An overview of the genetics, mechanisms and management of NAFLD and ALD

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ABSTRACT

Alcoholic liver disease (ALD) and, increasingly, non-alcoholic fatty liver disease (NAFLD) are common causes of advanced liver disease in many developed countries including the UK. Both diseases share parallel natural histories, progressing from steatosis, to steatohepatitis and fibrosis/cirrhosis; and are characterised by substantial interindividual variation in disease outcome. This article will provide an overview of disease mechanisms, genetic modifiers and management, focusing principally on NAFLD, while drawing parallels between the two conditions where appropriate.

KEYWORDS: NAFLD, fatty liver, alcohol, cirrhosis, gene

Introduction

As highlighted by the recent Lancet Commission report, liver-associated mortality and morbidity are rising at an alarming pace.[1] In the UK, while the standardised mortality rates for other top-five conditions (ischaemic heart disease, stroke, respiratory disease and cancer) are stable or declining, the rate for liver disease has risen by more than 400% since 1970 and continues to rise.[1] Historically liver disease has mainly been attributable to infectious causes, predominantly in the form of the viral hepatitides and alcohol use. However, as the epidemic of obesity, type-2 diabetes mellitus (T2DM) and the metabolic syndrome spreads, non-alcoholic fatty liver disease (NAFLD) is beginning to surpass these other causes and may become the most common cause of liver disease worldwide.[2] Furthermore, NAFLD is recognised to be an independent risk factor, not only for progressive liver disease, but also for development of T2DM and cardiovascular disease (reviewed[3]). NAFLD may therefore be considered the hepatic manifestation of the metabolic syndrome,[4] which is a constellation of cardiovascular risk factors comprising increased body mass index (BMI), waist circumference, blood pressure, triglycerides and fasting glucose.[3,5]

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NAFLD and ALD are both steatohepatitic processes and share many common features. Advances in our understanding of the pathogenic mechanisms that favour progression from steatosis to steatohepatitis and fibrosis in NAFLD also offer new insights into the processes that may operate in ALD. Indeed, it appears that the pathogenesis of ALD and NAFLD may converge to follow a common destructive pathway to cirrhosis, and in some circumstances onto hepatocellular carcinoma (HCC).[6] This similarity has paved the way for new lines of research, translating findings in NAFLD to ALD; ultimately it is hoped that the clinical application of these discoveries will contribute to more effective management of both conditions.

Occurring on a background of obesity and insulin resistance, NAFLD is a spectrum of disease encompassing hepatic fat accumulation (simple steatosis), through to hepatocyte inflammation and necrosis (non-alcoholic steatohepatitis (NASH)), and on to fibrosis and cirrhosis.[4] The accepted definition of NAFLD is fat accumulation in >5% hepatocytes observed upon histological examination, when all other causes of steatosis have been excluded.[4] The harmful effects of excessive alcohol consumption are well documented, with fibrosis and cirrhosis the familiar end result of chronic, daily, excessive alcohol use.[1] Histologically, there is little difference between the appearances of alcoholic and non-alcoholic steatosis and steatohepatitis, so specific diagnosis of NAFLD versus ALD is made clinically, based upon recognition of associated features of the metabolic syndrome (T2DM, obesity, hypertension and dyslipidaemia) and exclusion of daily alcohol consumption above 20 g for females and 30 g for males.

Although NAFLD is a common condition, affecting up to 25% of the UK population, only a relatively small proportion of patients will progress beyond steatosis to steatohepatitis, fibrosis, cirrhosis and/or HCC.[4] What drives the progression to more advanced disease states has been the focus of much research activity as it may guide individualised risk stratification in the clinic.[7] While our understanding of NAFLD pathogenesis is still incomplete, research has revealed promising new insights into the environmental and genetic mediators of disease progression, uncovering common disease mechanisms and specific genes associated with advanced disease in both NAFLD and ALD. The factors which lead patients to develop cirrhosis in NAFLD are likely also to be some of the same factors which contribute to disease progression as a consequence of excessive alcohol consumption.

Epidemiology

In the UK, mortality and morbidity associated with liver disease are trending upwards, predicting a significant burden of poor health and rising demands on the NHS.[1] There is a consistent relationship between socioeconomic deprivation, obesity and liver disease, with those areas of highest deprivation also having the highest rates of liver disease due to ALD and NAFLD.[8] Overall, more liver-related deaths are attributable to ALD than NAFLD,[8] however the rising prevalence of NAFLD is reflective of the rise in obesity, metabolic syndrome and T2DM.[9] It is noteworthy that there is a significant interaction between BMI and liver damage attributed to high alcohol consumption with a 5.5-fold increased relative risk in liver-related death observed among overweight drinkers consuming >15 units/week and so the two conditions can be difficult to separate.[10]

Patients with metabolic syndrome have significantly more liver fat than those without, even when age, gender and BMI are controlled for.[5] Estimates of NAFLD prevalence vary both by the population that is studied (for example studies using patients with different ethnicities, gender and comorbidities) and the sensitivity of the diagnostic technique used.[4,11,12] Because a definitive diagnosis optimally requires liver biopsy and histological analysis to detect steatohepatitis, there have been difficulties in accurately quantifying population prevalence rates. Large-scale cohort studies, such as the Dallas Heart Study and the Italian Dionysus study, using sensitive non-invasive modalities, such as computed tomography (CT), proton magnetic resonance spectroscopy (^{1}H-MRS) or magnetic resonance imaging (MRI), report figures of 25–30%, thus NAFLD prevalence in Western countries is estimated to be approximately 20–30%.[9,13] Liver biochemistry correlates weakly with the presence of NAFLD or indeed disease severity and so may underestimate prevalence.[14,15]

Mechanisms of disease

Steatosis is the initial stage in the pathway for both NAFLD and ALD. In isolation this has been considered a relatively benign state with low associated morbidity and mortality, although recent data suggest that this may not be always the case and that steatosis is as likely to progress to more advanced disease as steatohepatitis.[18] Fat accumulation is regarded as the first hit in the original NAFLD 'two-hit hypothesis', making the liver susceptible to a range of 'second hits', such as oxidative stress, cytokine release and endotoxin from intestinal bacteria, that can subsequently initiate inflammatory and fibrogenic processes, leading to steatohepatitis.[19] As our understanding of pathogenesis has evolved, this hypothesis has been revised to take account of new research.[20] For example, evidence that hepatic triglyceride accumulation may not itself be directly harmful to the liver is neatly demonstrated in a murine model of NAFLD where it was observed that despite a reduction in hepatic fat accumulation, liver damage was in fact exacerbated by inhibition of triglyceride synthesis.[21] If storage of triglyceride in the liver confers protective effects by 'buffering' other potentially toxic forms of lipid, then this prompts the question of what is it that triggers a departure from this physiological state to progress onto the pathological states of steatohepatitis and fibrosis?

Among a cohort of non-diabetic patients, liver fat was significantly increased in those with metabolic syndrome compared to those without, and importantly this was the case when age, gender and BMI were all controlled for.[5] To a large extent this relationship may be accounted for by insulin resistance. In healthy people insulin is responsible for maintaining the homeostasis of both glucose and free fatty acids.[3] Insulin acts in the fed state to increase glycogenesis (the uptake and storage of glucose as glycogen), and it drives lipogenesis (the uptake and storage of lipids which are esterified and stored as triglyceride in both adipose tissue and the liver). In the presence of insulin resistance, adipose tissue hormone sensitive lipase is disinhibited. Stored triacylglycerol is enzymatically degraded into glycerol and fatty acids which are released into the circulation. Diabetes is therefore intrinsically linked to NAFLD due to the disruption of normal lipid homeostasis, but there are wider reaching consequences beyond simply the presence of excess fat. While both obesity and diabetes are associated with steatosis, diabetes in particular is predictive of subsequent disease progression in NAFLD.[3,18] In a recent dual-biopsy cohort study, progression to NASH and fibrosis among patients with steatosis and mild inflammation was predicted by T2DM status, providing strong evidence of the crucial role of insulin resistance in NAFLD pathogenesis as well as subsequent disease progression.[18]

Inflammatory pathways are complex and involve many different key players, including cytokines, adipokines, nuclear receptors, oxidative stress, mitochondria, hepatocytes, adipocytes and other cell types. There is growing evidence which suggests that progression to steatohepatitis occurs as a result of reduced esterification of fatty acids in the liver, with preferential diversion to oxidative pathways.[22] Oxidation of fatty acids produces reactive oxygen species creating oxidative stress, which is a trigger for inflammatory immune responses, recruitment of cytokines and immune mediators of inflammation, necrosis and apoptosis. What factors mediate the diversion of fatty acids away from the adaptive and protective response that is esterification and storage as triglycerides, towards a pathway of oxidation and thus oxidative stress and inflammation, remains elusive.

Following inflammation and cell injury, fibrosis is the next step in disease progression with hepatic stellate cells (HSCs) taking a lead role. Activated HSCs are responsible for deposition of collagen matrix and scar tissue formation in response to liver injury and thus they are primary agents of fibrogenesis in the liver.[23]

Environmental factors

NAFLD and ALD are complex disease traits that develop through a combination of environmental (dietary) and genetic factors. Importantly, when obesity and high alcohol consumption coexist, there is a significant synergistic effect with an increase in relative excess risk of liver disease that exceeds that for either alone.[10] This implies that, on a background of increasing adiposity across the UK population, more individuals that have had borderline or high levels of alcohol consumption may develop progressive liver disease due to 'dual-aetiology' fatty liver disease. A high-fat, high-sugar diet receives much attention for a contribution to obesity and metabolic syndrome, and is thus a risk factor for NAFLD; although any calorific

excess will be contributory. It is also important to recognise that nutritional status at the opposite end of the spectrum, in the form of malnutrition, frequently occurs in many alcohol-dependent patients and so may co-exist with ALD.[25] Other environmental factors that are reported to be correlated with disease progression in NAFLD include obstructive sleep apnoea[26] and the constitution of the gut microbiome.[27]

Genetic factors

It is of clinical relevance to identify causative environmental and genetic factors that contribute to this complex disease trait as they may in the future assist with risk stratification or offer novel therapeutic targets. Although the presence of fatty liver is common among chronic alcohol users, and similarly in the obese/diabetic population, only a small number of these people will go on to develop significant disease.[3,9] The tremendous variation in presence and severity of liver disease among individuals with a high BMI or significant alcohol use cannot be explained by environmental factors alone. There is a significant genetic component to the pathogenesis of ALD and NAFLD. Two different research approaches to establishing the genetic contribution to disease are candidate gene studies and genome-wide association studies (GWAS). Candidate gene studies attempt to identify genes that play a role in disease based upon prior knowledge of specific genes and their functions, and exploring whether differences exist between subjects with and without disease. Despite great efforts exploring genes which are known to be important in detoxification processes and alcohol metabolism in the liver, candidate gene studies in ALD have not identified many robustly validated modifier genes affecting ALD development and severity.[6] In NAFLD, candidate genes known to play a role in insulin regulation, fatty acid and triglyceride metabolism, oxidative stress, endotoxin and cytokine activity, and fibrogenesis have all been explored with greater success (see Fig 1).[28]

The most significant advances in identifying genetic contributors to NAFLD have come from GWAS with many subsequently being shown to have an effect in ALD as well. Of these, a non-synonymous single nucleotide polymorphism, rs738409 (c.444 C>G, I148M) in patatin-like phospholipase domain-containing 3 (*PNPLA3*), encoding the adiponutrin protein, has consistently shown a strong correlation with NAFLD and also ALD.[6,29] Romeo *et al*[30] first demonstrated the strong correlation between hepatic triglyceride content (steatosis) and the I148M PNPLA3 variant. Furthermore, this variant has been implicated in disease progression from steatosis to steatohepatitis, fibrosis, cirrhosis and HCC in NAFLD.[31,32] Much effort has been directed towards uncovering exactly what PNPLA3 does and how this is subverted in the development of steatosis and NASH. The results are contentious, however, PNPLA3 appears to possess enzymatic functions as a triacylglycerol lipase and is highly expressed in the liver.[28,33] Some researchers report findings that suggest the I148M variant is a loss of function mutation that results in a decrease in triglyceride hydrolysis and thus its accumulation in the liver, while others argue there is a gain of function that increases triglyceride synthesis. Studies which have examined the effects of overexpression of wild-type PNPLA3 have consistently failed to show a reduction in hepatic fat content, however studies using overexpression of the I148M variant show an increase in liver fat accumulation, and suggest PNPLA3 mediates remodelling of lipid droplets.[34] Overexpression of PNPLA3 I148M specifically in hepatocytes of mice, but not in adipocytes, resulted in hepatic steatosis.[34] However, despite the presence of fat accumulation, there was no inflammation or fibrosis. Given that there is a large body of evidence showing a high correlation between the I148M PNPLA3 variant and NASH, further mechanistic studies are needed to clarify this apparent discrepancy. PNPLA3 has also been implicated in disease progression in ALD and associated with increased risk of HCC.[35] While we have gained some insights into the role of PNPLA3, its enzymatic activity and differential effects upon hepatic steatosis, exactly how PNPLA3 contributes to disease progression in NAFLD and ALD warrants further study.

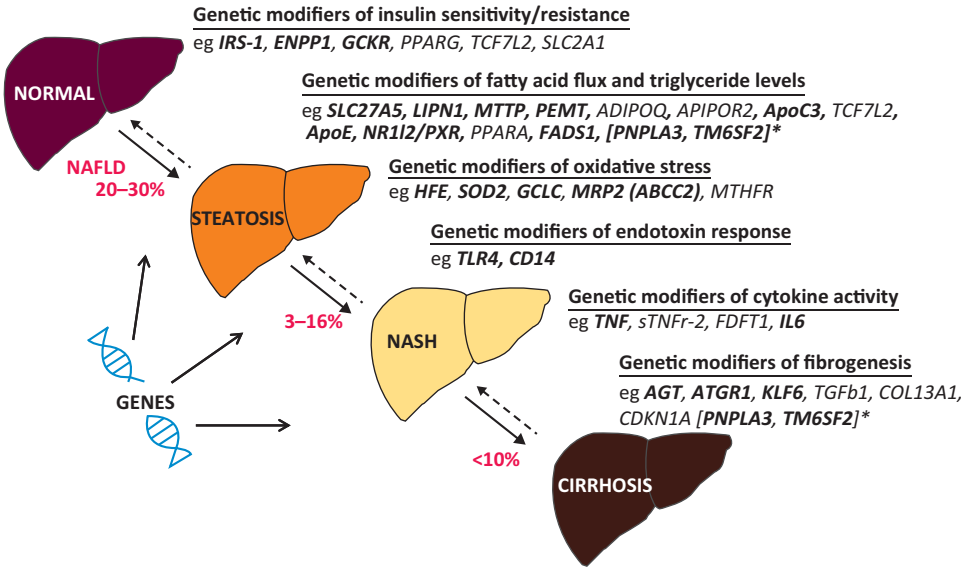

Fig 1. Genetic modifiers associated with NAFLD. Numerous genetic modifiers have been associated with progression of NAFLD from steatosis to advanced fibrosis and cirrhosis. Of these, the best validated are *PNPLA3* and *TM6SF2*, which have been associated with all stages for disease progression. *Identified with GWAS not candidate gene studies. GWAS = genome-wide association study; NAFLD = non-alcoholic fatty liver disease; NASH = non-alcoholic steatohepatitis; PNPLA3 = patatin-like phospholipase domain-containing 3; TM6SF2 = transmembrane 6 superfamily member 2.

Another genetic variant recently identified by GWAS as having a strong association with NAFLD and disease progression to fibrosis and cirrhosis is transmembrane 6 superfamily member 2 (*TM6SF2*) (rs58542926 c.449 C>T, E167K). This gene appears to play a role in lipid homeostasis with the less common variant being associated with increased hepatic fat accumulation but a decrease in free serum levels of lipids, reduced circulating very-low-density lipoprotein and lower cardiovascular disease risk.[36,37] *TM6SF2* may influence whether consequences of the metabolic syndrome will be manifest as hepatic or cardiovascular end-organ damage and has been described as a 'master regulator of metabolic syndrome outcome'.[38] Further work to understand the physiological and pathophysiological role of *TM6SF2* is ongoing.

Clinical perspectives

Although ALD and NAFLD are often identified through incidental findings of raised liver enzymes, particularly raised ALT,[17] levels of liver enzymes are poorly correlated with disease severity. For example, in NAFLD, ALT levels do not help differentiate between steatosis and NASH, or identify advanced fibrosis stage.[9] Full discussion of non-invasive testing for NAFLD falls outside the scope of this article but has recently been reviewed elsewhere.[7] Routine imaging methods such as ultrasound, CT and MRI are unable to differentiate between steatosis and steatohepatitis, and they cannot identify fibrosis until there is already advanced cirrhosis. Other imaging methods have been developed to identify liver fibrosis, including transient elastography (Fibroscan).[7] Numerous scores have

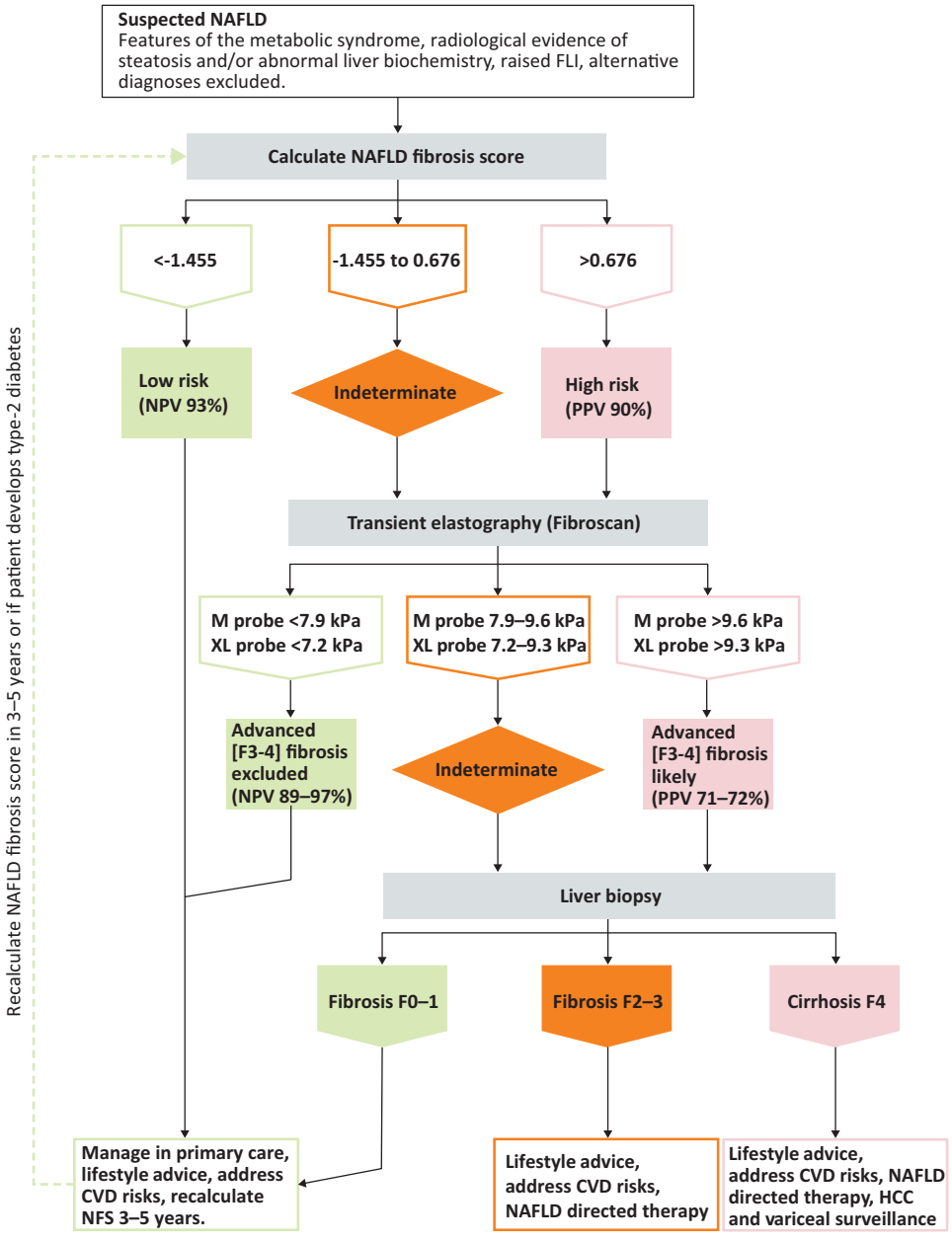

Fig 2. An algorithm used for clinical risk stratification of NAFLD in our centre. CVD = cardiovascular disease; FLI = fatty liver index; HCC = hepatocellular carcinoma; NAFLD = non-alcoholic fatty liver disease; NFS = NAFLD fibrosis score; NPV = negative predictive value; PPV = positive predictive value.

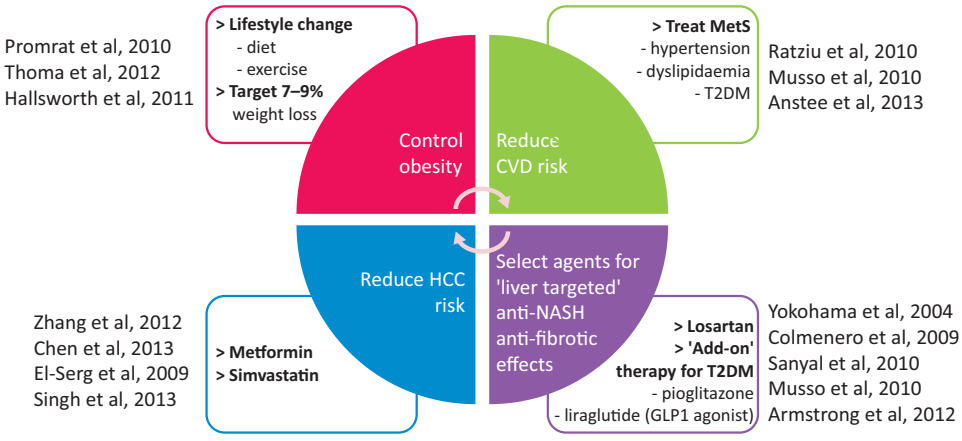

Fig 3. Management priorities in NAFLD. There are currently no licensed medical treatments for NAFLD. The first priority is weight loss through lifestyle change (diet and exercise). Medical therapies targeting individual features of the MetS should be used, selecting those that may have additional liver-directed beneficial effects based on current evidence. GLP-1 = glucagon-like peptide-1; HCC = hepatocellular carcinoma; MetS = metabolic syndrome; NAFLD = non-alcoholic fatty liver disease; NASH = non-alcoholic steatohepatitis; T2DM = type-2 diabetes mellitus.

been proposed to aid risk stratification. Of these, the NAFLD fibrosis score (online calculator at www.nafldscore.com) and FIB-4 score are probably the best established, incorporating factors such as age, BMI, diabetes and routine blood tests. Although their positive predictive value for advanced liver fibrosis is modest, both have good negative predictive values and can be used to effectively rule out the presence of advanced fibrosis.[40–42] The NAFLD fibrosis score has also been shown to have value as a prognostic indicator for long-term outcomes, including death and transplantation requirement,[43] and so is now widely adopted to help triage those patents that require further investigation. Despite the development of various new imaging methods, assessment tools and scores, the gold standard remains a histological diagnosis, by liver biopsy[4]. The sequential application of non-invasive tests to triage selected patients for liver biopsy is generally recommended (see Fig 2).

The key principles for treatment and management of ALD and NAFLD begin with lifestyle modifications, respectively targeting abstinence from alcohol and weight loss through dietary restriction. In NAFLD, diet and exercise are important modifiers of body weight and insulin resistance, helping to improve cardiovascular risk, steatosis and inflammation, although the evidence for an impact upon fibrosis is less strong.[44] Given that many patients with NAFLD will die of cardiovascular disease, treatment to reduce cardiovascular risk by addressing diabetic control, hypertension and dyslipidaemia are of major importance (Fig 3). Statins are generally considered safe in NAFLD. Although metformin has limited beneficial effects upon fibrosis progression in NAFLD,[45] longitudinal data suggest that it can reduce the risk of HCC by up to 7% per annum.[46] Use of glitazones is evidenced to improve steatosis, inflammation and possibly fibrosis.[45] There are several on-going trials exploring the use of a variety of novel agents that target various sites in the pathogenesis of NAFLD and NASH, and offer the prospect of improved liver-directed therapies in the near future.

Summary and conclusions

In summary, ALD and NAFLD are significant challenges in day-to-day clinical practice. Both exhibit substantial interindividual variation in disease outcomes and share many common pathogenic features. Substantial advances are being made in understanding disease pathogenesis. Current research, especially in NAFLD, is focusing on improved techniques for patient risk stratification and development of targeted therapies to prevent progression to advanced liver disease. ■

References

1 Williams R, Aspinall R, Bellis M *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* 2014;384:1953–97.
2 Bedogni G, Miglioli L, Masutti F *et al.* Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* 2005;42:44–52.
3 Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol* 2013;10:330–44.
4 Anstee QM, McPherson S, Day CP. How big a problem is non-alcoholic fatty liver disease? *BMJ* 2011;343:d3897.
5 Kotronen A, Westerbacka J, Bergholm R *et al.* Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab* 2007;92:3490–7.
6 Stickel F, Hampe J. Genetic determinants of alcoholic liver disease. *Gut* 2012;61:150–9.
7 Dyson JK, McPherson S, Anstee QM. Non-alcoholic fatty liver disease: non-invasive investigation and risk stratification. *J Clin Pathol* 2013;66:1033–45.
8 Public Health England. *Liver disease profiles.* London: PHE, 2014. Available online at http://fingertips.phe.org.uk/profile/liver-disease [Accessed 16 June 2015].
9 Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis* 2010;28:155–61.
10 Hart CL, Morrison DS, Batty GD *et al.* Effect of body mass index and alcohol consumption on liver disease: analysis of data from two prospective cohort studies. *BMJ* 2010;340:c1240.
11 Cobbold JF, Anstee QM, Taylor-Robinson SD. The importance of fatty liver disease in clinical practice. *Proc Nutr Soc* 2010:1–10.
12 Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011;43:617–49.
13 Argo CK, Caldwell SH. Epidemiology and natural history of non-alcoholic steatohepatitis. *Clin Liver Dis* 2009;13:511–31.
14 Mofrad P, Contos MJ, Haque M *et al.* Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. *Hepatology* 2003;37:1286–92.

15 Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharm Therap* 2011;34:274–85.
16 Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011;43:617–49.
17 Dyson JK, McPherson S, Anstee QM. Republished: Non-alcoholic fatty liver disease: non-invasive investigation and risk stratification. *Postgrad Med J* 2014;90:254–66.
18 McPherson S, Hardy T, Henderson E *et al.* Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: Implications for prognosis and clinical management. *J Hepatol* 2015;62:1148–55.
19 Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998;114:842–5.
20 Anstee QM, Daly AK, Day CP. Genetics of alcoholic and nonalcoholic fatty liver disease. *Seminars in liver disease* 2011;31:128–46.
21 Yamaguchi K, Yang L, McCall S *et al.* Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology* 2007;45:1366–74.
22 Anstee QM, Goldin RD. Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. *Int J Exp Pathol* 2006;87:1–16.
23 Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev* 2008;88:125–72.
24 Zeybel M, Hardy T, Wong YK *et al.* Multigenerational epigenetic adaptation of the hepatic wound-healing response. *Nature Med* 2012;18:1369–77.
25 McClain CJ, Barve SS, Barve A, Marsano L. Alcoholic liver disease and malnutrition. *Alcohol Clin Exp Res* 2011;35:815–20.
26 Misra VL, Chalasani N. Obstructive sleep apnea and nonalcoholic fatty liver disease: causal association or just a coincidence? *Gastroenterology* 2008;134:2178–9.
27 Le Roy T, Llopis M, Lepage P *et al.* Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. *Gut* 2013;62:1787–94.
28 Anstee QM, Day CP. The genetics of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013;10:645–55.
29 Anstee QM, Daly AK, Day CP. Genetic modifiers of non-alcoholic fatty liver disease progression. *Biochim Biophys Acta* 2011;1812:1557–66.
30 Romeo S, Kozlitina J, Xing C *et al.* Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008;40:1461–5.
31 Valenti L, Al-Serri A, Daly AK *et al.* Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2010;51:1209–17.
32 Liu YL, Patman GL, Leathart JB *et al.* Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol* 2014;61:75–81.
33 He S, McPhaul C, Li JZ *et al.* A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J Biol Chem* 2010;285:6706–15.
34 Li JZ, Huang Y, Karaman R *et al.* Chronic overexpression of PNPLA3I148M in mouse liver causes hepatic steatosis. *J Clin Invest* 2012;122:4130–44.
35 Nischalke HD, Berger C, Luda C *et al.* The PNPLA3 rs738409 148M/M genotype is a risk factor for liver cancer in alcoholic cirrhosis but shows no or weak association in hepatitis C cirrhosis. *PLoS One* 2011;6:e27087.
36 Kozlitina J, Smagris E, Stender S *et al.* Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014;46:352–6.
37 Holmen OL, Zhang H, Fan Y *et al.* Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. *Nat Genet* 2014;46:345–51.
38 Kahali B, Liu YL, Daly AK *et al.* TM6SF2: Catch-22 in the Fight Against Nonalcoholic Fatty Liver Disease and Cardiovascular Disease? *Gastroenterology* 2015;148:679–84.
39 Liu YL, Reeves HL, Burt AD *et al.* TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nature Commun* 2014;5:4309.
40 Angulo P, Hui Marchesini JM *et al.* The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007;45:846–54.
41 McPherson S, Anstee QM, Henderson E, Day CP, Burt AD. Are simple noninvasive scoring systems for fibrosis reliable in patients with NAFLD and normal ALT levels? *Eur J Gastroenterol Hepatol* 2013;25:652–8.
42 McPherson S, Stewart SF, Henderson E *et al.* Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut* 2010;59:1265–9.
43 Angulo P, Bugianesi E, Bjornsson ES *et al.* Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2013;145:782–9.e4.
44 Promrat K, Kleiner DE, Niemeier HM *et al.* Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* 2010;51:121–9.
45 Musso G, Gambino R, Cassader M, Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology* 2010;52:79–104.
46 Chen HP, Shieh JJ, Chang CC *et al.* Metformin decreases hepatocellular carcinoma risk in a dose-dependent manner: population-based and in vitro studies. *Gut* 2013;62:606–15.

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