

Original paper

Identification of novel therapeutic targets for liver fibrosis, cirrhosis, and hepatocellular carcinoma via dual-omics analysis and preliminary exploration of regulatory mechanisms

Jian Chen^{1*}, Rongge Cao^{1*}, Zhiyuan He², Zifan Li¹, Yanan Hong¹, Chuanzeng Ren¹, Ying Wang¹, Jiazhao Wu¹, Yongqing Liu¹, Honghai Xia¹

**Jian Chen and Rongge Cao contributed equally to this work.*

¹Department of Emergency Medicine, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, China

²Department of Allergy, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, China

Abstract

Aim of the study: Liver fibrosis (LF), cirrhosis, and hepatocellular carcinoma (HCC) are strongly correlated and impose a heavy burden on the global healthcare system. Currently, there are no effective treatments, and thus we performed dual-omics analysis to identify new targets and regulatory mechanisms.

Material and methods: A two-sample Mendelian randomization (MR), with cis-expression quantitative trait locus (cis-eQTL) as the genomic variables and cis-protein quantitative trait locus (cis-pQTL) as the proteomic variables, was performed to identify candidate therapeutic targets for LF, cirrhosis, and HCC at the gene and protein levels. Colocalization analysis was performed to screen for significant candidate targets. Moreover, the results were validated using summary-data-based MR (SMR) analysis. To further explore the downstream regulatory mechanisms of the targets, we performed two-step MR, with circulating metabolites, immune cells, gut microbiota, and inflammatory proteins as mediating variables.

Results: Through the two-sample MR, we identified 9 candidate targets for LF, 6 targets for cirrhosis, and 12 targets for HCC. Two significant therapeutic targets for LF, 3 targets for cirrhosis, and 1 target for HCC were identified through colocalization analysis. Among them, DMWD, CDK13, ATRAID, SLC5A6, and CD300LD were validated through SMR. Further analysis revealed that ATRAID might inhibit cirrhosis by upregulating threonine levels. In contrast, CDK13 was predicted to promote LF by suppressing CD20 on naive-mature B cells.

Conclusions: In this study, dual-omics analysis identified new therapeutic targets for LF, cirrhosis, and HCC, as well as potential regulatory mechanisms; these results lay the foundation for further investigations into the disease mechanisms and new drug development.

Key words: liver fibrosis, cirrhosis, hepatocellular carcinoma, dual-omics, targets.

Address for correspondence:

Honghai Xia, Department of Emergency Medicine, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Lujiang Road, No. 17, Luyang District, Hefei City, Anhui Province, 230001, China, e-mail: honghaixia6@sina.com

Introduction

Liver diseases have continued to impose a heavy burden on the global healthcare system. Particularly,

under the International Agency for Research on Cancer (IARC), it was estimated that there were 865,269 newly diagnosed liver cancer patients in 2022, accounting for 4.3% of the new cases of all cancers, making

it the sixth most common cancer and contributing to 757,948 deaths, accounting for 7.8% of the total mortality rate among all cancers, ranking as the third most lethal cancers [1]. Currently, hepatocellular carcinoma (HCC) is the most common liver cancer and thus needs to be extensively studied. As precursor stages of liver cancer, the pathogenesis of liver fibrosis (LF) and cirrhosis warrants further exploration. LF is a dynamic process characterized by excessive accumulation of the extracellular matrix caused by hepatic stellate cell activation [2]. If untreated, LF can progress to cirrhosis or HCC, which increases the risk of death [3]. The incidence of LF has been on the rise globally, but there are no effective drugs for preventing or inhibiting it [4]. As the end stage of chronic liver disease, cirrhosis is the 11th leading cause of death globally [5], and is often associated with the development of LF [6], which, in turn, develops into liver cancer [7]. This calls for investigations into the development of novel therapeutic approaches for LF, cirrhosis, and HCC.

Notably, the development and testing of novel drugs is an expensive and time-consuming process with a high risk of failure [8]. To address the shortcomings of these traditional approaches of drug development, several research tools, including genomics, transcriptomics, proteomics, and other omics, have been designed in recent years, leading to the era of big data [9]. Using multi-omics data, researchers have been able to identify new drug targets for diseases, suggesting that multi-omics analyses shorten the development time and cost of developing new medicines.

In recent years, MR has increasingly been applied to study disease mechanisms using data from genome-wide association studies (GWAS). MR uses genetic variation data from GWAS to probe causal relationships between exposures and outcomes, and is considered to be similar to randomized controlled trials, presenting strong evidence because alleles are randomly assigned to the offspring at conception [10]. Recently, several researchers have investigated therapeutic targets for various diseases through genomic or proteomic analyses based on cis-expression quantitative trait locus (cis-eQTL) or cis-protein quantitative trait locus (cis-pQTL) data by MR [11–13]. In this study, we used genomic and proteomic analyses to identify the therapeutic targets of LF, cirrhosis, and HCC through MR. Subsequently, potential targets were identified through colocalization analysis. In addition, we conducted external validation for the identified targets using summary-data-based MR (SMR). To further provide important information to guide the development of effective drugs, we conducted a pre-

liminary exploration of the regulatory mechanisms of the key targets.

Material and methods

Ethics statement

There were no ethical issues involved in our study as the data sources for this study were opensource databases, and ethical approvals for them have been obtained.

Study design

In this study, novel therapeutic targets for liver diseases (here, LF, cirrhosis, and HCC) were screened using dual-omics analysis at the gene and protein levels. An overview of this study is presented in Figure 1. Initially, we explored the causal association of druggable genes and proteins with liver diseases through a two-sample MR analysis based on cis-eQTL and cis-pQTL to identify potential therapeutic targets for these diseases. Subsequently, colocalization analyses were performed to determine whether the candidate drug targets shared genetic variation with liver diseases to select significant candidate targets. The SMR approach was employed to validate the causal relationships between significant candidate drug targets and liver diseases. Finally, a preliminary exploration of the regulatory mechanisms of the selected targets was conducted through a two-step MR, and the mediating roles of circulating metabolites, immune cells, gut microbiota, and inflammatory proteins were analyzed.

Exposure data

The druggable genes included in the MR analysis were obtained from the Drug-Gene Interaction Database (DGIdb, <https://www.dgldb.org/>) [14] and the study of Finan *et al.* [15]. DGIdb is a database containing drug-gene interactions and is widely used in genetics and pharmacology research [14]. The DGIdb was screened to download the latest “interactions” data updated in May 2024. A total of 5012 druggable genes were extracted from it. Moreover, 4479 druggable genes were extracted from the study by Finan *et al.* The total druggable genes obtained from both sources were merged, totaling 6888 druggable genes.

To identify the candidate therapeutic targets for the diseases at the gene level, MR analysis was performed based on the expression quantitative trait locus (eQTL). Generally, eQTL are divided into cis and trans, and the exposure data were the cis-eQTL data, representing genes

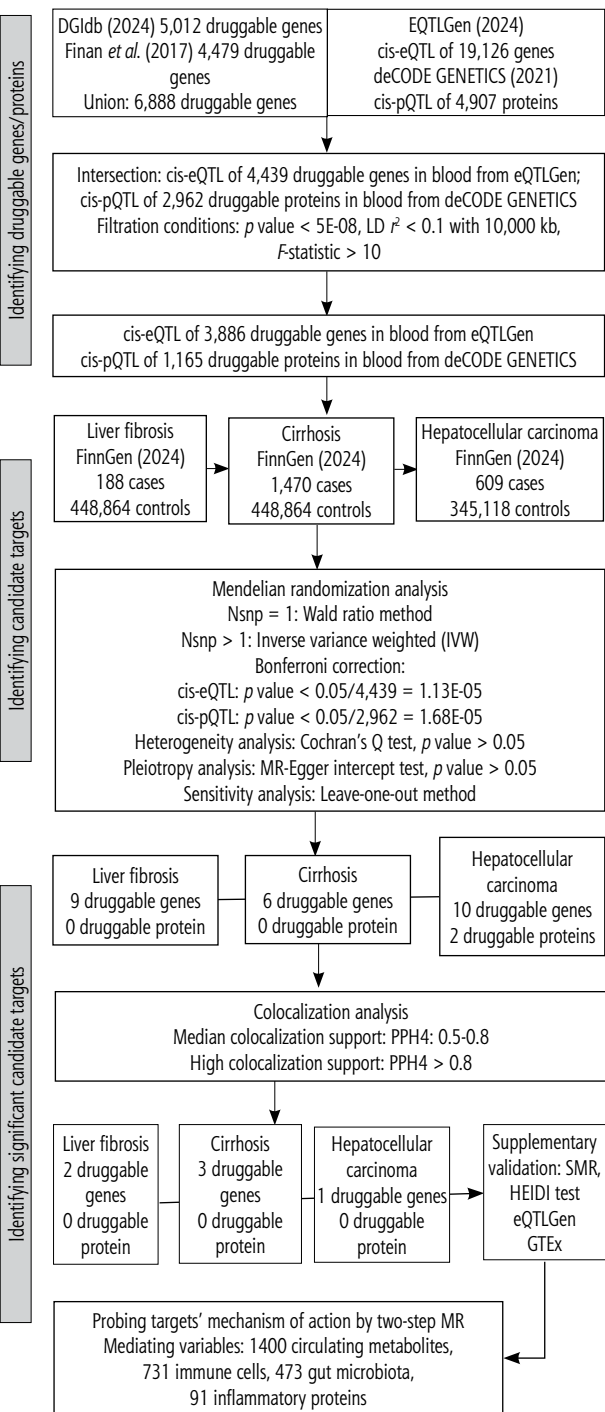


Fig. 1. Flow chart of this study

normally located in the upstream and downstream 1 Mb regions of their regulatory genes. The updated eQTLGen Consortium (<https://eqtlgen.org/>) contained cis-eQTL data in blood samples for 19,126 genes. The intersection of these genes with 6,888 druggable genes yielded cis-eQTL data for 4,439 druggable genes, which served as the exposure data in this study.

To identify the candidate therapeutic targets for the selected diseases at the protein level, we selected pQTL as the exposure variable. The deCODE GENETICS (<https://www.decode.com/>) was screened to download the protein quantitative trait locus (pQTL) data for 4,907 proteins. Intersection of the retrieved data with the previously reported 6,888 druggable genes yielded 2,962 druggable proteins encoded by druggable genes. Subsequently, cis-pQTLs were selected as the exposure variable, including genes from the 1 Mb upstream and downstream regions.

All the genetic exposure data were derived from the European populations, and are presented in Table 1.

Instrumental variable selection

Genetic variation data generated from GWAS were selected as instrumental variables (IVs). The selected IVs met the following three criteria [16]: 1) they are significantly associated with the exposure factors; 2) they should not be linked to the confounders of outcomes; 3) they affect outcomes only through exposure and not through other pathways. Therefore, we performed correlation analyses on the exposure data, setting the threshold of significance at $p < 5 \times 10^{-8}$. Independent exposure SNPs were selected after removal of linkage disequilibrium (LD) based on $r^2 < 0.1$ and kb = 10,000 [17, 18]. The F -test excluded weak IVs with an F -value > 10.

Outcome data

Datasets containing the genetic variations in LF, cirrhosis, and HCC were used as outcome variables and were obtained from the FinnGen Consortium (<https://www.finnngen.fi/en>) – a large-scale Finnish genomic project established in 2017 to integrate genomic data with digital health records from 500,000 Finns. A key strength is leveraging Finland's unique genetic history: its relatively isolated population harbors distinct genetic variations compared to other Europeans. This study utilized outcome data from FinnGen release R11; for LF, there were 188 cases and 448,864 controls; for cirrhosis, there were 1,470 cases and 448,864 controls; and for HCC, there were 609 cases and 345,118 controls. Cases in the LF, cirrhosis, and HCC cohorts were identified using ICD codes from the Finnish National Health Registry (covering hospitalizations, outpatient visits, medications, and mortality records). The general cohort comprised individuals without the specific target disease (LF, cirrhosis, or HCC) but potentially having other conditions, reflecting a more realistic population genetic background. Participants

Table 1. Characteristics of included GWAS data

Trait	Consortium	Sample size	Population	Year
Exposure				
Druggable genes	DGIdb	5,012 genes	European	2024
	Finan <i>et al.</i>	4,479 genes	European	2017
eQTL	eQTLGen	19,126 genes	European	2024
pQTL	deCODE	4,907 proteins	European	2021
SMR	eQTLGen	–	European	2024
	GTEx	–	European	2024
Outcomes				
Liver fibrosis	FinnGen	188 cases, 448,864 controls	European	2024
Cirrhosis	FinnGen	1,470 cases, 448,864 controls	European	2024
Hepatocellular carcinoma	FinnGen	609 cases, 345,118 controls	European	2024
Mediators				
Circulating metabolites	Chen <i>et al.</i>	1,400 metabolites	European	2023
Immune cells	Valeria <i>et al.</i>	731 immune cells	European	2020
Gut microbiota	Qin <i>et al.</i>	473 gut microbiota	European	2022
Inflammatory proteins	Zhao <i>et al.</i>	91 inflammatory proteins	European	2023

had a relatively high median age (63 years), and a large proportion were recruited from hospitals. Table 1 provides detailed descriptions of the study data.

Statistical analysis

Two-sample Mendelian randomization

In the MR analysis, the Wald ratio method was calculated for IVs with one SNP [17], and for IV with two or more SNPs, the inverse variance weighted (IVW) was employed to determine the causal relationships between the exposure and outcomes [19]. To adjust for multiple testing, we adopted the Bonferroni correction, setting a threshold p value at $1.13\text{E}-05$ ($0.05/4,439$) for gene-level analysis and $1.68\text{E}-05$ ($0.05/2,962$) for protein-level analysis. The MR-Egger intercept test was performed to explore the existence of pleiotropy [20], whereas the Cochran's Q test was conducted to test for heterogeneity. The p values in the above two analyses were set at > 0.05 . To test the stability of the results, the leave-one-out method was employed to carry out sensitivity analyses.

Colocalization analysis

For candidate drug targets found to be significantly causally associated with liver diseases (LF, cirrhosis, and HCC), we further performed colocalization analyses to identify common genetic variants between them and liver diseases. The following posterior prob-

abilities for the five hypotheses were derived from the colocalization analysis [21]: PPH0, which was not associated with either candidate drug targets or diseases; PPH1, which was associated with the candidate drug targets but not with diseases; PPH2, which was associated with diseases but not with the candidate drug targets; PPH3, which was associated with both candidate drug targets and diseases with different causal variants; PPH4, which was associated with both candidate drug targets and diseases, with the common causal variant. PPH4: 0.5–0.8 reflected moderate evidence of colocalization, and PPH4 > 0.8 reflected high evidence [22]. The candidate drug targets that were colocalized with the diseases were considered significant candidate drug targets.

All analyses were performed using the packages “TwoSampleMR”, “Mendelian Randomization”, and “coloc” in the R software (version 4.3.2).

Supplementary validation

Additional validation tests for causality of significant candidate targets with LF, cirrhosis and HCC were conducted using summary-data-based MR (SMR) [23], based on data from eQTLGen and GTEx (<https://www.gtexportal.org/>). The heterogeneity in dependent instruments (HEIDI) test was employed to differentiate causal associations between genes and diseases from common genetic variants or linkage disequilibrium [13]. For SMR, $p < 0.05$ was set as the threshold of statistical significance; for the HEIDI test,

$p < 0.05$ was set as the threshold of statistical significance for the causal association that was driven by linkage disequilibrium. These analyses were performed using the SMR v1.3.1 package. The details of SMR data are presented in Table 1.

Preliminary exploration of regulatory mechanisms

To predict the pathways involved in the mechanisms of disease modulation by the identified candidate targets, we performed a two-step MR. The mediating variables were 1400 circulating metabolites (GCST-90199621–GCST90201020) [24], 731 immune cells (ebi-a-GCST90001391–ebi-a-GCST90002121) [25], 473 gut microbiota (GCST90032172–GCST0032644) [26], and 91 inflammatory proteins (GCST90274758–GCST90274848) [27]. Their sources are detailed in Table 1. The above data were analyzed and screened using similar criteria as for the two-step MR. The causal effect between exposure and outcome variables was calculated as the total effect (Beta all), followed by determination of the causal effects between the exposure and mediating variables (Beta 1), and between the mediating and outcome variables (Beta 2). Finally, the indirect effects, also known as the mediator effects (Beta 1 * Beta 2) and direct effects (Beta all – Beta 1 * Beta 2) were calculated [28].

Results

Two-sample MR identified candidate therapeutic targets

The two-sample MR analysis revealed 9 druggable genes, which were significantly causally associated with LF and considered as candidate therapeutic targets for LF. These targets included ERCC3, PISD, RABEP1, P2RX4, BCR, SENP6, CA6, DMWD, and CDK13 (Fig. 2A, Supplementary Table 1). Among them, ERCC3, PISD, P2RX4, BCR, SENP6, CA6, and CDK13 were associated with increased risk of LF, whereas RABEP1 and DMWD decreased the risk. Supplementary Table 1 demonstrates that the p values of Cochran's Q test and the MR-Egger intercept test were > 0.05 , suggesting that none of the targets had heterogeneity and pleiotropy. Supplementary Figure 1 display the results of sensitivity analysis, indicating that, after each SNP was removed, the MR analysis was re-performed and the results were not significantly altered, confirming that the results were stable.

Six druggable genes that were causally associated with cirrhosis were identified, with KLHL8 and ATRAID reducing the risk of cirrhosis, while IL7R, SLC5A6, TNFSF14, and SERPINB10 were predicted to increase the risk (Fig. 2B, Supplementary Table 2). These targets had no heterogeneity and pleiotropy (Supplementary Table 2), and sensitivity analyses con-

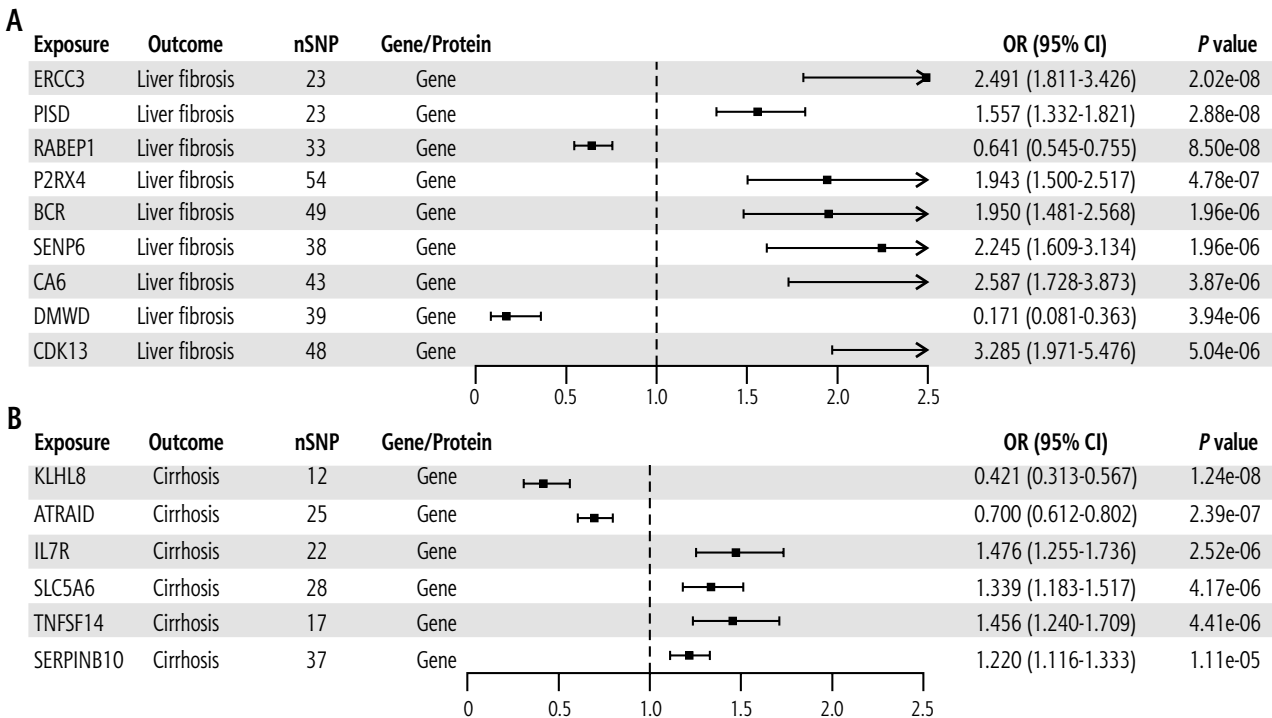


Fig. 2. Results of Mendelian randomization analysis of LF, cirrhosis and HCC based cis-eQTL and cis-pQTL. **A)** Forest plot of 9 candidate targets for LF. **B)** Forest plot of 6 candidate targets for cirrhosis

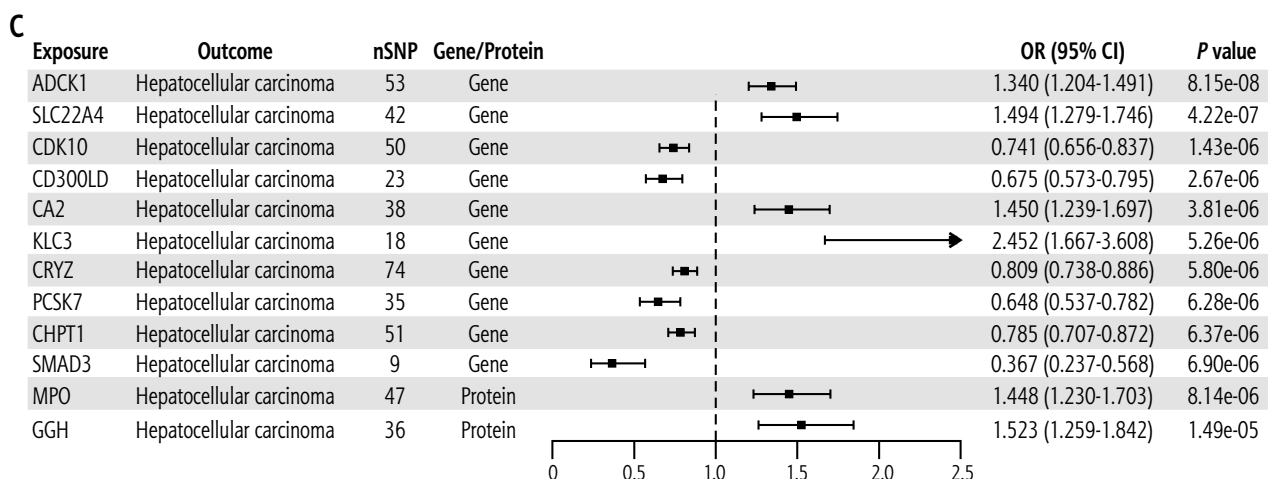


Fig. 2. Cont. **C**) Forest plot of 12 candidate targets for HCC. Multiple inspection criteria: p value $< 0.05/4,439 = 1.13\text{E-}05$ (cis-eQTL), p value $< 0.05/2,962 = 1.68\text{E-}05$ (cis-pQTL)

firmated that the results were stable (Supplementary Fig. 2).

For HCC, 12 candidate therapeutic targets were identified, including 10 druggable genes and 2 druggable proteins. Among the genes, ADCK1, SLC22A4, CA2, and KLC3 were associated with increased risk of HCC, whereas CDK10, CD300LD, CRYZ, PCSK7, CHPT1, and SMAD3 were associated with reduced risk. The two druggable proteins MPO and GGH increased the risk of HCC (Fig. 2C, Supplementary Table 3). Notably, all these candidate therapeutic targets had no significant heterogeneity or pleiotropy (Supplementary Table 3), and sensitivity analyses confirmed that the results were robust (Supplementary Fig. 3).

Table 2 displays the above candidate targets and their official full names.

Colocalization analysis identified significant candidate therapeutic targets

The colocalization analysis identified 2 candidate therapeutic targets (DMWD and CDK13) sharing genetic variants with LF, 3 candidate therapeutic targets (KLHL8, ATRAID, and SLC5A6) sharing genetic variants with cirrhosis, and 1 candidate therapeutic target (CD300LD) sharing genetic variants with HCC. Colocalization analysis further revealed causal relationships between the targets and diseases, and 6 targets were considered to be significant candidate targets for liver diseases. The detailed colocalization analysis results are shown in Figure 3.

Further analysis revealed that DMWD shared the genetic variant SNP rs6211759 with LF (Fig. 4A), CDK13 and LF shared the common genetic variant

rs13239106 (Fig. 4B); KLHL8, ATRAID, and SLC5A6 shared genetic variants rs10026594, rs2384575 and rs2384575 with cirrhosis (Fig. 4C-E); rs55981110 was the common genetic variant of CD300LD and HCC (Fig. 4F).

Supplementary validation for significant candidate therapeutic targets

Through the SMR analysis, we validated the causal association of significant candidate targets with liver diseases using two data sets (eQTLGen and GTEx). The data volume in GTEx is relatively small, and hence we could only validate the results of ATRAID, SLC5A6, and CD300LD in the GTEx group. The validation results are shown in Figure 5. In the eQTLGen group, DMWD was predicted to reduce the risk of LF, whereas CDK13 increased the risk of LF, ATRAID reduced the risk of cirrhosis, while SLC5A6 increased the risk of cirrhosis, CD300LD reduced the risk of HCC, and there was no causal relationship between KLHL8 and cirrhosis. Except for KLHL8, the associations of the other targets with liver diseases were consistent with the results of the two-sample MR analysis. In the GTEx group, ATRAID was predicted to reduce the risk of cirrhosis, while SLC5A6 increased the risk of cirrhosis, and CD300LD reduced the risk of HCC. These results were consistent with the two-sample MR and the results obtained for the eQTLGen group. Data shown in Figure 5 indicate that the p values of the HEIDI test for all targets in the two data sets, except KLHL8, were greater than 0.05, indicating that the causal relationship between these targets and liver diseases was driven by the common genetic variation rather than linkage disequilibrium.

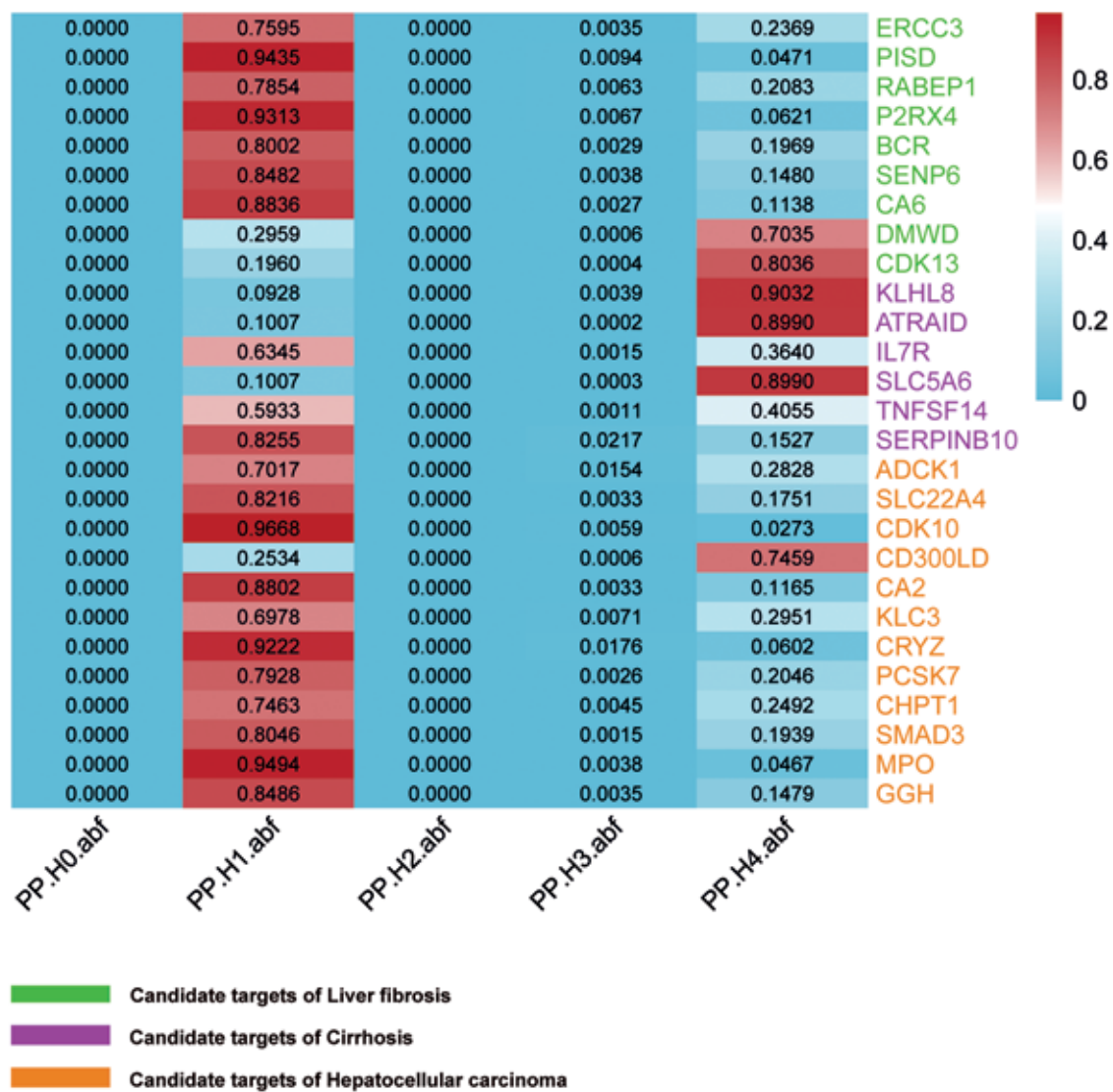


Fig. 3. Colocalization analysis for the significant candidates. DMWD and CDK13 share genetic variants with LF and are significant candidate targets for LF; KLHL8, ATRAID, and SLC5A6 have common genetic variants with cirrhosis and are significant candidate targets for cirrhosis; CD300LD shows common genetic variation with HCC and is a significant candidate target for HCC (Median colocalization support: PPH4: 0.5-0.8. High colocalization support: PPH4 > 0.8)

Preliminary exploration of regulatory mechanisms

To identify the regulatory mechanisms, a two-step MR was performed for significant candidate targets DMWD, CDK13, ATRAID, SLC5A6, and CD300LD. As shown in Figure 6A, mediation analysis was performed with 1400 circulating metabolites, 731 immune cells, 473 gut microbiota, and 91 inflammatory proteins. Among the circulating metabolites, ATRAID was predicted to inhibit cirrhosis by upregulating threonine ($p = 0.044$, mediation effect: 11.97%) (Fig. 6B, Supplementary Table 4). Moreover, CDK13 was found to promote liver fibrosis by inhibiting CD20 on naive-mature B cells ($p = 0.046$, mediation effect: 22.14%) (Fig. 6B, Supplementary Table 5). However, none of 473 gut microbiota

and 91 inflammatory proteins was involved in the regulation of liver diseases by the 5 targets (Supplementary Tables 6 and 7).

Discussion

In this study, we identified 9 candidate targets for LF, 6 candidate targets for cirrhosis and 12 candidate targets for HCC through the two-sample MR. Colocalization analysis uncovered 2 significant candidate targets for LF, 3 significant candidate targets for cirrhosis, and 1 significant candidate target for HCC. The results of the supplementary validation analysis using SMR were consistent with those of the two-sample MR, except for KLHL8. Finally, two-step MR was employed

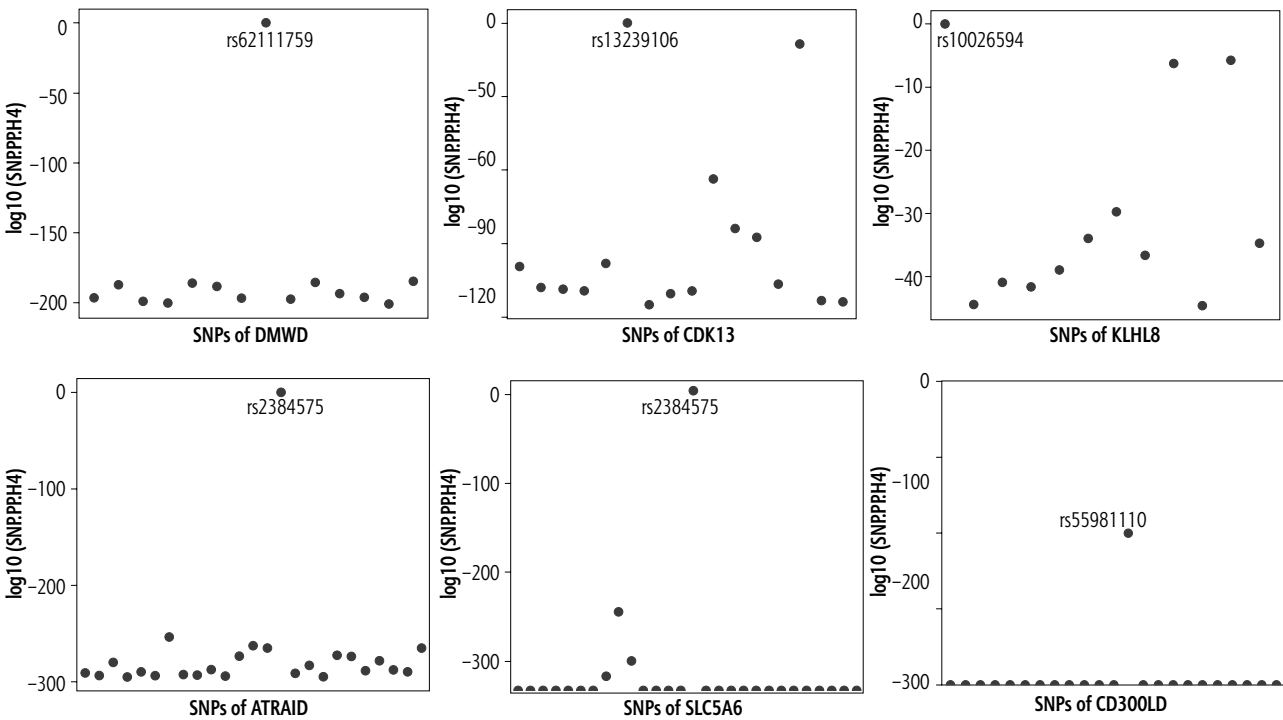


Fig. 4. Common genetic variants for significant candidate targets and diseases. **A)** Rs62111759 is the common SNP of DMWD and LF; **B)** Rs13239106 is the common SNP of CDK13 and LF; **C)** Rs10026594 is the common SNP of KLHL8 and cirrhosis; **D)** Rs2384575 is the common SNP of ATRAID and cirrhosis; **E)** Rs2384575 is the common SNP of SLC5A6 and cirrhosis; **F)** Rs55981110 is the common SNP of CD300LD and HCC

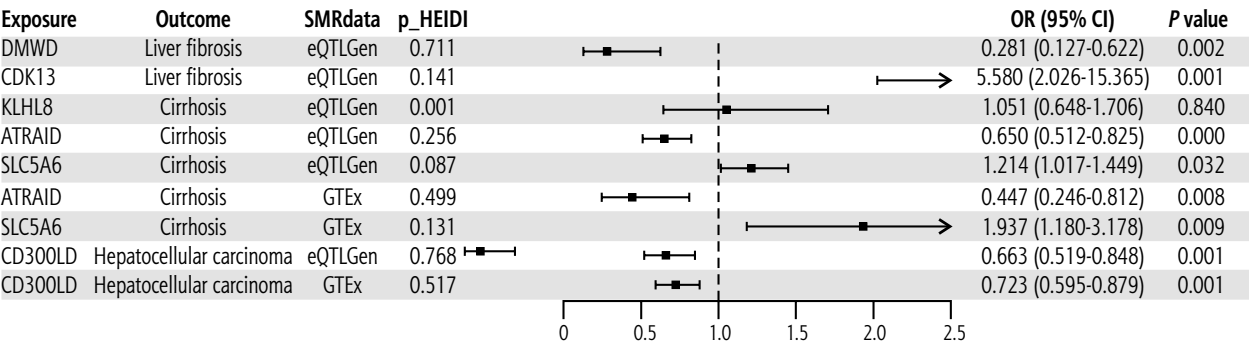


Fig. 5. Validation of significant candidate targets by SMR. No causal relationship was found between KLHL8 and cirrhosis. The validation results for the remaining targets were consistent with the results of two-sample MR

to explore the regulatory mechanisms of the validated targets, which revealed that ATRAID inhibited cirrhosis by reducing the levels of threonine, and CDK13 was predicted to promote LF by inhibiting CD20 on naive-mature B cells. To our knowledge, this is the first study to identify the therapeutic targets of LF, cirrhosis, and HCC, as well as their regulatory mechanisms through dual-omics.

For the LF, we identified 9 candidate targets (ERCC3, PISD, RABEP1, P2RX4, BCR, SENP6, CA6, DMWD, and CDK13). Ding *et al.* reported that activation of the EGF-EGFR/CHKA/PI3K-PDK1-AKT signaling cascade enhances the expression of ERCC3-regulated oncogenes to promote the progression of nonalcoholic

fatty liver disease to HCC [29]. Moreover, the ERCC3 gene was predicted to increase the risk of LF; however, further investigations are needed to clarify whether ERCC3 can enhance the progression of LF to HCC. Ma *et al.* found that the exosome circCDK13 can inhibit LF via the miR-17-5p/KAT2B axis, and our study showed that increased expression of CDK13 may promote LF, and thus there may be a mechanistic link between the two findings that need to be further explored [30]. None of the remaining targets have previously been reported to be associated with liver fibrosis. Furthermore, we performed colocalization analysis to identify the significant candidate targets DMWD and CDK13, both of which were confirmed by the SMR method. In

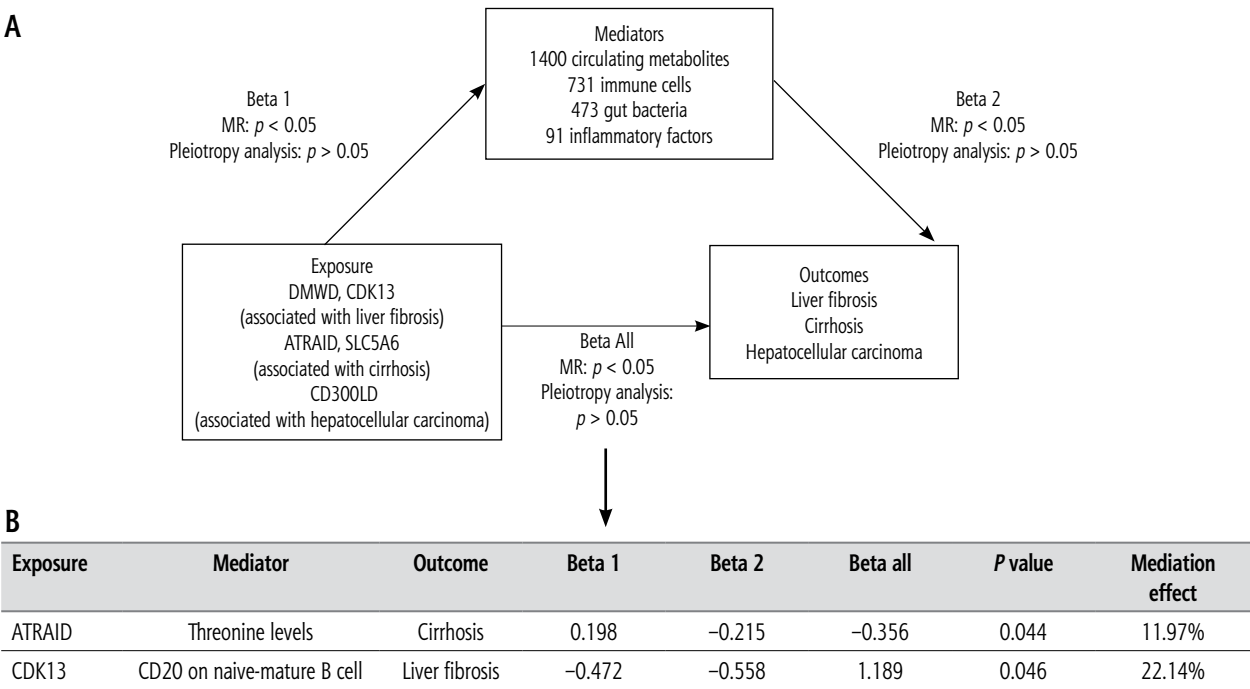


Fig. 6. Analysis of downstream regulatory mechanisms of DMWD, CDK13, ATRAID, SLC5A6, and CD300LD. **A)** Flowchart showing two-step MR. **B)** ATRAID may inhibit cirrhosis by upregulating threonine levels, and CDK13 promotes liver fibrosis by inhibiting CD20 on naive-mature B cells

the analysis of regulatory mechanisms of the two targets, we found that CDK13 may promote liver fibrosis by inhibiting CD20 on naive-mature B cells. Numerous studies have shown that B cells are associated with the occurrence and development of LF [31-33], and the present findings reveal a new mechanism for the role of the immune system in LF development.

For cirrhosis, 6 candidate targets (KLHL8, ATRAID, IL7R, SLC5A6, TNFSF14, and SERPINB10) were identified by the two-sample MR. *Ex vivo* experiments demonstrated that the IL7R expression accelerated the progression of cirrhosis [34], which is consistent with our finding. Elsewhere, Mells *et al.* identified IL7R as a genetic risk factor for the occurrence of primary biliary cirrhosis [35]; therefore, IL7R is likely to be an important target for cirrhosis. A previous study showed that splenectomy could effectively reduce the expression level of TNFSF14 in the blood, thereby reducing liver fibrosis and cirrhosis in patients [36]. Notably, TNFSF14 has been linked to the development of fibrosis in several organs, including the heart, lung, and kidney [37-39]. Therefore, we speculated that TNFSF14 may be a promising candidate target for investigation of cirrhosis. To our knowledge, no study has explored the association of KLHL8, ATRAID, SLC5A6, and SERPINB10 with cirrhosis or liver fibrosis. Here, three candidate targets – KLHL8, ATRAID, and SLC5A6 – were identified through colocalization analysis, among

which ATRAID and SLC5A6 are considered to be significant genes. The preliminary analysis of the target mechanisms revealed that ATRAID may inhibit cirrhosis by reducing threonine levels; however, this result needs to be further validated through basic experiments.

As for HCC, 12 candidate targets (ADCK1, SLC22A4, CDK10, CD300LD, CA2, KLC3, CRYZ, PCSK7, CHPT1, SMAD3, MPO and GGH) were identified in the preliminary analysis. Zhong *et al.* reported that decreased CDK10 expression was positively associated with the occurrence and progression of HCC [40], which is consistent with our findings. Overexpression of SMAD3 mediates the inhibitory effect of transforming growth factor β 1 (TGF- β 1) on the catalytic subunits, thereby promoting lipid peroxidation in HCC cells [41], whereas NOP2/Sun RNA methyltransferase 5 has been reported to accelerate the progression of HCC by upregulating SMAD3 expression [42]. TRIP13 silencing as a tumor suppressor in HCC can inhibit cell growth and metastasis *via* activating the TGF- β 1/SMAD3 signaling pathway [43], therefore regulating HCC through the SMAD3 complex. Available studies have demonstrated that MPO overexpression favors HCC progression [44, 45], which is consistent with our findings. Among them, ADCK1, SLC22A4, CD300LD, CA2, KLC3, CRYZ, PCSK7, CHPT1, and GGH are new unreported targets in HCC. Further

analyses showed that CD300LD was a key target for driving cancer immunotherapy [46, 47]. This indicates that further research is needed to explore the therapeutic potential of CD300LD in HCC.

In this study, novel therapeutic targets for LF, cirrhosis, and HCC were revealed by MR with dual-omics analysis at the gene and protein levels. No evidence of pleiotropy or heterogeneity was found in all targets analyses, and sensitivity analyses suggested that all results were stable. To predict the therapeutic value of the targets, we used genetic variation data of druggable genes and druggable proteins as exposure variables, from the latest and most comprehensive datasets. Next, colocalization analyses were conducted to identify the common genetic variants driving the targets and diseases. Additional validation of the targets was also carried out using SMR from multiple sources. This led to the identification of CDK13 and ATRAID as key targets influencing LF and cirrhosis through immune cells and metabolites, providing ideas for the development of new targeted drugs.

This study has several limitations. Given the limitations of accessing original data, all participants were European; therefore, the conclusions may not be applicable to other populations. Second, the proteomic data were smaller in scale compared to genomic data, and therefore fewer protein targets were identified. Thirdly, the targets and mechanisms identified in this study need to be further explored in basic experiments.

Conclusions

In this study, novel therapeutic targets for LF, cirrhosis, and HCC were identified through dual-omics analysis, colocalization, and validation. The downstream regulatory mechanisms were probed, with CDK13 and ATRAID identified for LF and cirrhosis. This study provides important reference data to guide future investigations into disease mechanisms and the development of new targeted drugs.

Acknowledgements

We sincerely thank all participants in GWASs cited in this paper and providers of the GWAS data.

Disclosures

This research received no external funding.
Institutional review board statement: Not applicable.

The authors declare no conflict of interest.

Supplementary material is available on the journal's website.

References

- Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2024; 74: 229-263.
- Wu WH, Yang YL, Wang T, et al. Ginsenoside compound K restrains hepatic fibrotic response by dual-inhibition of GLS1 and LDHA. *Phytomedicine* 2024; 135: 156223.
- Ramachandran P, Henderson NC. Antifibrotics in chronic liver disease: tractable targets and translational challenges. *Lancet Gastroenterol Hepatol* 2016; 1: 328-340.
- Zhang N, Yao H, Zhang Z, et al. Ongoing involers and promising therapeutic targets of hepatic fibrosis: The hepatic immune microenvironment. *Front Immunol* 2023; 14: 1131588.
- Israelsen M, Francque S, Tsochatzis EA, Krag A. Steatotic liver disease. *Lancet* 2024; 404: 1761-1778.
- Tang B, Jin C, Li M, et al. A novel pectin-like polysaccharide from *Crocus sativus* targets Galectin-3 to inhibit hepatic stellate cells activation and liver fibrosis. *Carbohydr Polym* 2025; 348: 122826.
- Ginès P, Krag A, Abraldes JG, et al. Liver cirrhosis. *Lancet* 2021; 398: 1359-1376.
- Wouters OJ, McKee M, Luyten J. Estimated research and development investment needed to bring a new medicine to market, 2009-2018. *Jama* 2020; 323: 844-853.
- Ouyang Y, Chen Y, Chen K, et al. Mendelian randomization and colocalization analysis reveal novel drug targets for myasthenia gravis. *Hum Genomics* 2024; 18: 43.
- Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* 2003; 32: 1-22.
- Wu J, Mei Y, Li X, et al. PRCP is a promising drug target for intracranial aneurysm rupture supported via multi-omics analysis. *Stroke Vasc Neurol* 2024; 10: e003076.
- Li X, Shen A, Zhao Y, Xia J. Mendelian randomization using the druggable genome reveals genetically supported drug targets for psychiatric disorders. *Schizophr Bull* 2023; 49: 1305-1315.
- Sun J, Zhao J, Jiang F, et al. Identification of novel protein biomarkers and drug targets for colorectal cancer by integrating human plasma proteome with genome. *Genome Med* 2023; 15: 75.
- Freshour SL, Kiwala S, Cotto KC, et al. Integration of the drug-gene interaction database (DGIdb 4.0) with open crowdsourcing efforts. *Nucleic Acids Res* 2021; 49: D1144-d51.
- Finan C, Gaulton A, Kruger FA, et al. The druggable genome and support for target identification and validation in drug development. *Sci Transl Med* 2017; 9: eaag1166.
- Burgess S, Scott RA, Timpson NJ, et al. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *Eur J Epidemiol* 2015; 30: 543-552.
- Liu Z, Peng Z, Lin H, et al. Identifying potential drug targets for idiopathic pulmonary fibrosis: a mendelian randomization study based on the druggable genes. *Respir Res* 2024; 25: 217.
- Gkatzionis A, Burgess S, Newcombe PJ. Statistical methods for cis-Mendelian randomization with two-sample summary-level data. *Genet Epidemiol* 2023; 47: 3-25.
- Burgess S, Dudbridge F, Thompson SG. Combining information on multiple instrumental variables in Mendelian random-

- ization: comparison of allele score and summarized data methods. *Stat Med* 2016; 35: 1880-1906.
20. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol* 2016; 40: 304-314.
21. Foley CN, Staley JR, Breen PG, et al. A fast and efficient colocalization algorithm for identifying shared genetic risk factors across multiple traits. *Nat Commun* 2021; 12: 764.
22. Zhang LY, Chu YH, You YF, et al. Systematic druggable genome-wide mendelian randomization identifies therapeutic targets for functional outcome after ischemic stroke. *J Am Heart Assoc* 2024; 13: e034749.
23. Wu Y, Zeng J, Zhang F, et al. Integrative analysis of omics summary data reveals putative mechanisms underlying complex traits. *Nat Commun* 2018; 9: 918.
24. Orrù V, Steri M, Sidore C, et al. Complex genetic signatures in immune cells underlie autoimmunity and inform therapy. *Nat Genet* 2020; 52: 1036-1045.
25. Chen Y, Lu T, Pettersson-Kymmer U, et al. Genomic atlas of the plasma metabolome prioritizes metabolites implicated in human diseases. *Nat Genet* 2023; 55: 44-53.
26. Qin Y, Havulinna AS, Liu Y, et al. Combined effects of host genetics and diet on human gut microbiota and incident disease in a single population cohort. *Nat Genet* 2022; 54: 134-142.
27. Zhao JH, Stacey D, Eriksson N, et al. Genetics of circulating inflammatory proteins identifies drivers of immune-mediated disease risk and therapeutic targets. *Nat Immunol* 2023; 24: 1540-1551.
28. Carter AR, Sanderson E, Hammerton G, et al. Mendelian randomisation for mediation analysis: current methods and challenges for implementation. *Eur J Epidemiol* 2021; 36: 465-478.
29. Ding J, Liu H, Zhang X, et al. Integrative multiomic analysis identifies distinct molecular subtypes of NAFLD in a Chinese population. *Sci Transl Med* 2024; 16: eadh9940.
30. Ma J, Li Y, Chen M, et al. hMSCs-derived exosome circCDK13 inhibits liver fibrosis by regulating the expression of MFGE8 through miR-17-5p/KAT2B. *Cell Biol Toxicol* 2023; 39: 1-22.
31. Kotsiliti E, Leone V, Schuehle S, et al. Intestinal B cells license metabolic T-cell activation in NASH microbiota/antigen-independently and contribute to fibrosis by IgA-FcR signalling. *J Hepatol* 2023; 79: 296-313.
32. Zhang L, Zhao C, Dai W, et al. Disruption of cholangiocyte-B cell crosstalk by blocking the CXCL12-CXCR4 axis alleviates liver fibrosis. *Cell Mol Life Sci* 2023; 80: 379.
33. Kang X, Chen S, Pan L, et al. Deletion of Mettl3 at the pro-B stage marginally affects B cell development and profibrogenic activity of B cells in liver fibrosis. *J Immunol Res* 2022; 2022: 8118577.
34. Lu Y, Li M, Zhou Q, et al. Dynamic network biomarker analysis and system pharmacology methods to explore the therapeutic effects and targets of Xiaoyaosan against liver cirrhosis. *J Ethnopharmacol* 2022; 294: 115324.
35. Mells GF, Floyd JA, Morley KI, et al. Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis. *Nat Genet* 2011; 43: 329-332.
36. Liang QS, Xie JG, Yu C, et al. Splenectomy improves liver fibrosis via tumor necrosis factor superfamily 14 (LIGHT) through the JNK/TGF- β 1 signaling pathway. *Exp Mol Med* 2021; 53: 393-406.
37. Wu Y, Zhan S, Chen L, et al. TNFSF14/LIGHT promotes cardiac fibrosis and atrial fibrillation vulnerability via PI3K γ /SGK1 pathway-dependent M2 macrophage polarisation. *J Transl Med* 2023; 21: 544.
38. da Silva Antunes R, Mehta AK, Madge L, et al. TNFSF14 (LIGHT) exhibits inflammatory activities in lung fibroblasts complementary to IL-13 and TGF- β . *Front Immunol* 2018; 9: 576.
39. Li Y, Tang M, Han B, et al. Tumor necrosis factor superfamily 14 is critical for the development of renal fibrosis. *Aging (Albany NY)* 2020; 12: 25469-25486.
40. Zhong XY, Xu XX, Yu JH, et al. Clinical and biological significance of Cdk10 in hepatocellular carcinoma. *Gene* 2012; 498: 68-74.
41. Kim DH, Kim WD, Kim SK, et al. TGF- β 1-mediated repression of SLC7A11 drives vulnerability to GPX4 inhibition in hepatocellular carcinoma cells. *Cell Death Dis* 2020; 11: 406.
42. Han H, Zhang C, Shi W, et al. NSUN5 facilitates hepatocellular carcinoma progression by increasing SMAD3 expression. *Adv Sci (Weinh)* 2024; 2024: e2404083.
43. Yao J, Zhang X, Li J, et al. Silencing TRIP13 inhibits cell growth and metastasis of hepatocellular carcinoma by activating of TGF- β 1/smad3. *Cancer Cell Int* 2018; 18: 208.
44. Nahon P, Sutton A, Rufat P, et al. Myeloperoxidase and superoxide dismutase 2 polymorphisms comodule the risk of hepatocellular carcinoma and death in alcoholic cirrhosis. *Hepatology* 2009; 50: 1484-1493.
45. Nahon P, Sutton A, Rufat P, et al. A variant in myeloperoxidase promoter hastens the emergence of hepatocellular carcinoma in patients with HCV-related cirrhosis. *J Hepatol* 2012; 56: 426-432.
46. Wang C, Zheng X, Zhang J, et al. CD300ld on neutrophils is required for tumour-driven immune suppression. *Nature* 2023; 621: 830-839.
47. Zhang LZ, He X, Zhu HD. Targeting CD300ld to normalize the tumor microenvironment: an emerging insight in cancer immunotherapy. *MedComm (2020)* 2024; 5: e607.