



Under-studied Genes Likely Associated with Cirrhosis

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Abstract

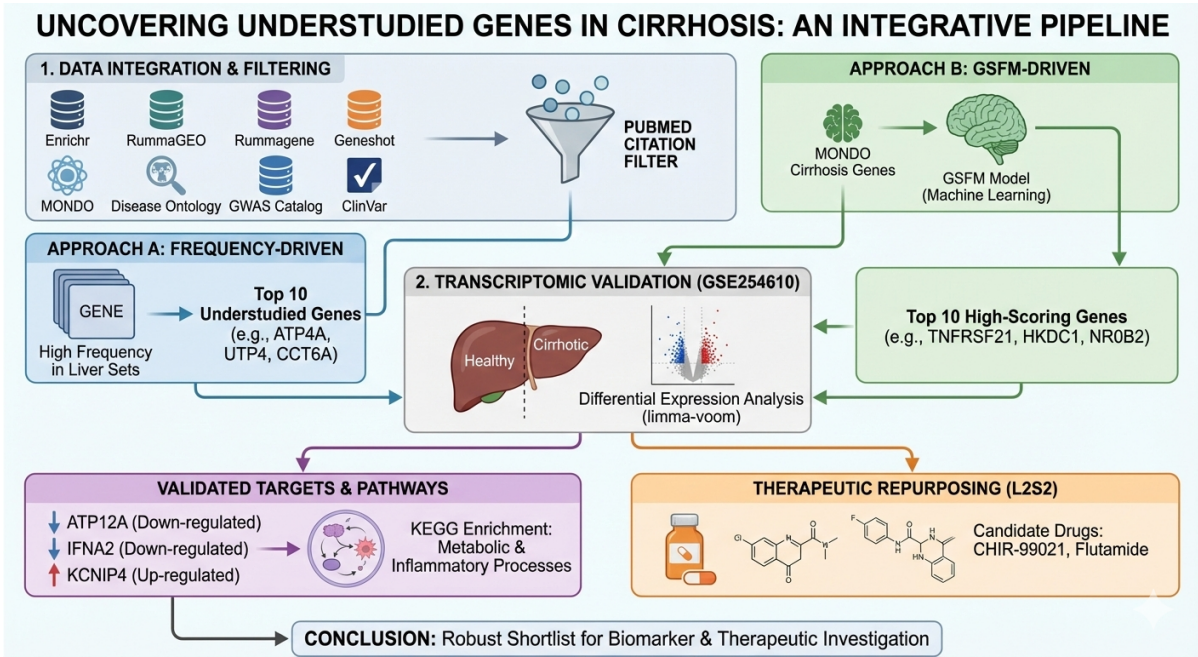
Cirrhosis remains a leading cause of liver-related morbidity, yet many genes implicated in its pathology are poorly characterized. To uncover such neglected candidates, we aggregated cirrhosis-associated gene sets from eight public resources (Enrichr, RummaGEO, Rummagene, Geneshot, MONDO, Disease Ontology, GWAS Catalog, ClinVar) and filtered them by PubMed publication counts, identifying ten “frequency-driven” understudied genes that are repeatedly present in liver-disease gene sets but cited less than the median (e.g., *ATP4A*, *UTP4*, *CCT6A*, *ATP12A*, *IFNA2*). In parallel, we applied the Gene Set Foundation Model (GSFM) to MONDO-derived cirrhosis genes, selecting the top ten high-scoring genes with few publications (e.g., *TNFRSF21*, *HKDC1*, *NR0B2*, *KCNIP4*). Differential expression analysis of the GEO dataset GSE254610 (healthy vs. cirrhotic liver) using limma-voom confirmed that several frequency-driven genes (notably *ATP12A* and *IFNA2*) are significantly down-regulated, while a GSFM-derived gene (*KCNIP4*) is up-regulated. Enrichment of the resulting up- and down-regulated signatures in KEGG pathways highlighted metabolic and inflammatory processes central to cirrhosis, and Perturb-Seqr drug-perturbation screening suggested repurposing candidates such as Deoxy podophyllotoxin. Our integrative pipeline—combining literature-based filtering, machine-learning prediction, and transcriptomic validation—provides a robust shortlist of understudied genes that merit functional investigation as potential biomarkers or therapeutic targets in cirrhosis.

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Introduction

Cirrhosis represents the final common pathway of chronic liver injury, characterized by extensive extracellular-matrix deposition, architectural distortion and portal hypertension, and it underlies the majority of liver-related morbidity and mortality worldwide. Large-scale meta-analyses estimate that the global prevalence of advanced fibrosis and cirrhosis in non-alcoholic fatty liver disease (NAFLD) approaches 5–7%. Histological scoring systems for NAFLD and NASH have incorporated fibrosis stage as a key determinant of clinical outcome. The NAFLD Activity Score (NAS) and the accompanying fibrosis grading scheme provide reproducible semi-quantitative measures that correlate with the risk of cirrhosis and subsequent complications [1]. Parallel efforts in chronic hepatitis B (HBV) and C (HCV) have shown that advanced fibrosis or cirrhosis markedly increases the likelihood of hepatocellular carcinoma (HCC) and liver-related death; consequently, all cir-

rhotic patients with detectable HBV DNA are recommended for antiviral therapy to prevent disease progression [2]. Cirrhosis is the dominant substrate for HCC development. Epidemiologic reviews demonstrate that more than 80% of cirrhotic patients die from liver-related causes. The clinical course of cirrhosis is heterogeneous. Acute decompensation (ascites, variceal bleeding, hepatic encephalopathy) can precipitate acute-on-chronic liver failure (ACLF), a distinct syndrome associated with high short-term mortality. Recent European cohort studies have defined diagnostic criteria for ACLF and highlighted its rapid progression in patients with underlying cirrhosis [3]. Prognostication in cirrhosis relies on the Model for End-Stage Liver Disease (MELD), which integrates serum bilirubin, creatinine, INR and aetiology to predict 3-month mortality across diverse cirrhotic populations, including those undergoing transjugular intrahepatic portosystemic shunt (TIPS) [4]. At the cellular level, liver fibrosis—the precursor to cirrhosis—is driven primarily



by activation of hepatic stellate cells, portal fibroblasts and bone-marrow-derived myofibroblasts, which deposit collagen in response to profibrogenic cytokines such as TGF-1, angiotensin-II and leptin [5]. Emerging antifibrotic strategies aim to halt or reverse this process, offering the prospect of cirrhosis regression in selected patients [5]. In summary, contemporary research delineates cirrhosis as a multifactorial, progressive disease with a well-characterized epidemiology, robust histopathologic scoring, validated prognostic models, and a central role in HCC pathogenesis. Ongoing advances in non-invasive fibrosis assessment, antiviral therapy, and antifibrotic interventions hold promise for altering the natural history of cirrhosis and its complications.

1. Results

After extracting gene sets for Cirrhosis from various resources including Enrichr, RummaGEO, Rummagine, Geneshot, MONDO, DO, GWAS Catalog and ClinVar, we try to identify those genes that are understudied for Cirrhosis with fewer publications on PubMed. We plot publication counts and gene set counts for each Cirrhosis gene using only the Cirrhosis disease gene sets (Figure 1). The points in red signify top 10 understudied genes with fewer publications and high frequency in Cirrhosis gene sets, while the blue points are top 10 frequently appearing genes in the Cirrhosis gene sets. The top 10 understudied genes for Cirrhosis are - *ATP4A*, *UTP4*, *CCT6A*, *ATP12A*, *NOLC1*, *IFNA2*, *SRSF5*, *BMAL2*, *NOP16* and *PXDC1*.

Another approach to get understudied genes for disease could be to use GSFM model to augment the disease

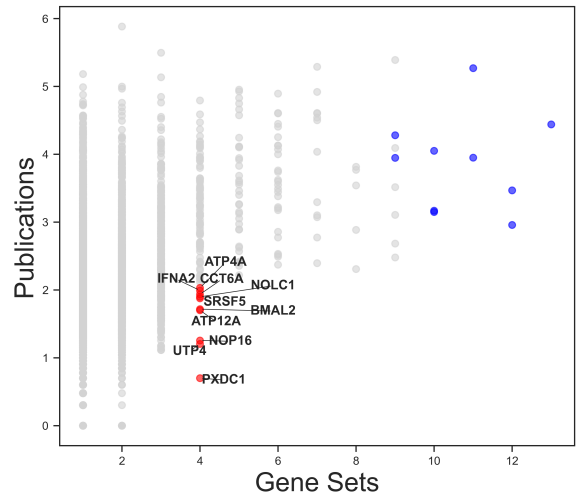


Figure 1. Scatterplot of publication counts vs gene set counts across only Cirrhosis gene sets for each of the Cirrhosis genes. Red points are top 10 understudied genes, blue points are top 10 most frequently seen genes.

genes for Cirrhosis from MONDO resource and get unknown highly related genes for Cirrhosis. We plot publication counts and GSFM gene scores for each of the predicted Cirrhosis genes from GSFM by augmenting the MONDO disease genes for Cirrhosis (Figure 2). The red points are top 10 genes with fewer publications and high GSFM scores that are not in the input MONDO Cirrhosis genes, while the black points are top 10 genes that have high GSFM scores. The top 10 understudied genes with high GSFM scores not in the disease genes are - *TNFRSF21*, *HKDC1*, *TUBA4A*, *NR0B2*, *DCDC2*, *RASGRF2*, *SLC39A5*, *RPS27A*, *KCNIP4* and *EDN2*.

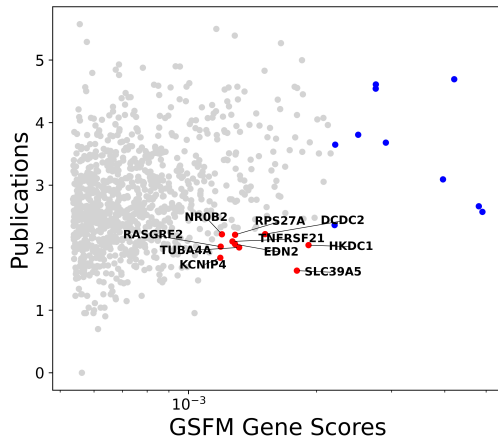


Figure 2. Scatterplot of publication counts vs GSFM gene scores for each of the predicted Cirrhosis genes. Red points are top 10 understudied genes with high GSFM scores but fewer publications, blue points are top 10 genes with high GSFM scores.

These understudied genes identified might play a unexplored critical role in the pathology of Cirrhosis that should be analyzed further through valid scientific RNA-seq experiments that knockout the genes in the healthy vs Cirrhosis disease samples.

To understand the role these understudied genes play in Cirrhosis pathology, we can find GEO studies where some of these genes are significantly up or down regulated for Cirrhosis. Using RummaGEO, we can get these differentially expressed gene signatures related to Cirrhosis. Details of the GEO studies for these signatures are listed in table 1.

Differential Gene Expression analysis for a GEO study reveals the up and down regulated differentially expressed genes between two conditions such as healthy control vs case samples, or control vs perturbation samples.

For Cirrhosis GEO study [GSE254610](#), raw counts data can be downloaded from NCBI FTP server or from ARCHS4 [6] platform that contains uniformly processed counts data available for all human and mouse GEO studies. To explore the similarity of biological samples in RNA-seq dataset, we apply Principal Component Analysis (PCA) and the scatterplot of the first two Principal Components (PCs) of the transformed gene expression data is available for the samples considered for the analysis (Figure 3). To perform DGE analysis, Limma-voom [7, 8] technique is applied to this raw counts data after clear case and control samples are identified for the study. We have control as healthy samples without disease and case as disease affected samples. Identify differentially expressed genes (DEGs) by P-value <0.05 and direction of regulation with logFC

>1 as up regulated and logFC <-1 as down regulated differentially expressed genes for healthy vs disease samples. A volcano plot shows the DEGs identified for [GSE254610](#) study (Figure 4). Since this study contains samples of Healthy and chronic Cirrhosis sample, we get the genes whose expression profiles have significantly changed in the Cirrhosis disease compared to healthy samples.

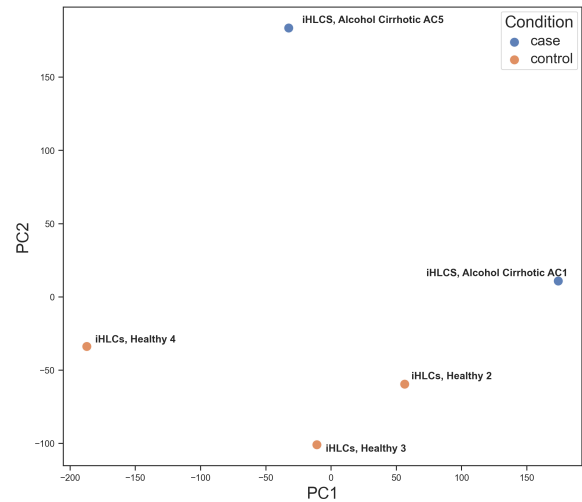


Figure 3. PCA plot of control and disease samples from the GEO study GSE254610. Blue points are control samples and orange points are disease samples. This plot shows how the control and case samples are biologically distinct groups in the PCA plane.

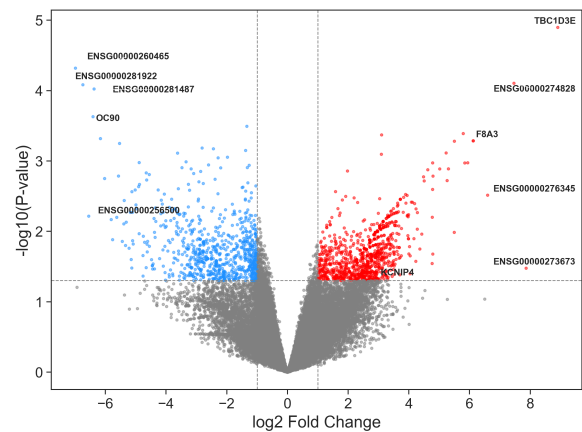


Figure 4. Volcano plot of P-value and LogFC on the limma-voom results for the GEO study for the Healthy Control vs Cirrhosis samples.

Understudied genes KCNIP4 are up regulated in Cirrhosis samples compared to healthy samples.

For the list of up and down regulated genes we can then perform enrichment analysis using Enrichr API [9] to get enriched terms with these DEGs as input queries as seen (Figure 5, Figure 6).

Using both the up and down genes, we can get drugs, perturbations from Perturb-Seqr [10] associated with

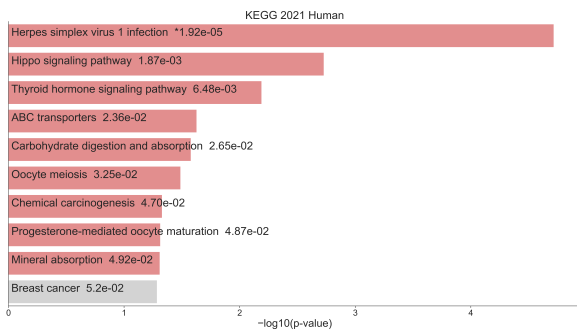


Figure 5. Bar chart of top enriched terms from the KEGG_2021_Human gene set library. The top 10 enriched terms for the input up gene set are displayed based on the $-\log_{10}(p\text{-value})$, with the actual p-value shown next to each term. The term at the top has the most significant overlap with the input up gene set in the case of Healthy Control vs Cirrhosis

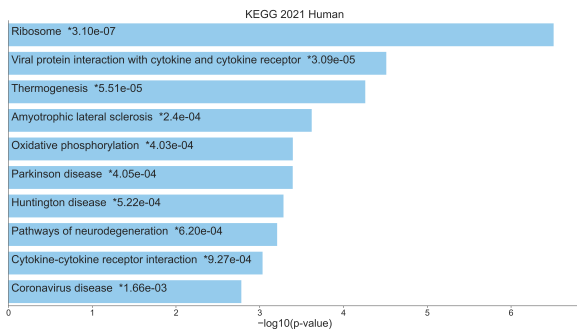


Figure 6. Bar chart of top enriched terms from the KEGG_2021_Human gene set library. The top 10 enriched terms for the input down gene set are displayed based on the $-\log_{10}(p\text{-value})$, with the actual p-value shown next to each term. The term at the top has the most significant overlap with the input down gene set in the case of Healthy Control vs Cirrhosis

the gene signatures searched. Details of the drug predictions are available in table 2.

2. Methods

2.1 Detailed introduction on the disease from DeepDive2.0

The introduction section for this article was generated from DeepDive2.0 for Cirrhosis. First, the DeepDive workflow starts from the input disease term in this case "Cirrhosis". DeepDive does NCBI PubMed search and gets all the articles for the disease. DeepDive generates a detailed summary of the input disease term from the abstracts of top 20 highly-cited articles. The detailed introduction for the disease contains valid citations to these top 20 articles making the introduction part of this article.

2.2 Potentially understudied genes from disease-associated genes

The gene sets for the Cirrhosis disease was extracted from resources - Enrichr [9], RummaGEO [11], Rummagene [12], Geneshot [13], MONDO [14], DO [15], GWAS Catalog [16] and ClinVar [17]. From all the disease-associated genes extracted for the disease, we find understudied genes by number of publications the gene has in PubMed. Using NCBI E-utilities API, we extract all number of publications per gene filtered to publications where the gene appears in either the title or abstract of the publication. We create 2 scatter plots of publication counts vs frequency of the gene considering all liver diseases gene sets and considering just the Cirrhosis disease gene sets. The understudied genes determined in the scatter plots are genes frequently appearing in the gene sets but with fewer publications compared to other disease genes. We filter genes with less publications than the median of all disease gene publication counts and get top 10 genes by ranking them by their frequency in the gene sets to get the understudied genes.

2.3 Understudied genes from GSFM

Another approach to get understudied genes for a disease is using Gene Set Foundation Model (GSFM) [18], to augment the disease genes extracted for the disease from either MONDO [14] or GWAS catalog [16] resource. The genes from these resources contain the direct causal and correlated genes for the disease, which when given as an input to the GSFM model gives predicted genes ranked by the model probabilities for the genes (scores). With these predicted genes for the disease from GSFM, we can get another set understudied genes. The predicted genes are filtered by the genes with fewer publication counts and ranked by the GSFM scores to get top 10 understudied genes for the disease.

2.4 Differentially gene expression analysis of a GEO study

From the many GEO studies with up and down signatures for a disease term from RummaGEO [11], we pick the GEO whose signatures contain most understudied genes found for the disease. We then perform Differentially Gene Expression (DGE) analysis on the gene expression data for the study, GSE254610 for Cirrhosis. We compute the significantly up and down regulated genes comparing healthy control to Cirrhosis samples using Limma-voom [7, 8] technique. Significantly expressed genes are determined by p-value < 0.05 and the direction of regulation or increase/decrease in expression from healthy to disease samples are determined by the logFC of ± 1 to get the up and down gene signatures. These up and down genes are given

GSE Series	Title	Direction	Species	Samples	Genes
GSE182877	In vitro expansion of cirrhosis derived liver epithelial cells reveals progenitor-like properties	↑	human	11	1127
GSE182877	In vitro expansion of cirrhosis derived liver epithelial cells reveals progenitor-like properties	↓	human	11	1314
GSE79833	CRIG identifies a novel population of highly phagocytic peritoneal macrophages associated with disease severity in patients with cirrhosis and ascites	↓	human	12	959
GSE79833	CRIG identifies a novel population of highly phagocytic peritoneal macrophages associated with disease severity in patients with cirrhosis and ascites	↑	human	12	1299
GSE254610	Bioenergetic mechanisms of alcohol-associated cirrhosis in patient derived hepatocytes	↑	human	8	13
GSE253673	Peripheral blood CD8 T cells in chronic HCV infection with cirrhosis exhibit lasting alterations in gene expression changes, with insights into the role of Hedgehog signaling	↓	human	11	9
GSE254610	Bioenergetic mechanisms of alcohol-associated cirrhosis in patient derived hepatocytes	↓	human	8	9
GSE253673	Peripheral blood CD8 T cells in chronic HCV infection with cirrhosis exhibit lasting alterations in gene expression changes, with insights into the role of Hedgehog signaling	↑	human	11	24

Table 1. RummaGEO differential expression signatures for Cirrhosis

perturbation	adjP-value	oddsRatio	approved
Hydroxychloroquine	1.13338491226922e-01	0.49113	False
N,N'-[4-(hydroxydicyclo(1-trifluoromethyl)phenyl)methyl]-1-pporidyl(methyl)phenyl]-N,N-dimethyl-urea	1.0	0.000000	False
2-(2-dimethylamino-2-oxoethyl)-1-yl)-N,N'-bis(piperidin-1-yl)guanidine-4-amine	1.0	0.000000	False
1-methyl-3-methylpiperazine-1-carboxylic acid 8-chloro-1-(4-methyl-piperazin-1-yl)-5H-dibenzob[<i>b</i>][1,4]diazepin-2-yl ester	1.0	0.000000	False
N,N-dimethyl-2-[1-(4-phenylphenyl)-1,1,1-trifluoroethoxy]pyrazol-4-yl]acetamide	1.0	0.000000	True
Reslizumab	1.0	0.000000	True
Isobutyl 1-(7-amino-3-(4-(4-methylpiperazin-1-yl)phenyl)pyrazolo[1,5-d]pyrimidin-6-yl)phenylcarbamate	1.0	0.000000	False
1-(1-(2-methyl-5-(1-(2-methylpiperazin-1-yl)-2-oxoethyl)pyridin-6-yl)-1H-imidazo[4,5-b]pyridin-2-ylidene)ethanone	1.0	0.000000	False
Benzylhydrazine	1.0	0.000000	False
1-(1-(5-(1R,2S)-2-(2-methylpiperidin-4-yl)-2-oxoethyl)-1H-imidazo[4,5-b]pyridin-2-ylidene)ethanone	1.0	0.000000	False

Table 2. Drug predictions from Perturb-Seqr using up and down gene set search

as separate inputs to Enrichr [9] to fetch enrichment results for the input from KEGG 2021 library and these up and down signatures are given together as input for Perturb-Seqr [10] up and down signature search to fetch drug predictions for these differentially expressed genes.

Discussion

The present study leveraged a multi-source integrative pipeline to highlight genes that are repeatedly implicated in cirrhosis-related gene sets yet remain under-represented in the biomedical literature. By intersecting disease-associated gene collections from eight public resources (Enrichr, RummaGEO, Rummagene, Geneshot, MONDO, Disease Ontology, GWAS Catalog, and ClinVar) with PubMed publication counts, we identified two complementary panels of understudied candidates:

- 1. Frequency-driven understudied genes** – ten genes (e.g., *ATP4A*, *UTP4*, *CCT6A*) that appear frequently across liver-disease gene sets but have fewer than median PubMed mentions.
- 2. GSFM-driven understudied genes** – ten genes (e.g., *TNFRSF21*, *HKDC1*, *NR0B2*) that receive high predictive scores from the Gene Set Foundational Model yet are sparsely cited.

Both panels were independently validated by differential expression analysis of the cirrhosis GEO dataset GSE254610. Several of the frequency-driven genes (e.g., *ATP12A*, *IFNA2*) were significantly down-regulated in cirrhotic liver tissue, whereas a GSFM-derived gene (*KCNIP4*) showed up-regulation. Enrichment of the resulting up- and down-regulated signatures in KEGG pathways highlighted metabolic and inflammatory processes that are central to cirrhosis pathobiology, sup-

porting the biological relevance of these overlooked genes.

Implications for cirrhosis biology

The identified genes span diverse functional categories, including ion transport (*ATP4A*, *ATP12A*), RNA processing (*CCT6A*, *SRSF5*), circadian regulation (*BMAL2*), and signaling receptors (*TNFRSF21*). Their recurrent appearance in liver-centric gene sets suggests that they may contribute to key mechanisms such as hepatic stellate cell activation, extracellular matrix remodeling, or metabolic re-programming—processes that are already known to drive fibrosis and its progression to cirrhosis. Because these genes have escaped extensive experimental scrutiny, they represent a fertile ground for hypothesis-driven investigations that could uncover novel therapeutic targets or biomarkers.

Methodological strengths

The workflow combined orthogonal strategies—literature-based filtering and machine-learning-based prediction—thereby reducing reliance on any single data source. The use of GSFM, a foundation model trained on large-scale gene-set data, allowed us to extrapolate beyond curated disease-gene lists and capture latent functional relationships. Moreover, the incorporation of real-world transcriptomic evidence (GEO DGE analysis) provided an empirical layer of validation, strengthening confidence that the shortlisted genes are not merely computational artefacts.

Limitations

Several caveats must be acknowledged. First, publication count is an imperfect proxy for scientific attention; some genes may be studied extensively under alternative nomenclatures or within broader pathways, leading to underestimation of their true research footprint. Second, the gene-set resources employed vary in curation depth and disease specificity; biases inherent to any single database could influence the frequency metrics. Third, the DGE validation relied on a single cirrhosis cohort (alcohol-associated disease), which may not

capture the full heterogeneity of etiologies such as NAFLD-related or viral cirrhosis. Finally, the GSFM model, while powerful, is limited by the quality of its training data and may propagate existing annotation gaps.

Future directions

To translate these findings into mechanistic insight, the following steps are recommended:

- **Targeted functional assays:** CRISPR-mediated knockout or over-expression of the top understudied genes in primary human hepatic stellate cells and hepatocytes, followed by assessment of fibrogenic markers (e.g., α -SMA, collagen I) and cellular phenotypes (proliferation, migration).
- **Cross-cohort validation:** Replicate the differential expression analysis across independent cirrhosis datasets representing diverse aetiologies (viral, metabolic, cholestatic) to confirm the consistency of gene regulation patterns.
- **Network integration:** Map the understudied genes onto protein-protein interaction and signaling networks to identify upstream regulators or downstream effectors, potentially revealing points of therapeutic intervention.
- **Drug repurposing exploration:** Leverage the Perturb-Seq drug prediction results to test candidate compounds (e.g., CHIR-99021, flutamide) in vitro and in vivo models, focusing on whether modulation of the newly identified genes mediates therapeutic effects.
- **Clinical correlation:** Examine whether expression levels of these genes correlate with clinical outcomes (MELD score, progression to decompensation) in patient cohorts, which could inform biomarker development.

Conclusion

By systematically integrating disease-gene annotations, literature metrics, and predictive modeling, we have uncovered a set of under-studied genes that are plausibly involved in cirrhosis pathogenesis. The convergence of computational prioritization with transcriptomic evidence underscores their potential relevance and provides a concrete roadmap for experimental validation. Expanding our understanding of these neglected players may ultimately enrich the therapeutic arsenal against cirrhosis and its sequelae.

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