ARTICLE TITLE

Genetic and environmental susceptibility to alcoholic hepatitis

AUTHOR NAMES AND DEGREES

Marsha Y Morgan MD

Moksh Sharma BSc

Stephen R Atkinson MD, PhD

AUTHOR AFFILIATIONS

MYM: Professor, UCl Institute for Liver & Digestive Health, Division of Medicine, Royal Free Campus, University College London, UK

MS: Medical Student UCl Institute for Liver & Digestive Health, Department of Medicine, Royal Free Campus, University College London, UK

SRA: Senior Clinical Fellow, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, UK

AUTHOR CONTACT INFORMATION

Mailing address as above

marsha.morgan@ucl.ac.uk

moksh.sharma.17@ucl.ac.uk

stephen.atkinson@imperial.ac.uk

CORRESPONDING AUTHOR

Marsha Y Morgan

DISCLOSURE STATEMENT

The Authors have nothing to disclose.

KEY WORDS

- Alcoholic hepatitis
- Alcohol-related cirrhosis
- Alcohol-related liver disease
- Alcohol-related steatohepatitis
- Environmental risk factors
- Genetic risk factor
- Host susceptibility

KEY POINTS

- Prolonged alcohol misuse is associated with the development of a spectrum of liver injury ranging from steatosis through steatohepatitis, fibrosis and cirrhosis, to hepatocellular carcinoma.
- Cirrhosis only develops in the minority of excessive drinkers and its clinical course is usually insidious, often presenting only with the onset of hepatic decompensation.
- A small proportion of patients with evolving or established alcohol-related liver disease develop the clinical syndrome of alcoholic hepatitis which manifests as the comparatively rapid onset of, often profound, liver failure.
- A number of constitutional, environmental, and genetic factors have been identified as associated with the risk for developing alcohol-related cirrhosis.
- The same risk factors may play a role in the development of alcoholic hepatitis but the possibility that additional more specific risk factors exist remains to be delineated.

SYNOPSIS

Only the minority of people who misuse alcohol will develop cirrhosis. A number of constitutional, environmental and genetic risk factors have been identified which influence the development of significant liver injury. The key risk factor is the amount of alcohol consumed and whether excessive drinking continues or ceases after identification of the presence of liver damage. Female sex, ethnicity, obesity, coffee consumption, cigarette smoking and exposure to other injurious liver agents have also influence the risk for developing significant disease. Recent large-scale genome-wide association studies have identified several genetic loci consistently associated with the risk for developing alcohol-related cirrhosis - chief amongst them variants in the gene *PNPLA3.* A small number of people with evolving/established alcohol-related liver disease develop the clinical syndrome of alcoholic hepatitis, which has a high associated mortality. It is unclear, at present, whether additional risk factors are involved in the development of this syndrome but there is some suggestive genetic evidence.

Introduction

The development of advanced liver injury in patients who misuse alcohol, even those who consume the greatest quantities, is not inevitable. Indeed, only a comparative minority of heavy drinkers develop significant liver disease. Furthermore the rate of progression shows significant heterogeneity. Considerable effort has been invested in trying to determine the environmental and genetic factors that influence the development of advanced alcohol-related liver disease, especially the risk factors which predispose to the clinical syndrome of alcoholic hepatitis.

Steatohepatitis and alcoholic hepatitis: nomenclature

There is considerable confusion over the nomenclature of alcohol-related liver disease and, in particular, the terms steatohepatitis and alcoholic hepatitis. The recent definitions provided by the European Association for the Study of the Liver (EASL) will be adopted here (**Table 1**).[1]

The vast majority of people who develop alcohol-related cirrhosis progress through the stages of steatosis, steatohepatitis, and fibrosis to reach this end-point. Most do so silently and often do not present until their liver disease finally decompensates. However, a comparatively small number of people with a history of prolonged heavy and ongoing alcohol misuse develop the clinical syndrome of alcoholic hepatitis which manifests as the recent onset of jaundice frequently accompanied by other features of liver failure such as hepatic encephalopathy, coagulopathy and ascites.[2] These people show histological features of steatohepatitis, often florid, and a high proportion have established cirrhosis. This condition has a high associated mortality of 15 to 30% in the first month and upwards of 50% within one year of presentation.[3]

Table 1: Nomenclature of alcohol-related liver disease

Term	Definition	Old nomenclature
Alcohol-related liver disease	Any form of liver disease, irrespective of severity, attributable to excess alcohol consumption. Comprises the entire spectrum of disease from steatosis through to cirrhosis	Alcoholic liver disease
Alcohol-related cirrhosis	End-stage liver disease attributable to excess alcohol consumption	Alcoholic cirrhosis
Steatohepatitis due to alcohol-related liver disease	A histologically defined lesion typified by the presence of features including steatosis, ballooning degeneration of hepatocytes, inflammatory infiltrates, Mallory-Denk bodies and megamitochondria	Alcoholic steatohepatitis
Alcoholic hepatitis	A clinical syndrome typified by the recent onset of progressive and typically profound jaundice, often with additional clinical features of liver failure, in patients with a history of chronic, excess alcohol misuse	Alcoholic hepatitis

Adapted from: EASL Clinical Practice Guidelines: Management of alcohol-related liver disease.[1] J Hepatol 2019; 69:154-181; with permission

The presence of steatohepatitis is considered a necessary pre-requisite for the development of progressive liver disease leading to cirrhosis. However, it is only infrequently accompanied by the clinical syndrome of alcoholic hepatitis and this distinction is extremely important. Thus, exploring

the environmental and genetic risk factors for the development of alcoholic hepatitis is a two step process as the majority of people with this syndrome already have established cirrhosis, indicating phenotypic overlap. In consequence, the risk factors that predispose to the development of alcohol-related liver disease must first be delineated and then factors that are additionally or specifically associated with the risk for developing the clinical syndrome of alcoholic hepatitis can be explored.

Natural history of alcohol-related liver disease

Excess alcohol consumption is associated with the development of liver injury. Early liver biopsy studies identified a spectrum of change associated with sustained alcohol ingestion progressing from steatosis to steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) (**Fig. 1**). These stages are not mutually exclusive and often co-exist. The prevalence of biopsy-proven liver disease among drinkers is largely unknown although a recent meta-analysis reported that within cohorts of hazardous drinkers, approximately 15% had normal liver histology, 27% had hepatic steatosis, 24% had steatohepatitis while 26% had cirrhosis.[4] The annualized rates of progression of pre-cirrhotic disease to cirrhosis were 1% (0–8%) for patients with normal histology, 3% (2–4%) for hepatic steatosis, 10% (6–17%) for steatohepatitis and 8% (3–19%) for fibrosis.[4]

Overall, it is estimated that only 15 to 20% of people drinking excessively will develop cirrhosis, no matter how much they drink nor for how long.[5,6] The prevalence of cirrhosis among individuals abusing alcohol varies from 9-18% in autopsy studies and from 12-31% based on liver biopsy series.[6-8] In a recent meta-analysis based on 38 observational studies, the incidence of alcohol-related cirrhosis in cohorts with alcohol problems ranged from 7-16% after 8–12 years.[9]

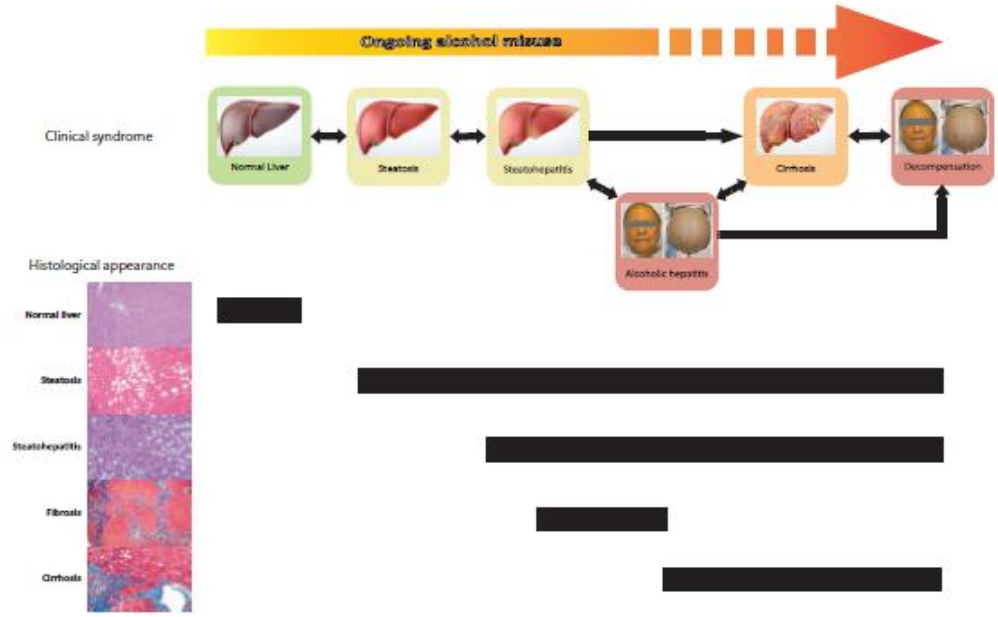

Fig. 1: Natural history of alcohol-related liver disease

Top panel: The development of alcohol-related cirrhosis progress through the stages of steatosis, steatohepatitis, and fibrosis. The majority of patients do not present until their liver disease finally decompensates. A comparatively small number of people develop the clinical syndrome of alcoholic hepatitis which manifests as the recent onset of often profound liver failure.

Bottom panel: Although the histological stages of alcohol-related liver disease are described separately they often coexist. Thus, patients with alcohol-related cirrhosis may also have steatosis while patients with alcoholic hepatitis have a steatohepatitis, often florid, but may also have established cirrhosis.

Modulating effects of drinking behaviour

The effects of drinking behaviour on the evolution of alcohol-related liver disease are substantial (**Fig. 2**). Steatosis usually resolves within 2-4 weeks of cessation of alcohol consumption.[10] Steatohepatitis develops in only a proportion of drinkers even after decades of alcohol abuse and is assumed to be a precirrhotic lesion, although its natural history is not well understood.

Thus, Galambos [11] in a serial liver biopsy study in individuals with steatohepatitis showed that 38% developed cirrhosis, 52% retained the hepatitic lesion, while in 10% the liver lesion regressed despite continued alcohol use. Abstinence did not guarantee regression of the lesion; of those that

maintained abstinence 27% had normal histology, 55% retained the hepatitic lesion and 18% developed cirrhosis.[11]

Parés and coworkers[12] showed that in men with mild to moderate steatohepatitis subsequent drinking behaviour was the major factor influencing outcome whereas in women and in individuals with severe steatohepatitis, of both sexes, progression to cirrhosis was more likely to occur and was less influenced by subsequent drinking behaviour. Data from two long term serial biopsy studies confirmed that the effects of abstinence from alcohol on the progression of steatohepatitis are not entirely predictable and provided evidence that over a given threshold the risk of developing cirrhosis was unrelated to the average daily intake of alcohol. [13,14] In addition they confirmed that progression to cirrhosis was more likely in women and in individuals with severe steatohepatitis, irrespective of subsequent drinking behaviour.[14] Although cirrhosis is an irreversible lesion abstinence from alcohol can have a significant beneficial effect on outcome even in patients with established disease.[15]

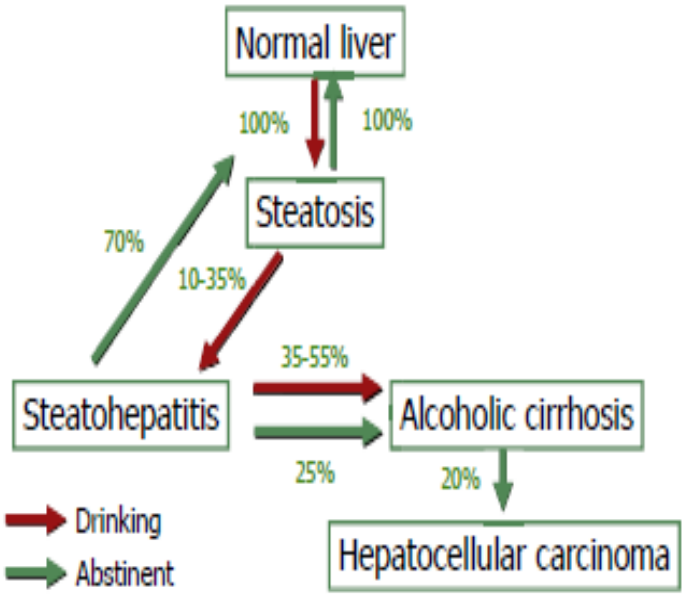

Fig. 2: Effects of continued drinking and abstinence from alcohol on the evolution of alcohol-related liver disease

Risk factors for the development of alcohol-related cirrhosis

Environmental and host related factors

Drinking behaviour

Excess alcohol consumption is the major epidemiological factor determining the risk for developing alcohol-related cirrhosis.[16] However, the precise nature of the relationship between alcohol consumption and cirrhosis risk is debated.[17] Results from early seminal studies [18-23] and a later meta-analysis of 15 studies [24] showed that the risk for cirrhosis was significantly increased with alcohol intakes of approximately 25-30 g/day and that thereafter the risk increased, almost exponentially, with greater daily as well as cumulative alcohol consumption. However, a Danish group reported that there was a risk threshold at approximately 60 g/day beyond which there was no further dose-response relationship and no additional risk with drinking greater amounts of alcohol.[17,25] Conversely, a more recent Danish study reported a clear dose-dependent association between the level of alcohol intake and risk for developing alcohol-related cirrhosis among both men and women drinking > 24 g/day.[26] A recent meta-analysis of seven studies reported a steadily increasing dose-response relationship in women from as little as 12 g/day and some evidence for a threshold effect in men with alcohol intakes greater than 84g/day.[27]

A number of studies have reported that daily drinking is associated with a higher cirrhosis risk than binge drinking.[13,22,25-28] However, failing to take account of the total amount of alcohol consumed when comparing regular and binge drinkers may confound these findings.[28,29] There is some evidence that drinking wine, as opposed to other beverage types, is associated with a lower cirrhosis risk.[26,30] However, it is the amount of contained alcohol that is the key factor;[31] apparent

differences in cirrhosis risk, by beverage, are likely confounded by variations in dietary and lifestyle factors.[32,33] Consuming alcohol together with food may reduce the cirrhosis risk,[23] whereas regular consumption of a diet high in fat but low in carbohydrate and protein may increase the risk.[32]

Sex

Men consume significantly more alcohol than women and consequently have higher alcohol-related cirrhosis rates.[20,34] However, women have a significantly higher relative risk of developing alcohol-related liver disease than men for a given level of alcohol consumption.[34] This difference has been consistently reported across individual studies [20,24,34,35] and in a meta-analysis involving 1,477,887 individuals (**Fig. 3**).[36] The thresholds above which alcohol consumption should be considered harmful are, accordingly, lower in women at 20-40 grams/day than in men >60 grams/day.[31] The difference in susceptibility primarily relates to sex-related differences in body composition which result in a smaller apparent volume of distribution of alcohol in women and consequently higher blood alcohol concentration, for a given weight-adjusted ingested dose, and greater tissue levels of exposure.[37]

Ethnicity

There are notable interethnic differences in alcohol-related cirrhosis risk. A study in the UK reported that non-Muslim men of South Asian descent present with alcohol-related cirrhosis at a younger age and with a higher than expected frequency compared to their white British counterparts, while Afro-Caribbean men were significantly underrepresented in this patient group.[38] In the USA white men and women of Hispanic, predominantly Mexican ancestry, have a higher risk for cirrhosis mortality compared with black and white non-Hispanic men and women.[39]

Individuals of Hispanic origin have also been shown to present with alcohol-related cirrhosis up to 10 years earlier than their white Caucasian counterparts.[40] However, these differences could represent constitutional differences in alcohol metabolism or differences relating to the amounts and types of alcohol consumed, dietary intake, socioeconomic status, and access to health care.

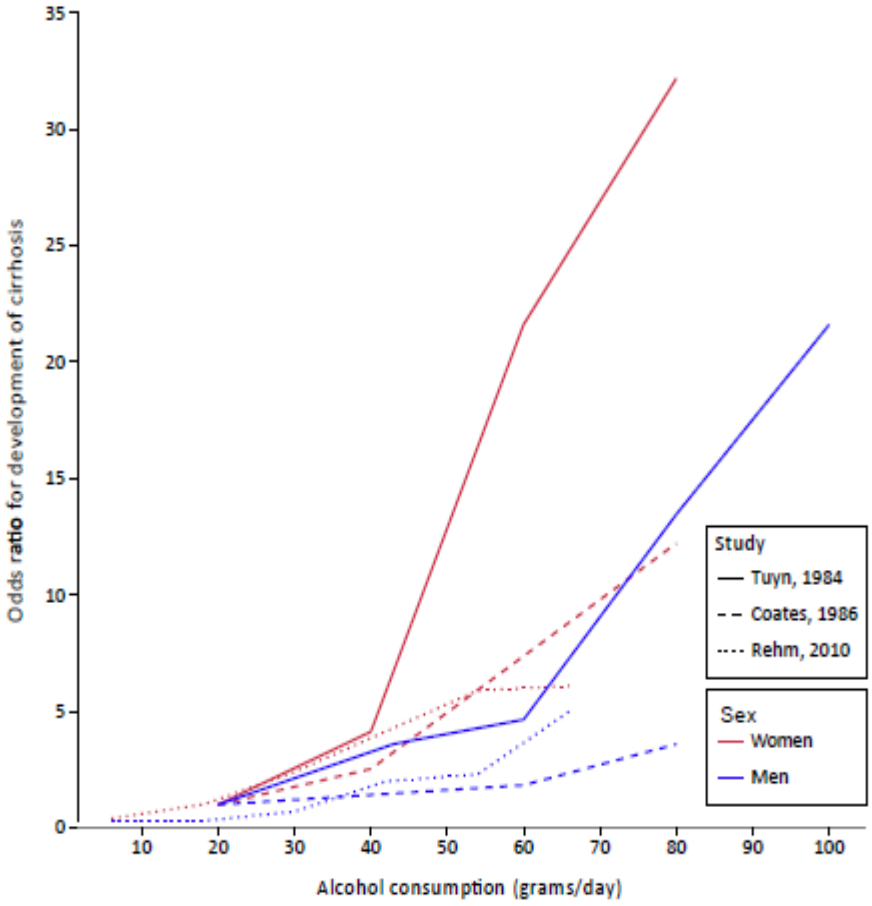

Fig. 3: Odds ratios for the development of cirrhosis at different levels of alcohol consumption, by sex

Data are drawn from two case-control studies[20,35] and a meta-analysis[36]. Although the odds ratios vary between studies, potentially as a function of estimation of alcohol consumption and case acquisition, within studies the risk of cirrhosis for a given level of alcohol consumption is consistently higher in women compared to men.

An interesting paradox is the indirect protection against the development of alcohol-related cirrhosis afforded to individuals of east Asian ancestry who carry the SNP rs671 in *ALDH2* and, as a consequence, develop high levels of circulating acetaldehyde following alcohol ingestion and so tend to avoid alcohol. A meta-analysis of published studies identified a significant and robust association between possession of this variant and the development of alcohol-related physical harm, including cirrhosis.[41] There is, however, no evidence that this is anything other than an indirect effect.

Body weight and composition

Both alcohol misuse and obesity are independent risk factors for the development of cirrhosis. Whether obesity is an independent risk factor for the development of alcohol-related cirrhosis is debated. Overweight or obese white, middle-aged women in the UK who consume low to moderate amounts of alcohol are at increased risk for developing cirrhosis compared to women with a body mass index (BMI) of 22.5 - 25; the risk increases by about 28% for each five unit increase in BMI.[42] The absolute increase in cirrhosis rates with increasing BMI is substantially greater in women drinking 150 g of alcohol per week or more than in those drinking 70 g a week (**Table 2**). However, compared with the other known risk factors for cirrhosis, the effect of obesity in this study was moderate but none of the participants drank excessively. The resultant liver injury likely represents the combined insults of both alcohol and obesity.

However, Naveau and colleagues[43] undertook a detailed study of patients with a history of prolonged harmful use of alcohol, in whom the degree of liver injury was confirmed histologically. They reported that those who had been overweight for at least 10 years, defined as a BMI 25 or higher for women and 27 or higher for men, were 2.15 times more likely to have cirrhosis than

their counterparts who were not overweight. In a later study the same group reported that the visceral abdominal fat level, assessed using abdominal height in the supine position, is an independent risk factor for the development of fibrosis in patients with a history of prolonged harmful use of alcohol, regardless of total body fat content assessed using the BMI.[44]

Table 2: Cirrhosis risk in women drinking low to moderate amounts of alcohol, by Body Mass Index.[42]

Alcohol intake (g/week)	< 70		≥ 150	
Body Mass Index	22.5-25	≥ 30	22.5-25	≥ 30
Absolute risk (95% confidence interval) of cirrhosis/1000 women over 5 years	0.8 (0.7-0.9)	1.0 (0.9-1.2)	2.7 (2.1-3.4)	5.0 (3.8-6.6)

Adapted from Liu B, Balkwill A, Reeves G, et al. Body mass index and risk of liver cirrhosis in middle aged UK women: prospective study. BMJ 2010; 340:c912; with permission.

Tobacco and cannabis

The prevalence of tobacco smoking, amongst individuals who drink excessively, is high.[45] Cigarette smoking is independently associated with the risk for developing alcohol-related cirrhosis with smokers of a pack or more per day at treble the risk of non-smokers.[46] This risk is particularly high amongst women.[45] Thus, in a very large Danish population-based study, mean follow-up time of 21 years, approximately 26% of cases of alcohol-related cirrhosis in women and 8% of cases in men could be attributed to smoking, after adjustment for alcohol intake.[45] Conversely, the GenomALC consortium recently reported a lower prevalence of smoking in cases with alcohol-related cirrhosis than in controls drinking excessively with no liver disease.[47]

In a large and complex analysis of the 2014 Healthcare Cost and Utilization Project-Nationwide Inpatient Sample, involving 319,514 participants with a past or current history of alcohol abuse, Adejumo and colleagues[48] reported that additional cannabis use was associated with a 55% reduction in the likelihood of developing alcohol-related cirrhosis; the higher the intake of cannabis the greater the reduction in the likelihood of cirrhosis development.[48] The GenomALC consortium reported that in individuals with a history of alcohol abuse prolonged regular cannabis use was three times commoner in controls with no liver disease (27%) than in cases with alcohol-related cirrhosis (9%); the prevalence differences between cases and controls were highly significant up to the age of 60 years.[47]

Coffee drinking

Coffee drinking is inversely related to alcohol related cirrhosis risk suggesting a protective effect;[46] people drinking four or more cups a day have one-fifth the risk of developing cirrhosis of non-coffee drinkers.[46] Kennedy and colleagues[49] undertook a meta-analysis of nine studies to further characterize the relationship between coffee consumption and cirrhosis risk. They confirmed the protective effect of coffee on cirrhosis risk and the dose-related effect. They concluded that an increase in coffee consumption of two cups per day is associated with a near halving of the cirrhosis risk. In the GenomALC study controls with no liver disease were more likely than cases with alcohol-related cirrhosis to have been coffee drinkers during the time they were drinking alcohol heavily and to have drunk more coffee per day.[47]

Co-morbid liver disease

Alcohol may act synergistically with other injurious agents to increase the risk for developing significant liver injury. Thus, individuals who drink heavily and have features of the metabolic syndrome, [43,50] chronic hepatitis B and C viral infection,[51,52] or HIV infection[53] display accelerated rates of hepatic fibrosis. People with chronic HCV infection whose alcohol intake exceeds 50 g/day have a significantly higher risk of advanced fibrosis than those who drink less or not at all.[54] Patients with hereditary haemochromatosis who drink more than 60 g alcohol per day are approximately nine times more likely to develop cirrhosis than those who drink less than this amount.[55]

Genetic risk factors

Familiarity and heritability

There are very few epidemiological studies relating to the familiarity and heritability of alcohol-related cirrhosis.

No formal family studies have been conducted in people with alcohol-related liver disease. However, in the GenomALC study cirrhosis risk was significantly increased in individuals with a history of prolonged excessive alcohol consumption if their father also drank excessively and died of liver disease.[47, 56] These findings are potentially supportive of a genetic contribution but the effects of a shared environment or of inherited epigenetic changes, such as imprinting or other mechanisms of selective transmission cannot be excluded.

Available estimates of the heritability of alcohol-related cirrhosis are available from a single study of 15,924 male twin pairs.[57] The concordance for alcohol-related cirrhosis was three times higher in monozygotic than in dizygotic twins and this was confirmed in a second analysis undertaken over a decade later.[58] The heritability estimates for alcohol-related cirrhosis ranged from 21% to 67%. There was some disagreement between the two reports in relation to the proportion of the genetic variance that was independent of the heritability of alcohol dependence.[58]

Candidate gene studies

The selection of candidates for genetic studies in alcohol-related liver disease has, in the main, been based on assumptions relating to the mechanisms of the liver injury. Thus associations, have been sought, in case-control studies, between the development of alcohol-related liver injury and functional variants in loci implicated in alcohol and lipid metabolism; endotoxin–mediated inflammation and cytokines; immune responses; oxidative stress and fibrogenesis, amongst others.[59-61]

These studies have, with few exceptions, shown no evidence of association between the variants of interest and the risk for developing alcohol-related cirrhosis.[59-61] However, meta-analyses of the association studies involving *Tumor Necrosis Factor-α (TNFA)* and *Glutathione S-transferase 1* (*GSTM1*) suggest that variants at these loci may be associated with an increased risk for developing alcohol-related liver disease.[62,63] However, no significant associations with these loci have been identified in genome-wide association studies (GWAS).[64-66]

In 2008, Romero and colleagues[67] reported that rs738409:G in *Patatin-like Phospholipase Domain-Containing Protein 3* (*PNPLA3*) was strongly associated with the risk for developing non-alcoholic

fatty liver disease (NAFLD). Evaluation of this locus, using a candidate gene approach, identified and validated robust associations between this variant in *PNPLA3* and the risk for developing alcohol-related cirrhosis both in individual studies and meta-analyses.[68-72] The effect sizes were in the range expected for a relatively frequent single nucleotide polymorphism (SNP) in a complex disease while the population-attributable risk for progression to alcohol-related cirrhosis conferred by carriage of *PNPLA3* rs738409:G was 26.6%, suggesting that other modifiers are likely to exist.[73] Studies have also shown that carriers of *PNPLA3:* rs738409:G (i) present with cirrhosis after a shorter drinking history;[74] (ii) develop decompensated cirrhosis at an earlier stage of their disease history;[75] and, (iii) are more likely to die of their liver disease.[76]

In 2018, Abul-Husn and colleagues[77] identified a splice variant rs72613567 in *Hydroxysteroid 17-Beta Dehydrogenase13* (*HSD17B13*), which appeared to protect against the development of alcohol-related cirrhosis in people of European decent although the total number of cases was very small. This variant also appeared to attenuate the risk for developing progressive liver injury associated with carriage of rs738409:G in *PNPLA3.* A case-control study, undertaken by Stickel and colleagues,[78] involving 6,171 participants, found that carriage of *HSD17B13* rs72613567:TA was associated with a lower risk for developing both alcohol-related cirrhosis (OR, 0.79; 95% CI, 0.72-0.88; $P = 8.13 \times 10^{-6}$) and HCC (OR, 0.77; 95% CI, 0.68-0.89; $P = 2.27 \times 10^{-4}$). They further reported that carriage of *HSD17B13* rs72613567:TA attenuated the risk for developing alcohol-related cirrhosis associated with *PNPLA3* rs738409:G in both men and women, but the protective effect against the subsequent development of HCC was observed only in men.[78]

Finally, in 2019, Strnad and coworkers[79] identified a significant association between heterozygous carriage of the Pi*Z (rs28929474) variant in *serpin family A member 1 (SERPINA1)* and the risk for

developing alcohol-related cirrhosis (adjusted OR=5.8 [95% CI 2.9 to 11.7]). Homozygous carriage of Pi*Z is associated with severe alpha 1 antitrypsin deficiency which results in damage to the lungs and liver.

Thus, case–control studies have identified variants in *PNPLA3, HSD17B13 and SERPINA1,* which differentially affect the risk for developing advanced alcohol-related liver disease.

Genome-wide association studies

Three GWAS in alcohol-related cirrhosis have been undertake to date.[64-66] These studies have confirmed the associations between cirrhosis risk and variants at loci encoding *PNPLA3, HSD17B13* and *SERPINA1* at genome-wide significance. Additionally, they have identified several novel variants, also at genome-wide significance that are associated with either an increased risk for developing advanced alcohol-related liver disease, for example, rs58542926 in *Transmembrane 6 Superfamily Member 2* (*TM6SF2)*; rs641738 in *Membrane-bound O-Acyltransferase Domain Containing 7* (*MBOAT7*), and rs15052 in *Heterogeneous Nuclear Ribonucleoprotein U like 1 (HNRNPUL1)* or else provide protection against its development for example rs2642438 in *Mitochondrial Amidoxime Reducing Component 1 (MARC1*) and rs374702773 in *Fas Associated Factor Family Member 2* (*FAF2*) (**Table 3**).

It is highly likely that larger studies and further meta-analyses will identify additional risk and protective factors associated with the development of alcohol-related cirrhosis.

Table 3: Genes identified in genome wide association studies which associated with the risk for developing alcohol-related cirrhosis at genome-wide significance

Study	Phenotypes	Gene	Lead Single Nucleotide Polymorphism (SNP)[a]
Buch et al [64] 2015	Alcohol-related cirrhosis (n=712) Heavy drinking controls (n=1426)	*PNPLA3* *SUGP1* *MBOAT7*	rs738409:G (OR 2.03, p= 1.54×10^{-48})[b] rs10401969:C (OR 1.57, p=7.89×10^{-10})[d,f] rs626283:C (OR 1.33, p=1.03×10^{-9})[d]
Innes et al [65] 2020	APRI, FIB-4, Forns index, serum ALT and AST in hazardous drinkers from UK Biobank (n=35 839)	*PNPLA3* *SUGP1* *HNF1A* *ARHGEF3* *SERPINA1* *TRIB1* *HNRNPUL1* *MARC1* *HSD17B13*	rs738408:T (β=0.803, p=2.21×10^{-51})[b,e] rs10401969:C (β=0.660, p=1.21×10^{-15})[b,f] rs11065384:T (β=0.199, p=1.01×10^{-4})[b] rs11925835:T (β=-0.134, p=6.64×10^{-3})[b] rs28929474:T (β=0.717, p=2.77×10^{-5})[b] rs2954038:C (β=0.140, p=8.75×10^{-3})[b] rs15052:C (β=0.220, p=1.06×10^{-3})[b] rs2642438:A (β=-0.223, p=4.51×10^{-5})[b] rs72613567:TA (β=-0.237, 1.38×10^{-5})[b]
Schwantes-An et al. [66] 2020	Alcohol-related cirrhosis (n=1128) Heavy drinking controls (n=614)	*PNPLA3* *HSD17B13* *FAF2* *SERPINA1* *SUGP1*	rs2294915:T (OR 2.07, p=1.28×10^{-53})[b,e] rs10433937:G (OR 0.78, p=2.85×10^{-9})[b,a] rs11134977:C (OR=0.79, p=1.56×10^{-8})[c] rs28929474:T (OR 1.90, p=1.99×10^{-8})[d] rs10401969:C (OR 1.49, p=2.40×10^{-9})[d,f]

[a]The most significantly associated SNP at a given locus reported by the study authors.
SNPs, risk alleles, odds ratios, beta estimates and p-values are based on the final reported meta-analyses of study results
[b]Genome-wide significance reported in primary analysis
[c]Genome-wide significance reported in conditional analysis
[d]Genome-wide significance reported in meta-analysis with replication or additional cohorts
[e]In strong LD with *PNPLA3*:rs738409
[f]In strong LD with *TM6SF2*:rs5854926

Epigenetics

Epigenetic modifications are hereditable changes that impact on gene expression without altering the nucleotide sequence. Examples include: DNA methylation, histone modifications and RNA silencing by microRNAs (miRNAs). Epigenetic mechanisms which are deregulated by alcohol in the liver may contribute to the pathogenesis and progression of alcohol-related liver disease but their role as possible risk factors for the development of alcohol-related liver disease needs further exploration.

Specific risk factors for the clinical syndrome of alcoholic hepatitis

Very little information is available on possible environmental and genetic risk factors, additionally or specifically associated with the risk for developing the clinical syndrome of alcoholic hepatitis.

Drinking behaviour

There does not appear to be a direct relationship between the amount or pattern of drinking and the development of alcoholic hepatitis beyond the generally accepted threshold of >40 grams/day for women and >60 grams/day for men for a minimum of 6 month.[80-82]

Anecdotally, many patients with alcoholic hepatitis report a reduction in food intake in the weeks or months before presentation, together with an increase in alcohol intake or additional binge drinking. This observation is supported by data in mice showing that chronic ethanol feeding plus a single binge dose of ethanol exacerbates hepatic steatosis and neutrophilic inflammation over and above the effects of chronic or binge ethanol feeding alone.[83] However, it is not supported by available observational data, albeit limited, from the Translational Research and Evolving Alcoholic

Hepatitis Treatment (TREAT) consortium; they reported that the total amount of alcohol consumed in the 30 days prior to presentation and the rates of binge drinking in cases with alcoholic hepatitis were significantly lower than in heavy drinking controls with no liver disease.[81]

Case definition in this study was based largely on clinical evaluation and on laboratory findings of a serum bilirubin >2 mg/dL (34 μmol/L and serum aspartate aminotransferase (AST) >50 U/L but the mean (± 1SD) MELD score of 22 ± 7.1 and the mean Maddrey Discriminant Function of 41.6 ± 29.1 would indicate that a high proportion of the included cases did not have severe disease. Thus, the relationship between the recency and nature of alcohol consumption and the risk for developing alcoholic hepatitis remains unclear.

Demographic and lifestyle factors

Older patients recruited by the TREAT consortium were less likely to present with severe alcoholic hepatitis; indeed the age at presentation was significantly and inversely associated with the severity of the disease.[81,82] Female sex has also been reported to be an independent risk factor for the development of alcoholic hepatitis.[43]

Excess weight is a risk factor for the development of alcohol-related liver disease *per se* and has also been shown to be an independent risk factor for the development of steatohepatitis on liver biopsy.[43] Thus, people drinking excessively who have been overweight for at least 10 years, defined by a BMI of 25 or greater in women and 27 or greater in men, are three times more likely to develop biopsy-proven steatohepatitis than those who are not overweight, even after adjusting for age, sex, and drinking behaviour.[43] A report from the TREAT consortium also confirmed that BMI is an independent predictor of severe alcoholic hepatitis.[81]

In contrast, coffee consumption may be protective.[82] Thus, the TREAT consortium found that regular coffee consumption was independently associated with a significantly lower risk for developing severe alcoholic hepatitis (OR = 0.26; 95% CI, 0.15-0.46); only 20% of patients with this syndrome drank coffee regularly compared to 44% of heavy drinkers with no liver disease (P<.001).[82]

Genetic factors

Patients with alcoholic hepatitis often have a background of cirrhosis or else rapidly progress towards cirrhosis irrespective of their subsequent drinking behaviour. This suggests that both presentations have a similar genetic background but as severe alcoholic hepatitis only develops in a minority of patients with alcohol-related liver injury there may be additional genetic variants that are exclusively associated with the risk for developing this syndrome.

A small candidate gene study published in 2011 identified rs738409:G in *PNPLA3* as a risk factor for developing severe alcoholic hepatitis.[84] This finding was confirmed in a much larger study involving the STOPAH cohort.[85] A later reanalysis of the STOPAH data identified a significant interaction between the rs738409 genotype and sex in relation to medium-term mortality which was independent of the return to drinking.[86] Thus, men who were homozygous for *PNPLA3* rs738409:G had a significantly reduced medium term mortality if they remained abstinent from alcohol whereas their female counterparts had a significantly improved medium term survival even if they continued to drink.[86]

Carriage of *HSD17B13:*rs72613567:TA decreases the risk for developing alcohol-related cirrhosis and HCC and attenuates the disease risks associated with carriage of *PNPLA3:*rs738409:G. A

recent candidate gene study reported that carriage of rs72613567:TA was also associated with a decreased risk for developing severe alcoholic hepatitis and likewise attenuated the risk associated with carriage of *PNPLA3:*rs738409:G.[87] In addition, carriage of rs72613567:TA was associate with less severe liver dysfunction, lower disease severity scores and a reduction in serum markers of hepatocellular injury.[87]

In 2017, the TREAT consortium reported the result of a preliminary GWAS looking for genetic variability between heavy drinkers with and without alcoholic hepatitis.[88] Their study cohort was of European decent and comprised of 90 cases with alcoholic hepatitis and 93 controls drinking excessively but had no evidence of liver disease. No single genetic marker was associated with the risk for developing alcoholic hepatitis at genome-wide significance; an association signal was observed for *PNPLA3* rs738409 ($p = 0.01$, OR 1.9, 95% CI 1.1 – 3.1). A variety of bioinformatics techniques were used to identify gene sets and pathways which might be of importance in the development of this syndrome; several of the identified pathways were involved in lymphocyte activation, chemokine signaling, and ethanol degradation. This study had significant limitations in terms of statistical power; thus these preliminary findings need further exploration in much larger population cohorts.[88]

The results of a more recent GWAS support the concept that the development of severe alcoholic hepatitis may be associated with additional genetic risk factors.[89] The study population was of white British/Irish descent and comprised of 812 cases with severe alcoholic hepatitis from the STOPAH trial and 936 controls with a history of alcohol dependence but without evidence of significant liver injury. The results confirmed the previously described pivotal role of *PNPLA3*:rs738409 in determining the risk for developing severe alcoholic hepatitis.[85] In addition,

potential risk loci were identified in *ATP2C2 (ATPase Secretory Pathway Ca2+ Transporter)*, which encodes a Mn+/Ca2+ transporter and is highly expressed in the gastrointestinal tract; *PHYH (Phytanoyl-CoA 2-Hydroxylase)* which is implicated in phytanic acid metabolism; phytanic acid binds to and/or activates the transcription factors peroxisome proliferator-activated receptors (PPAR)-α and retinoid X receptor; and *ANGPT1* (*Angiopoietin 1*) which encodes the angiogenic promoter angiopoietin 1. Gene set enrichment analysis delineated significant associations between the susceptibility to develop severe alcoholic hepatitis and pathways involved in lipid metabolism and inflammation particularly gene groups involved in sterol regulator element binding protein signalling, interleukin 17 secretion and regulation of natural killer T cell proliferation. These findings will need further validation.

Clinical implications of genetic testing

There is some evidence that knowledge of the genetic risk factors for advanced alcohol-related liver disease might facilitate the management of individuals misusing alcohol. Thus, carriage of *PNPLA3* rs738409 and *TM6SF2* rs58542926 accounts for half of the attributable risk for HCC in patients with alcohol-related cirrhosis and it has been suggested that genotyping these two SNPs would facilitate HCC risk-stratification in this population.[90] Similarly a genetic risk score based on allelic variants in *PNPLA3, TM6SF2* and *HSD17B13* has been shown to be useful in identify individuals in the general population at higher risk for cirrhosis and HCC.[91] Nevertheless, it is unlikely that genetic testing will play a role in the management of people with alcohol-related liver disease at the present time. However, an increased understanding of the genetic basis of alcohol-related liver disease may provide insights into it pathogenesis and help identify potential therapeutic targets.

Summary

The development of advanced alcohol-related liver disease is associated with a complex interplay between constitutional, environmental and genetic risk factors (**Fig. 4**). The duration and quantity of alcohol consumption are clear determinants of advanced disease, with female sex increasing the risk of liver disease for any given level of consumption; the role of ethnicity is less clear. Lifestyle factors such as diet, body weight, tobacco and cannabis use play a role as does the presence of other risk factors for liver disease such as obesity and chronic infection with hepatitis Band C. Recent large-scale GWAS have now identified several genetic loci consistently associated with the risk for developing alcohol-related cirrhosis. Of particular interest is the fact that genes at these loci appear to be predominantly related to lipid metabolism and many are also risk loci for the development of non-alcohol related fatty liver disease. However, the precise biological mechanisms resulting in the development of alcohol-related liver disease remain to be elucidated. The association between these factors and alcohol-related cirrhosis is taken as a proxy indicator for their association with the development of alcoholic steatohepatitis in the absence of studies evaluating this histological phenotype. There is some evidence that demographic, lifestyle and genetic factors may play a role in the development of the clinical syndrome of alcoholic hepatitis but further delineation of these risk factors is required.

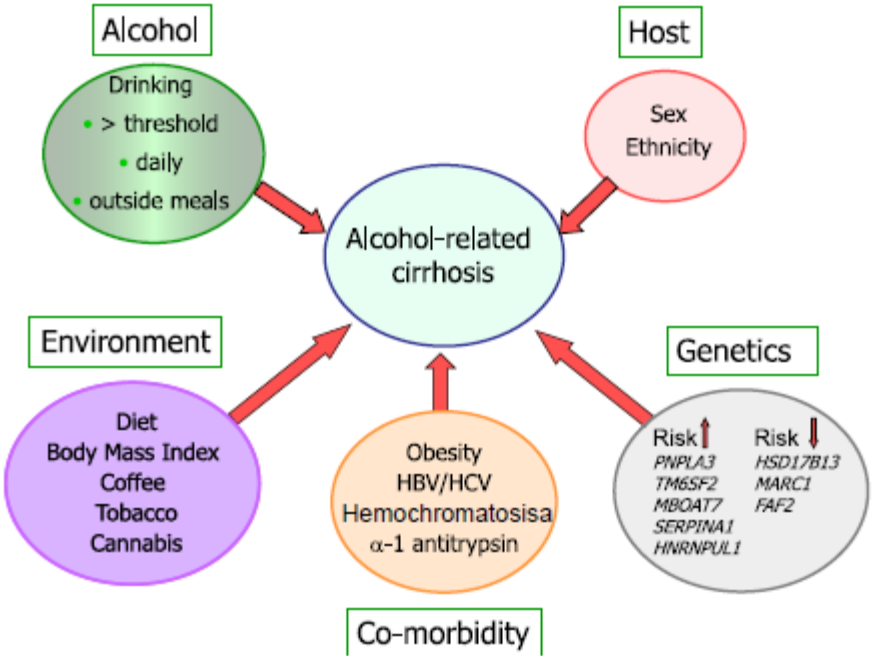

Fig. 4: Risk factors for the development of alcohol-related cirrhosis

Clinical Care Points

- Only a minority of individuals who drink heavily will develop significant alcohol-related liver disease but susceptible individuals can not be identified, with any certainty, at the present time.
- The main management goal in people drinking at hazardous and harmful levels is o significantly reduce their alcohol consumption
- Additional attention should be paid to other modifiable risk factors for the development of advanced liver disease; thus encouraging weight loss smoking cessation and increasing coffee consumption may be beneficial

- The popularity of genetics home testing may prompt enquires from patients about their particular risk for developing alcohol-related liver disease; this may provide an opportunity to advise modification of any identifiable risk factors

References

1. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of alcohol-related liver disease. J Hepatol 2018;69:154-181.

2. Sandahl TD, Jepsen P, Thomsen KL, et al. Incidence and mortality of alcoholic hepatitis in Denmark 1999-2008: a nationwide population based cohort study. J Hepatol 2011;54:760-4.

3. Thursz MR, Richardson P, Allison M, et al. Prednisolone or pentoxifylline for alcoholic hepatitis. N Engl J Med 2015;372:1619–28.

4. Parker R, Aithal G, Becker U, et al. Natural history of histologically proven alcohol-related liver disease: A systematic review. J Hepatol 2019;71:586-93.

5. Lelbach WK. [Liver damage in chronic alcoholism. Results of a clinical, clinical-chemical and bioptic-histological study in 526 alcoholic patients during a low-calorie diet in an open drinking sanatorium]. Acta Hepatosplenol 1966;13:321-49.

6. Leevy CM. Cirrhosis in alcoholics. Med Clin North Am 1968;52:1445–51.

7. Lelbach WK. Leberschaden bei chronischem Alcoholismuss I-III. Acta Hepatosplenologica 1966; 13: 321–49.

8. Galambos, JT, ed. Major Problems in Internal Medicine, Vol. 17, Cirrhosis. Philadelphia: WB Saunders, 1979.

9. Askgaard G, Kjær MS, Tolstrup JS. Opportunities to prevent alcoholic liver cirrhosis in high-risk populations: a systematic review with meta-analysis. Am J Gastroenterol 2019;114:221-32.

10. Leevy CM. Fatty liver: a study of 270 patients with biopsy proven fatty liver and a review of literature. Medicine 1962;41:249-76.

11. Galambos JT. Natural history of alcoholic hepatitis. 3. Histological changes. Gastroenterology 1972;63:1026-35.

12. Parés A, Caballería J, Bruguera M, et al. Histological course of alcoholic hepatitis. Influence of abstinence, sex and extent of hepatic damage. J Hepatol 1986;2:33–42.

13. Sørensen TI, Orholm M, Bentsen KD, et al. Prospective evaluation of alcohol abuse and alcoholic liver injury in men as predictors of development of cirrhosis. Lancet 1984;2:241-4.

14. Marbet UA, Bianchi L, Meury U, et al. Long-term histological evaluation of the natural history and prognostic factors of alcoholic liver disease. J Hepatol 1987;4:364-72.

15. Saunders JB, Walters JR, Davies AP, et al**.** A 20-year prospective study of cirrhosis. BMJ 1981;282:263-6.

16. Lelbach WK. [Dose-effect relation in alcoholic liver lesions]. Dtsch Med Wochenschr 1972;97:1435-6.

17. Sørensen TI. Alcohol and liver injury: dose-related or permissive effect? Liver 1989;9:189-97.

18. Péquignot G. [The role of alcohol in the aetiology of liver cirrhoses in France; results and significance of a systematic survey]. Munch Med Wochenschr 1961;103:1464-8.

19. Péquignot G, Tuyns AJ, Berta JL. Ascitic cirrhosis in relation to alcohol consumption. Int J Epidemiol 1978;7:113-20.

20. Tuyns AJ, Péquignot G. Greater risk of ascitic cirrhosis in females in relation to alcohol consumption. Int J Epidemiol 1984;13:53-7.

21. Lelbach WK. Cirrhosis in the alcoholic and its relation to the volume of alcohol abuse. Ann N Y Acad Sci 1975;252:85–105.

22. Lelbach WK. Epidemiology of alcoholic liver disease. Prog Liver Dis 1976;5:494-515.

23. Bellentani S, Saccoccio G, Costa G, et al. Drinking habits as cofactors of risk for alcohol induced liver damage. Gut 1997;41:845–50.

24. Corrao G, Bagnardi V, Zambon A, et al. A metaanalysis of alcohol consumption and the risk of 15 diseases. Prev Med 2004;38:613–9.

25. Kamper-Jørgensen M, Grønbæk M, Tolstrup J, et al. Alcohol and cirrhosis: dose–response or threshold effect? J Hepatol 2004;41:25–30.

26. Askgaard G, Grønbæk M, Kjær MS, et al. Alcohol drinking pattern and risk of alcoholic liver cirrhosis: a prospective cohort study. J Hepatol 2015;62:1061–7.

27. Roerecke M, Vafaei A, Hasan OSM, et al. Alcohol consumption and risk of liver cirrhosis: a systematic review and meta-analysis. Am J Gastroenterol 2019;114:1574-86.

28. Rehm J, Roerecke M. Patterns of drinking and liver cirrhosis - what do we know and where do we go? J Hepatol 2015;62:1000–1.

29. Aberg F, Helenius-Hietala J, Puukka P, et al. Binge drinking and the risk of liver events: a population-based cohort study. Liver Int 2017;37:1373-81

30. Becker U, Gronbaek M, Johansen D, et al. Lower risk of alcohol induced cirrhosis in wine drinkers. Hepatology 2002;35:868–75.

31. Tuyns AJ, Estève J, Péquignot G. Ethanol is cirrhogenic, whatever the beverage. Br J Addict 1984;79:389-93.

32. Rotily M, Durbec JP, Berthezene P, et al. Diet and alcohol in liver cirrhosis: a case-control study. Eur J Clin Nutr 1990;44:595–603.

33. Johansen D, Friis K, Skovenborg E, et al. Food buying habits of people who buy wine or beer: cross sectional study. BMJ 2006;332:519–22.

34. Becker U, Deis A, Sørensen TI, et al. Prediction of risk of liver disease by alcohol intake, sex, and age: a prospective population study. Hepatology 1996;23:1025-9.

35. Coates RA, Halliday ML, Rankin JG, et al. Risk of fatty infiltration or cirrhosis of the liver in relation to ethanol consumption: a case-control study. Clin Invest Med 1986;9:26-32.

36. Rehm J, Taylor B, Mohapatra S, et al. Alcohol as a risk factor for liver cirrhosis: a systematic review and meta-analysis. Drug Alcohol Rev 2010;29:437-45.

37. Marshall AW, Kingstone D, Boss M, et al. Ethanol elimination in males and females: relationship to menstrual cycle and body composition. Hepatology 1983;3:701–6.

38. Douds AC, Cox MA, Iqbal TH, et al. Ethnic differences in cirrhosis of the liver in a British city: alcoholic cirrhosis in South Asian men. Alcohol Alcohol 2003;38:148–50.

39. Stinson FS, Grant BF, Dufour MC. The critical dimension of ethnicity in liver cirrhosis mortality statistics. Alcohol Clin Exp Res 2001;25:1181–7.

40. Levy RE, Catana AM, Durbin-Johnson B, et al. Ethnic differences in presentation and severity of alcoholic liver disease. Alcohol Clin Exp Res 2015;39:566–74.

41. Li D, Zhao H, Gelernter J. Strong association of the alcohol dehydrogenase 1B gene (ADH1B) with alcohol dependence and alcohol-induced medical diseases. Biol Psychiatry 2011;70:504–12.

42. Liu B, Balkwill A, Reeves G, et al. Body mass index and risk of liver cirrhosis in middle aged UK women: prospective study. BMJ 2010; 340:c912.

43. Naveau S, Giraud V, Borotto E, et al. Excess weight risk factor for alcoholic liver disease. Hepatology 1997;25:108–11.

44. Naveau S, Dobrin AS, Balian A, et al. Body fat distribution and risk factors for fibrosis in patients with alcoholic liver disease. Alcohol Clin Exp Res 2013;37:332-8

45. Dam MK, Flensborg-Madsen T, Eliasen M, et al. Smoking and risk of liver cirrhosis: a population-based cohort study. Scand J Gastroenterol 2013;48:585–91.

46. Klatsky AL, Armstrong MA. Alcohol, smoking, coffee, and cirrhosis. Am J Epidemiol 1992;136:1248–57.

47. Whitfield JB, Masson S, Liangpunsakul S, et al. Obesity, diabetes, coffee, tea, and cannabis use alter risk for alcohol-related cirrhosis in 2 large cohorts of high-risk drinkers. Am J Gastroenterol 2021;116:106-15.

48. Adejumo AC, Ajayi TO, Adegbala OM, et al. Cannabis use is associated with reduced prevalence of progressive stages of alcoholic liver disease. Liver Int 2018;38:1475–86.

49. Kennedy OJ, Roderick P, Buchanan R, et al. Systematic review with meta-analysis: coffee consumption and the risk of cirrhosis. Aliment Pharmacol Ther 2016;43:562–74.

50. Aberg F, Helenius-Hietala J, Puukka P, et al. Interaction between alcohol consumption and metabolic syndrome in predicting severe liver disease in the general population. Hepatology 2018;67:2141-9.

51. Noda K, Yoshihara H, Suzuki K, et al. Progression of type C chronic hepatitis to liver cirrhosis and hepatocellular carcinoma--its relationship to alcohol drinking and the age of transfusion. Alcohol Clin Exp Res 1996;20:95a-100a.

52. Serfaty L, Poujol-Robert A, Carbonell N, et al. Effect of the interaction between steatosis and alcohol intake on liver fibrosis progression in chronic hepatitis C. Am J Gastroenterol 2002;97:1807-12.

53. Canan CE, Lau B, McCaul ME, et al. Effect of alcohol consumption on all-cause and liver-related mortality among HIV-infected individuals. HIV Med 2017;18:332-41.

54. Monto A, Patel K, Bostrom A, et al. Risks of a range of alcohol intake on hepatitis C-related fibrosis. Hepatology 2004;39:826–34.

55. Fletcher LM, Dixon JL, Purdie DM, et al. Excess alcohol greatly increases the prevalence of cirrhosis in hereditary hemochromatosis. Gastroenterology 2002;122:281-9.

56. Whitfield JB, Rahman K, Haber PS, et al. Brief report: genetics of alcoholic cirrhosis-GenomALC multinational study. Alcohol Clin Exp Res 2015;39:836–42.

57. Hrubec Z, Omenn GS. Evidence of genetic predisposition to alcoholic cirrhosis and psychosis - twin concordances for alcoholism and its biological end-points by zygosity among male veterans. Alcohol Clin Exp Res 1981;5:207–15.

58. Reed T, Page WF, Viken RJ, et al. Genetic predisposition to organs specific endpoints of alcoholism. Alcohol Clin Exp Res 1996;20:1528–33.

59. Anstee QM, Daly AK, Day CP. Genetics of alcoholic liver disease. Semin Liver Dis 2015;35:361-74.

60. Stickel F, Moreno C, Hampe J, et al. The genetics of alcohol dependence and alcohol-related liver disease. J Hepatol 2017;66:195-211.

61. Meroni M, Longo M, Rametta R, et al. Genetic and epigenetic modifiers of alcoholic liver disease. Int J Mol Sci 2018;19:3857-78.

62. Marcos M, Gomez-Munuera M, Pastor I, et al. Tumor necrosis factor polymorphisms and alcoholic liver disease: a HuGE review and meta-analysis. Am J Epidemiol 2009;170:948-56.

63. Marcos M, Pastor I, Chamorro AJ, et al. Meta-analysis: glutathione-S-transferase allelic variants are associated with alcoholic liver disease. Aliment Pharmacol Ther 2011;34:1159-72.

64. Buch S, Stickel F, Trépo E, et al. A genome-wide association study confirms *PNPLA3* and identifies *TM6SF2* and *MBOAT7* as risk loci for alcohol-related cirrhosis. Nat Genet 2015;47:1443-8.

65. Innes H, Buch S, Hutchinson S, et al. Genome-wide association study for alcohol-related cirrhosis identifies risk loci in *MARC1* and *HNRNPUL1*. Gastroenterology 2020;159:1276-89.

66. Schwantes-An TH, Darlay R, Mathurin P, et al. Genome-wide association study and meta-analysis on alcohol-related liver cirrhosis identifies novel genetic risk factors. Hepatology 2021; 73: 1920-31.

67. 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.

68. Chamorro AJ, Torres JL, Miron-Canelo JA, et al. Systematic review with meta-analysis: the I148M variant of patatin-like phospholipase domain-containing 3 gene (*PNPLA3*) is significantly associated with alcoholic liver cirrhosis. Aliment Pharmacol Ther 2014;40:571-81.

69. Singal AG, Manjunath H, Yopp AC, et al. The effect of *PNPLA3* on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. Am J Gastroenterol 2014;109:325-34.

70. Trépo E, Nahon P, Bontempi G, et al. Association between the *PNPLA3* (rs738409 C>G) variant and hepatocellular carcinoma: evidence from a meta-analysis of individual participant data. Hepatology 2014;59:2170–7.

71. Salameh H, Raff E, Erwin A, et al. *PNPLA3* gene polymorphism Is associated with predisposition to and severity of alcoholic liver disease. Am J Gastroenterol 2015;110:846-56.

72. Li JF, Zheng EQ, Xie M. Association between rs738409 polymorphism in *patatin-like phospholipase domain-containing protein 3 (PNPLA3)* gene and hepatocellular carcinoma susceptibility: evidence from case-control studies. Gene 2019;685:143-8.

73. Stickel F, Buch S, Lau K, et al. Genetic variation in the *PNPLA3* gene is associated with alcoholic liver injury in Caucasians. Hepatology 2011;53:86-95.

74. Burza MA, Molinaro A, Attilia ML, et al. *PNPLA3* I148M (rs738409) genetic variant and age at onset of at-risk alcohol consumption are independent risk factors for alcoholic cirrhosis. Liver Int 2014;34:514–20.

75. Friedrich K, Wannhoff A, Kattner S, et al. *PNPLA3* in end-stage liver disease: alcohol consumption, hepatocellular carcinoma development, and transplantation-free survival. J Gastroenterol Hepatol 2014;29:1477–84.

76. Valenti L, Motta BM, Soardo G, et al. *PNPLA3* I148M polymorphism, clinical presentation, and survival in patients with hepatocellular carcinoma. PLoS One 2013;8:e75982.

77. Abul-Husn NS, Cheng X, Li AH, et al. A protein-truncating *HSD17B13* variant and protection from chronic liver disease. N Engl J Med 2018;378:1096-106.

78. Stickel F, Lutz P, Buch S, et al. Genetic variation in *HSD17B13* reduces the risk of developing cirrhosis and hepatocellular carcinoma in alcohol misusers. Hepatology 2020;72:88-102.

79. Strnad P, Buch S, Hamesch K, et al. Heterozygous carriage of the alpha1-antitrypsin Pi*Z variant increases the risk to develop liver cirrhosis. Gut 2019;68:1099-107.

80. Crabb DW, Bataller R, Chalasani NP, et al. Standard definitions and common data elements for clinical trials in patients with alcoholic hepatitis: recommendation from the NIAAA Alcoholic Hepatitis Consortia. Gastroenterology 2016;150:785-90.

81. Liangpunsakul S, Puri P, Shah VH, et al. Effects of age, sex, body weight, and quantity of alcohol consumption on occurrence and severity of alcoholic hepatitis. Clin Gastroenterol Hepatol 2016;14:1831-8.

82. Lourens S, Sunjaya DB, Singal A, et al. Acute alcoholic hepatitis: natural history and predictors of mortality using a multicenter prospective study. Mayo Clin Proc Innov Qual Outcomes 2017;1:37-48.

83. Bertola A, Park O, Gao B. Chronic plus binge ethanol feeding synergistically induces neutrophil infiltration and liver injury in mice: a critical role for E-selectin. Hepatology 2013;58:1814-23.

84. Nguyen-Khac E, Houchi H, Dreher M-L, et al. Is *PNPLA3* polymorphism involved in severe acute alcoholic hepatitis. Hepatology 2011;54(S1):976A.

85. Atkinson SR, Way MJ, McQuillin A, et al. Homozygosity for rs738409:G in *PNPLA3* is associated with increased mortality following an episode of severe alcoholic hepatitis. J Hepatol 2017;67:120-7.

86. Atkinson SR, Way MJ, McQuillin A, et al. Reply to: ‘‘The *PNPLA3* SNP rs738409:G allele is associated with increased liver disease-associated mortality but reduced overall mortality in a population-based cohort.’’ J Hepatol 2018;68: 840–66.

87. Atkinson SR, Buckley T, Strnad P, et al. Genetic variation in *HSD17B13* reduces the risk for developing severe alcoholic hepatitis. J Hepatol 2020;73 (Suppl1):S61-62.

88. Beaudoin JJ, Long N, Liangpunsakul S, et al. An exploratory genome-wide analysis of genetic risk for alcoholic hepatitis. Scand J Gastroenterol 2017;52:1263-9.

89. Sharma M, Atkinson S, McQuillin A, et al. A Genome-wide Association Study of severe alcoholic hepatitis. Gut 2020;69 (Suppl 1):A4-A5:O8.

90. Stickel F, Buch S, Nischalke HD, et al. Genetic variants in *PNPLA3* and *TM6SF2* predispose to the development of hepatocellular carcinoma in individuals with alcohol-related cirrhosis Am J Gastroenterol 2018;113:1475-83.

91. Gellert-Kristensen H, Richardson TG, Davey Smith G, et al. Combined effect of *PNPLA3, TM6SF2*, and *HSD17B13* variants on risk of cirrhosis and hepatocellular carcinoma in the general population. Hepatology 2020;72:845-56.