Review Article

Genes in Alcoholic Liver Disease: A Complex Interplay

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Abstract

Alcohol-related liver disease is a major public health problem, with a varying clinical course and often devastating consequences. Its spectrum can vary from alcohol-related fatty liver, alcoholic hepatitis, cirrhosis, and hepatocellular carcinoma. Multiple environmental, genetic, socio-economic and epigenetic factors are intrinsically involved, affecting disease behavior and progression. Recent advances in genome-wide association studies have identified multiple genetic polymorphisms that may affect the pathophysiological process, and in turn, affect disease progression. Although the present understanding of this complex process remains nascent at best, there is an ardent need to incorporate available genetic markers into diagnostic and prognostic algorithms. This may pave the way for future research into targeted therapies.

Introduction

The burden of alcoholic liver disease (ALD) is a pertinent health-related issue. In 2017, 23.6 million people developed compensated cirrhosis, while another 2.5 million people developed decompensated cirrhosis worldwide due to alcohol abuse.[1] In India, the prevalence of alcohol-related chronic liver disease varied within 10.9–31.9%, with a predicted mortality rate of 10–22%.[2]

Alcoholism or the harmful consumption of alcohol, especially among the younger age group, is particularly noteworthy. This is especially true for India, which presently happens to be the most populous nation in the world. Drinking patterns and the higher per capita consumption of alcohol in a population, which is already predisposed to diabetes and non-alcoholic steatohepatitis (NASH), has opened up a Pandora's Box for healthcare professionals. Understanding the spectrum of ALD in the younger population assumes significance, because this can adversely impact a country's

economy and healthcare resources. In addition, this is a major cause of premature death and disability.[3,4]

A literature search was conducted using the following MeSH terms in the PubMed database: "Alcohol abuse", "Alcoholic liver disease", "Alcoholic cirrhosis of liver", and "Genetic modifiers". When the genetic modifiers implicated in the development of ALD were identified, the literature search was conducted using the individual genes in the PubMed, EMBASE, and Web of Science databases. The identified titles, abstracts, and full text articles were employed by two independent reviewers in writing the present review. The majority of the articles included in the present review were published over the last 15 years. In addition, some landmark articles predating 15 years, which were pertinent to explain the evolution of genetic research in ALD, were included. Genes with a proven role in the development of ALD were described. Since the disease shares much of its characteristics with NASH, a number of genes were identified to have a pathological role in the development of NASH, but the pathogenic role in ALD remains debatable. Hence, these genetic alterations were not summarized in the present review. Furthermore, a number of epigenetic modifications occur in parenchymal and non-parenchymal hepatic cells due to alcohol consumption. These modifications are heritable, which alter the gene expression, but do not alter the nucleotide sequence. Hence, these were beyond the preview of the present review.

Natural history of ALD

The spectrum of ALD passes through the following stages: (a) alcohol-related fatty liver (occurs in 90% of heavy drinkers, and may appear as early as 3–7 days following heavy alcohol consumption; (b) alcoholic hepatitis (AH) tends to occur in 10–35% of heavy alcohol consumers, and AH, in particular, has a poor prognosis: (c) alcohol-related cirrhosis (50% of individuals hospitalized for AH

may have underlying cirrhosis, while 27% of individuals with mild AH may develop cirrhosis over a mean follow up of 4–8 years). The risk of progression to cirrhosis increases to 68% with severe alcoholic hepatitis.[5,6] The National Institute of Alcohol Abuse and Alcoholism defines heavy alcohol consumption as having more than four drinks a day or more than 14 drinks per week for men, and more than three drinks a day or more than seven drinks per week for women.[7]

ALD involves the complex interplay between genetic and environmental factors. The advent of human genome sequencing and genome-wide association studies (GWAS) have opened the doors for the identification of novel foci, which have previously not been implicated in disease pathogenesis.

The investigators attempted to explain the complex association of the genetic polymorphisms involved in the development of ALD by classifying these genetic polymorphisms, in terms of the pathophysiological processes involved in the development of ALD. The triggering event in this disease process is the harmful consumption of alcohol/alcohol abuse. Thus, genes that affect neurotransmission and the hepatic metabolism of alcohol assume great significance. Once the pathophysiological changes have set in, the next step is disease progression. Genes that affected the oxidative and inflammatory process, hepatic lipid deposition, and fibrogenesis were described. The eventual outcome was the development of cirrhosis. Hepatocellular carcinoma (HCC) was the final outcome, and genes that played a critical role in carcinogenesis in patients of ALD were described.

Genetics of alcohol abuse

ALD by itself may be termed as an "avoidable disease". The disease progression depends on the amount and pattern of alcohol consumption. A family history of alcohol use disorder may predict an individual's risk of developing addiction. This may involve the influence of both environment and genetic factors. An elegant study that analyzed evidence obtained from family, adoption, and twin studies highlighted the incremental effects of genetic influence on alcohol addiction (the estimates varied from 0.30 to 0.70).[8] Among these, two aspects of alcohol dependence have been the most widely studied: those that affect neurotransmission, and those that affect alcohol metabolism.

Genes involved in neurotransmission in individuals with alcohol abuse

Gamma amino butyric acid (GABA) is the principal inhibitory neurotransmitter that influences alcohol consumption and withdrawal. Four specific chromosomal clusters located in chromosomes 4, 5, 15 and X modulate the GABA activity in humans. Two GWAS and the "Collaborative Study on the Genetics of Alcoholism" provided valuable evidence that implicates role of the 4q13-q11 GABA cluster located in the *GABRA2* gene in modulating alcohol consumption, and the development of symptoms of alcohol withdrawal.[9–11]

The Ras protein specific nucleotide release factor 2 (*RasGRF2*) gene, which is located in chromosome 5,5q14.1, has been implicated in the neurotransmission of alcohol. Individuals with single nucleotide polymorphism (SNP) rs26907 tend to experience a "reward induced alcohol reaction variation" and greater alcohol use. RasGRF2 directs the Ras protein, which acts via dopamine receptors to activate mitogen-activated protein kinase and extracellular signal-regulated kinases, and activate the "reward mechanism" associated with alcohol use.[12–15]

Genes that affect alcohol metabolism

Ethanol undergoes two stages of enzymatic metabolism in the liver. First, alcohol dehydrogenase (ADH) oxidizes ethanol to acetaldehyde, and this is further converted to acetate by aldehyde dehydrogenase (ALDH). ADH has isozymes (ADH1, ADH2, ADH3 and ADSH4) that are expressed in chromosome 4q21-23 ADH1 alleles ADH1B*2 and ADH1B*3 confer high metabolic activity, thereby increasing acetaldehyde production. ADH1B*2 was associated with lower risk of alcohol consumption in Jewish and European populations.[16–18] On the other hand, ADH2*1 and ADH3*2 isozymes confer an increased risk of alcohol dependence in individuals with alcohol abuse, except for those with East Asian ancestry.[19] ALDH has two isoforms: ALDH1 and ALDH2. ALDH2 is located in chromosome 12q24. A mutation on the single base pair in the gene that codes ALDH2 confers low activity for isozyme ALDH2*2. Furthermore, 30–50% of East Asian populations have reported a carriage of ALDH2*2 allele, and its presence has caused exaggerated unpleasant sensations upon alcohol consumption.[18,20] Figure 1 summarizes the genes involved in the hepatic metabolism of alcohol.

Pathophysiology of ALD

Before elucidating the causative association of genes, it is imperative to understand the basic pathophysiology that causes apoptotic cell death and the initiation of fibrosis in ALD. Generation of toxic compounds: "aldehyde adducts", in addition to "damage associated molecular pattern molecules" and "pathogen-associated molecular pattern molecules", initiate oxidative stress and free radical production. This initiates the vicious cycle of cluster differentiation (CD14)/toll-like receptor-induced inflammation, the activation of hepatic innate immunity (Kupffer cells and macrophage activation), cytokine release, and finally, hepatocyte necrosis and apoptotic cell death. These processes are common in ALD and non-alcoholic fatty liver disease (NAFLD). Thus, understanding the genetic basis of ALD derives substantive inference from NAFLD.[21–24]

Genes that modify the progression of ALD

Well-established facts have supported the hypothesis that genetic factors, in addition to environmental influences, play a prominent role in propagating hepatic damage in individuals with sustained alcohol consumption. Human females tend to present with rapid progression and severity of ALD, when compared to males who consume similar quantities of alcohol. Within this species, there are well-known ethnic differences: Hispanics are more prone to develop ALD vs. individuals of European or African ancestry. Furthermore, monozygotic twins have an increased concordance for ALD cirrhosis, when compared to dizygotic twins.[25–28]

Two genes, which were initially identified to play a pathophysiological role in the progression of NAFLD, have been associated with the development of ALD. GWAS have identified SNP rs738409, which occurs in human patatin-like phospholipase domain-containing 3 gene (PNPLA3), to be strongly associated with NAFLD. PNPLA3, which is present in chromosome 22, and encodes "adiponutrin", is closely associated with adipose triglyceride lipase (triglyceride hydrolase in adipose tissues). One of the variants of PNPLA3, the 1148M variant, predisposes to progression of ALD. These findings have been confirmed in Mexican and European cohorts of patients.[29,30] The findings reported by a systemic review and meta-analysis concluded that individuals with
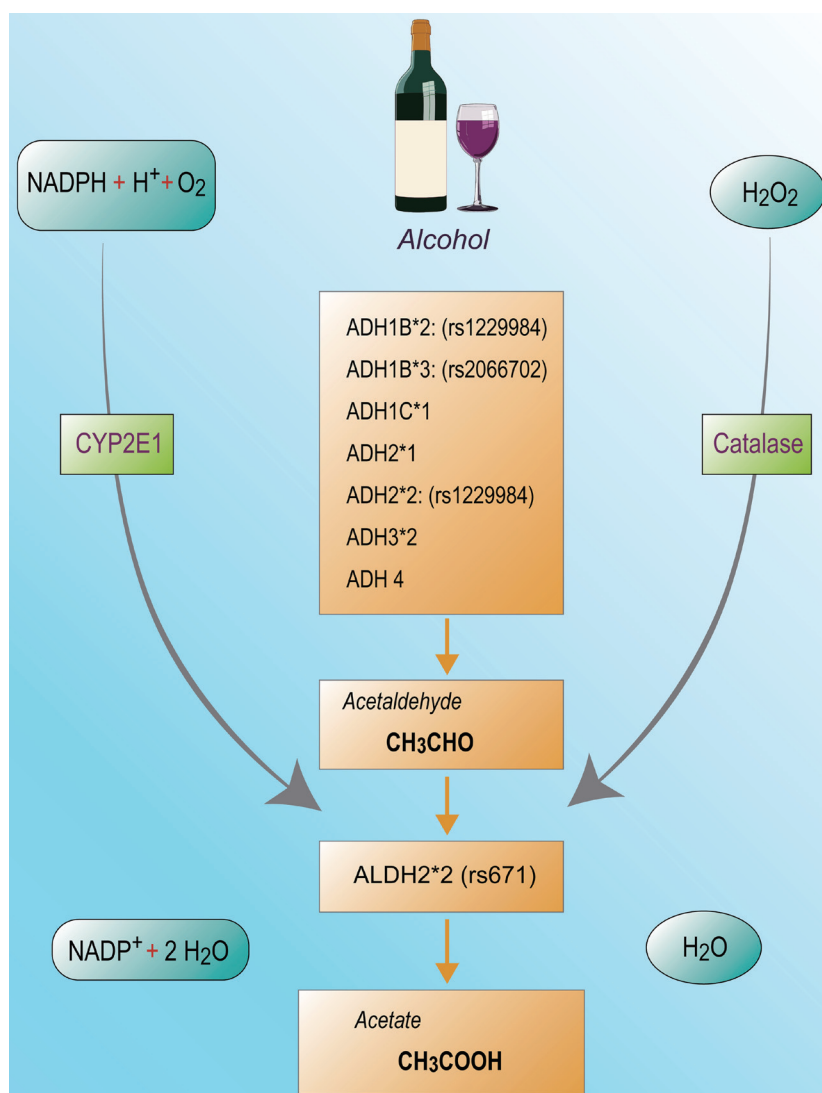
Fig. 1. Genes involved in the hepatic metabolism of alcohol. ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP2E1, cytochrome P450 2E1 subfamily; NADPH, nicotinamide adenine dinucleotide phosphate; H_2O_2, hydrogen peroxide; H_2O, water; O_2, oxygen.

rs738409 CG heterozygotes for PNPLA3 have an odds ratio (OR) of 2.09 (95% confidence interval [CI]: 1.79–2.44) for developing ALD cirrhosis vs. the OR of 3.37 (95% CI: 2.49–4.58) for those with minor-allele homozygotes.[31]

Another candidate gene is transmembrane 6 superfamily member 2 (TM6SF2), which is located in chromosome 19p13.11. Preliminary results have revealed that the carriage of the rs58542926 variant of TM6SF2 is associated with ALD cirrhosis.[32,33]

Although some genetic polymorphisms modify the effect of environmental factors, other genes independently increase the risk of progression of ALD, independent of age, gender, diabetes, or socio-economic factors. One of these genes is membrane bound O-acyltransferase domain-containing 7/transmembrane channel-like 4 (MBOAT7/TMC4). The GWAS conducted for individuals with European ancestry identified the rs641738 C>T SNP in the MBOAT7/TMC4 locus as an independent risk factor that increases the risk of developing cirrhosis in individuals with a definite history of alcohol abuse.[34]

It is noteworthy that all three genes described in this section (*PNPLA3*, *TM6SF2* and *MBOAT7*) modify the genetic expression in patients with ALD and NAFLD, possibly via altered hepatic lipid remodeling.

Genetic basis of oxidative stress in ALD

Enzymes in the mitochondrial oxidative chain: Cytochrome p450 (CYP2E1) and cytosolic enzymes, such as xanthine oxidase and aldehyde oxidase, generate reactive oxygen species (ROS). This is counteracted by enzymes, such as glutathione-s-transferases (GSTs) and superoxide dismutase. Although the role of oxidative enzymes in the development of ALD remains controversial, GSTs may conjugate with ROS, leading to the formation of toxic xenobiotics. Evidence was provided by studies that involved patients with hereditary hemochromatosis, in which the isozyme GST1 "valval" variant is associated with the development of cirrhosis. However, similar studies that evaluated the role of GSTs in the progression

of ALD have been inconclusive.[35,36]

Studies that analyzed the pathological role of superoxide dismutase in ALD have provided conflicting results. Mitochondrial manganese superoxide dismutase (Mn SOD) converts ROS to hydrogen peroxide and water. The "Ala sequence of Mn SOD" has a variation in codon 16 of the precursor protein, in which either Alaline or Valine appears in amino acid position 9. This induces the increased translocation of protein into the mitochondria. Individuals who are homozygous for "Ala" in the mitochondrial target sequencing of Mn SOD have three times increased propensity for micro vesicular steatosis, six times increased risk of alcoholic hepatitis, and 10 times increased risk of cirrhosis.[37] Although the initial results were promising, the follow-up study that involved individuals with ALD cirrhosis vs. individuals with heavy alcohol consumption, but without cirrhosis, did not reveal any statistical difference in "heterozygote vs. homozygote Ala allele frequency".[38]

Protective role of nuclear factor erythroid 2-related factor 2 gene (NrF2) in oxidative stress

NrF2 is a 605 amino acid protein, which is present in the cytoplasm in a bound state, with its inhibitor protein, Kelch-like ECH-associated protein 1 (keap1). Under conditions of oxidative stress, this interaction is altered, causing Nrf2 to stabilize and accumulate in the nucleus. Nrf2 forms a complex with the small musculoaponeurotic fibrosarcoma oncogene homolog (sMAF) protein. The Nrf2-sMaf complex induces the transcription of genes that act against oxidative damage. Murine models have provided evidence that Nrf2 regulates glutathione metabolism, thereby providing a protective response against alcohol-induced oxidative stress.[39] In mice exposed to alcohol, the treatment with 3H-1,2-dithiole-3-thione upregulated NrF2, and decreased the production of alcohol-related ROS and apoptosis. Further research has highlighted the protective role of the activation of the NrF2 pathway in alcoholic steatosis and alcohol-related fibrosis, while NrF2 knockdown mice suffered from the progression of alcohol-related hepatocyte necroptosis.[40]

Preliminary human studies, which evaluated the role of SNPs in the promoter region of the *NrF2* gene, revealed that -274A in SNP rs35652124, in terms of both homozygosity (A/A) and heterozygosity (A/G), may increase the risk for progression of ALD, while the -274G/G allele may confer some protection. However, a study did not reveal any correlation with the progression of hepatitis C virus cirrhosis.[41]

Genetic basis of lipid deposition in ALD

Hepatic steatosis is the most common pathological finding in individuals with heavy alcohol consumption. Peroxisome proliferator-activated receptors (PPARs) consist of three isoforms: PPARα, PPARδ and PPARγ. These are nuclear receptors that regulate cell differentiation, growth, and fat metabolism, in response to insulin secretion. The *PPARγ2* gene may undergo splice variation with a nucleotide substitution (C-G), followed by amino acid substitution (Pro12Ala). This modified gene includes an increase in translational activity of PPARγ.

Individuals with hepatic steatosis and a mutated PPAR have been identified to be associated with histologically proven necroinflammation.[42,43]

The altered hepatic lipid remodeling induced by the *PNPLA3*, *TM6SF2* and *MBOAT7* genes increases the risk of progression to the stage of liver cirrhosis. This was covered in the "Genes that modify the progression of ALD" section.

Genetic basis of endotoxin-mediated inflammation in ALD

The cluster of differentiation 14 (CD14) endotoxin receptor gene induces the increased expression of CD14. Individuals with a history of heavy alcohol consumption, who carry the genetic variants of this gene (CD14-159TT homozygous), possess an increased risk of developing cirrhosis, when compared to CC homozygotes.[44,45]

Interleukin (IL) 10 is another target molecule that has been investigated for individuals with ALD. IL10 is an anti-inflammatory cytokine that upregulates the IL1R antagonist, which further increases the collagenase expression and inhibits the collagen 1 transcription. For individuals with a history of heavy alcohol consumption, particularly in individuals with histological evidence of ALD, the "IL10-627a" allele expression has been documented in 50% vs. 34% of subjects with no histological evidence of ALD, and in 33% of healthy controls.[46] The tumor necrosis factor-α (*TNFα*) gene is another target molecule that has generated interest among researchers. SNPs at positions 238, 1,031, 863, 857, 851, 376 and 308, and SNPs at positions 308 and 238 have been identified to have a statistically significant association with ALD cirrhosis. In addition, individuals who carry the glutathione S-transferase (GST) M1 variant also known as the Val/ Val genotype have been found to have an increased risk of developing ALD.[47]

Genetic basis of hepatic fibrosis in ALD

The net result of necroinflammation is hepatic fibrogenesis. Angiotensin II can induce the activation of I kappa B kinase, which further phosphorylates nuclear factor kappa B (NF-kB) subunit Re1A. The modification of NF-kB polymorphisms is associated with an increased risk of developing cirrhosis for individuals with alcohol dependence.[48] Polymorphisms involving the transforming growth factor β (*TGβ1*) gene and matrix metalloproteinase 3 (*MMP-3*) gene have failed to demonstrate any functional association with the development of ALD cirrhosis.[49] Table 1 summarizes the genes that affect the pathophysiological changes, leading to the development of cirrhosis.

Genetic basis of the development of HCC in ALD

Polymorphisms in SOD, myeloperoxidase, and glutathione peroxidase have all been implicated in developing HCC in patients with underlying ALD. The methylenetetrahydrofolate reductase (*MTHFR*) gene is another gene that increases the risk of HCC in ALD.[50] A number of studies and meta-analyses have highlighted the role of the *PLPNA3* gene in increasing the risk of HCC in patients with underlying ALD.[51–54] Indeed, researchers have recommended that for patients with alcohol-related cirrhosis, the genotyping for the rs738409 PNPLA3 variant, in addition to USG screening, may be used for the more precise risk stratification of HCC.[55]

Similar to *PNPLA3* gene SNPs, another genetic polymorphism that involves the *TM6SF2* gene (TM6SF2 rs58542926) increases the risk of developing HCC in alcoholic cirrhosis: rs58542926 (OR: 1.66, CI 95%: 1.30–2.13). Indeed, the presence of both these polymorphisms in an individual with alcoholic cirrhosis worsens the risk of HCC.[56] The data obtained from an Italian cohort of patients with ALD and NAFLD, but without cirrhosis, implicated the pathogenic role of the MBOAT7 rs641738 T allele, increasing the risk of HCC by 80%. However, this risk disappeared when the process of fibrosis has already set in.[57] Table 2 highlights the various

Table 1. Genes involved in the propagation and development of cirrhosis in ALD

Event (Physiological/Pathological)	Genes involved	Implications
Neurotransmission	*4q13-q11GABA cluster*	Modulation of alcohol consumption and symptoms of alcohol withdrawal
	(RasGRF2) rs26907	Reward induced alcohol variation, greater alcohol use
Metabolism of alcohol	*ADH1B*2:ADH4*	Fastens oxidation of alcohol
	ALDH2*2 (rs671)	Protects against alcohol dependence
Inflammation and Fibrosis	*IL 10 C-627A (rs1800872)*	Increases the risk of inflammation and immune-mediated fibro-genesis
	IL1B -511	Increases the risk of fibrosis
	TNFα:G238A (rs361525)	Increases the risk of fibrosis
	Nrf2:-274A rs35652124	Increases risk of fibrosis
Lipogenesis and Fibrosis	*PNPLA3:I148M (rs738409)*	Alters lipid metabolism, increases the risk of fibrosis
	TM6SF2:E167K (rs58542926)	Alters lipid metabolism, increases the risk of decompensation and death
Hepatocellular carcinoma	*TM6SF2: (rs58542926)*	Increases the risk of HCC
	MBOAT7: (rs641738 T)	In patients of NAFLD and ALD, increases the risk of HCC

ALD, alcoholic liver disease; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; HCC, hepatocellular carcinoma; MBOAT7/TMC4, membrane bound O-acyltransferase domain containing 7/transmembrane channel-like 4; NrF2, nuclear factor erythroid 2-related factor 2 gene; PNPLA3, patatin-like phospholipase domain-containing 3; TM6SF2, transmembrane 6 superfamily member 2; Mn SOD, manganese superoxide dismutase.

Table 2. Highlights for the modifier genes with proven roles in ALD

Serial no.	Gene	SNP	Pathophysiological role	Clinical significance
1	Alcohol dehydrogenase (ADH)	ADH1B*2:(rs1229984)	Metabolism of alcohol	Hastens oxidation of ethanol in Asian ancestry, except Indians and Jews
		ADH1B*3:(rs2066702)		Hastens oxidation of ethanol in Native Americans and Africans
		ADH1C*1		Hastens oxidation of ethanol in Asians
		ADH 4		Increases risk of alcoholism
2	Aldehyde dehydrogenase (ALDH)	ALDH2*2 (rs671)	Metabolism of alcohol	Reduced oxidation of acetaldehyde Protective against alcohol dependence
3	Interleukin 10 (IL10)	C-627A (rs1800872)	Inflammation mediated liver injury	Low IL10 expression; Increases the risk of inflammation, immune mediation, and pro-fibrotic mechanism of ALD
4	Interleulin-1β (IL1B)	IL1B -511	Inflammation mediated liver injury	Increased risk of cirrhosis in a Japanese cohort
5	Patatin-like phospholipase domain-containing 3 (PNPLA3)	I148M (rs738409)	Hepatic lipid metabolism	Increases the risk of developing alcohol-related cirrhosis and risk of development of HCC in alcoholic cirrhosis
6	Transmembrane 6 superfamily member 2 (TM6SF2)	E167K (rs58542926)	Hepatic lipid metabolism	Increases risk of development of advanced chronic liver disease, and modifies the risk of hepatic decompensation and liver-related death, independent of baseline liver severity
7	Tumor necrosis factor-α (TNFα)	G-238A (rs361525)	Inflammation-mediated liver injury	Increases the risk of development of alcohol-related liver cirrhosis
8	Nuclear factor erythroid-related factor-2 (Nrf2)	-274A (rs35652124)	Inflammation-mediated liver injury and apoptosis	Increases the risk of progression of alcohol-related liver disease

ALD, alcoholic liver disease; HCC, hepatocellular carcinoma; SNP, single nucleotide polymorphism.

genetic polymorphisms with proven effects in the progression of ALD.

Shortcomings of genetic studies that evaluated the role of genetic polymorphisms in the development and progression of ALD

The shortcomings of the present study were, as follows: the majority of the studies carried out to date were case control studies, and the majority of these studies were single-center studies with definite referral bias; the sample size was small, and there was a lack of adequate statistical power, such as having less than 80% of minor allele frequencies; the presence of co-morbidities, especially obesity, in the studied populations, in addition to the inappropriate selection of controls, reduced the weight of these studies; studies that evaluated the protective role of genes remained limited to animal studies; a major portion of the world population that resides in developing and least developed countries has not been investigated.

Conclusion

The present review highlights the genetic alterations with a proven association with disease progression in ALD. There is an ardent need to modify present diagnostic protocols to include genetic polymorphism in investigative algorithms. The incorporation of proven genetic polymorphisms may be used to screen individuals with alcohol abuse disorders, and improve the prognostic scores for liver transplantation and screening protocols for HCC. In the future, these would be able to determine individuals who are likely to have disease progression, allowing them to consider genetic therapies to modify the natural history of the disease.

Conflict of interest

The authors have no financial/non-financial declarations of interests.

Author contributions

SD and MM: acquisition of data; SD and MM: drafting of the manuscript: MM: critical revision of the manuscript for important intellectual content. All authors have made a significant contribution to the study, and have approved the final manuscript.

References

[1] Asrani SK, Mellinger J, Arab JP, Shah VH. Reducing the global burden of alcohol-associated liver disease: A blueprint for action. Hepatology 2021;73(5):2039–2050. doi:10.1002/hep.31583, PMID:32986 883.

[2] Mondal D, Das K, Chowdhury A. Epidemiology of liver diseases in India. Clin Liver Dis (Hoboken) 2022;19(3):114–117. doi:10.1002/ cld.1177, PMID:35355840.

[3] Eashwar VMA, Gopalakrishnan S, Umadevi R, Geetha A. Pattern of alcohol consumption and its associated morbidity among alcohol consumers in an urban area of Tamil Nadu. J Family Med Prim Care 2019;8(6):2029–2035. doi:10.4103/jfmpc.jfmpc_226_19, PMID:313 34175.

[4] De A, Duseja A. Nonalcoholic Fatty Liver Disease: Indian Perspective. Clin Liver Dis (Hoboken) 2021;18(3):158–163. doi:10.1002/cld.1141, PMID:34691404.

[5] Maddrey WC, Boitnott JK, Bedine MS, Weber FL Jr, Mezey E, White RI Jr. Corticosteroid therapy of alcoholic hepatitis. Gastroenterology 1978;75(2):193–199. doi:10.1016/0016-5085(78)90401-8, PMID:352 788.

[6] Singal AK, Mathurin P. Diagnosis and treatment of alcohol-associated liver disease: A Review. JAMA 2021;326(2):165–176. doi:10.1001/ jama.2021.7683, PMID:34255003.

[7] Nehring SM, Freeman AM, Doerr C. Alcohol Use Disorder (Nursing). StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023. PMID:33760498.

[8] Wang K, Duan Y, Duan W, Yu Y, Zheng N, Hu J, He J, *et al*. Bibliometric insights in genetic factors of substance-related disorders: intellectual developments, turning points, and emerging trends. Front Psychiatry 2021;12:620489. doi:10.3389/fpsyt.2021.620489, PMID:341 35780.

[9] Williams JT, Begleiter H, Porjesz B, Edenberg HJ, Foroud T, Reich T, *et al*. Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. II. Alcoholism and event-related potentials. Am J Hum Genet 1999;65(4):1148–1160. doi:10.1086/302571, PMID:10486334.

[10] Long JC, Knowler WC, Hanson RL, Robin RW, Urbanek M, Moore E, *et al*. Evidence for genetic linkage to alcohol dependence on chromosomes 4 and 11 from an autosome-wide scan in an American Indian population. Am J Med Genet 1998;81(3):216–221. doi:10.1002/ (sici)1096-8628(19980508)81:3<216::aid-ajmg2>3.0.co;2-u, PMID:9603607.

[11] Reich T, Edenberg HJ, Goate A, Williams JT, Rice JP, Van Eerdewegh P, *et al*. Genome-wide search for genes affecting the risk for alcohol dependence. Am J Med Genet 1998;81(3):207–215. doi:10.1002/ (SICI)1096-8628(19980508)81:3<207::AID-AJMG1>3.0.CO;2-T, PMID:9603606.

[12] Stacey D, Bilbao A, Maroteaux M, Jia T, Easton AC, Longueville S, *et al*. RASGRF2 regulates alcohol-induced reinforcement by influencing mesolimbic dopamine neuron activity and dopamine release. Proc Natl Acad Sci USA 2012;109(51):21128–21133. doi:10.1073/ pnas.1211844110, PMID:23223532.

[13] Schwechter B, Rosenmund C, Tolias KF. RasGRF2 Rac-GEF activity couples NMDA receptor calcium flux to enhanced synaptic transmission. Proc Natl Acad Sci USA 2013;110(35):14462–14467. doi:10.1073/pnas.1304340110, PMID:23940355.

[14] Hsu TC, Rodrigues GOL, Winer H, Hixon JA, Li W, Tarasova NI, *et al*. A Focused Review of Ras Guanine Nucleotide-Releasing Protein 1 in Immune Cells and Cancer. Int J Mol Sci 2023;24(2):1652. doi:10.3390/ijms24021652, PMID:36675167.

[15] Feig LA. Regulation of neuronal function by Ras-GRF exchange factors. Genes Cancer 2011;2(3):306–319. doi:10.1177/1947601911408077, PMID:21779501.

[16] Whitfield JB, Nightingale BN, Bucholz KK, Madden PA, Heath AC, Martin NG. ADH genotypes and alcohol use and dependence in Europeans. Alcohol Clin Exp Res 1998;22(7):1463–1469. doi:10.1111/j.1530-0277, PMID:9802529.

[17] Yao P, Zhang Z, Liu H, Jiang P, Li W, Du W. p53 protects against alcoholic fatty liver disease via ALDH2 inhibition. EMBO J 2023;42(8):e112304. doi:10.15252/embj.2022112304, PMID:36825429.

[18] Edenberg HJ, Gelernter J, Agrawal A. Genetics of alcoholism. Curr Psychiatry Rep 2019;21(4):26. doi:10.1007/s11920-019-1008-1, PMID:308 52706.

[19] Zintzaras E, Stefanidis I, Santos M, Vidal F. Do alcohol-metabolizing enzyme gene polymorphisms increase the risk of alcoholism and alcoholic liver disease? Hepatology 2006;43(2):352–361. doi:10.1002/ hep.21023, PMID:16440362.

[20] Harada S, Agarwal DP, Goedde HW, Tagaki S, Ishikawa B. Possible

protective role against alcoholism for aldehyde dehydrogenase isozyme deficiency in Japan. Lancet 1982;2(8302):827. doi:10.1016/s0140-6736(82)92722-2, PMID:6126701.

[21] Tan HK, Yates E, Lilly K, Dhanda AD. Oxidative stress in alcohol-related liver disease. World J Hepatol 2020;12(7):332–349. doi:10.4254/wjh.v12.i7.332, PMID:32821333.

[22] Mandrekar P, Szabo G. Signalling pathways in alcohol-induced liver inflammation. J Hepatol 2009;50(6):1258–1266. doi:10.1016/j.jhep.2009.03.007, PMID:19398236.

[23] Malhi H, Gores GJ, Lemasters JJ. Apoptosis and necrosis in the liver: a tale of two deaths? Hepatology 2006;43(2 Suppl 1):S31–S44. doi:10.1002/hep.21062, PMID:16447272.

[24] Farrell GC, Larter CZ, Hou JY, Zhang RH, Yeh MM, Williams J, *et al*. Apoptosis in experimental NASH is associated with p53 activation and TRAIL receptor expression. J Gastroenterol Hepatol 2009;24(3):443–452. doi:10.1111/j.1440-1746.2009.05785.x, PMID:19226377.

[25] Sato N, Lindros KO, Baraona E, Ikejima K, Mezey E, Järveläinen HA, *et al*. Sex difference in alcohol-related organ injury. Alcohol Clin Exp Res 2001;25(5 Suppl ISBRA):40S–45S. doi:10.1097/00000374-200105051-00007, PMID:11391047.

[26] Stinson FS, Grant BF, Dufour MC. The critical dimension of ethnicity in liver cirrhosis mortality statistics. Alcohol Clin Exp Res 2001;25(8):1181–1187. doi:10.1111/j.1530-0277.2001.tb02333.x, PMID:11505049.

[27] Reed T, Page WF, Viken RJ, Christian JC. Genetic predisposition to organ-specific endpoints of alcoholism. Alcohol Clin Exp Res 1996;20(9):1528–1533. doi:10.1111/j.1530-0277.1996.tb01695.x, PMID:8986199.

[28] 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(2):207–215. doi:10.1111/j.1530-0277.1981.tb04890.x, PMID:7018299.

[29] Stickel F, Hampe J. Genetic determinants of alcoholic liver disease. Gut 2012;61(1):150–159. doi:10.1136/gutjnl-2011-301239, PMID:22110053.

[30] Semmler G, Balcar L, Oberkofler H, Zandanell S, Strasser M, Niederseer D, Feldman A, *et al*. PNPLA3 and SERPINA1 Variants Are Associated with Severity of Fatty Liver Disease at First Referral to a Tertiary Center. J Pers Med 2021;11(3):165. doi:10.3390/jpm11030165, PMID:33804385.

[31] Salameh H, Raff E, Erwin A, Seth D, Nischalke HD, Falleti E, *et al*. PNPLA3 Gene polymorphism is associated with predisposition to and severity of alcoholic liver disease. Am J Gastroenterol 2015;110(6):846–856. doi:10.1038/ajg.2015.137, PMID:25964223.

[32] Scott E, Anstee QM. Genetics of alcoholic liver disease and non-alcoholic steatohepatitis. Clin Med (Lond) 2018;18(Suppl 2):s54–s59. doi:10.7861/clinmedicine.18-2-s54, PMID:29700094.

[33] Nagata K, Suzuki H, Sakaguchi S. Common pathogenic mechanism in development progression of liver injury caused by non-alcoholic or alcoholic steatohepatitis. J Toxicol Sci 2007;32(5):453–468. doi:10.2131/jts.32.453, PMID:18198478.

[34] Buch S, Stickel F, Trépo E, Way M, Herrmann A, Nischalke HD, *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(12):1443–1448. doi:10.1038/ng.3417, PMID:26482880.

[35] Stickel F, Osterreicher CH, Datz C, Ferenci P, Wölfel M, Norgauer W, *et al*. Prediction of progression to cirrhosis by a glutathione S-transferase P1 polymorphism in subjects with hereditary hemochromatosis. Arch Intern Med 2005;165(16):1835–1840. doi:10.1001/archinte.165.16.1835, PMID:16157826.

[36] Khan AJ, Ruwali M, Choudhuri G, Mathur N, Husain Q, Parmar D. Polymorphism in cytochrome P450 2E1 and interaction with other genetic risk factors and susceptibility to alcoholic liver cirrhosis. Mutat Res 2009;664(1-2):55–63. doi:10.1016/j.mrfmmm.2009.02.009, PMID:19428381.

[37] Degoul F, Sutton A, Mansouri A, Cepanec C, Degott C, Fromenty B, *et al*. Homozygosity for alanine in the mitochondrial targeting sequence of superoxide dismutase and risk for severe alcoholic liver disease. Gastroenterology 2001;120(6):1468–1474. doi:10.1053/gast.2001.24051, PMID:11313317.

[38] Stewart SF, Leathart JB, Chen Y, Daly AK, Rolla R, Vay D, *et al*. Valine-alanine manganese superoxide dismutase polymorphism is not associated with alcohol-induced oxidative stress or liver fibrosis. Hepatology 2002;36(6):1355–1360. doi:10.1053/jhep.2002.36940, PMID:12447859.

[39] Galicia-Moreno M, Lucano-Landeros S, Monroy-Ramirez HC, Silva-Gomez J, Gutierrez-Cuevas J, Santos A, *et al*. Roles of Nrf2 in Liver Diseases: Molecular, Pharmacological, and Epigenetic Aspects. Antioxidants (Basel) 2020;9(10):980. doi:10.3390/antiox9100980, PMID:33066023.

[40] Zhou J, Zheng Q, Chen Z. The Nrf2 Pathway in Liver Diseases. Front Cell Dev Biol 2022;10:826204. doi:10.3389/fcell.2022.826204, PMID:35223849.

[41] Nunes Dos Santos K, Florentino RM, França A, Lima Filho ACM, Santos MLD, Missiaggia D, *et al*. Polymorphism in the promoter region of NFE2L2 gene is a genetic marker of susceptibility to cirrhosis associated with alcohol abuse. Int J Mol Sci 2019;20(14):3589. doi:10.3390/ijms20143589, PMID:31340446.

[42] Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. Nat Med 2004;10(4):355–361. doi:10.1038/nm1025, PMID:15057233.

[43] Rey JW, Noetel A, Hardt A, Canbay A, Alakus H, Zur Hausen A, *et al*. Pro12Ala polymorphism of the peroxisome proliferator-activated receptor γ2 in patients with fatty liver diseases. World J Gastroenterol 2010;16(46):5830–5837. doi:10.3748/wjg.v16.i46.5830, PMID:21155004.

[44] Järveläinen HA, Orpana A, Perola M, Savolainen VT, Karhunen PJ, Lindros KO. Promoter polymorphism of the CD14 endotoxin receptor gene as a risk factor for alcoholic liver disease. Hepatology 2001;33(5):1148–1153. doi:10.1053/jhep.2001.24236, PMID:11343243.

[45] Meiler C, Muhlbauer M, Johann M, Hartmann A, Schnabl B, Wodarz N, *et al*. Different effects of a CD14 gene polymorphism on disease outcome in patients with alcoholic liver disease and chronic hepatitis C infection. World J Gastroenterol 2005;11(38):6031–6037. doi:10.3748/wjg.v11.i38.6031, PMID:16273620.

[46] Bathgate AJ, Pravica V, Perrey C, Hayes PC, Hutchinson IV. Polymorphisms in tumour necrosis factor alpha, interleukin-10 and transforming growth factor beta1 genes and end-stage liver disease. Eur J Gastroenterol Hepatol 2000;12(12):1329–1333. doi:10.1097/00042737-200012120-00011, PMID:11192323.

[47] Marcos M, Gómez-Munuera M, Pastor I, González-Sarmiento R, Laso FJ. Tumor necrosis factor polymorphisms and alcoholic liver disease: a HuGE review and meta-analysis. Am J Epidemiol 2009;170(8):948–956. doi:10.1093/aje/kwp236, PMID:19755636.

[48] Marcos M, Pastor I, González-Sarmiento R, Laso FJ. A functional polymorphism of the NFKB1 gene increases the risk for alcoholic liver cirrhosis in patients with alcohol dependence. Alcohol Clin Exp Res 2009;33(11):1857–1862. doi:10.1111/j.1530-0277.2009.01023.x, PMID:19673747.

[49] Stickel F, Osterreicher CH, Halangk J, Berg T, Homann N, Hellerbrand C, *et al*. No role of matrixmetalloproteinase-3 genetic promoter polymorphism 1171 as a risk factor for cirrhosis in alcoholic liver disease. Alcohol Clin Exp Res 2008;32(6):959–965. doi:10.1111/j.1530-0277.2008.00654.x, PMID:18445105.

[50] Saffroy R, Pham P, Chiappini F, Gross-Goupil M, Castera L, Azoulay D, *et al*. The MTHFR 677C > T polymorphism is associated with an increased risk of hepatocellular carcinoma in patients with alcoholic cirrhosis. Carcinogenesis 2004;25(8):1443–1448. doi:10.1093/carcin/bgh147, PMID:15033905.

[51] Guyot E, Sutton A, Rufat P, Laguillier C, Mansouri A, Moreau R, *et al*. PNPLA3 rs738409, hepatocellular carcinoma occurrence and risk model prediction in patients with cirrhosis. J Hepatol 2013;58(2):312–318. doi:10.1016/j.jhep.2012.09.036, PMID:23069476.

[52] Nischalke HD, Berger C, Luda C, Berg T, Müller T, Grünhage F, *et al*. The PNPLA3 rs738409 148M/M genotype is a risk factor for liver cancer in alcoholic cirrhosis but shows no or weak association in hepatitis C cirrhosis. PLoS One 2011;6(11):e27087. doi:10.1371/journal.pone.0027087, PMID:22087248.

[53] Falleti E, Fabris C, Cmet S, Cussigh A, Bitetto D, Fontanini E, *et al*.

PNPLA3 rs738409C/G polymorphism in cirrhosis: relationship with the aetiology of liver disease and hepatocellular carcinoma occurrence. Liver Int 2011;31(8):1137–1143. doi:10.1111/j.1478-3231.2011.02534.x, PMID:21745286.

[54] Trepo E, Guyot E, Ganne-Carrie N, Degre D, Gustot T, Franchimont D, *et al*. PNPLA3 (rs738409 C>G) is a common risk variant associated with hepatocellular carcinoma in alcoholic cirrhosis. Hepatology 2012;55(4):1307–1308. doi:10.1002/hep.25518, PMID:22162034.

[55] Meroni M, Longo M, Rametta R, Dongiovanni P. Genetic and

Epigenetic Modifiers of Alcoholic Liver Disease. Int J Mol Sci 2018;19(12):3857. doi:10.3390/ijms19123857, PMID:30513996.

[56] Falleti E, Cussigh A, Cmet S, Fabris C, Toniutto P. PNPLA3 rs738409 and TM6SF2 rs58542926 variants increase the risk of hepatocellular carcinoma in alcoholic cirrhosis. Dig Liver Dis 2016;48(1):69–75. doi:10.1016/j.dld.2015.09.009, PMID:26493626.

[57] Anstee QM, Daly AK, Day CP. Genetics of alcoholic and nonalcoholic fatty liver disease. Semin Liver Dis 2011;31(2):128–146. doi:10.1055/s-0031-1276643, PMID:21538280.