9 or ICD 10 coding) was reported to be about 21.09% [95% 15.03-28.8], suggesting that diagnosis of NAFLD that is solely based on the blood testing or ICD coding can lead to under-reporting of its true prevalence.[1]

In the United States, the prevalence of NAFLD can vary by the ethnicity. In this context, the prevalence of NAFLD is reported to be highest in the Hispanic Americans, followed by Americans of European descent followed by African Americans.[3-11] Although still not fully resolved, a number of factors may explain these reported ethnic disparities in the prevalence of NAFLD in the United States. These include genetic factors, environmental factors, access to health care, and presence of chronic diseases such as metabolic syndrome.[1,6-8]

This issue is elucidated by the lower prevalence of NAFLD among African American than the Hispanic Americans.[5,8] This is especially surprising because of the higher prevalence of obesity and hypertension in African American subjects. [5,8]

In contrast, a study using the Third National Health and Nutrition Examination Survey (1988-1994) data reported that metabolic syndrome was the primary driver of NAFLD among the non-Hispanic Blacks and the Mexican Americans, but not for the White Americans. Despite some contradictory data regarding the interaction of NAFLD and components of metabolic syndrome in African Americans, this study suggests that the association of metabolic syndrome with NAFLD may be influenced by ethnicity.[12]

In another study using data from Non-alcoholic Steatohepatitis Clinical Research Network (NASH CRN), investigators compared Latino subjects with NASH to non-Latino White subjects with NASH.[13] The study found that Latinos with NASH were younger, had less physical activity, but higher carbohydrate intake. Furthermore, they reported that the effect of HOMA-IR on the risk of NASH was modified by ethnicity. In this context, HOMA-IR was not a significant risk factor for NASH among Latinos (odds ratio [OR] = 0.93; 95% confidence interval [CI]: 0.85-1.02), but was an important risk among non-Latino whites with NASH (OR, 1.06; 95% CI: 1.01-1.11). These data confirm that factors associated with NAFLD can be influenced by ethnic background of the patient.[13]

It is also important to recognize that even within a certain ethnic group in the United States; there may be differences in the prevalence of NAFLD. In fact, the prevalence of NAFLD among the Hispanic Americans can vary according to the country of origin.[8,14,15] In one such study, investigators compared the prevalence rates of NAFLD subjects between Hispanics of Mexican origin and Hispanics of Dominican and Puerto Rican origins (Caribbean area). Using data from the multi-ethnic study of atherosclerosis (MESA) cohort, the overall Hispanic prevalence of NAFLD was 29%.[14] On the other hand, Hispanics of Mexican origin had a significantly higher prevalence of NAFLD at 33% while Hispanics of Dominican origin had a prevalence rate of only 16% and Hispanics of Puerto Rican origin had a prevalence rate of 18%. After multivariate analysis, Hispanics of Mexican origin continued to remain at higher risk of having NAFLD than the individuals of Dominican and Puerto Rican origin.[14]

Although the ethnic and country of origin data regarding the prevalence of NAFLD is interesting, the exact explanation for these ethnic differences remains unknown. Some of these differences can be explained by the genetic factors that are described later in this review (nature) while others can be explained by environmental factors (nurture), such as diet, exercise and alcohol consumption.

Finally, the prevalence of the progressive form of NAFLD or NASH in the general population remains unknown. Nevertheless, there are indirect estimates for these rates by calculating prevalence of NASH in NAFLD and prevalence of NAFLD in the general population. In this context, the prevalence of NASH among

subjects with NAFLD in the United States is reported to be 21% (95% CI: 19.85%- 22.95%].[1] Using this rate, prevalence of NASH in the US population is estimated to be around 3-4%.[1] The corresponding prevalence rates of the comorbid conditions associated with NASH have been reported to be obesity 82%, type 2 diabetes mellitus (T2DM) 48%, hyperlipidemia 82%, metabolic syndrome 76%, and hypertension 70%.[1]

Prevalence of NAFLD in South America

The prevalence rate of NAFLD in South America seems to be higher than the rate reported for the United States. Specifically, NAFLD prevalence (using ultrasound) for South America has been estimated to be around 30.45% [95% CI: 22.74-39.4].[1] The majority of studies reporting the prevalence of NAFLD from South America have been performed in Brazil.[15-17] Nevertheless, in a study reported from Chile, the prevalence of NAFLD (using ultrasound) was estimated to be 23%.[18] Another study from Columbia also using ultrasound, reported a prevalence rate of 26.6% in male subjects.[19] Furthermore, the same investigators have estimated that the prevalence of "probable NAFLD" based on the rates of obesity in Peru, Argentina, Ecuador, Paraguay, and Uruguay could be as low as 13% (Peru) to as high as 24% (Uruguay).[19] Although there are estimates for the prevalence of NAFLD in South America, the data on the prevalence of NASH is even more scarce. Nevertheless, in one study, 61% of the patients with NAFLD in South America were found to have NASH which could make the prevalence of NASH from 6% to 18%.[20] These rates again can be influenced by genetic predisposition, as described later.

In summary, NAFLD prevalence rates do differ by ethnicity within the United States.[1,18-21] The Hispanic population has the highest prevalence while African Americans are reported to have the lowest prevalence, despite having higher prevalence rates of hypertension and obesity, both NAFLD risk factors. There are also ethnic differences noted within South America as well with Brazil reporting the highest prevalent rate and Peru the lowest.

Incidence of NAFLD and Future Projections the United States and in South America

Longitudinal studies of the general population are lacking in both the United States and South America. As a result, there is no true population-based incidence rates reported NAFLD. However, Kanwal and colleagues have suggested that the annual incidence of NAFLD within the USA should generally be stable at 2.2% to 3.2%.[10] On the other hand, we know that NAFLD is highly associated with several metabolic conditions [type 2 diabetes mellitus (T2DM), obesity, metabolic syndrome, hypertension, and hyperlipidemia].[22,23] Therefore, it is expected that the incidence of NAFLD should rise in parallel to the increasing incidence of obesity and T2DM.[24] In addition to the data about the true incidence rates for NAFLD, it is also important to determine the long -outcomes of patients with NAFLD. In this context, developing algorithms to define which patients with NAFLD will develop the progressive form of NASH, cirrhosis, liver-related mortality or cardiovascular mortality will be of great importance.

Epidemiology of NAFLD in Europe

In Europe a North-to-South gradient has been described for most chronic viral or non-viral hepatitis, but this does not hold true for NAFLD. Rather, the globalization of NAFLD runs parallel to the prevalence rates of obesity and varies accordingly, with degree of hepatic triglyceride accumulation being directly proportional to the severity of each element of the metabolic syndrome.[25] Although prevalence varies according to the modality used to detect NAFLD, approximately a quarter of the European population is affected by this liver disease. A recent meta-analysis reported an average prevalence of 23.71% in Europe, with variations ranging from 5% to 44% in different countries.[1]

Data from the Study of Health in Pomerania (SHIP) cohort in North-eastern Germany, estimates the prevalence of NAFLD to be around 30% when diagnosed by ultrasound.[26] A UK-based community study determined that NAFLD was the most common aetiology for asymptomatic abnormal liver biochemistry, accounting for 26.4% of cases (of whom 7.6% were predicted to have advanced liver disease).[27] Similarly, a French series of liver biopsies in subjects with unexplained abnormal liver tests reported simple steatosis in 26.8% of cases, of whom 32.7% had NASH.[28] In the Dyonisos Study, the prevalence of NAFLD assessed by ultrasound in Northern Italy was similar in subjects with and without suspected liver disease (25 vs. 20%, P =0.203), defined as altered liver enzymes or positivity of HBsAg and/or anti-HCV. Notably, only 54% of NAFLD cases occurred in patients with elevated ALT,[29] but the vast majority of them had many features of the metabolic syndrome. Epidemiological data from Spain describe similar rates, with a NAFLD prevalence of 25.8% in the adult population.[30] Only few studies are available from Eastern Europe. In Romania, NAFLD assessed by ultrasound has been found in 20% of 3005 hospitalized patients without liver disease,[31] while a study on healthy Hungarian adult confirmed a 22.6% overall prevalence of sonographically detected fatty liver.[32]

As expected, the prevalence of NAFLD increases substantially in “at-risk” groups such as patients with type 2 diabetes (T2DM).[2] The two major available studies, conducted on a large cohort of Italian patients with T2DM, reported NAFLD prevalence rates of 60–70%,[33,34] while UK data suggests that ultrasound detected

196 NAFLD is present in 42.6% of patients with T2DM.[35] Similarly, prevalence increases with body mass index
197 (BMI) so that 91% of obese patients (BMI ≥30 kg/m2), 67% of overweight (BMI 25-30 kg/m2) and 25% in
198 normal weight individuals had ultrasound evidence of NAFLD in an unselected Italian population sample. [36]
199 The prevalence of NAFLD among subjects matching at least one of the ATP III criteria for lipid alterations is
200 similarly high (78.8 %).[33]

201 Data regarding the prevalence of advanced forms of NAFLD and NASH in the general population is more
202 limited. A community-based study from the Netherlands using a transient elastography reading of ≥8kPa
203 for the diagnosis of liver fibrosis, estimated that clinically significant fibrosis was present in 5.6% (169/3041)
204 of total subjects and 8.4% (69/822) of those with NAFLD, and was positively associated with steatosis and
205 T2DM.[37] In this respect, the influence of T2DM on risk of progressive NAFLD is supported by a UK-based
206 paired-biopsy study that showed incident T2DM to be the strongest predictor of progressive disease.[38] A
207 Greek post-mortem study from 498 cases of ischaemic heart disease or traffic accident deaths revealed
208 simple steatosis in 31.3% and NASH in 39.8% of cases.[39] In a recent study performed on Spanish patients
209 with gallstone disease scheduled for cholecystectomy, 51.6% of them had histological evidence of NAFLD
210 and 19.8% of NASH.[40] Of note, in this cohort ultrasonography confirmed a fatty liver in only 67.6% of the
211 histologically diagnosed NAFLD subjects. Similar data are reported in healthy people evaluated in
212 Transplant Units as potential living liver donors. In a single retrospective study performed in a mixed
213 American and Italian cohort, the histological prevalence of steatosis was 48.5%, including a 15.5% of
214 steatohepatitis.[41] However, both NAFLD and NASH were more frequently found in Americans compared
215 with Italians (54% vs 34% for NAFLD and 17.6% vs 16.2% for NASH, respectively). The rates of NASH are
216 clearly increased in patients referred to tertiary centers for NAFLD. In a recent meta-analysis the pooled
217 NASH prevalence in Europe among NAFLD patients with an indication for biopsy was 69.25% (95% CI:
218 55.93-79.98).[1]

219 ***Incidence of NAFLD and future projections in Europe***

Only a small number of studies explored the incidence of NAFLD in the general population. Over a follow up of 8.5 years, the incidence of ultrasound detected NAFLD was 18.5 per 1,000 person-years in a sample representative of the general Italian population.[36] On the contrary, recent data are clarifying the natural history of histologically diagnosed NAFLD. In a Swedish cohort of 229 biopsy-proven NAFLD patients with a mean longitudinal follow-up of 26.4±5.6 years, compared to a matched reference population sample, NAFLD patients had significantly increased all-cause mortality (hazard ratio [HR] 1.29, 95%CI 1.04-1.59), exhibited an increased risk of cardiovascular disease (HR 1.55, 95%CI 1.11-2.15), hepatocellular carcinoma (HR 6.55, 95%CI 2.14-20.03) and cirrhosis (HR 3.2, 95%CI 1.05-9.81).[42] The presence of fibrosis was found to be the strongest prognostic factor for liver-related events and mortality.[42] Consistent with this, the burden of NAFLD-related hepatocellular carcinoma is also increasing dramatically. A study from North East England found that NAFLD associated HCC accounted for 35% of all cases (41/118) in 2010, representing a >10-fold increase in 10 years.[43] Finally, results from the UK NHS Blood and Transplant Agency show that decompensated NASH cirrhosis accounted for an increased proportion of patients undergoing liver transplant (12% in 2013 compared with 4% in 1995).[44]

Epidemiology of NAFLD in the Asia-Pacific and Africa

Within the Asia-Pacific Region, NAFLD prevalence varies widely as would be expected from a region of at least 55 countries with marked disparities in rates of development within the economic, political and educational spheres, and tied to this, variations in nutrition, lifestyle and sedentary behaviour. Further, there is a bias towards reporting studies that emanate from more affluent economics in the region with better health systems. Unlike from Europe and North America, data from the Asia Pacific and Africa is not as comprehensive both between and within countries and there is a total absence of this information from many countries.

Though there are no nationwide epidemiological surveys that include an assessment of liver fat, even within a single country such as China, there are striking differences according to region and over time in the prevalence of NAFLD. As an example, NAFLD prevalence in the populations of Chengdu (Southwest China), Shanghai (East China), Guangdong (South China) and central China were 12.5%, 15%, 17% and 24.5%, respectively.[45-47] On the other hand, a more recent ultrasound based study of 7152 employees from Shanghai suggested that NAFLD prevalence was as high as 38.17%.[48] In Hong Kong, a community-based study employing state of the art proton-magnetic resonance spectroscopy to quantify liver fat estimated a NAFLD prevalence of 28.8%; 19.3% in non-obese subjects and 60.5% among the obese.[49] Similarly in Taiwan, the prevalence of NAFLD was reported to be 11.4% in the general community [50] but higher in certain sub-populations including the elderly (50.1%) [51] and in those with a typically inactive lifestyle (66.4% in Taxi drivers).[52]

In the Far East, the community prevalence of NAFLD was ~25% in Japan, increasing from 12.6% before 1990 to 30.3% in 1998.[53] More recent reports suggest that 23-26% of subjects undergoing routine health screening have fatty liver by abdominal ultrasonography.[54] Using similar methodology, the reported prevalence of NAFLD in Korea in 141,610 subjects was 27.3%.[55]

South Asia and the Indian sub-continent are currently in the throes of rapid economic and social change, with trends towards urbanization and an urban/rural economic divide. Not unexpectedly, in rural India, a

region characterized by traditional diets and lifestyles, the prevalence of NAFLD is remarkably low (~9%), while it mimics western prevalence rates in urban populations, with rates varying between 16% and 32%.[56-58] A similar dramatic variation in NAFLD prevalence (5-30%) was observed from smaller surveys in Sri Lanka Malaysia, Singapore and Indonesia.[59-62]

Overall, while NAFLD prevalence rates are varied but increasing across Asia, given that this region is subject to the same global forces of change towards energy dense food consumption and reduced physical activity, NAFLD rates between the East and West are more similar than different, in the context of a similar obesogenic environment.

The scanty available data on the prevalence of NAFLD in Africa suggests that Africans tend to have lower prevalence, consistent with what has been reported in African Americans. In Nigeria, prevalence between 9.5%-16.7% in diabetics and 1.2%-4.5% in non-diabetics has been reported.[63,64] Similarly, in South Africa, the prevalence in obese and overweight was 45-50%.[65] A recent small population based study suggested a prevalence of 20% in the Sudanese population.[66]

Lean NAFLD

Initially described in Asian populations, NAFLD in the absence of obesity, i.e., the so-called 'lean' NAFLD, can develop in around 10%-20% of non-obese Americans and Caucasians [5,67] (Figure 2 and Supplementary Tables A and B)[45,49,50,56,67-78]. It deserves clinical attention as many physicians have a perception that lean NAFLD is more "benign" in nature. Lean NAFLD encompasses a heterogeneous spectrum of disease arising from different aetiologies, as listed in Table 1. Visceral obesity as opposed to general obesity, high fructose and high fat intake and genetic risk factors, including congenital defects of metabolism, may be associated with lean NAFLD. It is likely that a vast bulk of lean NAFLD cases belong to the phenotype of "metabolically obese normal weight" subjects,[79] described in at least 5% of the occidental population. It consists of a subgroup of non-obese, frequently sedentary subjects who display altered insulin sensitivity, increased cardiovascular risk and increased liver lipid, the consequence of decreased capacity for storing fat and reduced mitochondrial function in adipose tissue and increased de novo lipogenesis in liver.[79] When compared to overweight-obese NAFLD patients, lean NAFLD subjects are younger and have a lower prevalence of MetS (2%-48% versus 22-64% in overweight-obese).[67,80] However, these patients are usually insulin-resistant and have higher plasma triglycerides when compared to matched controls without NAFLD.[74,80] In a cohort of non-obese, non-diabetic subjects with biopsy-proven NAFLD, the metabolic pattern was similar to that observed in obesity, with adipose tissue IR playing an important role.[81]

Since lean NAFLD is usually present with less obesity-related comorbidities, it is commonly believed that this group would follow a relatively benign clinical course. Within the cohort of the National Health and Nutrition Examination Survey III (NHANES III),[79] mortality of metabolically-normal NAFLD patients was similar to the cohort without liver disease. Unfortunately, most reports are limited by the use of imaging modalities rather than liver biopsy to confirm the diagnosis of fatty liver.[5,74,79-84] In an Italian study [82] including 430 biopsy proven NAFLD, 55% of patients without visceral obesity had NASH and fibrosis ≥ F2 despite milder metabolic alterations. In a recent study [83] similar proportions of obese and non-obese

patients had NASH (51.9% versus 43.5%, P = 0.217), although the latter group had a lower degree of steatosis and hepatocyte ballooning. Consistent with earlier reports [79], the proportion of patients with advanced fibrosis at baseline was not different between obese and non-obese subjects, suggesting that once an individual declares him or herself as having NASH, obesity may not be the main driver of fibrosis progression. Genetic factors might be involved in lean NAFLD, however the presence of NASH in these subjects was not explained by mutations able to influence either IR (ENPP1 and IRS-1 polymorphisms) or the severity of steatosis (PNPLA3 and TM6SF2 polymorphisms).[82]

The longitudinal risk of mortality in lean NAFLD has been scarcely explored. In the above-mentioned study, [82] after a median follow-up of 49 months, clinical events occurred in 11.9% of obese patients and 8.3% of nonobese patients (P = 0.190). Cardiovascular events accounted for about two thirds of all major events in both groups. All deaths (n=6) occurred in the obese group, but definitive conclusions are difficult to make as follow-up was relatively short. An international cohort study including 483 cases with a mean follow-up period of over 11 year [85], published so far in abstract form, challenged the concept that the prognosis of patients with NAFLD who have normal BMI is benign. Despite presenting with a healthier metabolic profile and less advanced liver fibrosis, median survival free of liver transplantation was significantly shorter in lean than in non-lean patients (18.1 vs. 26.6 years, respectively, $p<0.001$).

The final question is how to manage lean subjects diagnosed with NAFLD, given that it might be harder to correct the underlying risk factors. Careful identification and correction of environmental causes, such as significant fructose consumption, may be effective particularly in young patients. Weight loss remains the background therapy in all cases with overweight/obesity, but in lean NAFLD patients habitual physical activity should also be emphasized. A call for more studies to understand the natural history of the disease but also for greater awareness among practitioners about the potential health risks associated with lean NAFLD is urgently needed.

Table 1

Causes of NAFLD in lean subjects
Environmental
• high-fructose diet, high fat diet
• DAFLD (Dual alcoholic and nonalcoholic fatty liver disease)
Metabolically Obese Normal Weight Subjects
Congenital and acquired lipodistrophy (HIV-HAART)
Genetic
• PNPLA3
• Congenital defects of metabolism (FHLB, LAL-D)
Endocrine disorders (PCOS, hypothyroidism, GH deficiency)
Drug-related (amiodarone, methotrexate, tamoxifen)
Jejunoileal bypass, starvation, TPN

FHLB, Familial Hypobetalipoprotein B; GH, Growth Hormone; LAL-D, Lysosomal Acid Lipase Deficiency; PCOS, Polycystic Ovary Syndrome; TPN, Total Parenteral Nutrition.

The future impact of pediatric NAFLD

Obesity in children has risen from 5.0% in 1960 to 16.9% in 2009-2010.[86] The obesity-related risk of future liver disease is alarming, as a weight gain during school-years carries a higher risk of NAFLD than weight-gain in late adulthood. In a large longitudinal Danish study,[87] a weight increase during childhood and early adolescence was related to all histological features of adult NAFLD even after adjusting for initial as well as attained BMI. Among children with similar attained BMIs at 13 years of age, the risk of cirrhosis in adulthood was increased by 16% per each unit gain in BMI z-score at every age from 7 through 13 years.[87] Similarly, also a weight gain during late adolescence is able to induce an increased susceptibility of developing NAFLD later in life. Another study tested the association of basal BMI on the development of End-Stage-Liver-Disease (ESLD) or liver-related death in a general population cohort of 44,248 men aged 18-20 years that attended military service in Sweden between 1969 and 1970.[88] After a follow-up of almost 38 years, being overweight in late adolescence increased the risk of liver-related outcomes by 64% compared with a low–normal range BMI, with a 5% increased risk for each unit of BMI above normal range.[88] Obesity early in life also increases the adulthood risk of HCC. Another Danish study including schoolchildren 7 through 13 years old [89] showed that each unit increase in BMI z-score increased by 20–30% the risk of liver cancer 30 years later. In other words, compared with an average height and weight 13-year-old boy, a boy of similar height but who weighed 6 kg more would have a 30% increased risk of liver cancer.[89] Besides the weight trajectory, other mechanisms appear to influence the spectrum of liver damage in NAFLD later in life. In the Cardiovascular Risk in Young Finns Study [90], after a follow-up of 31 years, adult NAFLD was predicted by modifiable as well as non-modifiable risk factors during childhood, including BMI and insulin levels, male sex, genetic background (that is, *PNPLA3* and *TM6SF2* variants) and low birth weight, an emerging risk factor for adulthood NAFLD probably related to intrauterine epigenetic regulations. Overall, this means that NAFLD and its complications, including HCC, are more likely to be anticipated at a fairly young age, foreseeing a possible reduction of life expectancy and an additional societal burden.

Risk factors: nature or nurture?

Evidence from patients that have undergone serial liver biopsies over an interval of several years demonstrates that the progression of NAFLD from steatosis to NASH and fibrosis is not linear and is likely to be more dynamic than previously thought.[38,91] Furthermore, evidence from familial aggregation and twin studies have shown a significant heritable component to NAFLD.[92,93] Interestingly, the genetic susceptibility for the development of steatosis and fibrosis may be shared.[94] Different ethnic groups have disparate propensities to advanced disease, with Hispanics being more susceptible than whites, while the lowest susceptibility is observed in blacks.[95] An interesting systematic review suggested that the leading explanations for the lowest incidence and prevalence of both NAFLD and NASH in African-Americans in the United States is related to genetic differences in lipid metabolism, i.e. lower triglyceride levels and significantly higher serum HDL-c in this ethnic group compared to Hispanics and Caucasians with NAFLD. [96]

In NAFLD, genome wide association studies (GWAS) have identified novel loci associated with disease severity phenotypes. A full discussion is beyond the scope of this article but the available literature has recently been reviewed elsewhere.[97] To date, non-synonymous SNPs in two genes in particular: *patatin-like phospholipase domain-containing 3 (PNPLA3)* and *transmembrane 6 superfamily member 2 (TM6SF2)* have most consistently been validated in separate large cohorts.[98,99] Figure 1 shows the distribution of *PNPLA3* genotypes in NAFLD patients according to geographical areas. *PNPLA3* is presented as MAF frequency. Amongst the emerging newly discovered risk loci, variants near the *membrane bound O-acyltransferase domain-containing 7 gene (MBOAT7*) and *transmembrane channel-like 4 gene (TMC4)* have been shown to be associated with development and severity of NAFLD in patients of European descent.[100] Similarly, within the Latino population in South America, the TM6SF2 E167K and PNPLA3 I148M gene variants seem to be responsible for susceptibility to progressive NASH.[101]

Whilst there have been major advances uncovering the genetic basis for the heritability of NAFLD, heritable mechanisms other than those encoded within the nucleotide sequence of genes are emerging. Small non-coding RNAs such as microRNAs (miRNAs), recently been shown to explain discordant NAFLD in genetically identical twins.[102] Epigenetic factors may also be a mechanism through which environmental exposures exert a heritable effect on disease risk. Remodelling of DNA methylation at key fibrosis modifier genes

underpinned murine ancestral protection to liver fibrosis.[103] Remarkably, similar remodelling occurred in NASH patients with mild fibrosis and there is intriguing data to suggest that epigenetic signatures present in the blood on circulating cell-free DNA may be a potential biomarker of this effect and thus disease severity.[103,104]

Genetic predisposition must be placed in the context of environmental factors that also play an important role. The most relevant factors are dietary habits, activity and socio-economic factors.

Although a large amount of data suggests that dietary composition may predispose subjects to NAFLD, evidence at the population level is less well characterised. In this context, a recent study reported that subjects with NAFLD tended to reside in areas with many food source options including grocery stores, restaurants and fast food places. Furthermore, those with NAFLD were more likely to report having the unhealthiest eating habits (eating foods with high fat, high salt, high sugar/corn syrup, processed foods) and reported eating more frequently at restaurants.[105] Other studies focused on the nutritional assessments of subjects with NAFLD have further documented consumption of low-nutrient, high sodium and high fat foods, especially diets with high-fat food from meat and lower amounts of fresh fruits.[106,107] In addition to the dietary habits, subjects with fatty liver were found to have very low physical activity levels and increased sitting times.[108-110]

The prevalence of NAFLD is also related to socio-economic factors, but their exact role is debated. In a study exploring the role of environmental factors in different ethnic groups with NAFLD, acculturation, educational level, healthcare use, and income along with dietary and lifestyle factors and sleep, were not found to be independently associated with risk of developing NAFLD, suggesting that the environmental factors may play a role on a background of genetic predisposition.[16]

Alcohol consumption in the context of NAFLD should be carefully considered. Data from the SHIP study demonstrates that the presence of obesity and alcohol consumption are not mutually exclusive. In subjects with radiologically diagnosed hepatic steatosis, 27.3% of males and 9.7% of females fulfilled criteria for both obesity and high alcohol consumption (i.e. Dual Aetiology Fatty Liver Disease, 'DAFLD').[111] Prospective

data from the UK in 9559 men with up to 42-years of follow up unequivocally show that alcohol consumption and the presence of obesity act synergistically to increase the risk of liver disease morbidity and mortality.[112] Ultimately, to fully address the impact of even moderate alcohol consumption on NAFLD will require prospective, longitudinal studies recording cumulative lifetime alcohol consumption.

Overall, these data confirm the concept that NAFLD is a complex disease and is affected by inter-related environmental factors and genetic predisposition. The exact contribution of each of the genetic or environmental components in the promoting the burden of NAFLD is not known and may vary in different regions of the world. Therefore, future studies need to focus on this gap of knowledge in order to better determine treatment and improve patient outcomes.

Global perspectives, healthcare challenges and prevention strategies

With an estimate of 64 million individuals affected in the United States and 52 million in European countries, [1,113] what clearly sets NAFLD apart from other common liver diseases is the sheer volume of patients. In this context, the major focus of clinical care is discerning NAFLD subjects at highest risk for liver-related complications. The recently released EASL-EASD-EASO guidelines recommend that individuals with obesity or any component of the metabolic syndrome should have an ultrasound, steatosis biomarkers and liver enzymes measured.[114] Further, a burning issue is the development of HCC in non-cirrhotic NASH. As the clinical consequences of NAFLD grow, the economic consequences will also increase. A recent model on the population of US and of four European countries (Germany, France, Italy, and United Kingdom) estimated the annual burden associated with all incident and prevalent NAFLD cases at $103 billion in the United States ($1,613 per patient) and at €35 billion in the Europe-4 countries (from €354 to €1,163 per patient).[113] In a study of Medicare NAFLD patients, the mean yearly inflation-adjusted charges from the outpatient setting increased from $2,624 ± $3,308 in 2005 to $3,608 ± $5,132 in 2010.[115] If we assume the annual rate of increase in the costs due to NAFLD to parallel the annual growth in the prevalence of obesity, the expected 10-year burden of NAFLD could increase to an estimated $1.005 trillion in the United States

and €334 billion in the Europe. [113] In addition to the direct annual cost of NAFLD, there is a societal cost related to the loss of QALYs and the burden of metabolic complications, including cardiovascular disease.

The main question is whether this enormous cost would be justified, provided it will be affordable. Cost-utility analysis of NASH screening is hampered by the lack of evidence around the early stages of the disease progression, uncertainties around the non-invasive markers of liver damage and the lack of effectiveness data relating to the impact of treatment in patients with NASH. Steatosis testing has not been recommended by the UK National Institute for Health and Care Excellence (NICE) NAFLD Guideline Committee (GC), due to the uncertainty both in the cost effectiveness results for all tests and in the clinical evidence base.[116] On the other hand, both the EASL-EASO-EASD and the UK NAFLD CG recommend biomarkers and transient elastography/ARFI to screen subjects with NAFLD for advanced fibrosis and cirrhosis.[114,116] In the end, screening for NASH will likely be cost-effective when medications with reasonable efficacy and side effects will be available.

Conclusions

NAFLD is now the leading cause of chronic liver disease in the United States and Europe and increasing worldwide, but there is a paucity of prospective population-based cohort studies from other geographical areas, including South America, Asia, Australia and Africa, which are needed to better understand the global burden of disease. Understanding the genetic and environmental risk factors of NAFLD and NASH and their distribution across different countries is of paramount importance to develop strategies to implement a multipronged public health policy and deal with this important chronic liver disease.

454

455 **Figure 1**: Worldwide estimated prevalence of NAFLD and the distribution of PNPLA3 genotypes. PNPLA3 is

456 presented as MAF frequency (red section of the pie chart).

457

458 **Figure 2**: A) The proportion of NAFLD in lean Subjects as compared to obese Subjects; B) The proportion of

459 NAFLD in lean Subjects. Data taken from references (45, 49, 50, 56 and 67-87).

460

461 **Supplementary Table A: Summary of studies showing the proportion of NAFLD in lean as compared to**
462 **obese patients.**

Country	Number	NAFLD prevalence			Method of diagnosis	Reference
		Overall	Non-obese	Obese		
India (lucknow)	280 subjects were screened and 150 were enrolled	53%	20%	80%	Ultrasonography	Bhat, et al. (68)
China (Heilongjiang)	2000 subjects were screened and 1779 were enrolled	44.9%	18.33	72.9%	Ultrasonography	Feng, et al. (69)
Japan (Kyoto)	5433 subjects were screened and 3271 were enrolled	24.6%	15.2%	68.5%	Ultrasonography	Nishioji, et al. (70)
South Korea (Seoul)	3123 subjects were screened and 2307 were enrolled	36%	22.4%	60.9%	Ultrasonography	Kim, et al. (71)
Hong Kong	3000 subjects were invited and 911 were enrolled	28.8%	19.3%	60.5%	^{1}H-MRS	Wei, et al. (49)
Japan (Nagasaki)	3579 subjects were screened and 3432 were enrolled	21.8%	11%	60%	Ultrasonography	Omagari, et al. (72)
South Korea (Seoul)	59,771 subjects were screened and 29,994 were enrolled	20.1%	12.6%	50.1%	Ultrasonography	Kwon, et al. (73)
China (Shanghai)	4205 subjects were screened and 3175 were enrolled	20.82%	21%	39%	Ultrasonography	Fan, et al. (45)
Taiwan (Shengang)	12,474 residents were invited and 3245 were enrolled	11.5%	4.2%	30.8%	Ultrasonography	Chen, et al. (50)

463

464 **Supplementary Table B: Summary of studies showing the proportion of NAFLD in lean patients.**

Country	Number	NAFLD prevalence in Non-obese	Method of diagnosis	Reference
Belgium	1,777	2.8% ((38%) of cryptogenic liver disease)	Liver biopsy	Vos, et al. (74)
USA	11,613 were considered, 2492 fulfilled the definition of NAFLD.	7.39%	Ultrasonography	Younossi, et al. (67)
Greece	185	12%	Ultrasonography and/or liver histology	Margariti, et al. (75)
Korea (Changwon)	2,058 subjects were considered, 1,711 fulfilled the definition of NAFLD	12.4%	Ultrasonography	Cho et al., (76)
Australia	422	14%	Liver biopsy	Personal communication
Spain	262	21%	Blood test (OWLiver® test)	Ortiz, et al. (78)
Korea (Seoul)	768	23.4%	Ultrasonography	Kim, et al. (77)
Korea (Seoul)	3,123 subjects were screened and 2307 were enrolled	30.3% at follow-up assessment. The mean duration of follow-up was 28.7 ± 13.2 months	Ultrasonography	Kim, et al. (71)
India (Kolkata)	1,911	75%	Ultrasonography and CT	Das, et al. (56)

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References

1 Younossi, Z. M. *et al.* Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* **64**, 73-84, doi:10.1002/hep.28431 (2016).

2 Anstee, Q. M., Targher, G. & Day, C. P. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol* **10**, 330-344, doi:10.1038/nrgastro.2013.41 (2013).

3 Lazo, M. *et al.* Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol* **178**, 38-45, doi:10.1093/aje/kws448 (2013).

4 Pan, J. J. & Fallon, M. B. Gender and racial differences in nonalcoholic fatty liver disease. *World J Hepatol* **6**, 274-283, doi:10.4254/wjh.v6.i5.274 (2014).

5 Browning, J. D. *et al.* Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* **40**, 1387-1395 (2004).

6 Schneider, A. L., Lazo, M., Selvin, E. & Clark, J. M. Racial differences in nonalcoholic fatty liver disease in the U.S. population. *Obesity (Silver Spring)* **22**, 292-299, doi:10.1002/oby.20426 (2014).

7 Sherif, Z. A. *et al.* Global Epidemiology of Nonalcoholic Fatty Liver Disease and Perspectives on US Minority Populations. *Dig Dis Sci* **61**, 1214-1225, doi:10.1007/s10620-016-4143-0 (2016).

8 Saab, S., Manne, V., Nieto, J., Schwimmer, J. B. & Chalasani, N. P. Nonalcoholic Fatty Liver Disease in Latinos. *Clin Gastroenterol Hepatol* **14**, 5-12; quiz e19-10, doi:10.1016/j.cgh.2015.05.001 (2016).

9 Balakrishnan, M., Kanwal, F., El-Serag, H. B. & Thrift, A. P. Acculturation and Nonalcoholic Fatty Liver Disease Risk Among Hispanics of Mexican Origin: Findings From the National Health and Nutrition Examination Survey. *Clin Gastroenterol Hepatol* **15**, 310-312, doi:10.1016/j.cgh.2016.09.149 (2017).

10 Kanwal, F. *et al.* Trends in the Burden of Nonalcoholic Fatty Liver Disease in a United States Cohort of Veterans. *Clin Gastroenterol Hepatol* **14**, 301-308 e301-302, doi:10.1016/j.cgh.2015.08.010 (2016).

11 Carroll, J. F. *et al.* Impact of race/ethnicity on the relationship between visceral fat and inflammatory biomarkers. *Obesity (Silver Spring)* **17**, 1420-1427, doi:10.1038/oby.2008.657 (2009).

12 Smits, M. M., Ioannou, G. N., Boyko, E. J. & Utzschneider, K. M. Non-alcoholic fatty liver disease as an independent manifestation of the metabolic syndrome: results of a US national survey in three ethnic groups. *Journal of gastroenterology and hepatology* **28**, 664-670, doi:10.1111/jgh.12106 (2013).

13 Bambha, K. *et al.* Ethnicity and nonalcoholic fatty liver disease. *Hepatology* **55**, 769-780, doi:10.1002/hep.24726 (2012).

14 Fleischman, M. W., Budoff, M., Zeb, I., Li, D. & Foster, T. NAFLD prevalence differs among hispanic subgroups: the Multi-Ethnic Study of Atherosclerosis. *World J Gastroenterol* **20**, 4987-4993, doi:10.3748/wjg.v20.i17.4987 (2014).

15 Karnikowski, M., Cordova, C., Oliveira, R. J., Karnikowski, M. G. & Nobrega Ode, T. Non-alcoholic fatty liver disease and metabolic syndrome in Brazilian middle-aged and older adults. *Sao Paulo Med J* **125**, 333-337 (2007).

16 Kallwitz, E. R. *et al.* Prevalence of suspected nonalcoholic fatty liver disease in Hispanic/Latino individuals differs by heritage. *Clin Gastroenterol Hepatol* **13**, 569-576, doi:10.1016/j.cgh.2014.08.037 (2015).

17 Oni, E. T. *et al.* Relation of physical activity to prevalence of nonalcoholic Fatty liver disease independent of cardiometabolic risk. *Am J Cardiol* **115**, 34-39, doi:10.1016/j.amjcard.2014.09.044 (2015).

18 Riquelme, A. *et al.* Non-alcoholic fatty liver disease and its association with obesity, insulin resistance and increased serum levels of C-reactive protein in Hispanics. *Liver international : official*

journal of the International Association for the Study of the Liver **29**, 82-88, doi:10.1111/j.1478-3231.2008.01823.x (2009).

19 Lopez-Velazquez, J. A. *et al.* The prevalence of nonalcoholic fatty liver disease in the Americas. *Ann Hepatol* **13**, 166-178 (2014).

20 Feijo, S. G. *et al.* The spectrum of non alcoholic fatty liver disease in morbidly obese patients: prevalence and associate risk factors. *Acta Cir Bras* **28**, 788-793 (2013).

21 Hernaez, R. *et al.* Association between variants in or near PNPLA3, GCKR, and PPP1R3B with ultrasound-defined steatosis based on data from the third National Health and Nutrition Examination Survey. *Clin Gastroenterol Hepatol* **11**, 1183-1190 e1182, doi:10.1016/j.cgh.2013.02.011 (2013).

22 Ballestri, S., Nascimbeni, F., Romagnoli, D. & Lonardo, A. The independent predictors of non-alcoholic steatohepatitis and its individual histological features.: Insulin resistance, serum uric acid, metabolic syndrome, alanine aminotransferase and serum total cholesterol are a clue to pathogenesis and candidate targets for treatment. *Hepatology research : the official journal of the Japan Society of Hepatology* **46**, 1074-1087, doi:10.1111/hepr.12656 (2016).

23 Stepanova, M. *et al.* Predictors of All-Cause Mortality and Liver-Related Mortality in Patients with Non-Alcoholic Fatty Liver Disease (NAFLD). *Dig Dis Sci* **58**, 3017-3023, doi:10.1007/s10620-013-2743-5 (2013).

24 Motamed, N. *et al.* Non-alcoholic fatty liver disease (NAFLD) and 10-year risk of cardiovascular diseases. *Clin Res Hepatol Gastroenterol* **41**, 31-38, doi:10.1016/j.clinre.2016.07.005 (2017).

25 Kotronen, A., Westerbacka, J., Bergholm, R., Pietilainen, K. H. & Yki-Jarvinen, H. Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab* **92**, 3490-3497, doi:10.1210/jc.2007-0482 (2007).

26 Haring, R. *et al.* Ultrasonographic hepatic steatosis increases prediction of mortality risk from elevated serum gamma-glutamyl transpeptidase levels. *Hepatology* **50**, 1403-1411, doi:10.1002/hep.23135 (2009).

27 Armstrong, M. J. *et al.* Presence and severity of non-alcoholic fatty liver disease in a large prospective primary care cohort. *J Hepatol* **56**, 234-240, doi:10.1016/j.jhep.2011.03.020 (2012).

28 de Ledinghen, V. *et al.* Diagnostic and predictive factors of significant liver fibrosis and minimal lesions in patients with persistent unexplained elevated transaminases. A prospective multicenter study. *Journal of hepatology* **45**, 592-599, doi:10.1016/j.jhep.2006.05.008 (2006).

29 Bedogni, G. *et al.* Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* **42**, 44-52, doi:10.1002/hep.20734 (2005).

30 Caballeria, L. *et al.* Prevalence and factors associated with the presence of nonalcoholic fatty liver disease in an adult population in Spain. *European journal of gastroenterology & hepatology* **22**, 24-32, doi:10.1097/MEG.0b013e32832fcdf0 (2010).

31 Radu, C. *et al.* Prevalence and associated risk factors of non-alcoholic fatty liver disease in hospitalized patients. *J Gastrointestin Liver Dis* **17**, 255-260 (2008).

32 Tarnoki, A. D. *et al.* Heritability of non-alcoholic fatty liver disease and association with abnormal vascular parameters: a twin study. *Liver international : official journal of the International Association for the Study of the Liver* **32**, 1287-1293, doi:10.1111/j.1478-3231.2012.02823.x (2012).

33 Soresi, M. *et al.* Nonalcoholic fatty liver and metabolic syndrome in Italy: results from a multicentric study of the Italian Arteriosclerosis society. *Acta Diabetol* **50**, 241-249, doi:10.1007/s00592-012-0406-1 (2013).

34 Targher, G. *et al.* Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* **30**, 1212-1218, doi:10.2337/dc06-2247 (2007).

35 Williamson, R. M. *et al.* Prevalence of and risk factors for hepatic steatosis and nonalcoholic Fatty liver disease in people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetes Care* **34**, 1139-1144, doi:10.2337/dc10-2229 (2011).

36 Bedogni, G. *et al.* Incidence and natural course of fatty liver in the general population: the Dionysos study. *Hepatology* **46**, 1387-1391, doi:10.1002/hep.21827 (2007).

37 Koehler, E. M. *et al.* Presence of diabetes mellitus and steatosis is associated with liver stiffness in a general population: The Rotterdam study. *Hepatology* **63**, 138-147, doi:10.1002/hep.27981 (2016).

38 McPherson, S. *et al.* Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: Implications for prognosis and clinical management. *J Hepatol* **62**, 1148-1155, doi:10.1016/j.jhep.2014.11.034 (2015).

39 Zois, C. D. *et al.* Steatosis and steatohepatitis in postmortem material from Northwestern Greece. *World J Gastroenterol* **16**, 3944-3949 (2010).

40 Garcia-Monzon, C. *et al.* Prevalence and risk factors for biopsy-proven non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in a prospective cohort of adult patients with gallstones. *Liver international : official journal of the International Association for the Study of the Liver* **35**, 1983-1991, doi:10.1111/liv.12813 (2015).

41 Minervini, M. I. *et al.* Liver biopsy findings from healthy potential living liver donors: reasons for disqualification, silent diseases and correlation with liver injury tests. *J Hepatol* **50**, 501-510, doi:S0168-8278(08)00796-4 [pii]10.1016/j.jhep.2008.10.030 (2009).

42 Ekstedt, M. *et al.* Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* **61**, 1547-1554, doi:10.1002/hep.27368 (2015).

43 Dyson, J. *et al.* Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *J Hepatol* **60**, 110-117, doi:10.1016/j.jhep.2013.08.011 (2014).

44 Williams, R. *et al.* Addressing liver disease in the UK: a blueprint for attaining excellence in health care and reducing premature mortality from lifestyle issues of excess consumption of alcohol, obesity, and viral hepatitis. *Lancet* **384**, 1953-1997, doi:10.1016/S0140-6736(14)61838-9 (2014).

45 Fan, J. G. *et al.* Prevalence of and risk factors for fatty liver in a general population of Shanghai, China. *J Hepatol* **43**, 508-514, doi:10.1016/j.jhep.2005.02.042 (2005).

46 Zhou, Y. J. *et al.* Prevalence of fatty liver disease and its risk factors in the population of South China. *World J Gastroenterol* **13**, 6419-6424 (2007).

47 Li, H. *et al.* Prevalence and risk factors of fatty liver disease in Chengdu, Southwest China. *Hepatobiliary Pancreat Dis Int* **8**, 377-382 (2009).

48 Hu, X. *et al.* Prevalence and factors associated with nonalcoholic fatty liver disease in Shanghai work-units. *BMC Gastroenterol* **12**, 123, doi:10.1186/1471-230X-12-123 (2012).

49 Wei, J. L. *et al.* Prevalence and Severity of Nonalcoholic Fatty Liver Disease in Non-Obese Patients: A Population Study Using Proton-Magnetic Resonance Spectroscopy. *Am J Gastroenterol* **110**, 1306-1314; quiz 1315, doi:10.1038/ajg.2015.235 (2015).

50 Chen, C. H. *et al.* Prevalence and risk factors of nonalcoholic fatty liver disease in an adult population of taiwan: metabolic significance of nonalcoholic fatty liver disease in nonobese adults. *J Clin Gastroenterol* **40**, 745-752 (2006).

51 Hung SC, L. S., Chen MC, Li PC, Lin KC. Prevalence and related factors of non-alcoholic fatty liver disease among the elderly in Taiwan. *Eur Geriatr Med* **4**, 78-81 (2013).

52 Tung, T. H. *et al.* Clinical correlation of nonalcoholic fatty liver disease in a Chinese taxi drivers population in Taiwan: Experience at a teaching hospital. *BMC Res Notes* **4**, 315, doi:10.1186/1756-0500-4-315 (2011).

53 Kojima, S., Watanabe, N., Numata, M., Ogawa, T. & Matsuzaki, S. Increase in the prevalence of fatty liver in Japan over the past 12 years: analysis of clinical background. *J Gastroenterol* **38**, 954-961, doi:10.1007/s00535-003-1178-8 (2003).

54 Hamaguchi, M. *et al.* The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. *Ann Intern Med* **143**, 722-728 (2005).

55 Jeong, E. H. *et al.* Regional prevalence of non-alcoholic fatty liver disease in Seoul and Gyeonggi-do, Korea. *Clin Mol Hepatol* **19**, 266-272, doi:10.3350/cmh.2013.19.3.266 (2013).

56 Das, K. *et al.* Nonobese Population in a Developing Country Has a High Prevalence of Nonalcoholic Fatty Liver and Significant Liver Disease. *Hepatology* **51**, 1593-1602, doi:10.1002/hep.23567 (2010).

57 Singh, S. P. *et al.* Prevalence of nonalcoholic fatty liver disease in coastal eastern India: a preliminary ultrasonographic survey. *Trop Gastroenterol* **25**, 76-79 (2004).

58 Amarapurkar, D. *et al.* Prevalence of non-alcoholic fatty liver disease: population based study. *Ann Hepatol* **6**, 161-163 (2007).

59 Dassanayake, A. S. *et al.* Prevalence and risk factors for non-alcoholic fatty liver disease among adults in an urban Sri Lankan population. *J Gastroenterol Hepatol* **24**, 1284-1288, doi:10.1111/j.1440-1746.2009.05831.x (2009).

60 Mohan, V., Farooq, S., Deepa, M., Ravikumar, R. & Pitchumoni, C. S. Prevalence of non-alcoholic fatty liver disease in urban south Indians in relation to different grades of glucose intolerance and metabolic syndrome. *Diabetes Res Clin Pract* **84**, 84-91, doi:10.1016/j.diabres.2008.11.039 (2009).

61 Goh, S. C., Ho, E. L. & Goh, K. L. Prevalence and risk factors of non-alcoholic fatty liver disease in a multiracial suburban Asian population in Malaysia. *Hepatol Int* **7**, 548-554, doi:10.1007/s12072-012-9359-2 (2013).

62 Chow, W. C. *et al.* Significant non-alcoholic fatty liver disease is found in non-diabetic, pre-obese Chinese in Singapore. *Singapore Med J* **48**, 752-757 (2007).

63 Onyekwere, C. A., Ogbera, A. O. & Balogun, B. O. Non-alcoholic fatty liver disease and the metabolic syndrome in an urban hospital serving an African community. *Ann Hepatol* **10**, 119-124 (2011).

64 Olusanya, T. O., Lesi, O. A., Adeyomoye, A. A. & Fasanmade, O. A. Non alcoholic fatty liver disease in a Nigerian population with type II diabetes mellitus. *Pan Afr Med J* **24**, 20, doi:10.11604/pamj.2016.24.20.8181 (2016).

65 Kruger, F. C. *et al.* Non-alcoholic fatty liver disease (NAFLD) in the Western Cape: a descriptive analysis. *S Afr Med J* **100**, 168-171 (2010).

66 Almobarak, A. O. *et al.* Non alcoholic fatty liver disease (NAFLD) in a Sudanese population: What is the prevalence and risk factors? *Arab J Gastroenterol* **15**, 12-15, doi:10.1016/j.ajg.2014.01.008 (2014).

67 Younossi, Z. M. *et al.* Nonalcoholic fatty liver disease in lean individuals in the United States. *Medicine (Baltimore)* **91**, 319-327, doi:10.1097/MD.0b013e3182779d49 (2012).

68 Bhat, G., Baba, C. S., Pandey, A., Kumari, N. & Choudhuri, G. Insulin resistance and metabolic syndrome in nonobese Indian patients with non-alcoholic fatty liver disease. *Trop Gastroenterol* **34**, 18-24 (2013).

69 Feng, R. N. *et al.* Lean-non-alcoholic fatty liver disease increases risk for metabolic disorders in a normal weight Chinese population. *World J Gastroenterol* **20**, 17932-17940, doi:10.3748/wjg.v20.i47.17932 (2014).

70 Nishioji, K. *et al.* Prevalence of and risk factors for non-alcoholic fatty liver disease in a non-obese Japanese population, 2011-2012. *J Gastroenterol* **50**, 95-108, doi:10.1007/s00535-014-0948-9 (2015).

71 Kim, N. H. *et al.* Clinical and metabolic factors associated with development and regression of nonalcoholic fatty liver disease in nonobese subjects. *Liver Int* **34**, 604-611, doi:10.1111/liv.12454 (2014).

72 Omagari, K. *et al.* Fatty liver in non-alcoholic non-overweight Japanese adults: incidence and clinical characteristics. *J Gastroenterol Hepatol* **17**, 1098-1105 (2002).

73 Kwon, Y. M. *et al.* Association of Nonalcoholic Fatty Liver Disease With Components of Metabolic Syndrome According to Body Mass Index in Korean Adults. *American Journal of Gastroenterology* **107**, 1852-1858, doi:10.1038/ajg.2012.314 (2012).

74 Vos, B. *et al.* Lean non-alcoholic fatty liver disease (Lean-NAFLD): a major cause of cryptogenic liver disease. *Acta Gastroenterol Belg* **74**, 389-394 (2011).

75 Margariti, E., Deutsch, M., Manolakopoulos, S. & Papatheodoridis, G. V. Non-alcoholic fatty liver disease may develop in individuals with normal body mass index. *Ann Gastroenterol* **25**, 45-51 (2012).

76 Cho, H. C. Prevalence and Factors Associated with Nonalcoholic Fatty Liver Disease in a Nonobese Korean Population. *Gut Liver* **10**, 117-125, doi:10.5009/gnl14444 (2016).

77 Kim, H. J. *et al.* Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. *Arch Intern Med* **164**, 2169-2175, doi:10.1001/archinte.164.19.2169 (2004).

78 Ortiz P, M. R., Pérez-Cormenzana M,Martínez-Arranz I, Martínez-Chantar M L, Lu SC; Mato JM. in *AASLD meeting.*
79 Conus, F., Rabasa-Lhoret, R. & Peronnet, F. Characteristics of metabolically obese normal-weight (MONW) subjects. *Appl Physiol Nutr Metab* **32**, 4-12, doi:10.1139/H07-926 (2007).
80 Younossi, Z. M., Otgonsuren, M., Venkatesan, C. & Mishra, A. In patients with non-alcoholic fatty liver disease, metabolically abnormal individuals are at a higher risk for mortality while metabolically normal individuals are not. *Metabolism* **62**, 352-360, doi:10.1016/j.metabol.2012.08.005 (2013).
81 Bugianesi, E. *et al.* Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia* **48**, 634-642, doi:10.1007/s00125-005-1682-x (2005).
82 Fracanzani, A. L. *et al.* Risk of nonalcoholic steatohepatitis and fibrosis in patients with nonalcoholic fatty liver disease and low visceral adiposity. *Journal of hepatology* **54**, 1244-1249, doi:10.1016/j.jhep.2010.09.037 (2011).
83 Leung, J. C. *et al.* Histological severity and clinical outcomes of nonalcoholic fatty liver disease in nonobese patients. *Hepatology* **65**, 54-64, doi:10.1002/hep.28697 (2017).
84 Kumar, R. *et al.* Clinicopathological characteristics and metabolic profiles of non-alcoholic fatty liver disease in Indian patients with normal body mass index: Do they differ from obese or overweight non-alcoholic fatty liver disease? *Indian J Endocrinol Metab* **17**, 665-671, doi:10.4103/2230-8210.113758 (2013).
85 Dela Cruz AC, B. E., George J, Day CP, Liaquat H, Charatcharoenwitthaya P, et al. . 379 characteristics and long-term prognosis of lean patients with nonalcoholic fatty liver disease. *Gastroenterology* **146**, doi:10.1016/S0016-5085(14)63307-2. (2014).
86 Ogden, C. L., Carroll, M. D., Kit, B. K. & Flegal, K. M. Prevalence of obesity and trends in body mass index among US children and adolescents, 1999-2010. *JAMA : the journal of the American Medical Association* **307**, 483-490, doi:10.1001/jama.2012.40 (2012).
87 Zimmermann, E. *et al.* Body mass index in school-aged children and the risk of routinely diagnosed non-alcoholic fatty liver disease in adulthood: a prospective study based on the Copenhagen School Health Records Register. *BMJ Open* **5**, e006998, doi:10.1136/bmjopen-2014-006998 (2015).
88 Hagstrom, H., Stal, P., Hultcrantz, R., Hemmingsson, T. & Andreasson, A. Overweight in late adolescence predicts development of severe liver disease later in life: A 39years follow-up study. *Journal of hepatology* **65**, 363-368, doi:10.1016/j.jhep.2016.03.019 (2016).
89 Berentzen, T. L., Gamborg, M., Holst, C., Sorensen, T. I. & Baker, J. L. Body mass index in childhood and adult risk of primary liver cancer. *Journal of hepatology* **60**, 325-330, doi:10.1016/j.jhep.2013.09.015 (2014).
90 Suomela, E. *et al.* Childhood predictors of adult fatty liver. The Cardiovascular Risk in Young Finns Study. *Journal of hepatology* **65**, 784-790, doi:10.1016/j.jhep.2016.05.020 (2016).
91 Singh, S. *et al.* Fibrosis Progression in Nonalcoholic Fatty Liver vs Nonalcoholic Steatohepatitis: A Systematic Review and Meta-analysis of Paired-Biopsy Studies. *Clin Gastroenterol Hepatol* **13**, 643-654 e649, doi:10.1016/j.cgh.2014.04.014 (2015).
92 Loomba, R. *et al.* Heritability of Hepatic Fibrosis and Steatosis Based on a Prospective Twin Study. *Gastroenterology*, doi:10.1053/j.gastro.2015.08.011 (2015).
93 Schwimmer, J. B. *et al.* Heritability of nonalcoholic fatty liver disease. *Gastroenterology* **136**, 1585-1592 (2009).
94 Cui, J. *et al.* Shared genetic effects between hepatic steatosis and fibrosis: A prospective twin study. *Hepatology* **64**, 1547-1558, doi:10.1002/hep.28674 (2016).
95 Anstee, Q. M. & Day, C. P. The genetics of NAFLD. *Nat Rev Gastroenterol Hepatol* **10**, 645-655, doi:10.1038/nrgastro.2013.182 (2013).
96 Foster, T., Anania, F. A., Li, D., Katz, R. & Budoff, M. The prevalence and clinical correlates of nonalcoholic fatty liver disease (NAFLD) in African Americans: the multiethnic study of atherosclerosis (MESA). *Dig Dis Sci* **58**, 2392-2398, doi:10.1007/s10620-013-2652-7 (2013).
97 Anstee, Q. M. & Day, C. P. The Genetics of Nonalcoholic Fatty Liver Disease: Spotlight on PNPLA3 and TM6SF2. *Semin Liver Dis* **35**, 270-290, doi:10.1055/s-0035-1562947 (2015).

98 Liu, Y. L. *et al.* TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nature communications* **5**, 4309, doi:10.1038/ncomms5309 (2014).
99 Valenti, L. *et al.* Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* **51**, 1209-1217, doi:10.1002/hep.23622 (2010).
100 Mancina, R. M. *et al.* The MBOAT7-TMC4 Variant rs641738 Increases Risk of Nonalcoholic Fatty Liver Disease in Individuals of European Descent. *Gastroenterology* **150**, 1219-1230 e1216, doi:10.1053/j.gastro.2016.01.032 (2016).
101 Chen, L. Z. *et al.* PNPLA3 I148M variant in nonalcoholic fatty liver disease: demographic and ethnic characteristics and the role of the variant in nonalcoholic fatty liver fibrosis. *World J Gastroenterol* **21**, 794-802, doi:10.3748/wjg.v21.i3.794 (2015).
102 Zarrinpar, A., Gupta, S., Maurya, M. R., Subramaniam, S. & Loomba, R. Serum microRNAs explain discordance of non-alcoholic fatty liver disease in monozygotic and dizygotic twins: a prospective study. *Gut* **65**, 1546-1554, doi:10.1136/gutjnl-2015-309456 (2016).
103 Zeybel, M. *et al.* Multigenerational epigenetic adaptation of the hepatic wound-healing response. *Nature Medicine* **18**, 1369-1377, doi:10.1038/nm.2893 (2012).
104 Hardy, T. *et al.* Plasma DNA methylation: a potential biomarker for stratification of liver fibrosis in non-alcoholic fatty liver disease. *Gut*, doi:10.1136/gutjnl-2016-311526 (2016).
105 Leslie, T. *et al.* Survey of health status, nutrition and geography of food selection of chronic liver disease patients. *Ann Hepatol* **13**, 533-540 (2014).
106 Kim, C. H. *et al.* Nutritional assessments of patients with non-alcoholic fatty liver disease. *Obesity surgery* **20**, 154-160, doi:10.1007/s11695-008-9549-0 (2010).
107 McCarthy, E. M. & Rinella, M. E. The role of diet and nutrient composition in nonalcoholic Fatty liver disease. *J Acad Nutr Diet* **112**, 401-409, doi:10.1016/j.jada.2011.10.007 (2012).
108 Gerber, L. *et al.* Non-alcoholic fatty liver disease (NAFLD) is associated with low level of physical activity: a population-based study. *Alimentary pharmacology & therapeutics* **36**, 772-781, doi:10.1111/apt.12038 (2012).
109 Hallsworth, K. *et al.* Non-alcoholic fatty liver disease is associated with higher levels of objectively measured sedentary behaviour and lower levels of physical activity than matched healthy controls. *Frontline gastroenterology* **6**, 44-51, doi:10.1136/flgastro-2014-100432 (2015).
110 Keating, S. E., George, J. & Johnson, N. A. The benefits of exercise for patients with non-alcoholic fatty liver disease. *Expert review of gastroenterology & hepatology* **9**, 1247-1250, doi:10.1586/17474124.2015.1075392 (2015).
111 Volzke, H. Multicausality in fatty liver disease: is there a rationale to distinguish between alcoholic and non-alcoholic origin? *World J Gastroenterol* **18**, 3492-3501, doi:10.3748/wjg.v18.i27.3492 (2012).
112 Hart, C. L., Morrison, D. S., Batty, G. D., Mitchell, R. J. & Davey Smith, G. Effect of body mass index and alcohol consumption on liver disease: analysis of data from two prospective cohort studies. *BMJ* **340**, c1240, doi:10.1136/bmj.c1240 (2010).
113 Younossi, Z. M. *et al.* The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. *Hepatology* **64**, 1577-1586, doi:10.1002/hep.28785 (2016).
114 European Association for the Study of the Liver . Electronic address, e. e. e., European Association for the Study of, D. & European Association for the Study of, O. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* **64**, 1388-1402, doi:10.1016/j.jhep.2015.11.004 (2016).
115 Younossi, Z. M. *et al.* Trends in outpatient resource utilizations and outcomes for Medicare beneficiaries with nonalcoholic fatty liver disease. *Journal of clinical gastroenterology* **49**, 222-227, doi:10.1097/MCG.0000000000000071 (2015).
116 National Institute for Health and Care Excellence. . *Non-alcoholic fatty liver disease (NAFLD): assessment and management. NICE clinical guideline NG49. London. National Clinical Guideline Centre* (2016).