DR. VINCENT LINGZHI CHEN (Orcid ID : 0000-0002-0157-6066)

Article type : Original Articles

Handling editor: Luca Valenti

Title Page

Title: Genetic variants that associate with liver cirrhosis have pleiotropic effects on human traits

(93 characters)

Authors: Vincent L. Chen,[1,2] Yanhua Chen,[1] Xiaomeng Du,[1] Samuel K. Handelman,[1,2] Elizabeth K. Speliotes[1,2]

1. Division of Gastroenterology and Hepatology, University of Michigan Health System, Ann Arbor, MI, USA
2. Department of Computational Medicine and Bioinformatics, University of Michigan Medical School, Ann Arbor, MI, USA

Correspondence:
Elizabeth K. Speliotes, MD, PhD, MPH
Keith Henley MD Collegiate Professor of Gastroenterology
Associate Professor of Medicine
Division of Gastroenterology and Hepatology
University of Michigan Health System
1150 West Medical Center Drive
Ann Arbor, Michigan 48109
Phone: 734-647-2964
Fax: 734-763-2535
E-mail: espeliot@med.umich.edu

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/LIV.14321

Word count: 3714 words

Figures: 3

Tables: 2

Abbreviations:

ALT, alanine aminotransferase. AP, alkaline phosphatase. APRI, AST-to-platelet ratio index. ICD, International Classification of Diseases. GWAS, genome-wide association study. MGI, Michigan Genomics Initiative. NAFLD, nonalcoholic fatty liver disease. PheWAS, phenome-wise association study. SNP, single nucleotide polymorphism.

Disclosures and conflicts of interest:

Vincent Chen, Yanhua Chen, Xiaomeng Du, Samuel Handelman, Elizabeth Speliotes: no financial conflicts of interest to disclose

Acknowledgements: **Acknowledgements**: V.L.C. was supported in part by a University of Michigan Training in Basic and Translational Digestive Sciences T32 grant (NIDDK 5T32DK094775) and The University of Michigan Department of Internal Medicine. EKS, SKH, XD, and YC are supported in part by R01 DK106621 (to EKS), R01 DK107904 (to EKS), and The University of Michigan Department of Internal Medicine. The authors acknowledge the University of Michigan Medical School Central Biorepository/Michigan Genomics Initiative for providing biospecimen storage, management, and distribution services in support of the research reported in this publication. Analyses in the UK BioBank were done under approved project 18120 (EKS).

Writing assistance: None

Author's contributions:

Guarantor of article: Dr. Elizabeth Speliotes

Vincent Chen: study design, data analysis and interpretation, and drafting of the manuscript

Yanhua Chen: data analysis and interpretation, and critical review of the manuscript

Xiaomeng Du: data analysis and interpretation, and critical review of the manuscript

Samuel Handelman: data analysis and interpretation, and critical review of the manuscript

Elizabeth Speliotes: concept development, study design, data analysis and interpretation, and critical revision of the manuscript.

All authors identified above have critically reviewed the paper and approve the final version of this paper, including the authorship statement.

Abstract:

Background and aims: Cirrhosis is characterized by extensive fibrosis of the liver and is a major cause of liver-related mortality. Cirrhosis is partially heritable but genetic contributions to cirrhosis have not been systemically explored. Here, we carry out association analyses with cirrhosis in two large biobanks and determine the effects of cirrhosis associated variants on multiple human disease/traits. Methods: We carried out a genome-wide association analysis of cirrhosis as a diagnosis in UK BioBank (UKBB; 1,088 cases vs. 407,873 controls) and then tested top-associating loci for replication with cirrhosis in a hospital-based cohort from the Michigan Genomics Initiative (MGI; 875 cases of cirrhosis vs. 30,346 controls). For replicating variants or variants previously associated with cirrhosis that also affected cirrhosis in UKBB or MGI we determined SNP effects on all other diagnoses in UKBB (PheWAS), common metabolic traits/diseases, and serum/plasma metabolites. Results: Unbiased genome-wide association study identified variants in/near *PNPLA3* and *HFE*, and candidate variant analysis identified variants in/near *TM6SF2*, *MBOAT7*, *SERPINA1*, *HSD17B13*, *STAT4*, and *IFNL4* that reproducibly affected cirrhosis. Most affected liver enzyme concentrations and/or aspartate transaminase-to-platelet ratio index. PheWAS, metabolic trait, and serum/plasma metabolite association analyses revealed effects of these variants on lipid, inflammatory, and other processes including new effects on many human diseases and traits. Conclusions: We identified eight loci that reproducibly associate with population-based cirrhosis and define their diverse effects on human diseases and traits.

228 words

Keywords: genetics, SNP, phenotype, fibrosis

Lay summary

Some genes cause cirrhosis, which is scarring of the liver that occurs after prolonged injury, but the genetics of cirrhosis have not been previously studied in an unbiased manner. We identified two genetic mutations that cause cirrhosis at genome wide significance and validated six other mutations previously suggested to affect cirrhosis in

small studies now in large biobanks. We show that these variants affect multiple human disease and traits which give us new insights into the diverse causes of cirrhosis.

Introduction

Cirrhosis is characterized by extensive scarring of the liver and can develop after many liver diseases. Cirrhosis is associated with medical complications including ascites, variceal bleeding, and hepatocellular carcinoma.[1] Cirrhosis accounts for nearly $10 billion in healthcare costs in the United States alone and over one million deaths worldwide in 2015.[1,2] While the most common causes of cirrhosis—viral hepatitis, non-alcoholic fatty liver disease (NAFLD), and alcoholic liver disease—are environmentally influenced, there is significant variation in whether people develop cirrhosis from these diseases.[3] Genetics contributes to development of cirrhosis with the heritability of liver fibrosis and cirrhosis estimated to be about 50%,[4] but the genetic determinants of disease have not been systematically explored.

The genetics of cirrhosis has so far been studied within small liver disease-specific populations. *PNPLA3* and *TM6SF2* variants associate with increased fibrosis in individuals with NAFLD, alcoholic liver disease, and hepatitis C virus infection.[5-7] Variants near *MBOAT7* associate with alcoholic cirrhosis.[6] Variants in or near *GCKR*, *LYPLAL1*, and *HSD17B13* associate with fibrosis in NAFLD patients.[8-11] In hepatitis C virus-infected patients, rs910049 (in the major histocompatibility complex region) and SNPs in *MERTK* and *TULP1* associated with liver fibrosis progression,[12,13] as does a SNP near *PCSK7* in individuals with hereditary hemochromatosis.[14] Finally, two monogenic liver diseases are caused by relatively common variants: hereditary hemochromatosis, which in 80-90% of cases is caused by rs1800562-A (*HFE* C282Y), and alpha-1 antitrypsin deficiency, which is caused by mutations in *SERPINA1*, the most common severely deleterious of which is rs28929474-T (E342K).[15,16] However, penetrance is incomplete for all these variants, including those in *HFE* and *SERPINA1*, and whether these variants lead to cirrhosis in the general population remains uncertain.

Here we aim to evaluate the genetics of all-cause cirrhosis in the population. We performed a GWAS for all cause cirrhosis (1,088 cases vs. 407,873 controls) in approximately 410,000 individuals of British-Caucasian descent from the UK BioBank (UKBB).[17] We test top associating variants and previously reported cirrhosis variants for replication with cirrhosis (875 cases vs. 30,346 controls) in the Michigan Genomics Initiative (MGI).[18] We determined the effects of all replicating variants on human disease/traits, on metabolic diseases from publically available GWAS analyses, and on serum metabolites.

Methods

Ethics statement

All research in this study was approved by the Institutional Review Board of the University of Michigan (Ann Arbor, MI). UKBB protocols were approved by the National Research Ethics Service Committee and all participants provided written informed consent. Analyses in this project were conducted under UKBB Resource Project 18120. All MGI participants provided written informed consent approved by the University of Michigan Institutional Review Board (Ann Arbor, MI).

Cohorts

The UKBB includes genotypic, clinical, and demographic information of over 400,000 individuals aged 40-69 at time of recruitment. Genotyping and data collection were previously described.[19] In brief, participants were genotyped on one of two custom arrays: UK BiLEVE Axiom Array (n = 50,520) or UK BioBank Axiom Array (n = 438,692) with >95% overlap. SNPs were imputed using the Haplotype Reference Consortium. For imputed SNPs, only SNPs with an imputation quality cutoff of 0.85 were used in analyses. SNPs with minor allele count < 20 were excluded. After quality control, 18,530,078 SNPs in 408,961 white-British individuals were included in analysis.

MGI is a prospective cohort with ongoing enrollment and at time of analysis included 35,888 subjects. All patients undergoing elective surgery at Michigan Medicine (Ann Arbor, MI) are potentially eligible for enrollment in this cohort. Participants underwent genotyping of peripheral blood on the Illumina HumanCoreExome v.12.1 array, a GWAS and exome array consisting of >500,000 SNPs.[18] In addition, full laboratory information and diagnosis codes are available. Imputation was performed as previously described.[20] In brief, samples were imputed to Haplotype Reference Consortium (release 1 for chromosomes 1-22 and 1.1 for X); SNPs were excluded if imputation quality was low ($r^2 < 0.3$) or minor allele count was < 4. After quality control, 30,751,457 imputed SNPs were available.

GWAS

This study had three stages (Fig. 1). We limited analyses to individuals of European ancestry as there were not enough individuals from other ancestries to carry out powered analyses. Stage 1 was a GWAS for cirrhosis as defined by International Classification of Diseases (ICD)-10 code K70.2-4, K71.7, or K74.X in UKBB. SNPs within 500 kilobases of a lead SNP with lower *p* value were removed to identify independent signals. In stage 2, independent SNPs with $p < 5 \times 10^{-6}$ in UKBB were tested for association with cirrhosis in MGI as defined by the presence of an ICD-9 code for cirrhosis (571.5, 571.2, and 571.6), ICD-10 code for cirrhosis (K74.X, K70.2-4, and K71.7), or for cirrhosis being present in pathology or radiology reports using text searching. Text search of

radiology and pathology reports was performed for the character "cirrho," and subjects with that character were included as having cirrhosis with the following exceptions: (1) if the word "without" or "no" appeared in the same sentence as "cirrho," subjects were considered controls; (2) if the words "primary biliary cirrhosis" appeared in a sentence, that sentence was ignored for text search purposes to avoid falsely identifying individuals with primary biliary cholangitis as having cirrhosis; and (3) if the words "evaluate," "assess," or "rule out" appeared in a sentence with "cirrho," that sentence was ignored for text search purposes. All subjects without ICD-9/-10 diagnosis code or positive text search were included as controls. A gastroenterologist (V.L.C.) manually reviewed 200 randomly-selected text strings and identified no false positive cirrhosis diagnoses. Only SNPs with a minor allele count >6 in MGI for cirrhosis were tested for replication as the others were underpowered to see an effect. Only lead SNPs were included for replication.

Association analyses in stage 1 and 2 were performed using Scalable and Accurate Implementation of GEneralized mixed model, which substantially reduces p value inflation otherwise seen in GWAS for rare traits,[21] with a logistic mixed model and the saddlepoint approximation. Covariates included in this analysis were age, age^2, sex, and principal components 1-10 to account for ethnic background. We used only a minimal number of covariates to maximize power and to avoid adjusting for potential mediators such as body mass index or diabetes mellitus.

Stage 3 analysis included replicating SNPs as well as SNPs that have been previously reported to associate with cirrhosis in subpopulations, as described in the introduction (Supp. Table 1).[5,8,9,12-16,22-32] As above, we included only SNPs with minor allele count > 6 in the European populations of UKBB and MGI, which corresponded to minor allele frequency > 0.006. For stage 3 SNPs, a cutoff of $p < 0.05$ in either UKBB or MGI was used because there was prior knowledge of their role in liver disease and fibrosis.

All SNP coordinates were reported based on GRCh38.p12 or, if updated during the most recent patch, p13.

C statistics for prediction of cirrhosis were calculated first using a minimal model that included only age, age^2, sex, and principal components 1-10, then adding cirrhosis-increasing SNPs. These were computed separately in MGI and UKBB.

Phenotype-wide association study (PheWAS)

To assess the effects of SNPs on other human diagnoses and diseases, we carried out PheWAS on all SNPs identified in stage 1-3 analysis based on published data on 778 traits (http://geneatlas.roslin.ed.ac.uk/phewas/).[17] For statistical significance of individual traits, a Bonferroni-corrected p value cutoff of 0.05 corrected for eight

SNPs and 778 traits was used ($p = 9.2 \times 10^{-6}$; $Z = 4.46$). Significant diagnoses with associated ICD-10 codes or blood cell traits are shown. Hierarchical clustering on SNPs (but not traits) was performed using the *gplot* package using default settings. We conducted sensitivity analysis where we adjusted for cirrhosis status the associations between each significant SNP-trait pair. We constructed our analyses to replicate those of the original PheWAS.[17] We conducted among unrelated Caucasian individuals in UKBB logistic regression (for binary traits) or linear regression (for quantitative traits) with each trait as the primary dependent variable and each SNP as the primary independent variable. Covariates were age, age^2, sex, principal components 1-20, genotyping batch, and assessment center, with or without cirrhosis status. A Z score change of >1.96 (corresponding to $P < 0.05$ for a difference between the associations) between the cirrhosis-adjusted and -unadjusted analyses was used as a cutoff for significant effect modification by cirrhosis.

We also tested the effect of all stage 1-3 SNPs on mean alanine aminotransferase (ALT), alkaline phosphatase (AP), and AST to platelet ratio index (APRI) in MGI. ALT, AP, and APRI were inverse-normally transformed and linear regression was performed for SNP effects, adjusted for age, age^2, sex, and principal components 1-10. As above, we used a minimal set of covariates to avoid including mediators and to maximize statistical power.

Metabolomic analysis

All SNPs identified in stage 1-3 analysis were tested for association with serum metabolites from previously-published data.[33] In brief, the authors performed genotyping and high-throughput nuclear magnetic resonance serum/plasma metabolomics on 14 datasets from ten European cohorts. Data on 123 metabolites in up to 24,925 individuals were included. Because the traits were highly cross-correlated (Supp. Fig. 1), data are only shown for certain representative metabolites. A Bonferroni-corrected significance cutoff of 0.05 corrected for eight SNPs and 123 metabolites was used ($p = 2.5 \times 10^{-5}$; $Z = 4.05$).

Cross-trait analyses

All SNPs identified in stage 1-3 analysis were tested for association with metabolic traits using publicly-available data from nine GWAS. These studies were: CARDIoGRAM-CAD[34] (coronary artery disease; http://www.cardiogramplusc4d.org/data-downloads), DIAGRAM[35] (type 2 diabetes; http://diagram-consortium.org/downloads.html), GCLC[36] (high- and low-density lipoproteins, total cholesterol, triglycerides; http://csg.sph.umich.edu/abecasis/public/lipids2013/), GIANT[37] (body mass index, height, waist-to-hip ratio, waist-to-hip ratio adjusted for body mass index; https://www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files), MAGIC[38] (fasting insulin and glucose levels; http://www.magicinvestigators.org/downloads/), a leptin GWAS,[39] and a body fat percentage GWAS[40] (https://walker05.u.hpc.mssm.edu for the last two). There is no adequately-powered GWAS

for blood pressure with full publicly-available data, so GWAS for systolic and diastolic blood pressure were performed on individuals of white-British ancestry in UK BioBank using Scalable and Accurate Implementation of GEneralized mixed model and a linear mixed model.[21] In addition, data from the GOLD consortium were used to determine the effect of these SNPs on hepatic steatosis, as measured by lower liver attenuation on computed tomography relative to a "phantom" control.[9] Heat maps and clustering was performed using Z scores of SNP-trait associations. Only traits that were associated with at least one SNP at nominal significance ($p < 0.05$) were included in the heat map, so only seven GWAS were included. For statistical significance a Bonferroni-adjusted p value cutoff of 0.05 divided by seven SNPs (one of the identified SNPs and its proxies were not available in any of the public GWAS data) and seventeen traits ($p = 4.2 \times 10^{-4}$; $Z = 3.52$) was used.

Tissue and pathway analyses

Analysis of pathway and tissue enrichment was performed using FUMA-GWAS as previously reported.[41] The background gene set used was "all". Tissue enrichment was based on Genome-Tissue Expression Project version 6 and pathway enrichment on Reactome and Gene Ontology biological process terms.

Results

Genome-wide association study

We carried out genome wide association analyses for ~ 70 million imputed SNPs with cirrhosis (n = 1088 cases identified using ICD-10 codes vs. 407,873 controls without this diagnosis) in UKBB controlling for age, gender, and the first ten principal components using a Scalable and Accurate Implementation of GEneralized logistic mixed model. There was no significant inflation of the association statistic with a lambda of 0.97 (Fig. 2A). Two SNPs associated with cirrhosis at genome-wide significance levels ($P < 5 \times 10^{-8}$) and an additional twelve had a suggestive association with $p < 5 \times 10^{-6}$ (Fig. 2B, Table 1, Supp. Table 2). We tested SNPs with suggestive association with cirrhosis ($P < 5 \times 10^{-6}$) in UK Biobank with cirrhosis in MGI (n = 875 cases based on ICD-10 codes and text search, vs. 31,221 controls). Only 8 SNPs were common enough to have enough power to be evaluated for association with cirrhosis in MGI (Table 1, Supp. Table 2). Of these, two were significantly associated with increased cirrhosis prevalence ($p < 0.05$) in MGI: rs738408-T (*PNPLA3* exon) and rs80215559-C (*SLC17A2* intron) (Table 1). Of note, rs80215559 is in complete linkage with rs1800562 ($r^2 = 1.0$ in CEU/GBR), which is also associated with cirrhosis at genome-wide significance levels ($p = 3.3 \times 10^{-8}$) and corresponds to the *HFE* C282Y mutation, the primary cause of hereditary hemochromatosis.[16] Conditional analysis of the effect of rs80215559 on cirrhosis conditional on rs1800562 eliminated its association with cirrhosis [odds ratio 1.61 (95% CI 1.33-1.84) to 1.00 (95% CI 1.00-1.00)], suggesting that its effect is due to the *HFE* C282Y mutation.

Candidate variant analysis

We also examined the effects of SNPs that have previously been associated with cirrhosis due to any cause of liver disease (Supp. Table 1). Rs738409-G (*PNPLA3*), rs1800562-G (*HFE*), rs28929474-C (*SERPINA1*), rs58542926-C (*TM6SF2*), rs6834314-A and rs72613567-(no insertion) (*HSD17B13*), rs641738-T and rs626283-G (*MBOAT7*), and rs7574865-T (*STAT4*) associated with cirrhosis in UK BioBank, and rs738409-G (*PNPLA3*), rs1800562-G (*HFE*), rs28929474-C (*SERPINA1*), rs6834314-A (*HSD17B13*), and rs12979860-T (*IFNL4*) associated with cirrhosis in MGI (Table 1). The SNP for each gene with the lowest p value in UK BioBank was included in subsequent analysis, except in the case of rs738409, which we chose in lieu of rs738408 as rs738409 is in high LD ($R^2 = 1$) and corresponds to the causal variant.[42]

The C statistics for a minimal model and a model including all eight cirrhosis-increasing SNPs were, respectively, 0.63 and 0.67 in UKBB and 0.61 and 0.63 in MGI.

Effects on liver enzymes

We investigated whether these SNPs had effects on markers of liver injury such as serum ALT, a marker of hepatocellular injury; AP, a marker of cholestasis/infiltrative disease; and APRI, a noninvasive marker of liver fibrosis.[43] Rs6834314 was used as a proxy for rs72613567 ($R^2 = 0.94$), as the latter was not available in MGI. Both rs738409-G (*PNPLA3*) and rs8021559-C (*HFE*) associated with increased APRI, consistent with a role in worsening fibrosis ($p = 1.75 \times 10^{-17}$ and 7.41×10^{-3}, respectively) (Table 2). In addition, rs738409-G (*PNPLA3*) associated with increased ALT ($p = 1.42 \times 10^{-14}$), but not AP, while rs80215559-C (*HFE*) associated with increased AP ($p = 8.46 \times 10^{-3}$) but not ALT (Table 2). *PNPLA3, HFE, SERPINA1, MBOAT7*, and *HSD17B13* variants increase AP while *TM6SF2* decreased AP ($p < 0.05$ for all). *PNPLA3, HFE*, *TM6SF2*, *HSD17B1*3 all increased ALT ($p < 0.05$ for all). *PNPLA3, HFE, SERPINA1,* and *HSD17B13* all associated with increased APRI ($p < 0.05$ for all). *STAT4* and *IFNL4* did not associate with abnormal liver enzymes in MGI.

Tissue/pathway analyses and PheWAS

The genes implicated by the variants were upregulated in liver (Supp. Fig. 2A).[41] Gene Ontology analysis showed that these same genes were enriched for effects on pathways related to inflammation, lipid synthesis, and protein complex synthesis (Supp. Fig. 2B). However, these analyses were based on previously-annotated pathways, and we sought to use a more unbiased approach to determine whether cirrhosis associated SNPs had effects on other human diseases and traits using PheWAS analysis of diagnoses/traits in UK Biobank (Supp. Table 3). Nearly all traits were either ICD-10 diagnoses (Fig. 3A) or blood cell traits (Fig. 3B). We replicated known disease processes: rs1800562-A (*HFE*) associated with numerous red blood cell-related traits and increased arthroses and

skin infections and rs2892947-T (*SERPINA1*) associated with increased emphysema. On PheWAS, *PNPLA3* and *TM6SF2* variants clustered together due to effect on various liver diseases as well as, unexpectedly, decreased neutrophil count, hemoglobin traits, and platelet traits. *TM6SF2* and *SERPINA1* variants had distinct effects on anthropometric traits: the former decreased peripheral fat while the latter increased it (Supp. Table 3). Multiple variants associated with blood cell traits; some of the unique associations included *HSD17B13* and basophil count/percentage, *STAT4* with eosinophil count/percentage, *TM6SF2* with neutrophil count, and *PNPLA3* with leukopenia (Fig. 3B).

Cirrhosis is itself associated with numerous physiological alterations including in lipid profiles, platelet count, and red blood cell count. Thus, as sensitivity analysis, we performed a PheWAS in UK BioBank adjusted for cirrhosis status. For continuous traits, none of the Z scores changed significantly (Z score change >1.96) after adjustment for cirrhosis (Supp. Table 4). For binary traits, associations between the SNPs and several liver-related traits (including esophageal varices and liver fibrosis/cirrhosis itself) changed in significance. However, no associations between SNPs and non-liver binary traits changed in significance; indeed, other than liver-related traits, no Z scores changed by >1 (Supp. Table 4). This analysis indicates that the associations between cirrhosis-increasing SNPs and non-liver diseases/traits are not due solely to the effect of these SNPs on cirrhosis.

Metabolic traits and metabolomics

We also characterized whether cirrhosis associated variants associated with any of 19 different metabolic phenotypes (Methods; Supp. Table 5; Fig. 3C). Due to missing data, we used rs10401969 as a proxy for rs58542926 (*TM6SF2*; $R^2 = 0.95$) and rs6834314 for rs72613567 ($R^2 = 0.94$); there were no acceptable linkage disequilibrium proxies ($R^2 > 0.5$) for rs28929474 (*SERPINA1*) in European populations with available data in the GWASs, so this SNP was not included in this analysis. Several variants with known metabolic effects clustered together: rs738409-G (*PNPLA3*) and rs5854292-T (*TM6SF2*) associated with decreased liver attenuation (increased liver fat) and altered lipid profiles. We also identified a separate cluster of variants which had not been thought to mediate liver disease through metabolic alterations, with effect on numerous metabolic traits. For example, rs1800562-A (*HFE*) decreased low density lipoprotein and total cholesterol, and increased height and blood pressure. Rs626283-C (*MBOAT7*) decreased serum triglycerides.

We finally determined the effect of cirrhosis-associated SNPs on 123 metabolites based on previously-published data (Fig. 3D, Supp. Table 6).[33] Because the metabolites were highly cross-correlated (Supp. Fig. 1), we performed hierarchical clustering and with metabolite clusters shown in Supp. Table 6. Consistent with our

previous report, the cirrhosis promoting allele rs58542926-T (*TM6SF2*) associated with decreased concentration of very low-density, intermediate, and high-density lipoproteins.[44] The cirrhosis promoting allele rs2892474-T (*SERPINA1*) also associated with decreased glycoproteins with several other suggestive associations. *MBOAT7* associated with fewer CH_2 groups in fatty acids.

Discussion

In this study, we identified common and low frequency genetic variants that associate with population based cirrhosis. PheWAS and metabolomic analysis identified distinct metabolic effects of cirrhosis associated variants suggesting that liver disease caused by many mechanisms can lead to liver cirrhosis.

Previously, rare diseases such as cirrhosis, which has an estimated prevalence of 1%, have been difficult to study at a population level.[2] With the use of large cohorts of densely-genotyped and well-characterized individuals, here we show that even previously-considered rare liver diseases such as alpha-1 anti-trypsin deficiency and hereditary hemochromatosis contribute to population based cirrhosis. Furthermore, using biobank PheWAS analysis, we verified known genetic pleiotropisms associated with liver cirrhosis promoting variants including dyslipidemia, diabetes, and body fat composition for six identified variants.[45-47] Others have reported associations between *HFE* C282Y and arthritis, diabetes, chronic pain, and brain iron accumulation,[48-50] and *SERPINA1* variants and hepatitis and fibrosis.[51-54] Another recent study identified a SNP in *MARC1* associated with cirrhosis, which was also associated with cirrhosis in UKBB ($p = 0.047$); differences in strengths of associations are likely due to differences in statistical power and definitions of cirrhosis.[55] However, we also identified previously-unreported associations such as increased body fat with *SERPINA1*, increased basophil count with *HSD17B13*, increased neutrophil count despite decreased overall leukocyte count with *PNPLA3*, and multiple sclerosis with *HFE* that might give new insights into disease pathophysiology caused by variants at these loci.

Most cirrhosis-increasing variants increased AP, suggesting a common pathway towards liver cholestasis with cirrhosis. *TM6SF2* was a strong notable exception where the cirrhosis promoting allele decreased AP. The mechanism of that is not clear but one intriguing possibility is that cholesterol (which cannot be excreted in very low-density lipoprotein in individuals with *TM6SF2* variants[56]) is shunted to bile synthesis which increases farsenoid X receptor agonism and lowers AP.[57] Notably, *PNPLA3* and *HSD17B13* variants did not associate with AP but did increase ALT, suggesting hepatocellular toxicity and potentially distinct disease mechanisms. Some variants (in *PNPLA3*, *HFE*, *SERPINA1*, and *HSD17B13*) also associated with AST-to-platelet ratio index,

suggesting that we may be able to use noninvasive scores to find more variants that associate with cirrhosis in populations where we do not have diagnoses or direct imaging or pathology verification of the disease.

Genetic variants implicated genes that were enriched for expression in liver which suggests likely tissue autonomous effect of these variants. *HFE* and *SERPINA1* were part of acute phase and inflammatory response pathways but these genes as well as all the other ones (*PNPLA3, TM6SF2, HSD17B13*, and *MBOAT7*) either were part of lipid metabolism affecting pathways or affected serum lipids in our metabolomics analyses. While cirrhosis itself alters lipid profiles,[58] there was a low prevalence of cirrhosis in both cohorts, so these lipid changes are unlikely to be driven by cirrhosis alone. Another interesting possibility is that disruptions in lipid signaling represent a final common pathway to cirrhosis. *PNPLA3* and *HSD17B13* for example may alter metabolism of vitamin A to retinoic acid to effect liver cirrhosis and is known to promote stellate cell activation and liver fibrosis.[8,59] Whether the effect of other variants will ultimately converge on this as a mechanism to all fibrosis remains to be determined. The biochemical effects of the variants that promote liver damage however are diverse and thus it is not likely that one solution to eliminating liver damage will be possible even if the final common fibrotic pathway is curbed.

Clustering of genes differs based on whether previously annotated or agnostic methods are used. When previously annotated pathways such as Reactome are used, *PNPLA3, TM6SF2, HSD17B13*, and *MBOAT7* cluster because they affect lipid traits, which is distinct from *HFE* and *SERPINA1* which more affect acute phase and inflammatory responses. Using more agnostic approaches we show here that *MBOAT7* also affects inflammation-related traits including neutrophil count and (at nominal significance) ankylosing spondylitis, peritonitis, and appendicitis. The effect of *MBOAT7* on the immune system has been suggested before—it is expressed at high levels in immune cells and *MBOAT7* variants are associated with increased inflammation with chronic hepatitis C infection[60]—but here we demonstrate this at the population level. Conversely, *HFE* and *SERPINA1* affect serum lipids and *SERPINA1* also affects body fat composition and weight.

Cirrhosis promoting variants protect against development of other human disease suggesting that simply reversing their effects may not be universally beneficial. For example, cirrhosis-causing variants decrease coronary artery disease/ischemic heart disease (*TM6SF2, SERPINA1*),[61,62] diabetes (*TM6SF2*), total cholesterol (*PNPLA3*, *HFE*, *TM6SF2*), and triglycerides (*MBOAT7*, *HSD17B13*, *TM6SF2*). These findings imply that medications may need to

be personalized based on individual risk factors and environmental exposures to be able to reverse fibrosis while maintaining an adequate cardiovascular safety profile.

NAFLD is a major cause of cirrhosis in the population. Notably, though, genes that predispose to hepatic steatosis do not necessarily increase risk of fibrosis. We previously showed that variants in/near *GCKR* and *LYPLAL1* increase nonalcoholic steatohepatitis/fibrosis,[9] but in this study we did not find that they increased cirrhosis in the population. This discrepancy could be in part because they had a weaker effect on fibrosis than variants in *TM6SF2*, *PNPLA3*, or *HSD17B13*. Also, *GCKR* and *LYPLAL1* variants may primarily affect steatosis or inflammation and not fibrosis: in our previous study most individuals with nonalcoholic steatohepatitis also had fibrosis, so we were not able to distinguish between effects on inflammation alone vs. fibrosis. It will be critical to distinguish between steatohepatitis and fibrosis in future studies investigating the effect of genetics on outcomes in NAFLD.

Limitations of our study include that it was only in individuals of European ancestry and may not have identified cirrhosis-increasing alleles that may be prevalent in only other ancestry groups. Interestingly, though, we replicated SNPs previously associated with cirrhosis in Asian populations, suggesting that many variants will have effects across populations. Further, the UKBB analyses rely on International Classification of Diseases codes, and while the codes we used for cirrhosis are specific, they are not sensitive, which may reduce overall sensitivity for identifying cirrhosis-altering variants.[63] This limitation may be why only two SNPs were identified on GWAS, whereas the rest were identified based on candidate variant analysis. Finally, rare diseases such as primary biliary cholangitis are under-represented in the general population, so genetic effects that require particular exposures to develop cirrhosis only in some but not all populations may not be detected.[64] On a related note, we lacked adequate power to subclassify patients with cirrhosis based on disease etiology. Strengths include that this is the largest GWAS for cirrhosis to date.

We demonstrate that eight loci promote liver cirrhosis in population-based data and define their effects on human diseases and traits including many novel pleiotropic effects. We identified previously unrecognized metabolic and immune effects of several of these SNPs and defined novel gene clustering. This work gives new insights into the pathophysiology of liver cirrhosis.

References

1. Mokdad AA, Lopez AD, Shahraz S, et al. Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. *BMC Medicine.* 2014;12(1):145.
2. Mellinger JL, Shedden K, Winder GS, et al. The high burden of alcoholic cirrhosis in privately insured persons in the United States. *Hepatology.* 2018;68(3):872-882.
3. Wong RJ, Aguilar M, Cheung R, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology.* 2015;148(3):547-555.
4. Loomba R, Schork N, Chen CH, et al. Heritability of Hepatic Fibrosis and Steatosis Based on a Prospective Twin Study. *Gastroenterology.* 2015;149(7):1784-1793.
5. Yang J, Trepo E, Nahon P, et al. PNPLA3 and TM6SF2 variants as risk factors of hepatocellular carcinoma across various etiologies and severity of underlying liver diseases. *Int J Cancer.* 2019;144(3):533-544.
6. Buch S, Stickel F, Trepo E, et al. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. *Nat Genet.* 2015;47(12):1443-1448.
7. Hernaez R, McLean J, Lazo M, et al. Association between variants in or near PNPLA3, GCKR, and PPP1R3B with ultrasound-defined steatosis based on data from the third National Health and Nutrition Examination Survey. *Clin Gastroenterol Hepatol.* 2013;11(9):1183-1190 e1182.
8. Ma Y, Belyaeva OV, Brown PM, et al. HSD17B13 is a Hepatic Retinol Dehydrogenase Associated with Histological Features of Non-Alcoholic Fatty Liver Disease. *Hepatology.* 2018.
9. 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(3):e1001324.
10. Pirola CJ, Garaycoechea M, Flichman D, et al. Splice variant rs72613567 prevents worst histologic outcomes in patients with nonalcoholic fatty liver disease. *J Lipid Res.* 2019;60(1):176-185.
11. Abul-Husn NS, Cheng X, Li AH, et al. A Protein-Truncating HSD17B13 Variant and Protection from Chronic Liver Disease. *N Engl J Med.* 2018;378(12):1096-1106.
12. Patin E, Kutalik Z, Guergnon J, et al. Genome-wide association study identifies variants associated with progression of liver fibrosis from HCV infection. *Gastroenterology.* 2012;143(5):1244-1252 e1241-1212.
13. Rueger S, Bochud PY, Dufour JF, et al. Impact of common risk factors of fibrosis progression in chronic hepatitis C. *Gut.* 2015;64(10):1605-1615.
14. Stickel F, Buch S, Zoller H, et al. Evaluation of genome-wide loci of iron metabolism in hereditary hemochromatosis identifies PCSK7 as a host risk factor of liver cirrhosis. *Hum Mol Genet.* 2014;23(14):3883-3890.

15. Greene CM, Marciniak SJ, Teckman J, et al. alpha1-Antitrypsin deficiency. *Nat Rev Dis Primers.* 2016;2:16051.
16. Radford-Smith DE, Powell EE, Powell LW. Haemochromatosis: a clinical update for the practising physician. *Intern Med J.* 2018;48(5):509-516.
17. Canela-Xandri O, Rawlik K, Tenesa A. An atlas of genetic associations in UK Biobank. *bioRxiv.* 2017.
18. Dey R, Schmidt EM, Abecasis GR, Lee S. A Fast and Accurate Algorithm to Test for Binary Phenotypes and Its Application to PheWAS. *Am J Hum Genet.* 2017;101(1):37-49.
19. Sudlow C, Gallacher J, Allen N, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med.* 2015;12(3):e1001779.
20. Maguire LH, Handelman SK, Du X, Chen Y, Pers TH, Speliotes EK. Genome-wide association analyses identify 39 new susceptibility loci for diverticular disease. *Nature Genetics.* 2018;50(10):1359-1365.
21. Zhou W, Nielsen JB, Fritsche LG, et al. Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies. *Nat Genet.* 2018;50(9):1335-1341.
22. Jiang DK, Ma XP, Wu X, et al. Genetic variations in STAT4,C2,HLA-DRB1 and HLA-DQ associated with risk of hepatitis B virus-related liver cirrhosis. *Sci Rep.* 2015;5:16278.
23. Attallah AM, Omran D, Marie MS, et al. IL-28B rs12979860 polymorphism affect the course of chronic hepatitis and the development of HCC in Egyptian patients with hepatitis C type 4. *Br J Biomed Sci.* 2018;75(4):157-162.
24. Besheer T, Arafa M, El-Maksoud MA, et al. Diagnosis of cirrhosis in patients with chronic hepatitis C genotype 4: Role of ABCB11 genotype polymorphism and plasma bile acid levels. *Turk J Gastroenterol.* 2018;29(3):299-307.
25. Medrano LM, Rallon N, Berenguer J, et al. Relationship of TRIM5 and TRIM22 polymorphisms with liver disease and HCV clearance after antiviral therapy in HIV/HCV coinfected patients. *J Transl Med.* 2016;14:257.
26. Ezzikouri S, Elfihry R, Chihab H, et al. Effect of MBOAT7 variant on hepatitis B and C infections in Moroccan patients. *Scientific Reports.* 2018;8(1):12247.
27. Huang L, Mo Z, Li S, Qin X. The association between PIN1 genetic polymorphisms and the risk of chronic hepatitis B and hepatitis B virus-related liver cirrhosis: A case-control study. *Medicine (Baltimore).* 2018;97(35):e12123.
28. El Sharkawy R, Thabet K, Lampertico P, et al. A STAT4 variant increases liver fibrosis risk in Caucasian patients with chronic hepatitis B. *Aliment Pharmacol Ther.* 2018;48(5):564-573.
29. Dai ZJ, Liu XH, Wang M, et al. IL-18 polymorphisms contribute to hepatitis B virus-related cirrhosis and hepatocellular carcinoma susceptibility in Chinese population: a case-control study. *Oncotarget.* 2017;8(46):81350-81360.

30. Zhang XQ, Hong XJ, Bai XJ. Susceptibility to active decompensated cirrhosis is associated with polymorphisms of intercellular adhesion molecule-1 (ICAM-1) in chronic HBV carriers. *Journal of Viral Hepatitis.* 2008;15(3):173-178.
31. Migita K, Maeda Y, Abiru S, et al. Polymorphisms of interleukin-1beta in Japanese patients with hepatitis B virus infection. *J Hepatol.* 2007;46(3):381-386.
32. Song QL, He XX, Yang H, et al. Association of a TANK gene polymorphism with outcomes of hepatitis B virus infection in a Chinese Han population. *Viral Immunol.* 2012;25(1):73-78.
33. Kettunen J, Demirkan A, Würtz P, et al. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. *Nature Communications.* 2016;7:11122.
34. the CDC. A comprehensive 1000 Genomes–based genome-wide association meta-analysis of coronary artery disease. *Nature Genetics.* 2015;47:1121.
35. the DGR, Meta-analysis C. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nature Genetics.* 2012;44:981.
36. Global Lipids Genetics C, Willer CJ, Schmidt EM, et al. Discovery and refinement of loci associated with lipid levels. *Nature Genetics.* 2013;45:1274.
37. Shungin D, Winkler TW, Croteau-Chonka DC, et al. New genetic loci link adipose and insulin biology to body fat distribution. *Nature.* 2015;518:187.
38. Dupuis J, Langenberg C, Prokopenko I, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nature Genetics.* 2010;42:105.
39. Kilpeläinen TO, Carli JFM, Skowronski AA, et al. Genome-wide meta-analysis uncovers novel loci influencing circulating leptin levels. *Nature Communications.* 2016;7:10494.
40. Lu Y, Day FR, Gustafsson S, et al. New loci for body fat percentage reveal link between adiposity and cardiometabolic disease risk. *Nature Communications.* 2016;7:10495.
41. Watanabe K, Taskesen E, van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. *Nat Commun.* 2017;8(1):1826.
42. Li JZ, Huang Y, Karaman R, et al. Chronic overexpression of PNPLA3I148M in mouse liver causes hepatic steatosis. *J Clin Invest.* 2012;122(11):4130-4144.
43. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology.* 2003;38(2):518-526.
44. Kim DS, Jackson AU, Li YK, et al. Novel association of TM6SF2 rs58542926 genotype with increased serum tyrosine levels and decreased apoB-100 particles in Finns. *J Lipid Res.* 2017;58(7):1471-1481.
45. Hyysalo J, Gopalacharyulu P, Bian H, et al. Circulating Triacylglycerol Signatures in Nonalcoholic Fatty Liver Disease Associated With the I148M Variant in PNPLA3 and With Obesity. *Diabetes.* 2014;63:312-322.

46. Palmer CN, Maglio C, Pirazzi C, et al. Paradoxical lower serum triglyceride levels and higher type 2 diabetes mellitus susceptibility in obese individuals with the PNPLA3 148M variant. *PLoS One.* 2012;7(6):e39362.
47. Speliotes EK, Butler JL, Palmer CD, et al. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. *Hepatology.* 2010;52(3):904-912.
48. Pilling LC, Tamosauskaite J, Jones G, et al. Common conditions associated with hereditary haemochromatosis genetic variants: cohort study in UK Biobank. *BMJ.* 2019;364:k5222.
49. Elliott LT, Sharp K, Alfaro-Almagro F, et al. Genome-wide association studies of brain imaging phenotypes in UK Biobank. *Nature.* 2018;562(7726):210-216.
50. Tamosauskaite J, Atkins JL, Pilling LC, et al. Hereditary Hemochromatosis Associations with Frailty, Sarcopenia and Chronic Pain: Evidence from 200,975 Older UK Biobank Participants. *J Gerontol A Biol Sci Med Sci.* 2019;74(3):337-342.
51. Clark VC, Marek G, Liu C, et al. Clinical and histologic features of adults with alpha-1 antitrypsin deficiency in a non-cirrhotic cohort. *J Hepatol.* 2018;69(6):1357-1364.
52. Kim RG, Nguyen P, Bettencourt R, et al. Magnetic resonance elastography identifies fibrosis in adults with alpha-1 antitrypsin deficiency liver disease: a prospective study. *Alimentary Pharmacology & Therapeutics.* 2016;44(3):287-299.
53. Hamesch K, Mandorfer M, Pereira VM, et al. Liver Fibrosis and Metabolic Alterations in Adults With alpha-1-antitrypsin Deficiency Caused by the Pi*ZZ Mutation. *Gastroenterology.* 2019;157(3):705-719 e718.
54. Tanash HA, Piitulainen E. Liver disease in adults with severe alpha-1-antitrypsin deficiency. *J Gastroenterol.* 2019;54(6):541-548.
55. Emdin CA, Haas M, Khera AV, et al. A missense variant in Mitochondrial Amidoxime Reducing Component 1 gene and protection against liver disease. *bioRxiv.* 2019:594523.
56. Mahdessian H, Taxiarchis A, Popov S, et al. TM6SF2 is a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content. *Proc Natl Acad Sci U S A.* 2014;111(24):8913-8918.
57. Trauner M, Gulamhusein A, Hameed B, et al. The nonsteroidal FXR agonist cilofexor (GS-9674) improves markers of cholestasis and liver injury in patients with PSC. *Hepatology.* 2019;0(ja).
58. Privitera G, Spadaro L, Marchisello S, Fede G, Purrello F. Abnormalities of Lipoprotein Levels in Liver Cirrhosis: Clinical Relevance. *Dig Dis Sci.* 2018;63(1):16-26.
59. Trepo E, Romeo S, Zucman-Rossi J, Nahon P. PNPLA3 gene in liver diseases. *J Hepatol.* 2016;65(2):399-412.

555 60. Thabet K, Asimakopoulos A, Shojaei M, et al. MBOAT7 rs641738 increases risk of liver inflammation
556 and transition to fibrosis in chronic hepatitis C. *Nat Commun.* 2016;7:12757.
557 61. Holmen OL, Zhang H, Fan Y, et al. Systematic evaluation of coding variation identifies a candidate
558 causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. *Nat Genet.*
559 2014;46(4):345-351.
560 62. Pirola CJ, Sookoian S. The dual and opposite role of the TM6SF2-rs58542926 variant in protecting
561 against cardiovascular disease and conferring risk for nonalcoholic fatty liver: A meta-analysis.
562 *Hepatology.* 2015;62(6):1742-1756.
563 63. Nehra MS, Ma Y, Clark C, Amarasingham R, Rockey DC, Singal AG. Use of administrative claims data
564 for identifying patients with cirrhosis. *Journal of clinical gastroenterology.* 2013;47(5):e50-e54.
565 64. Zhou WC, Zhang QB, Qiao L. Pathogenesis of liver cirrhosis. *World J Gastroenterol.* 2014;20(23):7312-
566 7324.

567

568 Table 1: Loci that associate with cirrhosis in UK BioBank or Michigan Genomics Initiative.

CHR:POS	Variant	Gene	Ref/ Alt	UK BioBank				Michigan Genomics Initiative			
				EAF	Beta	SE	P value	EAF	Beta	SE	P value
22: 43928850	rs738408	*PNPLA3* (m)	C/T	0.216	0.472	0.055	**6.73E-18**	0.233	0.269	0.058	**3.54E-06**
6: 25917997	rs80215559	*SLC17A2* (i), *HFE* (b)	T/C	0.077	0.477	0.083	**9.13E-09**	0.059	0.280	0.106	**8.39E-03**
22: 43928847	rs738409	*PNPLA3* (m)	C/G	0.216	0.470	0.055	**9.58E-18**	0.233	0.269	0.058	**3.56E-06**
6: 26092913	rs1800562	*HFE* (m)	G/A	0.078	0.454	0.082	**3.30E-08**	0.062	0.300	0.102	**3.09E-03**
19: 19268740	rs58542926	*TM6SF2* (m)	C/T	0.076	0.378	0.084	**6.01E-06**	0.075	0.086	0.093	3.54E-01
14: 94378610	rs28929474	*SERPINA1* (m)	C/T	0.020	0.727	0.162	**7.35E-06**	0.018	0.456	0.180	**1.11E-02**
4: 87310241	rs72613567	*HSD17B13* (s)	-/A	0.721	0.178	0.048	**2.30E-04**	NA			
19: 54173307	rs626283	*MBOAT7* (d)	G/C	0.440	0.121	0.044	**5.48E-03**	0.433	0.031	0.050	5.31E-01
2: 191099907	rs7574865	*STAT4* (i)	T/G	0.224	0.117	0.052	**2.38E-02**	0.224	0.077	0.059	1.87E-01
19:	rs12979860	*IFNL4* (i)	T/C	0.299	0.081	0.047	8.40E-02	0.317	0.115	0.053	**3.05E-02**

39248147											

CHR:POS, chromosome: position. Ref, reference allele. Alt, alternate allele. EAF, effect allele frequency. SE, standard error. NA Not available. (m), missense mutation. (i), intron. (b), biologically-relevant gene. (s), splice variant. UK BioBank had 1,088 cases and 407,874 controls, and Michigan Genomics Initiative had 875 cases and 30,346 controls. Additional variants are shown in Supp. Table 1.

Table 2: Association between liver enzymes and cirrhosis-affecting variants in Michigan Genomics Initiative.

Variant	Gene	Alanine aminotransferase			Alkaline phosphatase			AST-to-platelet ratio index		
		Beta	SE	P value	Beta	SE	P value	Beta	SE	P value
rs738409	*PNPLA3* (m)	0.091	0.012	**1.42E-14**	0.009	0.012	4.39E-01	0.102	0.012	**1.75E-17**
rs80215559	*SLC17A2* (i), *HFE* (b)	0.020	0.022	**3.55E-01**	0.058	0.022	**8.46E-03**	0.059	0.022	**7.41E-03**
rs1800562	*HFE* (m)	0.268	0.037	**3.93E-13**	0.059	0.021	**4.60E-03**	0.063	0.021	**2.60E-03**
rs28929474	*SERPINA1* (m)	0.268	0.037	**3.93E-13**	0.264	0.037	**9.67E-13**	0.214	0.037	**8.82E-09**
rs58542926	*TM6SF2* (m)	0.085	0.019	**8.00E-06**	-0.103	0.019	**6.28E-08**	0.027	0.019	1.54E-01
rs626283	*MBOAT7* (d)	0.009	0.010	3.92E-01	0.026	0.010	**1.27E-02**	0.004	0.010	6.70E-01
rs6834314	*HSD17B13* (u)	0.039	0.011	**1.11E-02**	-0.014	0.011	2.15E-01	0.036	0.011	**1.26E-03**
rs7574865	*STAT4* (i)	0.013	0.012	2.86E-01	-0.002	0.012	8.96E-01	0.019	0.012	1.03E-01
rs12979860	*IFNL4* (i)	0.004	0.011	6.85E-01	0.009	0.011	4.15E-01	0.001	0.011	9.14E-01

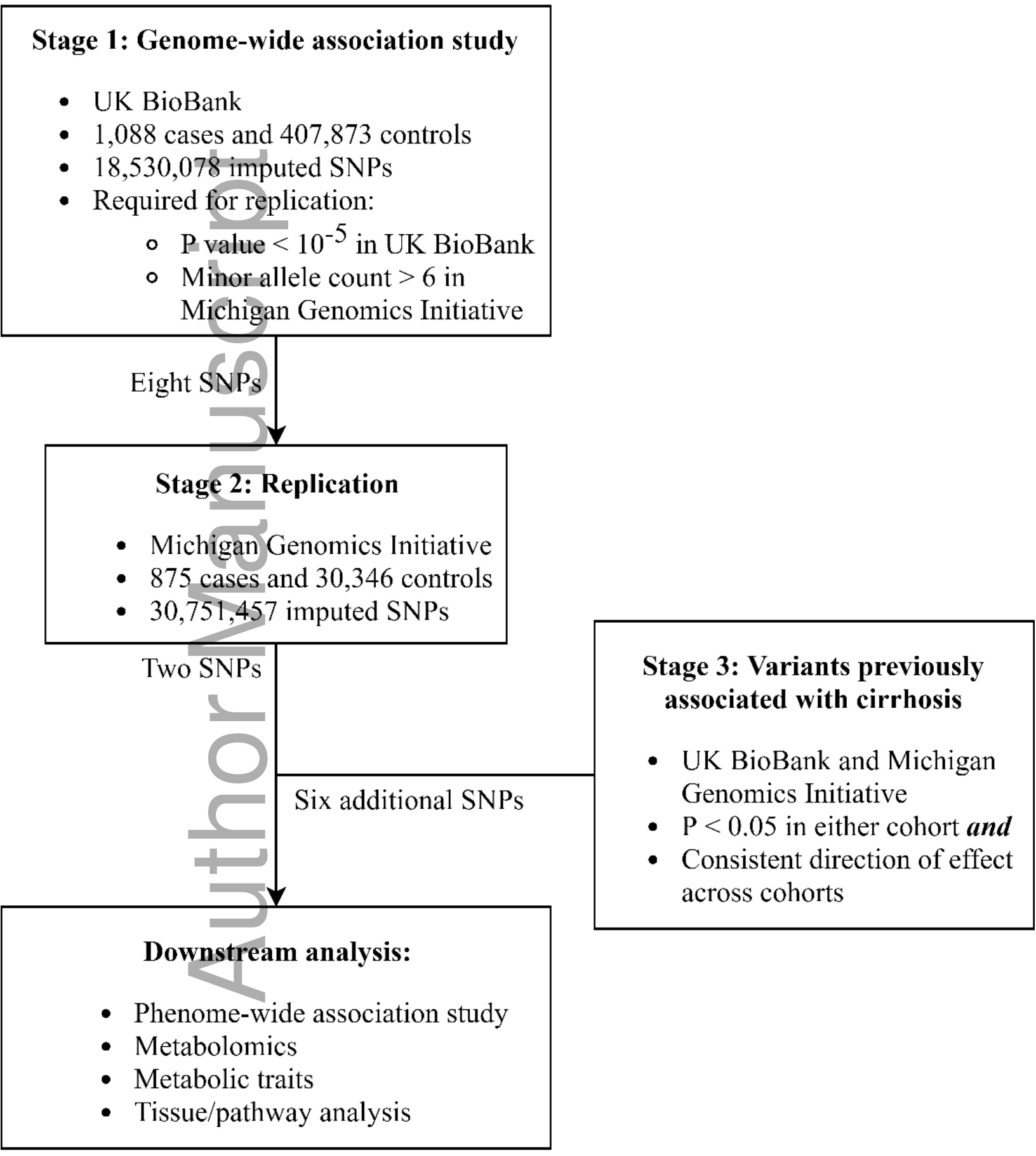
(m), missense mutation. (i), intron. (b), biologically-relevant gene. (d), downstream gene. N = 19,598 for alanine aminotransferase, 19,493 for alkaline phosphatase, and 19,269 for aspartate aminotransferase-to-platelet ratio index.

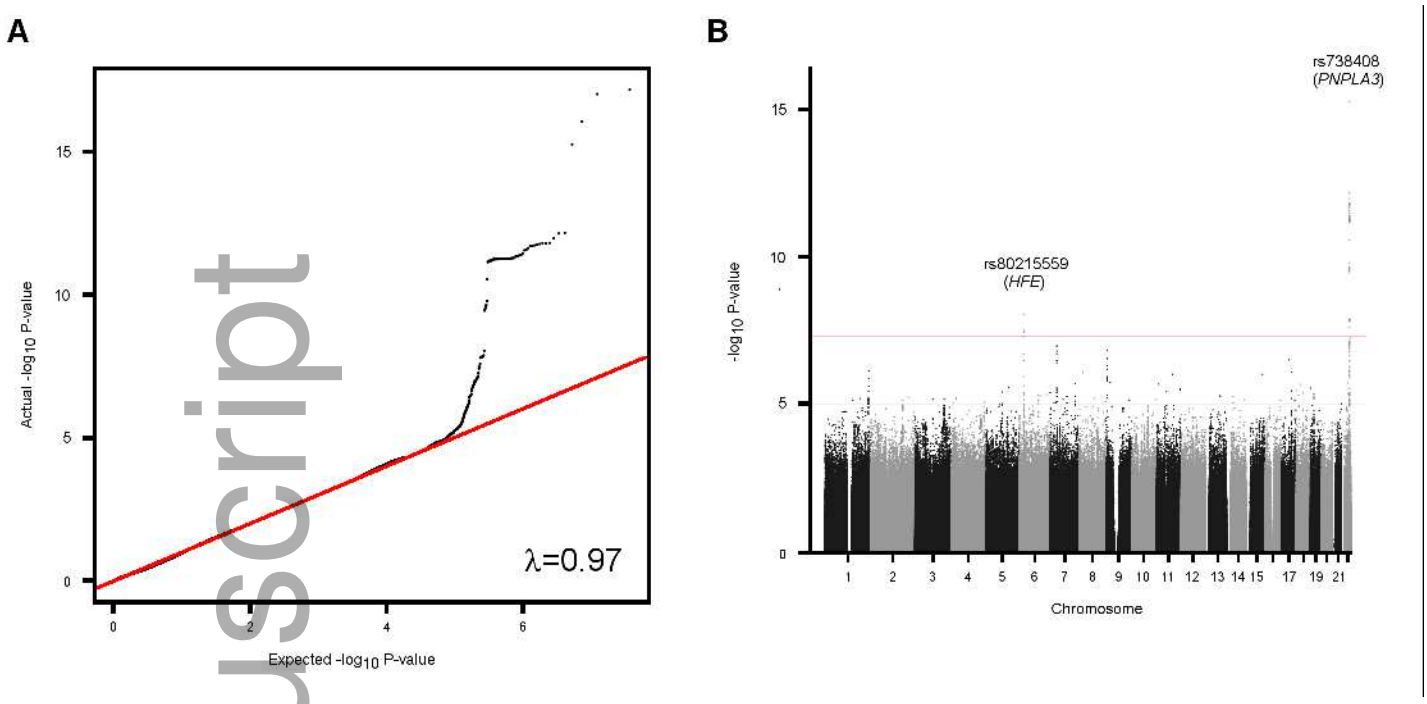
Figure Legends

Figure 1: Study design

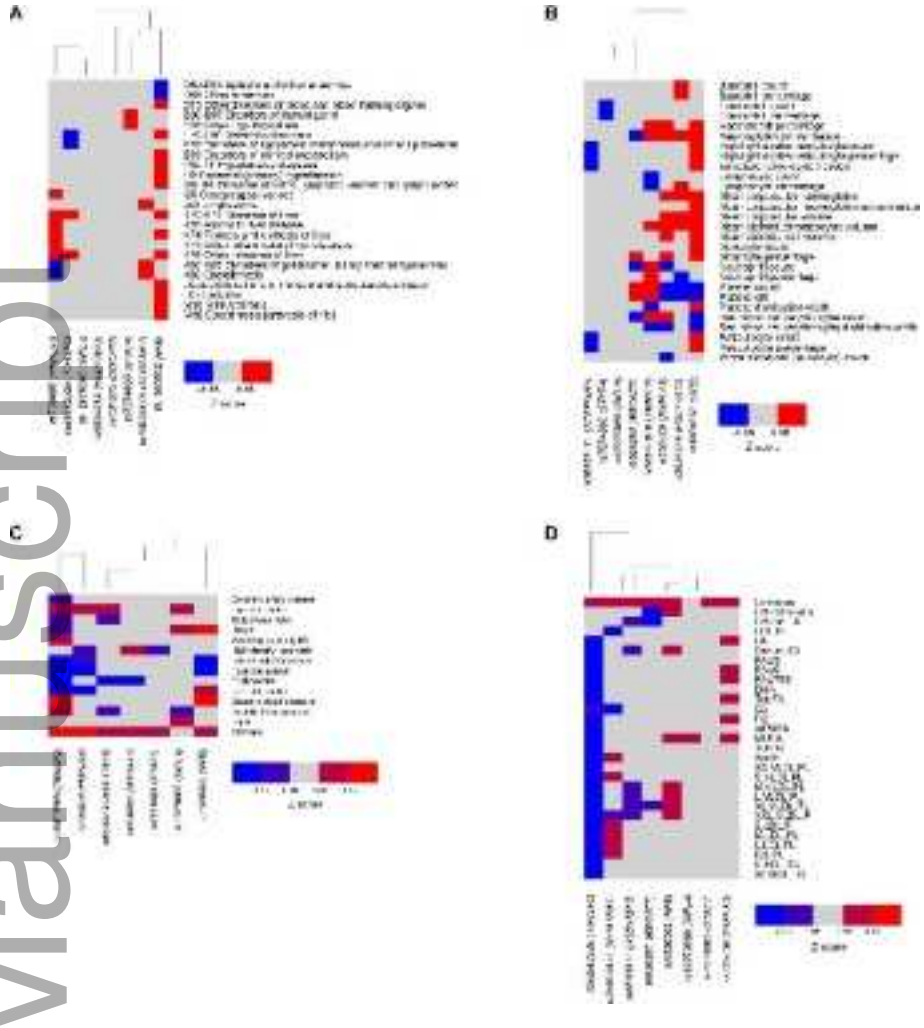
Figure 2: Quantile-quantile and Manhattan plots for GWAS of cirrhosis in UK BioBank. (A) Quantile-quantile plot. (B) Manhattan plot.

Figure 3: Representative pleiotropic effects of cirrhosis-increasing alleles. (A-B) Heat plot of the effect of cirrhosis-increasing alleles on phenome-wide association study in UK BioBank. Diagnoses are shown in (A) and blood cell traits in (B). (C) Heat plot of the effect of cirrhosis-increasing alleles on metabolic traits from publicly-available GWAS. (D) Heat plot of the effect of cirrhosis-increasing alleles on serum/plasma metabolites. "Cirrhosis" refers to cirrhosis diagnosis in UK BioBank (Methods). * *IFNL4* trended toward a significant association with cirrhosis in UK BioBank ($Z = 1.73$, $p = 0.08$). Full list of PheWAS analyses and metabolite labels are shown in Supp. Tables 3-5. CH2.DB.ratio, CH2 groups in fatty acids. CH2.in.FA, CH2 groups to double bonds ratio. LDL.D, LDL diameter. Serum.TG, Serum total triglycerides. FAw3, Omega-3 fatty acids. FAw6, Omega-6 fatty acids. DHA, 22:6 docosahexaenoic acid. Tot.FA, Total fatty acids. PC, Phosphatidylcholine and other cholines. Gp, Glycoprotein acetyls mainly a1-acid glycoprotein. MUFA, Mono-unsaturated fatty acids. otPUFA, Other polyunsaturated fatty acids than 18:2. TotPG, Total phosphoglycerides. ApoB, ApoB. XS.VLDL.PL, Phospholipids in very small very low-density lipoproteins. S.VLDL.PL, Phospholipids in small very low-density lipoproteins. M.VLDL.PL, Phospholipids in medium very low-density lipoproteins. L.VLDL.PL, Phospholipids in large very low-density lipoproteins. XL.VLDL.PL, Phospholipids in very large very low-density lipoproteins. XXL.VLDL.P, Concentration of chylomicrons and extremely large very low-density lipoproteins particles. S.LDL.P, Concentration of small low-density lipoprotein particles. M.LDL.PL, Phospholipids in medium low-density lipoproteins. L.LDL.PL, Phospholipids in large low-density lipoproteins. IDL.PL, Phospholipids in intermediate-density lipoprotein. S.HDL.TG, Triglycerides in small high-density lipoproteins. XL.HDL.TG, Triglycerides in very large high-density lipoproteins.





liv_14321_f2.tif



liv_14321_f3.tif