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Polymorphisms Associated With Metabolic Dysfunction-Associated Steatotic Liver Disease Influence the Progression of End-Stage Liver Disease

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Conflicts of Interest:

These authors disclose the following: Alejandro Soto-Gutierrez is an inventor on a patent application that describes the use of transcription factors to treat chronic liver failure (US20140249209). Edgar N. Tafaleng and Alejandro Soto-Gutierrez are inventors on a provisional patent application related to methods to enhance hepatic functions in human failing livers (PCT/US2020/055500). Alejandro Soto-Gutierrez is a co-founder and has a financial interest in Von Baer Wolff, Inc, a company focused on biofabrication of autologous human hepatocytes from stem cells technology. Alejandro Soto-Gutierrez and Alina Ostrowska are co-founders and have a financial interest in Pittsburgh ReLiver Inc, a company focused on reprogramming hepatocytes in liver failure. Jaideep Behari has received research grant funding from Gilead, Pfizer, and Endra Life Sciences. His institution has clinical research contracts with Intercept, Pfizer, Galectin, Exact Sciences, Inventiva, Enanta, Shire, Gilead, Allergan, Celgene, Galmed, Rhythm, and Genentech. All these interests are managed by the Conflict of Interest Office at the University of Pittsburgh in accordance with their policies. The remaining authors disclose no conflicts.

Ethical Statement:

The corresponding authors, on behalf of all authors, jointly and severally, certify that their institution has approved the protocol for any investigation involving humans and that all experimentation was conducted in conformity with ethical and humane principles of research.

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Declaration of Helsinki, SAGER.

Supplementary Materials

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Abstract

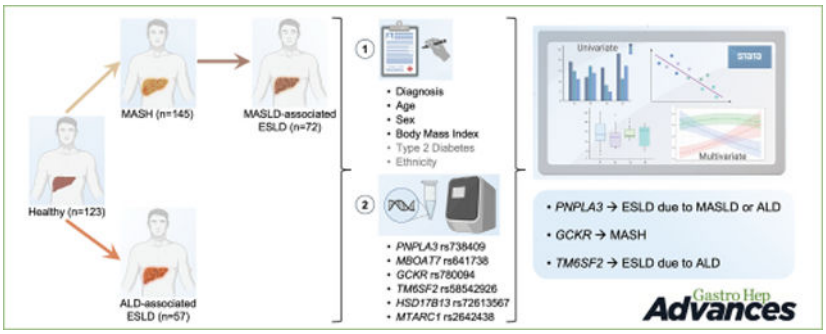
BACKGROUND AND AIMS: Chronic liver injury that results in cirrhosis and end-stage liver disease (ESLD) causes more than 1 million deaths annually worldwide. Although the impact of genetic factors on the severity of metabolic dysfunction-associated steatotic liver disease (MASLD) and alcohol-related liver disease (ALD) has been previously studied, their contribution to the development of ESLD remains largely unexplored.

METHODS: We genotyped 6 MASLD-associated polymorphisms in healthy (n = 123), metabolic dysfunction-associated steatohepatitis (MASH) (n = 145), MASLD-associated ESLD (n = 72), and ALD-associated ESLD (n = 57) cohorts and performed multinomial logistic regression to determine the combined contribution of genetic, demographic, and clinical factors to the progression of ESLD.

RESULTS: Distinct sets of factors are associated with the progression to ESLD. The *PNPLA3* rs738409:G and *TM6SF2* rs58542926:T alleles, body mass index (BMI), age, and female sex were positively associated with progression from a healthy state to MASH. The *PNPLA3* rs738409:G allele, age, male sex, and having type 2 diabetes mellitus were positively associated, while BMI was negatively associated with progression from MASH to MASLD-associated ESLD. The *PNPLA3* rs738409:G and *GCKR* rs780094:T alleles, age, and male sex were positively associated, while BMI was negatively associated with progression from a healthy state to ALD-associated ESLD. The findings indicate that the *PNPLA3* rs738409:G allele increases susceptibility to ESLD regardless of etiology, the *TM6SF2* rs58542926:T allele increases susceptibility to MASH, and the *GCKR* rs780094:T allele increases susceptibility to ALD-associated ESLD.

CONCLUSION: The *PNPLA3*, *TM6SF2*, and *GCKR* minor alleles influence the progression of MASLD-associated or ALD-associated ESLD. Genotyping for these variants in MASLD and ALD patients can enhance risk assessment, prompting early interventions to prevent ESLD.

Graphical Abstract



Keywords

End-stage Liver Disease; Metabolic Dysfunction-Associated Steatotic Liver Disease; Metabolic Dysfunction-Associated Steatohepatitis; Alcohol-related Liver Disease; Single Nucleotide Polymorphisms

Introduction

Chronic hepatic injury that leads to cirrhosis and end-stage liver disease (ESLD) represents a prominent cause of mortality around the world.[1,2] Furthermore, the global prevalence of ESLD is consistently rising due to steady increases in the frequency of individuals affected by the 2 leading nonviral etiologies for ESLD, metabolic dysfunction-associated steatotic liver disease (MASLD), previously known as nonalcoholic fatty liver disease (NAFLD),[3–5] and alcohol-related liver disease (ALD).[6]

Despite having distinct pathogenesis and timelines, MASLD and ALD share similar pathological progression. Both diseases typically begin with hepatic steatosis (fatty liver) that advance to steatohepatitis (fatty liver with inflammation), liver fibrosis (scarring), liver cirrhosis (severe scarring with hepatocyte dysfunction), and ultimately ESLD (liver failure) and/or hepatocellular carcinoma (HCC).[7]

Historically, the prevailing belief is that MASLD is predominantly caused by excessive caloric intake and a sedentary lifestyle, while ALD is entirely due to excessive alcohol consumption. However, it is now evident that while most individuals with such behaviors develop simple hepatic steatosis, only a small fraction progresses to more severe stages of liver disease.[8] Indeed, numerous studies have now recognized the complex pathophysiology of these disorders in which a combination of lifestyle, environmental, and genetic factors altogether induce or modify disease progression and survival of affected patients.[9–16]

Technological advancements in genomic research have revolutionized approaches for identifying the genetic factors involved in the development of MASLD and ALD. Genome-wide association studies and exome-wide association studies have identified several single nucleotide polymorphisms (SNPs) associated with the development of MASLD. Some of these MASLD-associated SNPs were subsequently found to be associated with the development of ALD.[17–19] Among these MASLD-associated variants, the *patatin-like phospholipase domain-containing 3 (PNPLA3)* rs738409:G variant,[20] the *membrane-bound*

O-acyltransferasedomain-containing7 (MBOAT7) rs641738:T variant,[21] the *glucokinase regulator (GCKR)* rs780094:T variant,[22,23] and the *transmembrane 6 superfamily member 2 (TM6SF2)* rs58542926:T variant[24,25] have been shown to be associated with increased risk for disease. In contrast, the *hydroxysteroid 17-β dehydrogenase 13 (HSD17B13)* rs72613567:TA variant[26,27] and the *mitochondrial amidoxime reducing component 1 (MTARC1)* rs2642438:A variant[28–30] have been found to be associated with reduced risk for disease.

Although the impact of MASLD-associated SNPs on the development and severity of MASLD and ALD has been analyzed in previous studies,[17–22,24–30] their contribution to the development of ESLD has been little explored.[31] ESLD, the most severe manifestation of chronic liver disease, necessitates orthotopic liver transplantation (OLT) as a vital intervention, as without it, mortality becomes an imminent inevitability.[1,32] Only one study has analyzed the impact of a MASLD-associated SNP on ESLD and found that the *PNPLA3* rs738409:G variant is associated with progression to HCC and reduced transplantation-free survival in ALD patients waitlisted for OLT.[31] In this study, the combined contribution of multiple MASLD-associated SNPs to the development of ESLD due to MASLD or ALD was investigated using genetic, demographic, and clinical data obtained from cohorts of healthy individuals and patients with metabolic dysfunction-associated steatohepatitis (MASH), previously known as nonalcoholic steatohepatitis (NASH),[3–5] MASLD-associated ESLD, and ALD-associated ESLD. Multinomial logistic regression (MLR) analysis uncovered distinct sets of genetic, demographic, and clinical risk factors associated with the progression from a healthy state to MASH, from MASH to MASLD-associated ESLD, and from a healthy state to ALD-associated ESLD. The findings indicate that when all other parameters are held constant, the presence of *PNPLA3* minor allele (rs738409:G) increases the overall risk for developing ESLD, the *TM6SF2* minor allele (rs58542926:T) enhances the risk for developing MASH, and the *GCKR* minor allele (rs780094:T) heightens the risk for developing ALD-associated ESLD. Taken together, MASLD-associated SNPs, in combination with demographic and clinical risk factors, have an impact on the progression of MASLD-associated and ALD-associated ESLD. Genotyping for these genetic variants in patients with MASLD or ALD can provide a more precise evaluation of their susceptibility to ESLD and might encourage early lifestyle modifications and/or clinical interventions before the disease progresses to liver failure.

Methods

Collection of Samples From Healthy Liver Donors

Cryopreserved primary hepatocytes from 123 healthy individuals were obtained from In Vitro ADMET Laboratories Inc (IVAL, Columbia, MD). These hepatocytes were isolated from liver specimens of donors who were negative for Hepatitis C virus, Hepatitis B virus, and human immunodeficiency virus. Vials of cryopreserved cells were stored in liquid nitrogen until DNA extraction and analysis.

Collection of Samples From MASH Patients

Patients with NASH/NAFLD were enrolled in the study between 2019 and 2022, prior to the adoption of the multisociety consensus definition of steatotic liver disease in June 2023.[3–5] Under the new nomenclature, NASH has been renamed as MASH, while NAFLD has been renamed as MASLD. All patients were enrolled from a tertiary care referral hepatology clinic after evaluation by a hepatologist.

The criteria for a diagnosis of NAFLD at the University of Pittsburgh Medical Center Fatty Liver, Obesity, and Wellness clinic, which is closely aligned with the 2023 consensus MASLD definition, required (1) evidence of steatosis after imaging by either ultrasound, computed tomography, or magnetic resonance imaging; (2) the absence of significant alcohol intake (<2 drinks per day for men or <1 drink per day for women) and the absence of a history of binge alcohol use (>5 drinks per day for men or >4 drinks per day for women); and (3) the presence of one or more NAFLD/MASLD-associated metabolic dysfunction (prediabetes or type 2 diabetes mellitus (T2DM), hypertension, dyslipidemia, or body mass index [BMI] >25 kg/m^2 [overweight to obese]). A few patients already had an established diagnosis of NASH/MASH prior to referral through a previous liver biopsy showing steatohepatitis. The most common reasons for a pre-existing diagnosis of NASH/MASH were an intraoperative liver biopsy performed during cholecystectomy or gastric bypass surgery, or a historic liver biopsy at another clinic prior to referral to the tertiary care center.

All patients diagnosed with NAFLD/MASLD were further risk-stratified using noninvasive tests (NITs) for liver fibrosis including (1) fibrosis-4 (FIB-4) index and (2) elastography-based imaging through either transient elastography or 2-dimensional shear wave elastography. Patients were offered a liver biopsy when (1) they had persistently elevated liver injury tests, (2) their NITs were suggestive of significant liver fibrosis (a FIB-4 index of ≥F2 or a liver stiffness measurement of >8 kPa by transient elastography or 2-dimensional shear wave elastography), (3) there is a concern for concurrent liver disease such as autoimmune hepatitis with positive AIH serology, or (4) their NITs were discordant, with one modality showing high risk of advanced fibrosis and the other showing low risk, and where magnetic resonance elastography was not covered by the patient's insurance or contraindicated for medical reasons.

Patient blood sample collection was performed in accordance with the Declaration of Helsinki with prior approval from the University of Pittsburgh Institutional Review Board Office of Research Protection (STUDY19080193). A total of 145 patients diagnosed with MASH at the University of Pittsburgh Medical Center Fatty Liver, Obesity, and Wellness clinic were included in the study. After obtaining informed written consent, peripheral blood samples were drawn from participants and stored at −80 °C until DNA extraction and analysis.

Collection of Samples From ESLD Patients

Human hepatocytes were obtained from liver explants in accordance with the Declaration of Helsinki with the approval of the University of Pittsburgh Institutional Review Board

Office of Research Protection (STUDY20090069). Primary hepatocytes were isolated from explanted liver specimens of 78 ESLD patients who received OLT for decompensated liver cirrhosis and were negative for Hepatitis C virus, Hepatitis B virus, and human immunodeficiency virus. Among these ESLD patients, 50 had decompensation due to MASLD and 28 were due to ALD. Liver tissue specimens were protected from ischemic injury by flushing with ice-cold University of Wisconsin solution immediately after vascular clamping and resection in the operating room, keeping the samples on ice, and transporting the specimens directly to the laboratory. Hepatocytes were isolated from encapsulated human liver segments (left lateral segment whenever possible) by a modified 3-step perfusion technique.[33] Briefly, the livers were flushed under a sterile biosafety hood through the portal vein, and hepatic vessels (recirculation technique) with 1 L of calcium-free Hank's balanced salt solution (Sigma, St. Louis, MO) supplemented with 0.5 mM ethylene glycol tetraacetic acid (ThermoFisher Scientific, Waltham, MA) prewarmed to 37 °C and then with collagenase/protease solution (VitaCyte, Indianapolis, IN) prewarmed to 37 °C until the tissue was fully digested. The digestion time for each preparation was 45–60 minutes. The digested liver was removed and immediately cooled with ice-cold Leibovitz's L-15 Medium (Invitrogen, Waltham, MA) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO). The final cell suspension was centrifuged twice at 65 × *g* for 3 minutes at 4 °C, and the medium was aspirated. The yield and viability of freshly isolated hepatocytes were estimated by trypan blue staining. Flash-frozen hepatocyte pellets were stored at −80 °C until DNA extraction and analysis.

In accordance with the Declaration of Helsinki and with prior approval from the Kyushu University Institutional Review Board (IRB 792–00), DNA samples were also collected from 51 ESLD patients who underwent living-donor liver transplantation at the Department of Surgery and Science, Kyushu University Hospital in Japan. Among these ESLD patients, 22 had decompensation due to MASLD and 29 were due to ALD.

DNA Isolation and Genotyping

Genomic DNA was isolated from hepatocyte or blood samples using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. DNA sample quantity and quality were measured using a NanoDrop Lite spectrometer (ThermoFisher Scientific, Waltham, MA). Genotyping reactions containing 1X TaqMan Genotyping Master Mix (Applied Biosystems, Foster City, CA), 1X TaqMan Genotyping Assays (Applied Biosystems, Foster City, CA), and 4.5 pg of genomic DNA were prepared in MicroAmp Fast Optical 96-well plates (Applied Biosystems, Foster City, CA). Genotyping polymerase chain reaction for *PNPLA3* rs738409, *MBOAT7* rs641738, *GCKR* rs780094, *TM6SF2* rs58542926, *HSD17B13* rs72613567, and *MTARC1* rs2642438 was performed using the StepOnePlus system (Applied Biosystems, Foster City, CA). Details of the TaqMan Genotyping Assays are listed in Table A1.

Statistical Analysis

Statistical analysis was performed using Stata SE version 18.0 (StataCorp LLC, College Station, TX). Data for continuous variables are presented as mean ± standard deviation, while data for categorical variables are presented as count (percentage). For continuous

variables, the dataset in each cohort were tested for normality based on skewness and kurtosis. For univariate analysis, differences across groups were determined using Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables. Pairwise correlations between variables were measured using Spearman's rank correlation coefficient (ρ) with Bonferroni-adjusted P values (P). For multivariate analysis, MLR was used to model the log odds of the clinical outcomes (healthy, MASH, MASLD-associated ESLD, or ALD-associated ESLD) as a function of the combination of all available genetic, demographic, and clinical variables (number of minor alleles for the 6 gene polymorphisms, age, sex, BMI, and T2DM status). For comparisons between cohorts, the less severe condition was selected as the base outcome for calculating the relative risk ratios (RRRs) and 95% confidence intervals (95% CIs). Variance inflation factor (VIF) and tolerance were determined using the collinearity diagnostics command (collin) written by Philip B. Ender of the Statistical Computing and Consulting of the UCLA Office of Academic Computing. RRR, 95% CI, and P were exported to GraphPad Prism version 9.5.1 (GraphPad Software Inc, San Diego, CA, USA) for the generation of Forest plots. For all analyses, a $P \leq .05$ was considered statistically significant.

Results

Study Design

In this retrospective study, we collected DNA samples as well as demographic and clinical information from a total of 411 individuals. From this group, we excluded 3 healthy liver donors because of missing BMI data. We also excluded 11 patients with ESLD who had mixed etiology, cryptogenic liver disease, or HCC to avoid misclassification. A total of 397 individuals, categorized into cohorts of healthy liver donors (n = 123), patients with MASH (n = 145), patients with ESLD due to MASLD (n = 72), and patients with ESLD due to ALD (n = 57), were ultimately analyzed. The demographic, clinical, and genetic data have been summarized for each cohort (Tables 1 and 2).

Development of Multinomial Logistic Regression (MLR) Models

Due to the lack of ethnicity data for 1 healthy individual, 50 MASLD-associated ESLD patients, and 28 ALD-associated ESLD patients (Table 1 and Table A2), we were unable to include ethnicity as a variable in the final MLR analysis. The influence of ethnicity on the development and progression of ALD and MASLD is worth considering given the disparities in genetics, diet, exercise, and environment among various ethnic groups. However, in the context of chronic liver disease, we assumed that the combination of BMI and genetics could effectively serve as a proxy for ethnicity. Due to the absence of T2DM data for the healthy cohort (Table 1), we were only able to include T2DM as a variable in the generation of the MLR model for the progression from MASH to MASLD-associated ESLD.

Before MLR analysis, we performed correlation analysis to assess multicollinearity among variables. Multicollinearity occurs when variables are highly correlated with each other, reducing the precision of estimated coefficients and weakening the power of the MLR models. We employed Spearman's rank correlation test with Bonferroni adjustment to

determine pairwise correlations between independent variables. We determined that the majority of pairwise comparisons showed no significant correlations with only 3 pairs of variables having significant but minimal correlation ($\rho < 0.2900$) (Tables A3 and A4).

We performed MLR to model the log odds of the clinical outcomes (healthy, MASH, MASLD-associated ESLD, or ALD-associated ESLD) as a function of the combination of all available genetic, demographic, and clinical variables. For simplicity, we assumed that MASH does not lead to ALD-associated ESLD and MASLD-associated ESLD does not lead to ALD-associated ESLD and vice versa (Figure A1A) and the risk for developing disease is proportional to the number of risk alleles carried by an individual (additive genetic model) (Figure A1B). We then computed the VIFs and tolerance to assess the multicollinearity of variables included in the final MLR models. The largest VIF was 1.16 and the lowest tolerance was 0.8600 suggesting that there was no significant multicollinearity in the MLR models (Tables A5 and A6). For comparison, we also generated MLR models for the recessive and dominant genetic models (Tables A7–A9). We ultimately selected the MLR models for the additive genetic model because these generated the largest *pseudo* R^2 indicating that these models had the greatest likelihood.

Progression From Healthy to MASH

We identified significant associations between some of the factors and the progression from a healthy state to MASH using the MLR model where the healthy state was selected as the base/reference outcome (Figure 1). Positive associations were observed for the *PNPLA3* ($P = .004$) and *TM6SF2* ($P = .004$) minor alleles, BMI ($P = .000$), and age ($P = .012$), indicating that individuals who carry these alleles, have higher BMI, or are older were more likely to develop MASH. Conversely, a negative association was detected for the male sex ($P = .039$), indicating that males had a lower likelihood for MASH progression compared to females.

We then analyzed the RRRs and 95% CIs to determine the magnitude and precision of these effects. The *PNPLA3* minor allele had an RRR of 1.875 (CI 1.217–2.887), indicating a 1.875-fold increased risk for MASH in individuals carrying this allele. Similarly, the *TM6SF2* minor allele showed an RRR of 2.959 (CI 1.402–6.245), corresponding to a 2.959-fold increased risk for MASH in individuals carrying this allele. The male sex had a protective effect, with an RRR of 0.554 (CI 0.316–0.971), indicating a 0.554-fold decreased risk for MASH in males compared to females. These factors exhibited relatively large magnitudes indicating strong effects on clinical outcome, but also showed wide intervals, denoting uncertainty in the estimates. BMI had an RRR of 1.105 (CI 1.065–1.147), indicating a modest increase in MASH risk per unit increase in BMI. Age had an RRR of 1.025 (CI 1.006–1.046), indicating a small increase in risk with advancing age. These factors exhibited relatively small magnitudes suggesting weak effects on clinical outcome, but also exhibited narrow intervals, indicating more precise estimates.

Progression From MASH to MASLD-associated ESLD

We likewise identified significant associations between some of the factors and the progression from MASH to MASLD-associated ESLD using the MLR model where MASH

was selected as the base/reference outcome (Figure 2). Positive associations were found for the *PNPLA3* minor allele ($P = .000$), age ($P = .000$), male sex ($P = .041$), and T2DM ($P = .006$) indicating that individuals who carry the *PNPLA3* minor allele, are older, are male, or are diabetic were more likely to progress from MASH to MASLD-associated ESLD. Conversely, BMI ($P = .001$) showed a negative association with the progression to MASLD-associated ESLD.

We then examined the RRRs and 95% CIs to determine the magnitude and precision of these effects. The *PNPLA3* minor allele had an RRR of 2.558 (CI 1.521–4.304), indicating a 2.558-fold increased risk for MASLD-associated ESLD among individuals carrying this allele. The male sex had an RRR of 2.164 (CI 1.033–4.534) corresponding to a 2.164-fold increased risk for males compared to females, while T2DM had an RRR of 2.883 (CI 1.361–6.107) indicating a 2.883-fold increased risk for diabetics compared to nondiabetics. These factors displayed relatively large magnitudes indicating strong effects on clinical outcome, but also showed wide intervals, indicating uncertainty in the estimates. Age had an RRR of 1.076 (CI 1.037–1.116), indicating a small increase in risk with aging. This factor showed a relatively small magnitude suggesting a weak effect on clinical outcome, but also exhibited a narrow interval denoting a more precise estimate.

Progression From Healthy to ALD-associated ESLD

We also identified significant associations between some of the factors and the progression from a healthy state to ALD-associated ESLD using the MLR model where the healthy state was selected as the base/reference outcome (Figure 3). Positive associations were found for the *PNPLA3* ($P = .000$) and GCKR ($P = .025$) minor alleles, age ($P = .002$), and male sex ($P = .003$) indicating that individuals who carry these minor alleles, are older, or are male were more likely to progress from a healthy state to ALD-associated ESLD. Conversely, BMI ($P = .001$) showed a negative association with the progression to ALD-associated ESLD.

We then analyzed the RRRs and 95% CIs to determine the magnitude and precision of these effects. The *PNPLA3* minor allele had an RRR of 3.479 (CI 1.969–6.149), indicating a 3.479-fold increased risk for ALD-associated ESLD among individuals carrying this allele. Similarly, the *GCKR* minor allele had an RRR of 1.976 (CI 1.084–3.247), indicating a 1.976-fold increased risk for ALD-associated ESLD among individuals carrying this allele. The male sex had an RRR of 3.277 (CI 1.480–7.258), indicating a 3.277-fold increased risk in males compared to females. These factors displayed relatively large magnitudes indicating strong effects on clinical outcome, but also showed wide intervals, indicating uncertainty in the estimates. Age had an RRR of 1.043 (CI 1.015–1.071), indicating a small increase in risk with advancing age. This factor exhibited a relatively small magnitude suggesting a weak effect on clinical outcome, but also exhibited a narrow interval indicating a more precise estimate.

Discussion

In this study, we investigated the impact of multiple MASLD-associated SNPs in conjunction with demographic and clinical factors on the progression of ESLD. To achieve this, we conducted an extensive analysis that involved genotyping for 6 MASLD-associated

variants in cohorts comprised of 123 healthy donors, 145 patients with MASH, 72 patients with MASLD-associated ESLD, and 57 patients with ALD-associated ESLD. Integrating this genetic data with age, sex, BMI, and T2DM status, we employed MLR, treating patient outcome as the dependent variable, to assess the collective impact of these factors on the progression of ESLD. We identified distinct sets of genetic, demographic, and clinical risk factors associated with the transition from a healthy state to MASH, from MASH to MASLD-associated ESLD, and from a healthy state to ALD-associated ESLD. Below, we provide a detailed discussion of the genetic, demographic, and clinical factors that exhibited significant associations with patient outcomes.

One of the genetic factors that showed significant associations was *PNPLA3*, which encodes for a lipase that converts lysophosphatidic acid into phosphatidic acid thereby promoting cellular lipid synthesis in hepatocytes.[34] The *PNPLA3* rs738409:G variant was initially discovered to be strongly associated with heightened hepatic fat levels and hepatic inflammation in the Dallas Heart Study.[20] Subsequent investigations further linked this variant to an increased risk of MASH,[22,28] MASH-associated fibrosis/cirrhosis,[28] MASH-associated HCC,[35,36] alcoholic steatosis,[17] and alcoholic cirrhosis.[18,19] Additionally, a study of ALD patients awaiting OLT revealed that the *PNPLA3* rs738409:G variant heightened the risk of HCC and diminished transplantation-free survival.[31] In line with previous findings, we confirmed a significant association between the *PNPLA3* minor allele and the progression from a healthy state to MASH. More importantly, we uncovered a significant association between the *PNPLA3* minor allele and the progression from MASH to MASLD-associated ESLD, as well as from a healthy state to ALD-associated ESLD. This novel finding suggests that the *PNPLA3* minor allele is associated with the progression to ESLD in patients with MASLD or ALD. All in all, these results underscore the strong involvement of this specific variant in both early and advanced stages of chronic liver disease regardless of etiology.

Another genetic factor that displayed a significant association was *TM6SF2*, which encodes for a protein with an undetermined function but is believed to be involved in the efflux of triglycerides from the liver into circulation.[37] The *TM6SF2* rs58542926:T variant has been linked to impaired hepatic secretion of large triglyceride-rich very-low-density lipoprotein, leading to intrahepatic triglyceride accumulation.[38] The variant has been associated with increased risk for MASH[28,39] and MASH-associated fibrosis/cirrhosis.[37,40] Consistent with previous reports, we identified a significant association between the *TM6SF2* minor allele and the progression from a healthy state to MASH. However, we did not observe a significant association between the *TM6SF2* minor allele and the progression from MASH to MASLD-associated ESLD nor did we identify a significant association between the *TM6SF2* minor allele and the progression from a healthy state to ALD-associated ESLD. This indicates that this particular variant is primarily involved in the earlier phases of MASLD rather than its progression to ESLD.

The last genetic factor that exhibited a significant association was *GCKR*, which encodes for a regulatory protein that modulates glucose storage and disposal and de novo lipogenesis by controlling glucokinase activity.[41] The *GCKR* rs780094:T variant has been associated with increased liver fibrosis and serum triglyceride levels in MASLD patients.[22,23] However,

in contrast to previous studies, we did not detect a significant association between the *GCKR* minor allele and the progression from a healthy state to MASH or from MASH to MASLD-associated ESLD. Interestingly, we identified a significant association between the *GCKR* minor allele and the progression from a healthy state to ALD-associated ESLD. This new finding suggests that this variant plays a role in the development of ALD-associated ESLD.

All available demographic and clinical factors analyzed in this study showed significant associations with the progression of MASLD and ALD. Age,[42] T2DM,[43–46] BMI,[47,48] and sex[49–51] have been known to affect predisposition to various liver diseases, including MASLD and ALD. Like previous studies, we determined that aging increases the risk of progressing from a healthy state to MASH, from MASH to MASLD-associated ESLD, and from a healthy state to ALD-associated ESLD. Similarly, we identified that T2DM increases the risk of progressing from MASH to MASH-associated ESLD.

The association between BMI and various stages of chronic liver disease is more complex and warrants careful interpretation because, unlike age and T2DM status, BMI is affected by physiological changes that occur during liver disease progression. Consistent with previous studies,[47,48] we observed a positive association between BMI and the progression from a healthy state to MASH. However, this relationship was reversed for the transition from MASH to MASLD-associated ESLD and from a healthy state to ALD-associated ESLD. We believe that this paradox can be explained by sarcopenia, the loss of muscle mass experienced by many patients during advanced stages of liver disease,[52] which leads to a decline in BMI. Our interpretation of these findings is that BMI contributes to disease progression during the initial phases of chronic liver disease, but as the disease advances, BMI itself becomes influenced by the condition.

Prior studies have also reported sex-based disparities in the susceptibility and progression of chronic liver disease. Specifically, males have been shown to exhibit reduced susceptibility to MASH,[50] yet have an elevated risk of progressing toward MASH-associated fibrosis[51] and, presumably, toward more advanced stages of MASLD. Moreover, males have an increased susceptibility for the development of ALD.[49] In line with previous findings, we determined that, compared to females, males had a reduced risk for progressing from a healthy state to MASH, but had an increased risk for progressing from MASH to MASLD-associated ESLD and from a healthy state to ALD-associated ESLD. Collectively, these findings suggest that females have a greater propensity for developing MASH, while males have an increased likelihood for progressing toward the development of fibrosis, cirrhosis, and hepatic dysfunction, processes that occur during advanced stages of chronic liver disease.

Conclusion

This study represents the first of its kind to comprehensively analyze the combined contribution of multiple MASLD-associated SNPs to the development of ESLD. Our findings provide compelling evidence of the significant role that genetic variants (*PNPLA3, TM6SF2,* and *GCKR*), demographic factors (age, sex), and clinical factors (T2DM, BMI)

play in the progression of chronic liver disease due to MASLD and ALD. Further research is warranted to explore the complex interplay between these factors and their potential implications for disease progression and treatment outcomes. Understanding the mechanisms by which these factors influence disease can contribute to better risk assessment, targeted interventions, and personalized management strategies for patients with MASLD and ALD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

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Data Transparency Statement:

The data supporting this article will be made available upon reasonable request to the corresponding authors.

Abbreviations used in this paper:

ALD	Alcohol-related liver disease
BMI	body mass index
CI	confidence interval
ESLD	end-stage liver disease
GCKR	glucokinase regulator
HCC	hepatocellular carcinoma
HSD17B13	hydroxysteroid 17-β dehydrogenase 13
MASH	metabolic dysfunction-associated steatohepatitis
MASLD	metabolic dysfunction-associated steatotic liver disease
MBOAT7	membrane bound O-acyltransferase domain containing 7
MLR	multinomial logistic regression
MTARC1	mitochondrial amidoxime reducing component 1
n	number

P	*P*-value
PNPLA3	patatin-like phospholipase domain containing 3
SD	standard deviation
SNP	single nucleotide polymorphism
T2DM	type 2 diabetes mellitus
TM6SF2	transmembrane 6 superfamily member 2

References

1. Habka D, Mann D, Landes R, et al. Future economics of liver transplantation: a 20-year cost modeling forecast and the prospect of bioengineering autologous liver grafts. PLoS One 2015;10:e0131764. [PubMed: 26177505]
2. Asrani SK, Devarbhavi H, Eaton J, et al. Burden of liver diseases in the world. J Hepatol 2019;70:151–171. [PubMed: 30266282]
3. American Association for the Study of Liver Diseases;, Electronic address: globalleadership@livernomenclature.org;, Latin American Association for the Study of the Liver; European Association for the Study of the Liver. A call for unity: the path towards a more precise and patient-centric nomenclature for NAFLD. Ann Hepatol 2023;28:101115. [PubMed: 37225637]
4. European Association for the Study of the Liver;, Electronic address: globalleadership@livernomenclature.org;, American Association for the Study of Liver Diseases; Latin American Association for the Study of the Liver. A call for unity: the path towards a more precise and patient-centric nomenclature for NAFLD. J Hepatol 2023;79:4–5. [PubMed: 37224924]
5. American Association for the Study of Liver Diseases;, Latin American Association for the Study of the Liver; European Association for the Study of the Liver. A call for unity: the path towards a more precise and patient-centric nomenclature for NAFLD. Hepatology 2023;78:3–5. [PubMed: 37192775]
6. Estes C, Razavi H, Loomba R, et al. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology 2018;67:123–133. [PubMed: 28802062]
7. Toshikuni N, Tsutsumi M, Arisawa T. Clinical differences between alcoholic liver disease and nonalcoholic fatty liver disease. World J Gastroenterol 2014;20:8393–8406. [PubMed: 25024597]
8. Schiavo L, Busetto L, Cesaretti M, et al. Nutritional issues in patients with obesity and cirrhosis. World J Gastroenterol 2018;24:3330–3346. [PubMed: 30122874]
9. Ayonrinde OT. Historical narrative from fatty liver in the nineteenth century to contemporary NAFLD - reconciling the present with the past. JHEP Rep 2021;3:100261. [PubMed: 34036255]
10. Collin de l'Hortet A, Takeishi K, Guzman-Lepe J, et al. Generation of human fatty livers using custom-engineered induced pluripotent stem cells with modifiable SIRT1 metabolism. Cell Metabol 2019;30:385–401 e9.
11. Duwaerts CC, Le Guillou D, Her CL, et al. Induced pluripotent stem cell-derived hepatocytes from patients with nonalcoholic fatty liver disease display a disease-specific gene expression profile. Gastroenterology 2021;160:2591–2594.e6. [PubMed: 33640436]
12. Gatzios A, Rombaut M, Buyl K, et al. From NAFLD to MAFLD: aligning translational in vitro research to clinical insights. Biomedicines 2022;10:161. [PubMed: 35052840]
13. Graffmann N, Ring S, Kawala MA, et al. Modeling nonalcoholic fatty liver disease with human pluripotent stem cell-derived immature hepatocyte-like cells reveals activation of PLIN2 and confirms regulatory functions of peroxisome proliferator-activated receptor alpha. Stem Cells Dev 2016;25:1119–1133. [PubMed: 27308945]

14. Gurevich I, Burton SA, Munn C, et al. iPSC-derived hepatocytes generated from NASH donors provide a valuable platform for disease modeling and drug discovery. Biol Open 2020;9:bio055087. [PubMed: 33268331]
15. Younossi ZM, Stepanova M, Negro F, et al. Nonalcoholic fatty liver disease in lean individuals in the United States. Medicine (Baltimore) 2012;91:319–327. [PubMed: 23117851]
16. Marroni CA, Fleck AM Jr, Fernandes SA, et al. Liver transplantation and alcoholic liver disease: history, controversies, and considerations. World J Gastroenterol 2018;24:2785–2805. [PubMed: 30018475]
17. Stickel F, Hampe J, Trepo E, et al. PNPLA3 genetic variation in alcoholic steatosis and liver disease progression. Hepatobiliary Surg Nutr 2015;4:152–160. [PubMed: 26151055]
18. Shen JH, Li YL, Li D, et al. The rs738409 (I148M) variant of the PNPLA3 gene and cirrhosis: a meta-analysis. J Lipid Res 2015;56:167–175. [PubMed: 25378656]
19. Stickel F, Buch S, Lau K, et al. Genetic variation in the PNPLA3 gene is associated with alcoholic liver injury in caucasians. Hepatology 2011;53:86–95. [PubMed: 21254164]
20. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40:1461–1465. [PubMed: 18820647]
21. Teo K, Abeysekera KWM, Adams L, et al. rs641738C>T near MBOAT7 is associated with liver fat, ALT and fibrosis in NAFLD: a meta-analysis. J Hepatol 2021;74:20–30. [PubMed: 32882372]
22. Speliotes EK, Yerges-Armstrong LM, Wu J, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet 2011;7:e1001324. [PubMed: 21423719]
23. Petta S, Miele L, Bugianesi E, et al. Glucokinase regulatory protein gene polymorphism affects liver fibrosis in non-alcoholic fatty liver disease. PLoS One 2014;9:e87523. [PubMed: 24498332]
24. Kozlitina J, Smagris E, Stender S, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2014;46:352–356. [PubMed: 24531328]
25. Prill S, Caddeo A, Baselli G, et al. The TM6SF2 E167K genetic variant induces lipid biosynthesis and reduces apolipoprotein B secretion in human hepatic 3D spheroids. Sci Rep 2019;9:11585. [PubMed: 31406127]
26. Motomura T, Amirneni S, Diaz-Aragon R, et al. Is HSD17B13 genetic variant a protector for liver dysfunction? Future perspective as a potential therapeutic target. J Pers Med 2021;11:619. [PubMed: 34208839]
27. Su W, Wang Y, Jia X, et al. Comparative proteomic study reveals 17beta-HSD13 as a pathogenic protein in nonalcoholic fatty liver disease. Proc Natl Acad Sci U S A 2014;111:11437–11442. [PubMed: 25028495]
28. Anstee QM, Darlay R, Cockell S, et al. Genome-wide association study of non-alcoholic fatty liver and steatohepatitis in a histologically characterised cohort. J Hepatol 2020;73:505–515. [PubMed: 32298765]
29. Innes H, Buch S, Hutchinson S, et al. Genome-wide association study for alcohol-related cirrhosis identifies risk loci in MARC1 and HNRNPUL1. Gastroenterology 2020;159:1276–1289.e7. [PubMed: 32561361]
30. Schneider CV, Schneider KM, Conlon DM, et al. A genome-first approach to mortality and metabolic phenotypes in MTARC1 p.Ala165Thr (rs2642438) heterozygotes and homozygotes. Med 2021;2:851–863.e3. [PubMed: 34258604]
31. Friedrich K, Wannhoff A, Kattner S, et al. PNPLA3 in end-stage liver disease: alcohol consumption, hepatocellular carcinoma development, and transplantation-free survival. J Gastroenterol Hepatol 2014;29:1477–1484. [PubMed: 25273282]
32. Miro JM, Laguno M, Moreno A, et al. Management of end stage liver disease (ESLD): what is the current role of orthotopic liver transplantation (OLT)? J Hepatol 2006;44(1 Suppl):S140–S145. [PubMed: 16352366]
33. Gramignoli R, Green ML, Tahan V, et al. Development and application of purified tissue dissociation enzyme mixtures for human hepatocyte isolation. Cell Transplant 2012;21:1245–1260. [PubMed: 22080793]

34. Trepo E, Romeo S, Zucman-Rossi J, et al. PNPLA3 gene in liver diseases. J Hepatol 2016;65:399–412. [PubMed: 27038645]
35. Liu YL, Patman GL, Leathart JB, et al. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. J Hepatol 2014;61:75–81. [PubMed: 24607626]
36. Trepo E, Nahon P, Bontempi G, et al. Association between the PNPLA3 (rs738409 C>G) variant and hepatocellular carcinoma: evidence from a meta-analysis of individual participant data. Hepatology 2014;59:2170–2177. [PubMed: 24114809]
37. Gellert-Kristensen H, Richardson TG, Davey Smith G, et al. Combined effect of PNPLA3, TM6SF2, and HSD17B13 variants on risk of cirrhosis and hepatocellular carcinoma in the general population. Hepatology 2020;72:845–856. [PubMed: 32190914]
38. Boren J, Adiels M, Bjornson E, et al. Effects of TM6SF2 E167K on hepatic lipid and very low-density lipoprotein metabolism in humans. JCI Insight 2020;5:e144079. [PubMed: 33170809]
39. Krawczyk M, Rau M, Schattenberg JM, et al. Combined effects of the PNPLA3 rs738409, TM6SF2 rs58542926, and MBOAT7 rs641738 variants on NAFLD severity: a multicenter biopsy-based study. J Lipid Res 2017;58:247–255. [PubMed: 27836992]
40. Liu YL, Reeves HL, Burt AD, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. Nat Commun 2014;5:4309. [PubMed: 24978903]
41. Beer NL, Tribble ND, McCulloch LJ, et al. The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. Hum Mol Genet 2009;18:4081–4088. [PubMed: 19643913]
42. Kim IH, Kisseleva T, Brenner DA. Aging and liver disease. Curr Opin Gastroenterol 2015;31:184–191. [PubMed: 25850346]
43. Coman LI, Coman OA, Badarau IA, et al. Association between liver cirrhosis and diabetes mellitus: a review on hepatic outcomes. J Clin Med 2021;10:262. [PubMed: 33445629]
44. Garcia-Compean D, Jaquez-Quintana JO, Gonzalez-Gonzalez JA, et al. Liver cirrhosis and diabetes: risk factors, pathophysiology, clinical implications and management. World J Gastroenterol 2009;15:280–288. [PubMed: 19140227]
45. Hsieh PH, Huang JY, Nfor ON, et al. Association of type 2 diabetes with liver cirrhosis: a nationwide cohort study. Oncotarget 2017;8:81321–81328. [PubMed: 29113391]
46. Tolman KG, Fonseca V, Dalpiaz A, et al. Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. Diabetes Care 2007;30:734–743. [PubMed: 17327353]
47. Ahn JC, Sundaram V. Obesity and liver decompensation. Clin Liver Dis (Hoboken) 2019;14:12–15. [PubMed: 31391930]
48. Yin Y, Li Y, Shao L, et al. Effect of body mass index on the prognosis of liver cirrhosis. Front Nutr 2021;8:700132. [PubMed: 34490322]
49. Erol A, Karpyak VM. Sex and gender-related differences in alcohol use and its consequences: contemporary knowledge and future research considerations. Drug Alcohol Depend 2015;156:1–13. [PubMed: 26371405]
50. Neuschwander-Tetri BA, Clark JM, Bass NM, et al. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. Hepatology 2010;52:913–924. [PubMed: 20648476]
51. Yang JD, Abdelmalek MF, Pang H, et al. Gender and menopause impact severity of fibrosis among patients with nonalcoholic steatohepatitis. Hepatology 2014;59:1406–1414. [PubMed: 24123276]
52. Son SW, Song DS, Chang UI, et al. Definition of sarcopenia in chronic liver disease. Life (Basel) 2021;11:349. [PubMed: 33923561]

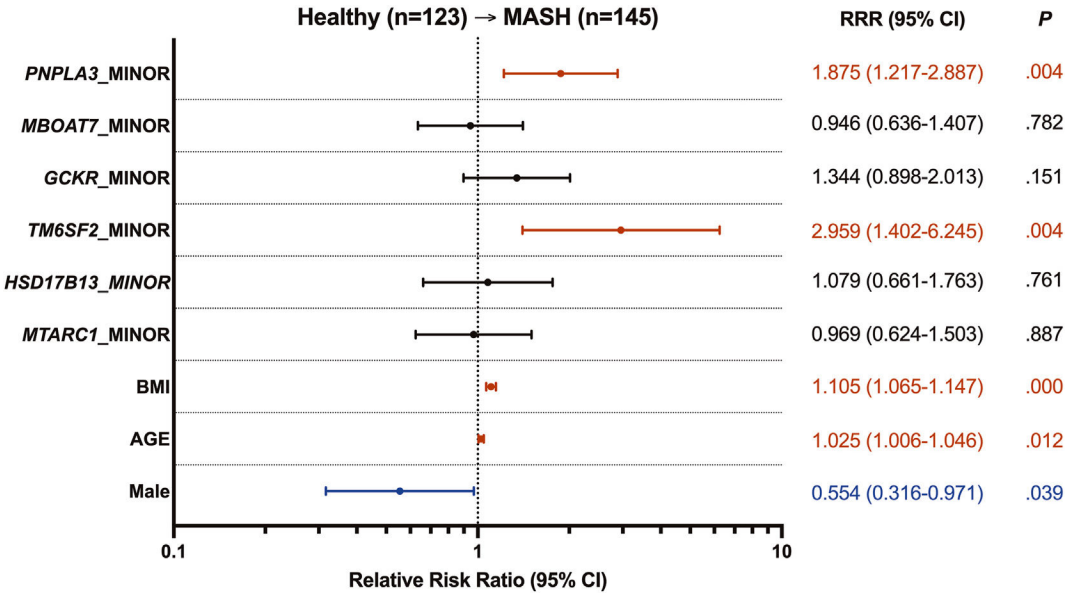

Figure 1.

Forest plot for the MLR model for the progression from a healthy state to MASH showing the genetic, demographic, and clinical factors on the y-axis and the relative risk ratio on the x-axis. Factors with significant associations are either in red (positive association) or blue (negative association). Values for the 95% CI and *P* are listed on the right side of the plot. MLR model *pseudo* R^2 = 0.2500.

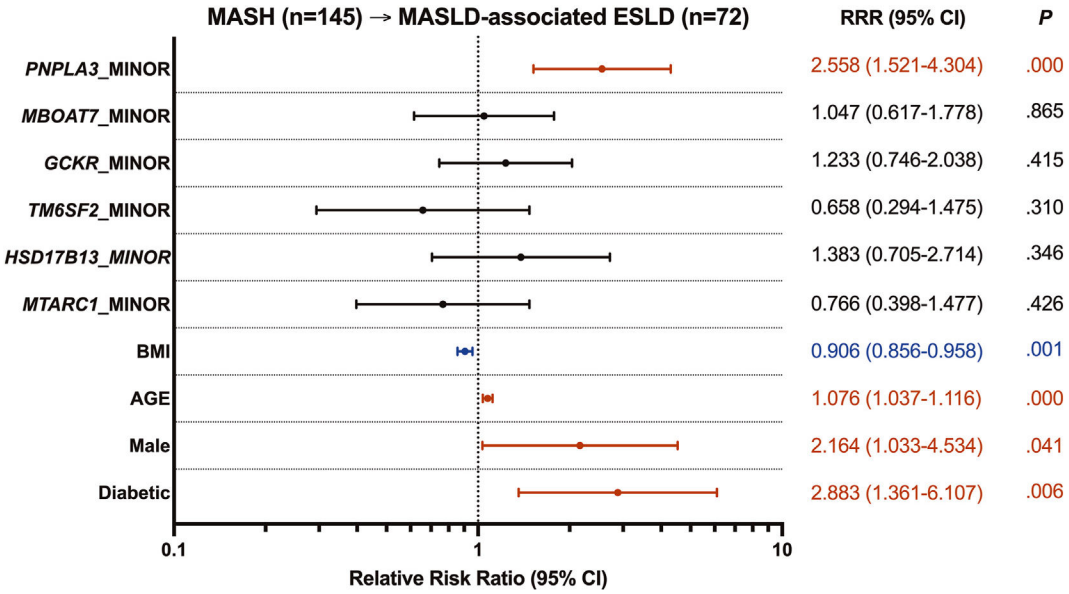

Figure 2.

Forest plot for the MLR model for the progression from MASH to MASLD-associated ESLD showing the genetic, demographic, and clinical factors on the y-axis and the relative risk ratio on the x-axis. Factors with significant associations are either in red (positive association) or blue (negative association). Values for the 95% CI and *P* are listed on the right side of the plot. MLR model *pseudo* R^2 = 0.3059.

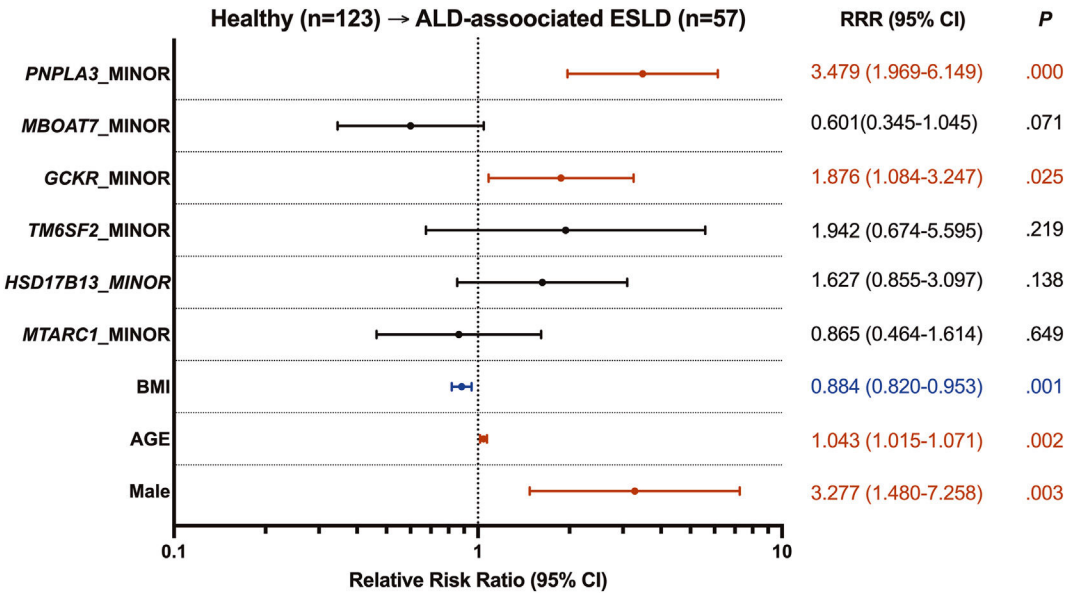

Figure 3.

Forest plot for the MLR model for the progression from a healthy state to ALD-associated ESLD showing the genetic, demographic, and clinical factors on the y-axis and the relative risk ratio on the x-axis. Factors with significant associations are either in red (positive association) or blue (negative association). Values for the 95% CI and *P* are listed on the right side of the plot. MLR model *pseudo* R^2 = 0.2500.

Table 1.

Summary of Demographic and Clinical Factors in Each Cohort (Total n = 397)

Characteristics	Healthy (n = 123)	MASH (n = 145)	MASLD-associated ESLD (n = 72)	ALD-associated ESLD (n = 57)	*P* [b]
Age[a] in y, mean ± SD	45 ± 16	51 ± 15	62 ± 7	54 ± 10	.000
range	1–77	18–78	41–74	36–71	
Sex					.000
Male, n (%)	62 (50.41%)	46 (31.72%)	33 (45.83%)	40 (70.18%)	
Female, n (%)	61 (49.59%)	99 (68.28%)	39 (54.17%)	17 (29.82%)	
BMI in kg/m^2, mean ± SD	29.62 ± 7.89	35.71 ± 8.07	30.64 ± 5.70	26.21 ± 4.09	.000
T2DM status					.001
Diabetic, n (%)	No data	50 (34.48%)	43 (59.72%)	20 (35.09%)	
Nondiabetic, n (%)	No data	95 (65.52%)	29 (40.28%)	37 (64.91%)	
Ethnicity					.000
White American, n (%)	87 (70.73%)	134 (92.41%)	0 (0.00%)	0 (0.00%)	
African American, n (%)	10 (8.13%)	6 (4.14%)	0 (0.00%)	0 (0.00%)	
Hispanic, n (%)	21 (17.07%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	
Asian, n (%)	0 (0.00%)	3 (2.07%)	22 (30.55%)	29 (50.88%)	
Other, n (%)	4 (3.25%)	2 (1.38%)	0 (0.00%)	0 (0.00%)	
No data, n (%)	1 (0.81%)	0 (0.00%)	50 (69.44%)	28 (49.12%)	

SD, standard deviation.

[a]For the Healthy and MASH cohort, age was recorded during liver/blood sample collection, while for the ESLD cohorts, age was recorded during OLT.

[b]Kruskal-Wallis test for continuous variables or Fisher's exact test for categorical variables.

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Table 2.

Number and Percentage of Individuals in Each Cohort That Carry No Copy, 1 Copy, or 2 Copies of Each MASLD-Associated Minor Allele

Number of copies of	Healthy (n = 123)	MASH (n = 145)	MASLD-associated ESLD (n = 72)	ALD-associated ESLD (n = 57)	*P* [a]
PNPLA3 rs738409:G allele					.000
No copy, n (%)	69 (56.10%)	58 (40.00%)	11(15.28%)	11 (19.30%)	
1 copy, n (%)	46 (37.40%)	64 (44.14%)	29 (40.28%)	29 (50.88%)	
2 copies, n (%)	8 (6.50%)	23 (15.86%)	32 (44.44%)	17 (29.82%)	
MBOAT7 rs641738:T allele					.039
No copy, n (%)	37 (30.08%)	38 (26.21%)	25 (34.72%)	33 (57.89%)	
1 copy, n (%)	59 (47.97%)	81 (55.86%)	30 (41.67%)	20 (35.09%)	
2 copies, n (%)	27 (21.95%)	26 (17.93%)	17 (23.61%)	4 (7.02%)	
GCKR rs780094:T allele					.047
No copy, n (%)	51 (41.46%)	46 (31.72%)	21 (29.17%)	12 (21.05%)	
1 copy, n (%)	57 (46.34%)	73 (50.34%)	35 (48.61%)	28 (49.12%)	
2 copies, n (%)	15 (12.20%)	26 (17.93%)	16 (22.22%)	17 (29.82%)	
TM6SF2 rs58542926:T allele					.010
No copy, n (%)	113 (91.87%)	112 (77.24%)	57 (79.17%)	51 (89.47%)	
1 copy, n (%)	9 (7.32%)	31 (21.38%)	14 (19.44%)	5 (8.77%)	
2 copies, n (%)	1 (0.81%)	2 (1.38%)	1 (1.39%)	1 (1.75%)	
HSD17B13 rs72613567:TA allele					.296
No copy, n (%)	78 (63.41%)	98 (67.59%)	49 (68.06%)	30 (52.63%)	
1 copy, n (%)	42 (34.15%)	41 (28.28%)	21 (29.17%)	22 (38.60%)	
2 copies, n (%)	3 (2.44%)	6 (4.14%)	2 (2.78%)	5 (8.77%)	
MTARC1 rs2642438:A allele					.575
No copy, n (%)	67 (54.47%)	81 (55.86%)	48 (66.67%)	37 (64.91%)	
1 copy, n (%)	47 (38.21%)	55 (37.93%)	22 (30.56%)	17 (29.82%)	
2 copies, n (%)	9 (7.32%)	9 (6.21%)	2 (2.78%)	3 (5.26%)	

[a] Fisher's exact test