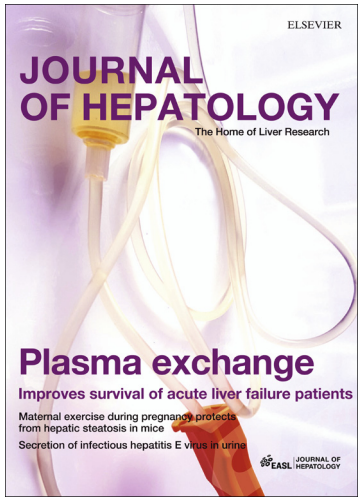

Review

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The Genetics of Alcohol Dependence and Alcohol-related Liver Disease

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Authors' contributions

FS conceived the paper structure, wrote the alcoholic liver disease part of the manuscript, and designed the figures, and critically revised the final version of the manuscript;

CM contributed to the drafting of the manuscript and critically revised the final version of the Manuscript;

JH contributed to the drafting of the manuscript and critically revised the final version of the Manuscript;

MYM conceived the paper structure, wrote the alcohol use disorders part of the manuscript, designed the figures, and critically revised the final version of the manuscript.

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Summary

The susceptibility to develop alcohol dependence and significant alcohol-related liver injury is determined by a number of constitutional, environmental and genetic factors, although the nature and level of interplay between them remains unclear. The familiarity and heritability of alcohol dependence is well-documented but, to date, no strong candidate genes have emerged with the exception of variants in alcohol dehydrogenase and acetaldehyde dehydrogenase, which confer protection predominantly in individuals of East Asian ancestry. Population contamination with confounder such as drug co-dependence and psychiatric and physical co-morbidity may explain the essentially negative genome wide association studies in this disorder. The familiarity and hereditability of alcohol-related cirrhosis is not as well–documented but three strong candidate genes *PNPLA3, TM6SF2* and *MBOAT7,* have been identified. The mechanisms by which variants in these genes confer risk and the nature of the functional interplay between them remains to be determined but, when elucidated, will undoubtedly increase our understanding of the pathophysiology of this disease. The way in which this genetic information could potentially inform patient management has yet to be determined and tested.

Key-points

- Alcohol misuse poses major problems for health and social agencies alike; it is responsible for 5.9% of the deaths and 5.1% of the burden of disease and injury worldwide.
- Not everyone who drinks excessively will develop alcohol dependence or alcohol-related cirrhosis; constitutional, environmental and genetic factors all contribute.
- There is strong evidence for an appreciably genetic contribution to alcohol dependence but no robust candidate genes have yet been discovered apart from rs1229984 in *ADH1B* in Europeans, East Asians and African Americans and rs671 in *ALDH2* in East Asians.
- Three loci have been identified which are associated with an increased risk of developing alcohol-related cirrhosis, *PNPLA3, TM6SF2* and *MBOAT7.*
- Genetic information has the potential to increase our understanding of the pathophysiology of both alcohol dependence and alcohol related cirrhosis, may allow identification of targets for drug development and may inform clinical management decisions

Introduction

Alcohol consumption is a major public health concern. In 2012 over three million deaths were attributed to alcohol consumption, corresponding to 5.9% of the global total or one in every twenty deaths world-wide [1]. In addition 5.1% of the global burden of disease and injury, as measured in Disability-Adjusted Life Years (DALYs) were attributable to alcohol consumption [1]. There is wide geographical variation in the proportion of alcohol-attributable deaths and DALYs, with the highest alcohol-attributable fractions reported in the WHO European Region [1]; estimates from this region indicate that harmful drinking, particularly when associated with alcohol dependence, is responsible for 1 in 7 deaths in men and 1 in 13 deaths in women aged 15 to 64 years [2].

Excess consumption of alcohol is associated with a wide-range of problems relating to physical health, either directly, or through contributions to other health conditions. It is the most frequent cause of cirrhosis in Europe; alcohol-related liver disease (ALD) is the most important cause of death due to alcohol in middle-aged men and women [3]. Mortality from alcoholic-related cirrhosis has declined over the past 30 years in most Western European countries, while it has increased in Eastern Europe, the United Kingdom, Southern Ireland and Finland [4]. Alcohol-related cirrhosis is now the second most common indication for liver transplantation, accounting for approximately 40% of all primary liver transplants in Europe and approximately 25% in the United States [5].

Other conditions directly attributable to excess alcohol consumption include alcohol-related injuries [6] alcohol-related pancreatitis [7], and the fetal alcohol syndrome [8]. In addition alcohol is an important co-factor in the development of cancers of the aerodigestive tract, liver, colorectal and breast [9]; and a major risk factor for the development of cardiovascular diseases [10]; and a range of neuropsychiatric disorders [11].

There is considerable variability in the outcomes of excessive alcohol consumption on an individual basis. The determinants of disease susceptibility are complex and reflect the

interplay of several constitutional, environmental and genetic factors. Technological advances in molecular genetics have provided a better understanding of the genetic background of alcohol-related disorders but the information is far from complete.

A better understanding of the genetic modulators of disease risk would potentially allow for better identification of risk groups, improved disease prevention and focused allocation of treatment resources; it would also help delineated the pathophysiology of alcohol-related disorders and the identification of potential drug targets for new therapies. The present review summarizes current knowledge of the genetic of alcohol use disorders and of alcohol-related liver disease, and provides a reasoned basis for future research direction.

Alcohol use disorders

A number of terms such as 'heavy drinking', 'harmful drinking', 'alcohol misuse/abuse', 'problem drinking' and 'dependent drinking' are used to describe drinking behaviour but often without clear or consistent characterization. The accurate definition of these behaviours is important if meaningful comparisons are to be made in genetic and epidemiological research. In the absence of biological phenotypes that can be used across studies, investigators tend to use criteria based on two separate but similarly structured systems: the *International Classification of Diseases* (ICD), published by the World Health Organization (WHO) [12] and the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) (ICD) published by the American Psychiatric Association [13]. These criteria are subject to revisions but at present the 4th edition of the DSM criteria (DSM-IV) and the 10th edition of the ICD criteria (ICD-10) are the ones most widely used for clinical and research purposes.

Both systems use questionnaire responses to determine the relationship with alcohol and to identify spectral differences in severity. *Alcohol dependence* is defined, in both systems, by the presence of tolerance to alcohol's neurobiological effects; the development of a physiological withdrawal syndrome; a preoccupation with alcohol; difficulty in controlling its use; and continued consumption despite harmful consequences (**Table 1**). Alcohol drinking behaviour which falls short of the definition of dependent drinking but which nevertheless can

cause damage to mental and/or physical health is termed '*harmful use*' in ICD-10 and '*alcohol abuse*' in DSM-IV (**Table 1**). The ICD-10 and DSM-IV diagnostic criteria for alcohol dependence overlap to a large degree and so they can be used interchangeably for research purposes but there is considerable discordance in the classification of harmful use and alcohol abuse [14,15].

The 5th edition of the DSM manual (DSM-5), published in 2013, has integrated alcohol abuse and alcohol dependence into a single category '*alcohol use disorder*', which has mild, moderate and severe sub-classifications (Supplementary **Table 1**). The 10th edition of the ICD is currently undergoing revision and it is likely that its criteria will mirror closely those of DSM-5 [16,17].

Risk factors for the development of alcohol use disorders

Environmental and host-mediated risk factors

A number of factors have been identified, at societal and individual levels, which affect the extent and patterns of alcohol consumption and hence the risk of developing an alcohol use disorder [18]. At a *societal* level, factors such as the degree of economic development, religious and cultural mores, the availability of alcohol, and the level and effectiveness of alcohol policies all play a part in determining population levels of alcohol consumption and hence of alcohol-related harm [18]. On an *individual* level, factors such as age, gender and socioeconomic status all play a role, in addition to behaviour and alcohol exposure.

Children, adolescents and the elderly are typically more vulnerable to alcohol-related harm than other age groups [19,20]. Initiation of alcohol use before the age of 14 years is associated with an increased risk of developing alcohol abuse and dependence in later life [1,21-23]. Parental alcohol problems and high trait anxiety are significant risk factors for alcohol dependence during this period [24]. Alcohol consumption generally declines with age, but older drinkers typically consume alcohol more frequently than other age groups. As people grow older, they are typically less able to handle the same levels and patterns of

alcohol consumption as in previous life years, leading to an increase in the burden of alcohol-related problems [25, 26].

Gender also plays an important determinant role. In 2012, higher proportions of men died of alcohol attributable causes than women (7.6% cf. 4.0%) and suffered proportionately higher rates of alcohol-related disease or injury (7.4% cf. 2.3%) [1]. These differences are explained mainly by the fact that men who drink alcohol do so more frequently and consume larger quantities than women. However, there is also evidence that for a given level of drinking, women may be more at risk of developing alcohol use disorders than their male counterparts [27,28]. The factors, which mediate this increased vulnerability, are complex, but differences in body composition, which are reflected in higher tissue doses of alcohol for a given amount of alcohol consumed, play a major role [29].

People with lower socioeconomic status appear to be more vulnerable to the consequences of alcohol consumption than those with higher status [30]. Thus, manual workers are more vulnerable to severe alcohol-related outcomes, including mortality, than non-manual workers for the same level of consumption [20,31]. There are several possible explanations including the fact that people with lower socioeconomic status are less likely to have a partner and tend to live less stable lives; they have fewer resources to protect themselves from the consequences of hazardous drinking and are less likely to seek help. They may also carry additional burdens relating to their childhood environment and an accumulation of other risk factors [32].

Studies showing differences in consumption or alcohol-related harm between various ethnicities within countries have underlined the importance of further research on culture-related vulnerabilities [33].

Heritability and genetic risk factors

'Alcohol use disorders' is a generic term covering a wide variety of drinking behaviours and their consequences. Even within the alcohol dependence phenotype, which is the most

easily identified, there is considerable variability in the presence and primacy of the defining features and hence substantial uncertainty about which features might be inherited. While it is generally agreed that inheritance is polygenic [34], identifying the genes involved and their relative contribution is difficult because of the considerable variations observed in the design of studies, population phenotypes, the type of data analysis, and because of a general failure to control for potential confounders such as co-morbid psychiatric conditions and co-occurring substance misuse [35,36].

Family, twin and adoption studies

Family studies statistically quantify whether a phenotype is present in related members of a family more often than would be expected by chance. In early studies people with a family history of alcohol misuse were found to be three to four times more likely to misuse alcohol than people without a family history [37]. More specifically the male and females siblings of a person with alcohol dependence had a life-time chance of developing alcohol dependence of 49.7% and 22.4% respectively [38]. Although a considerable number of family studies have been undertaken to date [39] they provide no information on phenotypic heritability because family members are typically exposed to the same environmental influences.

Twin studies are based on the assumption that additive genetic risk is completely shared between monozygotic twins while dizygotic twins share only half the risk. It follows that a higher concordance rate for a phenotype of interest in monozygotic than dizygotic twins implies genetic influence. Several large twin studies of alcohol-use phenotypes have been performed and provide heritability estimates for alcohol dependence ranging from -16% to 72% [39-53] (**Table 2**). These studies have demonstrated stronger evidence of hereditability in men but this may simply relate to the smaller sample sizes in the female twin-study cohorts [54]. Potential confounders of the twin study design are the assumptions that: parental mating is random not assortative [55, 56]; and that there is environmental equality [57,58].

Adoption studies compare the alcohol use phenotypes of individuals adopted in early childhood with those of their biological and adoptive parents. Three major adoption cohorts have been interrogated for information on alcohol use phenotypes but information on specifically defined conditions, for example alcohol dependence, is not available. Heritability estimates range from -2% to 52% (59-67) (**Table 3**). Criticisms have been levied at all of these cohort analyses for a number of reasons including: (i) the use of confusing and arbitrary classification criteria (59,60); (ii) the undertaking of assessments at too young an age for a true assessment of risk (59,60); and, (iii) reliance on second hand reporting of the prevalence of alcohol use disorders in biological parents (65,66).

Twin, family and adoption studies provide evidence for significant heritability of the alcohol dependence and alcohol misuse phenotypes. However, the magnitude of the effect is still debated. Verhulst et al. [53] undertook a meta-analysis of data from 12 twin and five adoption studies and provided an overall estimate of the heritability for alcohol use disorders of 49%. However, Walters [39] performed a meta-analysis of over 50 family, twin and adoption studies of alcohol misuse phenotypes and showed that there was significant heterogeneity across studies and provided a mean heritability estimate of 24%. The heritability was much stronger in men with severe alcoholism/alcohol dependence and in this cohort the heritability estimates were of the order of 30% to 36% [39].

These study cohorts continue to be interrogated, and with longevity and better design may yield data critical to understanding gene–environment interactions.

Candidate gene association studies

Numerous candidate gene association studies of alcohol dependence and related phenotypes have been undertaken but the results overall have been disappointing primarily because many of the identified associations fail to replicate [35,36]. The most consistent, replicable findings relate to functional variants in the genes encoding the alcohol metabolizing enzymes, alcohol dehydrogenase (*ADH*) and acetaldehyde dehydrogenase (*ALDH*). These variants, termed single nucleotide polymorphisms (SNPs), are associated

with changes in enzyme kinetics which affect production and removal of the toxic metabolite acetaldehyde [68] (Figure 1). Carriers of these variants accumulate acetaldehyde following alcohol consumption and develop unpleasant symptoms including: flushing, nausea, vomiting, tachycardia, hypotension, dyspnoea and headache. This acts as a deterrent to drinking and delivers 'protection' against alcohol use disorders and their sequelae [69].

The occurrence of these functional *ADH* and *ALDH* variants is considerably higher in East Asians that in other populations. Thus, the SNP rs1229984 in *ADH1B* is found in 19 to 91% of East Asians [70] but in zero to 10% of other populations [71]. Likewise the SNP rs671 in *ALDH2* is found in 30 to 50% of East-Asians and is almost exclusively confined to these populations [72]. Nevertheless the results of numerous studies and meta-analyses [73] have shown consistently that rs1229984 in *ADH1B* confer protection against alcohol use disorders in Europeans [74], Africans [74] and East Asians [70], while rs671 in *ALDH2* additionally protects in East Asians [75] **(Figure 1).**

Considerable interest has also centred on the possible association between functional variants in genes involved in γ-aminobutyric acid (GABA) neurotransmission and alcohol use disorder phenotypes, specifically in the association with the α2 $GABA_A$ receptor subunit gene (*GABRA2*) on chromosome 4p. In 2004, Edenberg et al. [76] reported significant associations between 31 SNPs in *GABRA2* and alcohol dependence and a significant association with a three-SNP haplotype. However, a reanalysis of these data in 2006 [77] showed that the association between *GABRA2* and alcohol dependence was essentially limited to individuals with co-occurring drug dependence. The $GABA_A$ receptor is a hetero pentamer so there are multiple potential variants in either *GABRA2* or other receptor subunits which might associate with the phenotypes of interest and there is also a high degree of linkage disequilibrium across the region. Further studies with controls exercised for potential confounders are clearly needed.

Genome-wide linkage studies

Genetic linkage studies are undertaken in families in which one or more members are

affected by the phenotype of interest. Genotyping is undertaken using microsatellites which are nucleotide tandem repeats in DNA sequences which have been mapped to known regions of the genome. Linkage is assumed when alleles of specific markers are non-randomly inherited in members of the family displaying the phenotype of interest. Linkage is statistically quantified by calculating the logarithm of the odds (LOD) score; scores of greater than 3 are indicative of linkage between a genomic region and a phenotype.

Several genome-wide linkage studies have been undertaken in families affected by alcohol dependence and other alcohol use disorders [78-86] (**Table 4**). However, the results are inconsistent and where evidence of linkage has been detected the LOD scores are invariably low. The genomic locations with the greatest evidence for linkage with alcohol dependence contain several plausible candidates such as the *ADH* and *GABA* receptor gene clusters, both of which are on chromosome 4.

Genome wide association studies

Genome-wide association studies (GWAS) entail the extensive parallel genotyping of hundreds of thousands of genomic markers, typically SNPS, which cover the majority of common genetic variation across the human genome. GWAS are performed in large, generally unrelated, populations in which either qualitative or quantitative phenotypic data are available. Genetic association is identified when an allele or genotype is associated with a phenotype at a specific significance threshold which takes into account the need for multiple testing and is typically set at $p<5x10^{-8}$. Independent verification through replication analysis in a separate population or cohort is then advised. It follows that large populations are required in order to ensure that GWAS are adequately powered. These studies are hypothesis-generating in that they are not based on *a priori* hypotheses.

Several GWAS of alcohol dependence and associated phenotypes have been undertaken (87-97), a large proportion of which are based on collaborative studies undertaken in the United States of America (USA) (**Table 5**). In most instances these cohorts are phenotypically heterogeneous, containing participants of multiple different, or mixed,

ancestries, a high proportion of whom have co-morbid psychiatric disorders and/or co-occurring drug dependence. Many of the GWAS undertaken to date have failed to identify genome-wide significant associations. However, meta-analyses and studies in populations with greater phenotypic surety have identified genome-wide significant associations between variants in the genes responsible for alcohol metabolism, for example *ALDH2* and *ADH1B* in East Asian ancestry populations [70,75] and *ADH1B* and *ADH1C* in European, African and East Asian ancestry populations [70,74]. Other significant associations, when identified, appear to be specific to individual studies. However, in a recent minor allele-based meta-analysis of four GWAS, multiple genes with significant or suggestive association with alcohol dependence were identified, some of which are supported by evidence from linkage and candidate gene studies [98].

Future directions

Alcohol has wide-spread adverse effects on a number of neurobiological systems. However, the effects of the genetic risk variants for alcohol dependence identified so far are small. It is highly unlikely that the inheritance of harmful drinking and alcohol dependence is simply controlled. It is more likely to be polygenic and complex as it probably involves transmission of one or more intermediate characteristics or endophenotypes which subsequently affect the risk for harmful drinking and dependence. Each of these endophenotypes is likely to reflect the actions of multiple genes and to reflect both genetic and environmental influences. Future studies should take advantage of improvements in technology, use large population consortia and avoid population heterogeneity and the confounding effects of co-morbid and co-occurring disorders [36].

Alcohol related liver disease

ALD is a term which encompasses a continuum of partly overlapping liver abnormalities ranging from hepatic steatosis to cirrhosis and hepatocellular carcinoma (HCC). Hepatic steatosis will develop in the majority of individuals who regularly consume alcohol in excess

of 40g/day. In 10% to 35% of harmful drinkers the presence of steatosis may be complicated by the development of inflammation and progressive fibrosis while cirrhosis develops in approximately 10% to 15% [99]. HCC develops in 1% to 2% of individuals with alcohol-related cirrhosis per annum [100]. The development of alcohol-related liver injury and its evolution to cirrhosis is generally asymptomatic. Symptomatic presentation is associated with the onset of hepatic decompensation in patients with established cirrhosis or, much less frequently, the development of severe alcoholic hepatitis. Abstinence from alcohol will result in reversal of many of the features of ALD short of cirrhosis and has a significant beneficial effect on mortality and morbidity even in those with established disease. Nevertheless, ALD is still responsible for the global deaths of over half a million people per annum [4]

The pathogenesis of ALD is impacted by multiple behavioral, environmental, and genetic factors. How these interact at a cellular and molecular level has recently been expertly reviewed but remains incompletely understood [101,102].

Risk factors for the development of ALD

Environmental and host-mediated risk factors

Excessive alcohol consumption is the major epidemiological factor determining the risk of developing alcohol-related cirrhosis [104]. However, there is still debate about the degree to which the amounts of alcohol and the pattern of drinking contribute to the risk. A number of large prospective cohort studies [105-108] and a systematic review and meta-analysis [109], have shown that above a threshold dose of alcohol, usually around 40 g/day for women and 60 g/day for men, there is a dose-dependent increase in cirrhosis risk.

The majority of epidemiological studies have shown that daily drinking is associated with a higher risk of cirrhosis than binge drinking [105,107,110]. However, these studies may be confounded by failing to take account of the total amount of alcohol consumed when comparing regular and irregular drinking [111]. Although it has been suggested that drinking wine is associated with a lower cirrhosis risk than with other beverages [110,112], it is the

amount of contained alcohol that is the key factor; apparent differences in cirrhosis risk, by beverage are likely to relate to lifestyle and dietary factors [113,114]. It has been shown that if alcohol is consumed with food this may reduce the risk for developing cirrhosis [106] whereas regular consumption of a high fat, low carbohydrate and protein diet may increase the risk [113].

Coffee drinking is inversely related to alcohol-related cirrhosis risk suggesting a protective effect [115.116]; people drinking four or more cups a day have one-fifth of the risk of developing cirrhosis as non-coffee drinkers [115]. Alternatively cigarette smoking has been shown to be independently related to the risk of developing alcohol-related cirrhosis [115,117] with smokers of a pack or more per day at treble the risk of non-smokers [115].

Gender plays an important role in determining cirrhosis risk. Women appear to be at greater risk of developing alcohol-related cirrhosis [27], even when differences in levels of alcohol consumption are accounted for [118,119]. These gender difference have been attributed variously to oestrogens and their putative synergism with oxidative stress and inflammation [120]; differences in expression patterns of the extra-hepatic alcohol-metabolizing enzymes [121]; and the smaller distribution volume of alcohol in women which results in higher tissue levels of exposure [29].

Co-morbid obesity has been shown to significantly increase the risk of developing alcohol-related fibrosis and cirrhosis potentially reflecting a synergistic interaction between alcohol and weight [122-124]. Likewise people with chronic hepatitis C who drink alcohol in excess of 50g/day have a significantly higher risk of advanced fibrosis than those who drink less or not at all [125]. Finally, low vitamin D levels are associated with increased liver damage and mortality in patients with ALD [126].

Heritability and genetic risk factors

There are very few epidemiological studies relating to the familiarity and heritability of alcohol-related cirrhosis. In one large, ongoing, prospective study alcohol misusers with

cirrhosis were more than twice as likely to report that a father with alcohol problems had died from liver disease compared with alcohol misusers without significant liver injury [127]. Estimate of the heritability of alcohol-related cirrhosis have been made in a single twin study undertaken in a population of 15,924 male twin pairs [128]; the concordance for alcohol-related cirrhosis was three times higher in monozygotic twins than dizygotic twins, which was confirmed in a second analysis undertaken over a decade later [48]. 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 genetic predisposition to alcohol dependence [48].

There are notable interethnic differences in alcohol-related cirrhosis risk. In the United Kingdom non-Muslim men of South Asian ancestry present with alcohol-related cirrhosis at a younger age and at a higher than expected frequency that their white British counterparts [129]. In the United States 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 [130]. Individuals of Hispanic origin have also been shown to present with alcohol-related cirrhosis up to 10 years earlier than their white/Caucasian counterparts [131]. 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.

One 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 in consequence tend to avoid alcohol. A meta-analysis of all published studies identified a significant and robust association between possession of this variant and the development of alcohol-related physical harm, including cirrhosis (P_{META} = 6 x10^{-19}, Odds Ratio (OR) = 0.25, 95% confidence interval (CI) [0.19–0.34]) [70]. There is, however, no evidence that this is anything other than an indirect effect.

Based on the somewhat limited studies available the heritability of alcohol-related cirrhosis risk appears to be modulated through polygenic and complex inheritance in the presence of several environmental risk factors.

Candidate gene association studies

Candidate gene association studies in ALD have been extensively and critically evaluated in past reviews and so will be summarized rather than extensively revisited [132-134]. The selection of candidates has, in the main, been based on an understanding of the biological mechanisms of liver injury. A number of functional variants have been studied in genes encoding proteins implicated in: alcohol metabolism; hepatic lipid turnover; modulation of endotoxin–mediated inflammation and cytokines; DNA damage and carcinogenesis; iron metabolism; immune responses; oxidative stress; and, tissue remodelling and fibrogenesis [132-134].

Most of these genetic association studies have been negative, or else the findings do not replicate or do not retain significance in meta-analysis [132-134]. However, three variants have shown rather more robust association: (i) the promoter variant rs361525 (-238) in tumour necrosis factor (TNF)-α, which is associated with alcohol-related liver disease when compared to population controls [135]; and (ii) the glutathione S-transferase mu (GSTM) 1 null allele, which is associated with alcohol-related liver disease when compared with alcohol dependent people with no liver diseases [136]. In both instances the effect size was small although significance was retained following meta-analysis. Nevertheless, neither was significantly associated with the risk of developing alcohol-related cirrhosis in a subsequent GWAS (*vide infra).*

There are two main reason why the candidate gene studies fail to show association in primary analyses or in replication: (i) this approach tends to be hypothesis-driven and based on variants in genes thought to be involved in the pathophysiology of the disease yet, the pathophysiology of ALD have not been clearly delineated and hence the right candidates are

likely to have eluded study; (ii) the major prerequisite of this approach is the need to ensure that both cases and controls have experienced the same environmental exposure to long-term harmful alcohol consumption and that the controls are free of liver disease. These are demanding condition rarely fulfilled in the studies undertaken to date.

There are a variety of other reasons why these candidate gene studies were compromised, including the use of small samples with consequent low statistical power and inadequate correction for confounding factors such as ethnic admixture. Studies were often from single centres and subject to selection bias and/or population stratification. The majority of studies lacked independent validation and allele/genotype frequencies were often not tested for deviation from Hardy-Weinberg equilibrium. Often, associations were reported but with little or no attempt to explore or determine functionality. Overall, a large number of 'positive' associations were published, while reluctance by researchers and journal editors has meant that negative replication studies often were not.

PNPLA3 and the risk of developing alcohol-related cirrhosis

Despite largely negative association the candidate gene approach, has proven successful, in identifying and validating the robust associations between the variant rs738409 in patatin-like phospholipase domain-containing 3 (*PNPLA3)* and the risk of developing alcohol-related cirrhosis [137] and subsequently HCC [137]. This variant became a candidate following discovery of a significant association with non-alcoholic fatty liver disease (NAFLD) [138] and its significant association with the risk for developing significant alcohol-related liver disease was soon established [139-149] (**Table 6**). A meta-analysis, which included the majority of the available studies, provided strong and unequivocal evidence for a significant role for rs738409 in *PNPLA3* in the progression of ALD [150] with effect sizes in the range expected for a relatively frequent SNP in a complex disease. The population-attributable risk for progression to alcohol-related cirrhosis conferred by carriage of the risk allele in *PNPLA3* was 26.6%, suggesting that other modifiers likely exist [142].

In patients with established alcohol-related cirrhosis carriage of rs738409 in *PNPLA3* is associated with an increased risk of developing HCC [144,148, 151-157] (**Table 7**). A meta-analysis of five of these studies, based on individual patient data, confirmed this association (OR of 2.2 (95% CI 1.8-2.67, $p=4.71x10^{-15}$), and showed that it was robust to adjustment for age, sex, and body mass index (OR=2.13; 95% CI: 1.73-2.61; $p=5.52 \times 10^{-13}$) [157].

Thus, carriers of the rs738409 in *PNPLA3* are at increased risk for developing cirrhosis and HCC but in addition it has been shown that they: (i) present with cirrhosis after a shorter drinking history [147]; (ii) develop decompensated cirrhosis at an earlier stage of their disease history [148] and (iii) are more likely to die of their liver disease [158].

The functional implications of the rs738409 in PNPLA3

Although the risk associations of rs738409 in *PNPLA3* are well-established the functional implications of this variant remain unclear. PNPLA3 is predominantly expressed in adipose tissue and is a member of the patatin-like phospholipase family of proteins which share homology with the broad acting lipase patatin [159]. Mammalian patatin-like phospholipases (PNPLAs) are involved in a number of processes such as maintenance of membrane integrity, lipid turnover and signalling, and regulation of energy homeostasis. Several are lipid hydrolases with substrate specificity for triacylglycerols, phospholipids, and retinol esters [160]. The structure of PNPLA3 has not been fully elucidated but the isoleucine to methionine substitution at position 148 in rs738409 likely results in a reduction in hydrolytic function and the accumulation of fat [161-163]. Cell culture experiments in HuH-7 cells [162] and in *Pnpla3* knock-out [164, 165] and knock in mice [166] provide convincing evidence for a 'lipid-trapping' effect of the rs738409 variant, which is further supported by studies in which overexpression of rs734809 in mice results in increased hepatic steatosis, especially of mono-unsaturated fatty acids [162, 167].

PNPLA3 is also highly expressed and synthesized in primary human HSCs and catalyzes the hydrolysis of retinyl esters [168]. The rs738409 variant abrogates this activity resulting in retinyl palmitate retention an effect confirmed in studies in knockout mice subjected to

induced endoplasmic reticulum stress [169]. This aspect of the altered functionality of rs734809 is of particular interest given its possible link to alcohol-driven fibrogenesis, alcohol-mediated retinoid depletion [170], and alcohol-induced retinoid hepatotoxicity [171].

Genome-Wide Scanning for Risk Genes in ALD

A number of liver diseases have been subjected to GWAS approaches including NAFLD [138] primary cholestatic disorders [172], autoimmune hepatitis [173], drug-induced liver injury [174], and haemochromatosis [175]. ALD has only recently been studied at genome-wide level in two studies focusing on the phenotypes 'alcohol-related cirrhosis' [176] and 'severe alcoholic hepatitis' [177].

Buch and coworkers [155] performed a two-step GWAS in >4,000 Europeans with alcohol-related cirrhosis with subsequent validation in two independent European cohorts. The strongest association signal was observed at the *PNPLA3* locus ($P=1.57\times10^{-34}$), while two hitherto unknown variants in *Membrane bound O-acyltransferase domain containing 7* (*MBOAT7*) ($P=9.25\times10^{-10}$) and *Transmembrane 6 superfamily member 2* (*TM6SF2)* ($P=1.73\times10^{-8}$) were identified as novel risk loci, although the *TM6SF2* locus had previously been identified as a risk factor for progressive non-alcohol-related steatohepatitis (NASH) [178]. Both *PNPLA3* and *TM6SF2* are implicated in hepatic lipid trapping, while *MBOAT7* has been linked to the transfer of fatty acid between phospholipids and lysophospholipids, a potent driver of hepatic inflammation [179].

The nature of the effects of these three variants suggests a pivotal role for dysfunctional lipid turnover in the pathogenesis of alcohol-related cirrhosis (**Fig 2**). One of the most interesting aspects of these findings and one which is in the process of exploration is the risk interplay between these variants. Thus, Falleti and coworkers [156] have recently confirmed that in patients with alcohol-related cirrhosis the rs738409 G/G genotype in *PNPLA3* is associated with the risk of developing HCC OR 2.85 ($p = 0.011$) but that a similar association is

observed with carriage of rs58542926 T/*genotype in *TM6SF2*, OR 2.57 (p = 0.035), with evidence of interplay between these two variants.

The GWAS in severe alcoholic hepatitis was performed as a spin-off project from the STOPAH trial, a large randomized trial of treatment for severe alcoholic hepatitis with prednisolone or pentoxifylline, or their combination [180]. Atkinson and associates [177] compared 332 cases with severe alcoholic hepatitis with 318 controls with no evidence of liver disease despite prolonged alcohol abuse by GWAS using the Illumina HumanCoreExome BeadChip, and validated the top hits in a second independent cohort of 528 cases and 873 controls [177]. The strongest signal was obtained for rs738409 in *PNPLA3,* in the identification and replication steps, most likely reflecting phenotypic overlap with alcohol-related cirrhosis. However, a novel association was identified with a SNP in the *Solute Carrier Family 38, Member A4* (*SLC38A4*) gene ($P_{replication}$ = 0.029; P_{meta} = $4.13x10^{-5}$; OR 1.32, the functional implications of which are as yet unknown. Further expansion and exploration of this cohort is underway.

Thus, these initial GWAS on alcohol-related cirrhosis and severe alcoholic hepatitis have identified a number of risk loci; further exploitation of the GWAS approach with larger population numbers and more complete genome coverage may yield further loci of interest. The availability of more refined genotyping tools such as whole exome or whole genome sequencing will likely help to detect rare variants with potentially strong effect sizes.

Further advances may be made by the GenomALC consortium (www.genomalc.org) which has implemented a prospective recruitment approach for an intended GWAS of alcohol-related cirrhosis which will allows in depth characterisation of cases and controls [127]. As such the envisaged data set will likely enable a wide panel of genotype-phenotype associations to be explored which is not usually possible using retrospective cohorts.

Future directions

Our understanding of the pathogenesis of alcohol-related liver disease and the relative roles of environmental and genetic factors is still at a relatively early stage. However, there are clear directional pathways:

1. There are no good animal models of human ALD [181] which hampers efforts to explore candidate gene functional mechanisms. Rodents are notoriously resistant to alcohol hepatotoxicity and only develop significant chronic liver injury, when exposed to alcohol in combination with a second toxin or dietary manipulation. Even then the liver injury is still not typical of the spectrum of ALD in man. Advances such as the *National institute on Alcohol Abuse and Alcoholism* (NIAAA) model of ALD combining binge drinking patterns with chronic alcohol exposure may herald the advent of more suitable models [181]. This appears particularly promising when combined with novel technologies to design genetically modified rodents such as CRISPR/Cas9 and to overcome species-related differences in alcohol susceptibility [182].

2. The application of more advanced computational handling of data. Polygenic scores have been used to summarise genetic effects among an ensemble of markers that do not individually achieve significance in large-scale association studies. This approach can be used to: (i) seek evidence of a genetic effect when no single markers are significant; (ii) establish a common genetic basis for related disorders; and, (ii) construct risk prediction models. More recently the technique been applied to data from GWAS to create genome-wide polygenic score which includes thousands of SNPs or even all of the SNPs on a DNA array weighted by the strength of their associations.

3. Further exploration of the biological action and functional implications of the genetic variation in *PNPLA3* and *TM6SF2*, and *MBOT7* will be important for the near future.

4. There is a clear need for well-funded, collaborative studies. Joint research collaboration between addition specialists and hepatologists should be encouraged to enable sufficiently powered studies to be undertaken which will encompass the phenotypic diversity of this population and allow controls to be exercised for possible confounding co-morbidities and co-

occurring disorders. Such successful coalescence of research activities across medical subspecialties will allow for the execution of studies on risk factors, markers of disease, prevention, and active treatment of patients at risk for or with established ALD.

Finally, thought should be given to how the current information available on the genetic basis of ALD can be translated into clinical practice for the benefit of patient. There are a number of feasible possibilities:

- The screening of high risk drinkers to determine their risk for developing cirrhosis
- Determination of the optimal screening intervals for HCC surveillance in patients with alcohol-related cirrhosis, by genotype
- The selection of patients with high priority for transplantation - both those with alcohol-related cirrhosis and those with unresponsive severe alcoholic hepatitis

No data are currently available on which to formulate answers to these questions and the quest for answers offers many research opportunities.

The immense impact of alcohol consumption on the overall disease burden stands in sharp contrast to its neglect in political and medical professional circles [182,183]. For the vast majority of health care physicians, the simple answer ‘just stop drinking’ has been the practical axiom which rendered ALD an orphan disease in its own right.

However, the genetic information gathered in recent years has shed new light on the inherited aspects of alcohol use disorders and alcohol-related cirrhosis which will hopefully promote the improvement of individualized approaches and allow delivery of optimal care for those with harmed in this way.

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Author names in **bold** designate shared co-first authorship

Legends to Figures

Figure 1: The effects of functional variants in the genes encoding the alcohol metabolizing enzymes alcohol dehydrogenase and acetaldehyde dehydrogenase

A Alcohol is metabolized in the liver primarily by alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH). Functional variants in the genes encoding the alcohol metabolizing enzymes are associated with changes in enzyme kinetics which affect the production and removal of the toxic metabolite acetaldehyde resulting in an increase in it circulating levels. The physical consequences of this act as a deterrent to drinking and hence 'protection' against alcohol use disorders and their sequelae.

B The single nuclear polymorphism (SNP) rs1229984 in *ADH1B* results in a gain of function and hence an increase in enzyme activity resulting in the production of excess acetaldehyde. It is found in 19 to 91% of East Asians [70] but in zero to 10% of other populations [71].

C The SNP rs671 in *ALDH2* results in a loss of function and hence a decrease in enzyme activity leading to the accumulation of acetaldehyde. It is found in 30 to 50% of East-Asians and is almost exclusively confined to these populations [72].

Figure 2: Involvement of the proteins coded by the identified risk loci in the development of alcohol-related steatosis as a first step in the evolution of alcohol-related liver injury

Green arrows indicate upregulation/stimulation; red drumsticks indicate down-regulation/inhibition.

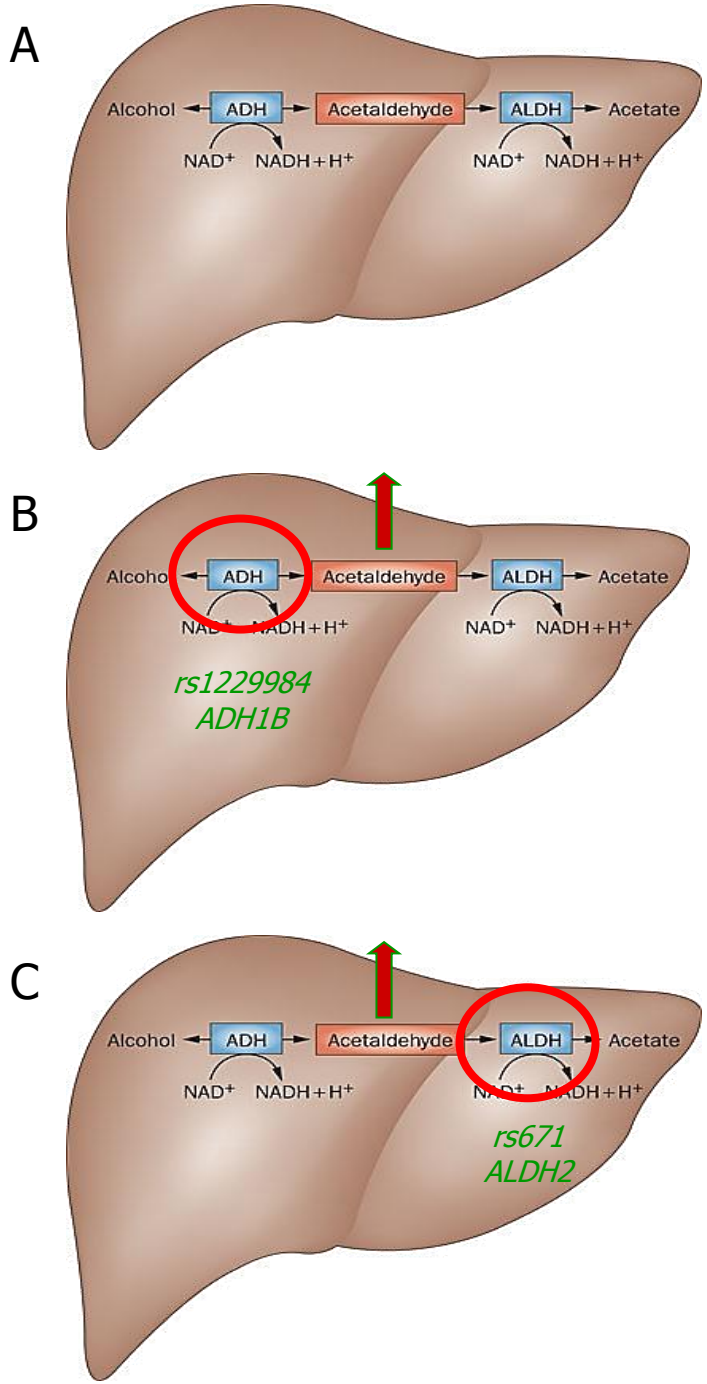
Alcohol can stimulate fat deposition through various mechanisms: (1) it increases NADH/NAD+ in hepatocytes, thereby disrupting β-oxidation, resulting in free fatty acid (FFA) and triglyceride (TG) accumulation; (2) it increases FFA and TG synthesis; (3) it enhances hepatic influx of FFA from adipose tissue and chylomicrons from the intestinal mucosa; and (4) inhibits assembly and secretion of VLDL by inhibition of microsomal triglyceride transfer protein. In addition alcohol contributes to (5) increased hepatic lipogenesis through up-regulation of sterol regulatory-binding protein 1c (SREBP-1c); (6) decreases lipolysis by downregulation of peroxisome proliferator-activated receptor (PPAR)-α; and, (7) downregulates adenosine monophosphate-activated protein kinase (AMPK). which inactivates acetyl-CoA carboxylase leading to reduced fatty acid synthesis and increased fatty acid oxidation.

Variants of the genes identified as putative risk factors for alcohol-related liver disease likely play an important role in this scenario.

Patatin-like phospholipase domain-containing 3 (PNPLA3) protein contributes significantly to TG hydrolysis and aberrant ('loss-off-function') activity would facilitate lipid accumulation. PNPLA3 is regulated by SREBP-1c through enhanced gene transcription and posttranscriptional stabilization. Whether alcohol, or its metabolite acetaldehyde (AA) impact on PNPLA3 hydrolytic activity is not known.

Transmembrane 6 superfamily member 2 (TM6SF2) protein is involved in the assembly and secretion of very low density lipoproteins (VLDL), similar to microsomal triglyceride transfer protein (MTTP). Thus, dysfunctional TM6SF2 is likely to lead to lower VLDL secretion from hepatocytes and hence more hepatic lipid accumulation but conversely a reduction in circulating VLDL and hence a reduction in cardiovascular risk.

Membrane bound O-acyltransferase domain containing 7 (MBOAT7) seems to be closely linked to inflammatory processes *via* interaction with arachidonic acid (ArA). *MBOAT7* encodes an enzyme with lysophosphatidylinositol acyltransferase activity and has been implicated in anti-inflammatory processes through regulating arachidonic acid levels in neutrophils, and the transfer of FFA between phospholipids and lysophospholipids modulating hepatic inflammation.


A
Alcohol
ADH
Acetaldehyde
ALDH
Acetate
NAD+
NADH+H+
NAD+
NADH+H+
B
Alcoho
ADH
Acetaldehyde
ALDH
Acetate
NAD+
NADH+H+
rs1229984
ADH1B
C
Alcohol
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ALDH
Acetate
NAD+
NADH+H+
rs671
ALDH2

Figure 2: Involvement of proteins coded by risk loci in alcoholic steatosis

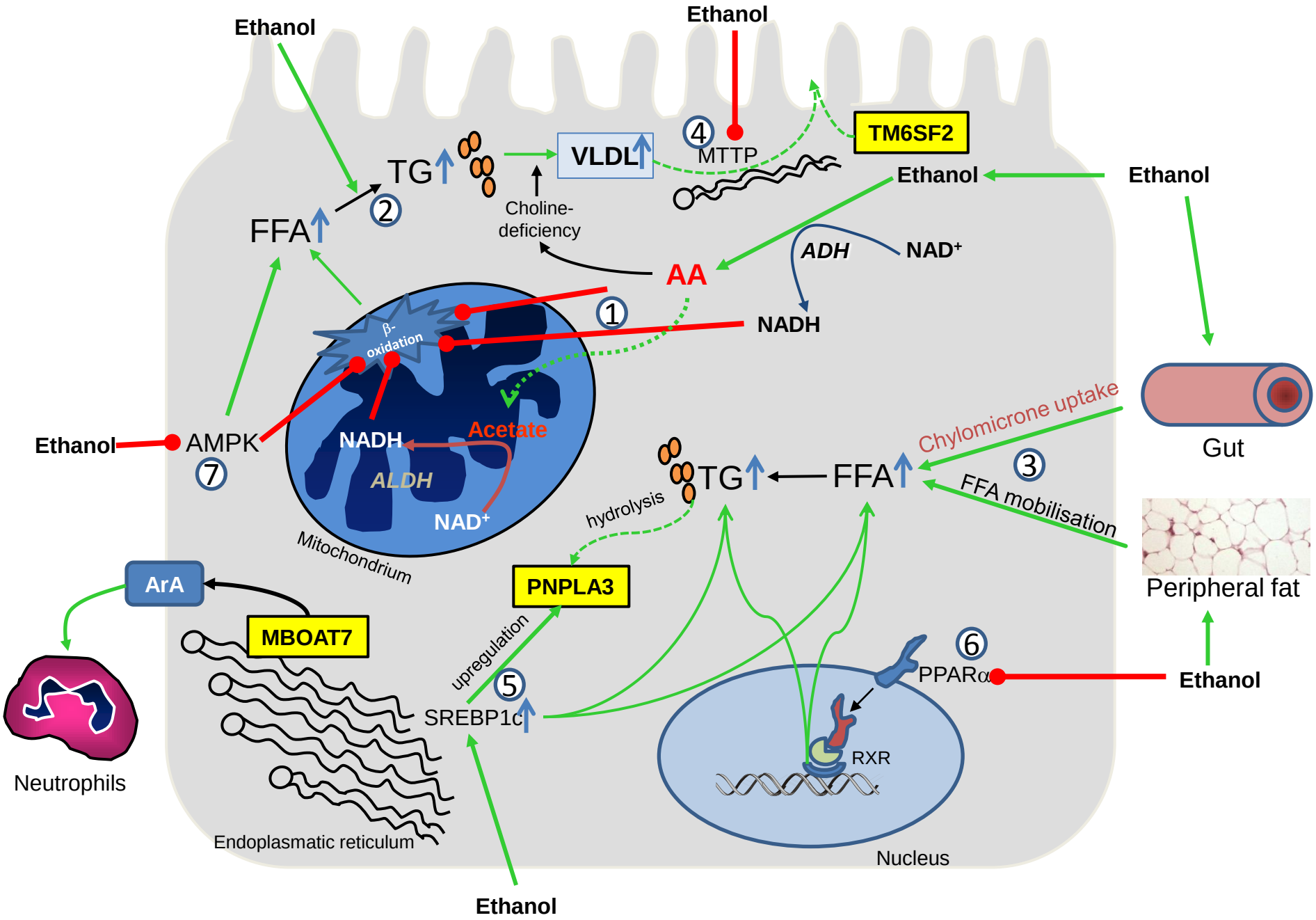

Table 1. Comparisons of the ICD-10 and DSM-IV diagnostic criteria for alcohol use disorders

	ICD-10*	DSM-IV*
Dependence	*Three or more of the following six symptoms occurring together for at least 1 month of if <1 month repeatedly during the last 12 – month period*	*A maladaptive pattern of alcohol use leading to clinically significant impairment or distress manifest by three or more of the following seven symptoms occurring in the same 12 month period*
Tolerance	Need for significantly increased amounts of alcohol to achieve intoxication/desired effect or markedly diminished effect with continued use	Need for significantly increased amounts of alcohol to achieve intoxication/desired effect or markedly diminished effect with continued use
Withdrawal	Characteristic physiological withdrawal syndrome or use of alcohol to relieve or off set withdrawal symptoms	Characteristic physiological withdrawal syndrome for alcohol. Alcohol often taken to relieve or avoid withdrawal symptoms
Impaired control	Difficulties in controlling use of alcohol in terms of onset, termination or levels of use; use of larger amounts or over a longer period than intended or a persistent desire or unsuccessful efforts to reduce or control use	Persistent desire or one or more unsuccessful efforts to cut down or control drinking Drinking in larger amounts or over a longer period than the person intended
Neglect of activities or time spent in alcohol-related activity	Preoccupation with alcohol use as manifested by important interests being given up or reduced or a great deal of time spent in activities necessary to obtain, take, or recover from the effects of the alcohol	Important social, occupational, or recreational activities given up or reduced because of drinking A great deal of time spent in activities necessary to obtain, to use or to recover from the effects of drinking
Continued use despite problems	Persistent alcohol use despite clear evidence of harmful physical or psychological consequences	Continued drinking despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to be caused or exacerbated by drinking
Compulsion	Strong desire or sense of compulsion to use the alcohol	Not included
	Harmful Use	**Alcohol Abuse**
	A pattern of drinking that can caused physical or psychological harm to the user endorsed by finding • Continued alcohol use despite the presence of related physical, psychological or cognitive problems • Use in situations where impairment could be dangerous • Detrimental behaviours and social problems related to its use • Interpersonal conflict attributed to its use	*A maladaptive pattern of alcohol use leading to clinically significant impairment or distress, during the last 12 months manifest by any of the following:* • Recurrent drinking resulting in inability to fulfill major role obligations at work, school, or home • Recurrent drinking in physically hazardous situations • Important social, occupational. or recreational activities given up/reduced because of drinking • Recurrent alcohol-related legal or interpersonal problems(e.g. arrests, traffic accidents, fights)

Abbreviations: ICD-10 - International Classification of Diseases, 10th Edition [12]
DSM-IV - Diagnostic and Statistical Manual of Mental Disorders, 4th Edition [13]

Table 2. Twin studies of alcohol dependence

First author & date [reference]	Location	Twin registry	Drinking behaviour	Monozygotic twins			Dizygotic twins			Heritability estimates* (%)
				N	Sex	Concordance (%)	N	Sex	Concordance (%)	
Kaij, 1960 [40]	Sweden	-	Chronic alcoholism	27	Male	71.0	60	Male	32.0	72
Allgulander, 1991 [41]	Sweden	Swedish Twin Registry I	Alcoholism	95	Male	12.6	187	Male	9.1	14
Kendler, 1997 [42]	Sweden	Swedish Twin Registry II	Temperance board registration	753	Male	31.3	1209	Male	21.6	22
Partanen, 1966 [43]	Finland	-	Alcoholism	172	Male	26.0	557	Male	12.0	30
Koskenvuo, 1984 [44]	Finland	Finish Twin Cohort	Alcoholism	69	Male	13.0	175	Male	5.7	24
				7	Female	0	20	Female	0	0
Pickens, 1991 [45]	USA	Minnesota Twin Registry	DSM-III Alcohol dependence	39	Male	59.0	47	Male	36.2	46
				24	Female	25.0	20	Female	5.0	54
Kendler, 1994 [46]	USA	Virginia Twin Registry	Alcohol dependence	203	Female	26.2	154	Female	11.9	34
Prescott,1999 [47]	USA	Virginia Twin Registry	DSM-IV Alcohol dependence	378	Male	31.7	436	Male	19.3	28
Reed, 1996 [48]	USA	-	Alcoholism	364	Male	26.7	571	Male	12.2	58
True, 1996 [49]	USA	Vietnam Era Twin Registry	Alcoholism	710	Male	53.2	588	Male	43.2	20
Prescott, 2005 [50]	USA	Washington University Twin Study of Alcoholism	Alcohol dependence	28	Male	40.0	26	Male	13.0	48
				48	Female	17.0	58	Female	24.0	10
Gurling,1981 [51]	UK	-	Alcoholism	15	Male	33.0	20	Male	30.0	8
				13	Female	8.0	8	Female	13.0	-16
Heath, 1997 [52]	Australia	Australian Twin Registry	Alcohol dependence	396	Male	38.9	231	Male	19.9	40
				932	Female	20.9	534	Female	9.2	22

Abbreviations: n – number; USA - United States of America; UK - United Kingdom; DSM - Diagnostic and Statistical Manual of Mental Disorders [13]
*Heritability estimates –calculated by doubling the mean effect size estimates of a correlation measure like the phi coefficient
Data adapted from Walters, 2002 [39] and Verhulst et al, 2015 [53]

Table 3. Adoption studies of alcohol use disorder phenotypes

First author & date [reference]	Location	Cohort	Drinking behaviour	Proband			Controls			Heritability estimates* (%)
				N	Sex	Outcome †	N	Sex	Outcome †	
	Denmark	Danish Adoption Cohort								
Goodwin, 1973 [59]			Alcoholism	55	Male	18.2	78	Male	5.1	42
Goodwin, 1973 [59]			Problem drinking	55	Male	9.1	78	Male	14.1	-16
Goodwin, 1977 [60]			Alcoholism	6	Female	33.3	90	Female	52.2	-18
	Sweden	Swedish Adoption Cohort								
Bohman, 1978 [61]			Alcohol abuse	89	Male	39.4	892	Male	13.1	42
Bohman, 1981 [62]			Alcohol abuse	172	Female	7.0	741	Female	2.6	20
Cloninger, 1981 [63]			Severe alcohol abuse	307	Male	7.8	555	Male	4.9	12
Sigvarsson, 1996 [64]			Alcohol abuse	108	Male	24.1	469	Male	12.8	24
Sigvarsson, 1996 [64]			Alcohol abuse	114	Female	0.9	546	Female	1.3	-2
	USA	Iowa Adoption Cohort								
Cadoret, 1980 [65]			Alcoholism	23	Men	13.0	69	Men	1.4	52
Cadoret, 1986 [66]			Alcohol abuse	39	Both	48.7	404	Both	13.9	5
Cadoret, 1994 [67]			Alcohol abuse	49	Both	70.6	34	Both	55.1	32

Abbreviations: N – number, USA – United States of America

†Percent of proband (alcohol abusing) and control (non-alcohol abusing) adoptees with at least one alcohol abusing biological parent

*Heritability estimates –calculated by doubling the mean effect size estimates of a correlation measure

Data adapted from Walters, 2002 [39] and Verhulst et al, 2015 [53]

Table 4. Genome-wide linkages studies of alcohol use phenotypes

First author & date [reference]	Location	Cohort	Families (n)	Ethnicity	Phenotype	Region of interest LOD>3	Potential regional candidates
Reich, 1998 [78]	USA	-	105	European	Alcohol dependence	-	-
Long, 1998 [79]	USA	-	172	Native American	Alcohol dependence	Chromosome 4p Chromosome11p	*GABRB1* *DRD4 & TH*
Ehlers, 2004 [80]	USA	-	100	Native American	Alcohol dependence	≈ Chromosome 4p	*ADH1B*
Wyszynski, 2003 [81]	USA	Framingham Heart Study	330	European	Heavy alcohol consumption	-	-
Wilhelmsen, 2005 [82]	USA	SMOFAM	158	European	Alcohol dependence	-	-
Prescott, 2006 [83]	Ireland	IASPSAD	474	European	Alcohol dependence/ alcohol misuse	Chromosome 4 q22 to q32	*ADH* cluster
Gelernter, 2009 [84]	USA	-	238	African America	Alcohol dependence	Chromosome 10 q23.3 to q24.1	-
Hansell, 2010 [85]	Australia	-	1690	European	Alcohol dependence	-	-
Gizer, 2011 [86]	USA	UCSF Family Alcoholism Study	713	European	Alcohol dependence	-	-

Abbreviations: USA - United States of America; SMOFAM - Smoking in Families Study; IASPSAD - Irish Affected Sib-Pair Study of Alcohol Dependence;
UCSF – University of California San Francisco; *DRD4* - dopamine receptor D_4; *TH* - tyrosine hydroxylase; *GABRB-1* - gamma-aminobutyric acid (GABA) A receptor, beta 1; *ADH* - alcohol dehydrogenase; LOD – Logarithm of the odds

Table 5. Genome-wide association studies of alcohol use phenotypes

First author & date [reference]	Location	Cohort	Ethnicity	Phenotype	Cases		Controls		Genes with significance $p \leq 5 \times 10^{-8}$
					N	Sex	N	Sex	
Treutlein, 2009 [87]	Germany	-	European	Alcohol dependence	1151	Male	2354	Male	*PECR*
Bierut, 2010 [88]	USA/ Germany	SAGE	African American European American	Alcohol dependence	1897	Both	1932	Both	-
Edenberg, 2010 [89]	USA	COGA	African American European American	Alcohol dependence	1192	Both	692	Both	-
Lind, 2010 [90]	Holland/ Australia	NESDA OZALC	European	Alcohol dependence	1823	Both	2763	Both	-
Heath, 2011 [91]	Australia	OZALC	European	Alcohol use disorder	2062	Both	3393	Both	-
Schumann, 2011 [92]	Pan-European	AlcGen	European	Alcohol consumption	47501	Both	-	-	*AUTS2*
Bail, 2011 [93]	South Korea	-	East Asian	Alcohol consumption	2834	Male	-	-	*ALDH2*
Zuo, 2012 [94]	USA	SAGE COGA	African American European American	Alcohol dependence	2090	Both	2016	Both	*KIAA0040*
Frank, 2012 [95]	Germany	-	European	Alcohol dependence	1333	Male	2168	Males	*ADH1B-ADH1C*
Park, 2013 [96]	South Korea	-	East Asian	Alcohol dependence	621	Both	750	Both	*ADH1B,ALDH2*
Gelernter, 2014 [97]	USA	GCD SAGE	African American European American	Alcohol dependence	7677	Both	6992	Both	*ADH1B, ADH1C,* LOC100507053 *METAP, PDLIM5*

Abbreviations: USA - United States of America; SAGE - Study of Addiction: Genetics and Environment, COGA – Collaborative Study on the Genetics of Alcoholism, NESDA – Netherlands Study of Depression and Anxiety; OZALC - Australian Twin-Family Study of Alcohol Use Disorder, AlcGen – Alcohol-GWAS consortium, GCD – GWAS discovery samples; *PERC*- peroxisomal trans-2-enoyl-coA reductase; *AUTS2* - autism susceptibility candidate 2 gene*; ALDH* acetaldehyde dehydrogenase; ADH-alcohol dehydrogenase; *METAP* - methionyl aminopeptidase; *PDLIM5* - PDZ and LIM domain 5

Table 6. Genetic association between rs738409 (G) in *PNPLA3* genotype and alcohol-related cirrhosis

First author & date [reference]	Ancestry	Cases (n)	Controls (n)	Odds ratio (95% CI)	Significance p
Tian, 2010 [137]	Mexican Mestizo (mixed European and Native American)	Alcohol-related cirrhosis (482)	Alcohol dependent: normal LFTs (305)	1.81 (1.36–2.41)	4.7×10^{-5}
Seth, 2010 [140]	British	Alcohol-related cirrhosis (266)	Heavy drinkers: normal LFTs (182)	2.2 (1.53-3.18)	2×10^{-5}
Trépo, 2011 [141]	Belgian and French	Alcohol-related liver disease -80% with cirrhosis (328)	Healthy controls (330)	1.54 (1.12-2.11)	8×10^{-3}
Stickel, 2011 [142]	German	Alcohol-related cirrhosis (210)	Alcohol dependent: no liver injury (439)	2.79 (1.55-5.04)	1.18×10^{-5}
Nguyen-Khac, 2011 [143]	French	Severe alcoholic hepatitis (65) Alcohol-related cirrhosis (40)	Healthy controls (105)	2.79 (1.39-5.64) 2.05 (1.0-4.19)	0.001 0.03
Nischalke, 2011 [144]	German	Alcohol-related cirrhosis (80)	Healthy controls (190)	1.92 1.28–2.86)	<0.002
Rosendahl, 2012 [145]	Dutch and German	Alcohol-related cirrhosis (135)	Healthy controls (2781)	2.2 (1.7-2.9)	<0.0001
Dutta, 2013 [146]	Indian	Alcohol-related cirrhosis (60)	Healthy controls (100)	2.12 (1.29-3.4)	0.037
Burza, 2014 [147]	Italian	Alcohol-related cirrhosis (84)	Alcohol dependence: no liver injury (300)	1.53 (1.07-2.19)	0.021
Friedrich, 2014 [148]	Caucasian on European transplant list	Alcohol–related cirrhosis (105)	Healthy controls (1950)	Not provided	<0.005
Way, 2014 [149]	British and Irish	Alcohol–related cirrhosis (323)	Population controls (1249) Alcohol dependent: no liver injury (331)	1.60 1.99	1.26×10^{-6} 2.54×10^{-7}

Odds ratios and significance levels are provided for cirrhosis vs. controls: CI, confidence interval; LFTs-liver function tests

Table 7. Genetic association between rs738409 (G) in *PNPLA3* genotype and HCC in patient with alcohol-related cirrhosis

First author & date [reference]	Ancestry	Cases (n)	Controls (n)	*Odds ratio (95% CI)	Significance p
†Falleti, 2011 [151]	Italian	Alcohol-related cirrhosis with HCC (66)	Alcohol-related cirrhosis (132)	1.64 (0.98-2.73)	5.74×10^{-2}
†Nischalke, 2011 [144]	German	Alcohol-related cirrhosis with HCC (77)	Alcohol-related cirrhosis (78)	2.68 (1.48-4.85)	0.0011
†Hamza, 2012 [152]	French	Alcohol-related cirrhosis with HCC (86)	Alcohol-related cirrhosis (85)	1.67 (0.96-2.89)	0.067
†Trépo, 2012 [153]	Belgian and French	Alcohol-related cirrhosis with HCC (145)	Alcohol-related cirrhosis (426)	2.51 (1.84-3.41)	4.30×10^{-9}
†Guyot, 2013 [154]	French	Alcohol-related cirrhosis with HCC (68)	Alcohol-related cirrhosis (211)	2.23 (1.44-3.45)	3.05×10^{-4}
Friedrich, 2014 [148]	Caucasian on European transplant list	Alcohol-related cirrhosis with HCC (29)	Alcohol-related cirrhosis (76)	CG: 3.92 (1.05-14.71) GG: 8.10 (1.82-36.11)	0.03 0.003
Nischalke, 2014 [155]	German	Alcohol-related cirrhosis with HCC (126)	Alcohol related cirrhosis (356)	2.32 (1.58- 3.41)	0.00002
Falleti, 2016 [156]	Italian	Alcohol-related cirrhosis with HCC (75)	Alcohol related cirrhosis (151)	CG 1.05 (0.52-2.12) GG 2.20 (1.03-4.64)	0.893 0.039

† Data extrapolated from Trépo et al 2014 [157] in which original trial data were reprocessed prior to meta-analyses based on individual participant data. HCC, hepatocellular carcinoma; CI, confidence interval;