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

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Key Words:

Prevalence, incidence, ethnicity, genes, environment, steatohepatitis, cost-effectiveness.

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.

29

30 **Key points:**

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

52

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.

85

86 **Prevalence of NAFLD in the United States of America**

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

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

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

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

109 In another study using data from Non-alcoholic Steatohepatitis Clinical Research Network (NASH CRN),
110 investigators compared Latino subjects with NASH to non-Latino White subjects with NASH.[13] The study
111 found that Latinos with NASH were younger, had less physical activity, but higher carbohydrate intake.
112 Furthermore, they reported that the effect of HOMA-IR on the risk of NASH was modified by ethnicity. In
113 this context, HOMA-IR was not a significant risk factor for NASH among Latinos (odds ratio [OR] = 0.93; 95%
114 confidence interval [CI]: 0.85-1.02), but was an important risk among non-Latino whites with NASH (OR,
115 1.06; 95% CI: 1.01-1.11). These data confirm that factors associated with NAFLD can be influenced by ethnic
116 background of the patient.[13]

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

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

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

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

138

139 **Prevalence of NAFLD in South America**

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

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

157

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

159 Longitudinal studies of the general population are lacking in both the United States and South America. As a
160 result, there is no true population-based incidence rates reported NAFLD. However, Kanwal and colleagues
161 have suggested that the annual incidence of NAFLD within the USA should generally be stable at 2.2% to
162 3.2%.[10] On the other hand, we know that NAFLD is highly associated with several metabolic conditions
163 [type 2 diabetes mellitus (T2DM), obesity, metabolic syndrome, hypertension, and hyperlipidemia].[22,23]
164 Therefore, it is expected that the incidence of NAFLD should rise in parallel to the increasing incidence of
165 obesity and T2DM.[24] In addition to the data about the true incidence rates for NAFLD, it is also important to
166 determine the long -outcomes of patients with NAFLD. In this context, developing algorithms to define
167 which patients with NAFLD will develop the progressive form of NASH, cirrhosis, liver-related mortality or
168 cardiovascular mortality will be of great importance.

169

170
171 **Epidemiology of NAFLD in Europe**

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

179 Data from the Study of Health in Pomerania (SHIP) cohort in North-eastern Germany, estimates the
180 prevalence of NAFLD to be around 30% when diagnosed by ultrasound.[26] A UK-based community study
181 determined that NAFLD was the most common aetiology for asymptomatic abnormal liver biochemistry,
182 accounting for 26.4% of cases (of whom 7.6% were predicted to have advanced liver disease).[27] Similarly, a
183 French series of liver biopsies in subjects with unexplained abnormal liver tests reported simple steatosis in
184 26.8% of cases, of whom 32.7% had NASH.[28] In the Dyonisos Study, the prevalence of NAFLD assessed by
185 ultrasound in Northern Italy was similar in subjects with and without suspected liver disease (25 vs. 20%, P
186 =0.203), defined as altered liver enzymes or positivity of HBsAg and/or anti-HCV. Notably, only 54% of
187 NAFLD cases occurred in patients with elevated ALT,[29] but the vast majority of them had many features of
188 the metabolic syndrome. Epidemiological data from Spain describe similar rates, with a NAFLD prevalence
189 of 25.8% in the adult population.[30] Only few studies are available from Eastern Europe. In Romania, NAFLD
190 assessed by ultrasound has been found in 20% of 3005 hospitalized patients without liver disease,[31] while a
191 study on healthy Hungarian adult confirmed a 22.6% overall prevalence of sonographically detected fatty
192 liver.[32]

193 As expected, the prevalence of NAFLD increases substantially in “at-risk” groups such as patients with type
194 2 diabetes (T2DM).[2] The two major available studies, conducted on a large cohort of Italian patients with
195 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***

220 Only a small number of studies explored the incidence of NAFLD in the general population. Over a follow up
221 of 8.5 years, the incidence of ultrasound detected NAFLD was 18.5 per 1,000 person-years in a sample
222 representative of the general Italian population.[36] On the contrary, recent data are clarifying the natural
223 history of histologically diagnosed NAFLD. In a Swedish cohort of 229 biopsy-proven NAFLD patients with a
224 mean longitudinal follow-up of 26.4±5.6 years, compared to a matched reference population sample,
225 NAFLD patients had significantly increased all-cause mortality (hazard ratio [HR] 1.29, 95%CI 1.04-1.59),
226 exhibited an increased risk of cardiovascular disease (HR 1.55, 95%CI 1.11-2.15), hepatocellular carcinoma
227 (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
228 be the strongest prognostic factor for liver-related events and mortality.[42] Consistent with this, the burden
229 of NAFLD-related hepatocellular carcinoma is also increasing dramatically. A study from North East England
230 found that NAFLD associated HCC accounted for 35% of all cases (41/118) in 2010, representing a >10-fold
231 increase in 10 years.[43] Finally, results from the UK NHS Blood and Transplant Agency show that
232 decompensated NASH cirrhosis accounted for an increased proportion of patients undergoing liver
233 transplant (12% in 2013 compared with 4% in 1995).[44]

234

235

236 **Epidemiology of NAFLD in the Asia-Pacific and Africa**

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

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

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

259 South Asia and the Indian sub-continent are currently in the throes of rapid economic and social change,
260 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]

276

277 **Lean NAFLD**

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

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

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

308 The longitudinal risk of mortality in lean NAFLD has been scarcely explored. In the above-mentioned study,
309 [82] after a median follow-up of 49 months, clinical events occurred in 11.9% of obese patients and 8.3% of
310 nonobese patients (P = 0.190). Cardiovascular events accounted for about two thirds of all major events in
311 both groups. All deaths (n=6) occurred in the obese group, but definitive conclusions are difficult to make
312 as follow-up was relatively short. An international cohort study including 483 cases with a mean follow-up
313 period of over 11 year [85], published so far in abstract form, challenged the concept that the prognosis of
314 patients with NAFLD who have normal BMI is benign. Despite presenting with a healthier metabolic profile
315 and less advanced liver fibrosis, median survival free of liver transplantation was significantly shorter in
316 lean than in non-lean patients (18.1 vs. 26.6 years, respectively, p<0.001).

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

324

325

326

327 **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

328 FHLB, Familial Hypobetalipoprotein B; GH, Growth Hormone; LAL-D, Lysosomal Acid Lipase Deficiency; PCOS,

329 Polycystic Ovary Syndrome; TPN, Total Parenteral Nutrition.

330

331 **The future impact of pediatric NAFLD**

332 Obesity in children has risen from 5.0% in 1960 to 16.9% in 2009-2010.[86] The obesity-related risk of future
333 liver disease is alarming, as a weight gain during school-years carries a higher risk of NAFLD than weight-
334 gain in late adulthood. In a large longitudinal Danish study,[87] a weight increase during childhood and early
335 adolescence was related to all histological features of adult NAFLD even after adjusting for initial as well as
336 attained BMI. Among children with similar attained BMIs at 13 years of age, the risk of cirrhosis in
337 adulthood was increased by 16% per each unit gain in BMI z-score at every age from 7 through 13 years.[87]
338 Similarly, also a weight gain during late adolescence is able to induce an increased susceptibility of
339 developing NAFLD later in life. Another study tested the association of basal BMI on the development of
340 End-Stage-Liver-Disease (ESLD) or liver-related death in a general population cohort of 44,248 men aged
341 18-20 years that attended military service in Sweden between 1969 and 1970.[88] After a follow-up of almost
342 38 years, being overweight in late adolescence increased the risk of liver-related outcomes by 64%
343 compared with a low–normal range BMI, with a 5% increased risk for each unit of BMI above normal
344 range.[88] Obesity early in life also increases the adulthood risk of HCC. Another Danish study including
345 schoolchildren 7 through 13 years old [89] showed that each unit increase in BMI z-score increased by 20–
346 30% the risk of liver cancer 30 years later. In other words, compared with an average height and weight 13-
347 year-old boy, a boy of similar height but who weighed 6 kg more would have a 30% increased risk of liver
348 cancer.[89] Besides the weight trajectory, other mechanisms appear to influence the spectrum of liver
349 damage in NAFLD later in life. In the Cardiovascular Risk in Young Finns Study [90], after a follow-up of 31
350 years, adult NAFLD was predicted by modifiable as well as non-modifiable risk factors during childhood,
351 including BMI and insulin levels, male sex, genetic background (that is, *PNPLA3* and *TM6SF2* variants) and
352 low birth weight, an emerging risk factor for adulthood NAFLD probably related to intrauterine epigenetic
353 regulations. Overall, this means that NAFLD and its complications, including HCC, are more likely to be
354 anticipated at a fairly young age, foreseeing a possible reduction of life expectancy and an additional
355 societal burden.

356 **Risk factors: nature or nurture?**

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

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

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

383 underpinned murine ancestral protection to liver fibrosis.[103] Remarkably, similar remodelling occurred in
384 NASH patients with mild fibrosis and there is intriguing data to suggest that epigenetic signatures present in
385 the blood on circulating cell-free DNA may be a potential biomarker of this effect and thus disease severity.
386 [103,104]

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

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

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

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

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

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

417

418 **Global perspectives, healthcare challenges and prevention strategies**

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

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

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

445

446 **Conclusions**

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

453

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)

465

466

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