Identification of Key Genes as Biomarkers for Autoimmune Hepatitis Using Comprehensive Bioinformatics Analysis

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ABSTRACT

Background: Autoimmune hepatitis(AIH) is a chronic progressive inflammatory disease of the liver. Although several genetic factors have been reported in the pathogenesis of AIH, many details remain unknown.

Methods: GSE140249 and GSE159676 datasets were downloaded from the GEO dataset. The Weighted Gene Co-expression Network Analysis (WGCNA) and LASSO algorithm were used to identify the miRNAs closely related to the occurrence of AIH. Both miRBase and miRWalk databases were used to predict potential mRNAs regulated by AIH related miRNAs, and the intersection was regarded as candidate genes.

Results: A total of 470 differentially expressed(DE) miRNAs were identified in GSE140249. The brown module of WGCNA was considered as the most critical module related to the occurrence of AIH, containing 100 miRNAs. The LASSO further identified thirteen miRNAs. Combined with the DE mRNAs in GSE159676, fourteen mRNAs were identified. Then, the ten candidate genes were found to be enriched in gene ontology (GO) database, mainly in biological process and cell components. In addition, seven pivotal genes, DYNC1H1, ALG1, PTPRB, SMAD2, MICA, GP2 and SPN were identified, and their otherness was confirmed in rat AIH model.

Conclusions: The integrative analysis firstly revealed that miRNA-mRNA might be a crucial susceptible factor for AIH and identified that DYNC1H1, ALG1, PTPRB, SMAD2, MICA and GP2 could be used as potential therapeutic targets and diagnostic biomarker.

Keywords: Autoimmune Hepatitis; Diagnostic Biomarker; WGCNA; LASSO; Bioinformatics Analysis

Abbreviations: AIH: Autoimmune Hepatitis; WGCNA: The Weighted Gene Co-expression Network Analysis; DE: Differentially Expressed; GO: Gene Ontology; GEO: Gene Expression Omnibus; LASSO: Least Absolute Shrinkage and Selection Operator; DEGs: Differentially Expressed Genes; FC: Fold Change; MM: Module Membership; GS: Gene Significance; MF: Molecular Function; BP: Biological Process; AST: Aspartate Transaminase; CC: Cellular Component; SD: Standard Deviation; EMT: Epithelial-Mesenchymal Transition; LIHC: Liver Hepatocellular Carcinoma; CDG: Congenital Disorders Of Glycosylation

Introduction

Autoimmune Hepatitis (AIH) is a world public health problem with a high risk of progression to liver cirrhosis [1]. It is mainly characterized by elevated autoantibodies, hyper gamma-globulinaemia and interface hepatitis [2]. AIH imposes a major health burden globally, owing to its high incidence in developed countries and the increasing prevalence in developing countries [3]. The pathophysiology of AIH is multifaceted and not completely clear. Environmental factors, genetic susceptibility, molecular mimicry and dysfunctional immune responses have been suggested to contribute to AIH pathogenesis [4]. Generally speaking, genetic susceptibility is considered to be the most common pathogenic factor leading to AIH [5]. Furthermore, some studies have been conducted in a large sample to explore new and existing AIH gene biomarkers [6]. A number of different methodologies are used to identify AIH related candidates, ranging from single-gene studies to genomics analyses [7]. It is worth considering that new methodologies are developed to explore more AIH biomarkers, which will be helpful to understand the biology of AIH by using genetic, epigenetic, and transcriptome investigations. Another area of concern is that AIH is often found in the stage of cirrhosis, missed the optimal treatment opportunity. Most patients with AIH respond well to standard immunosuppressive therapy with steroids and azathioprine, while about 10%-20% of patients have insufficient response to standard treatment or have intolerable side effects [8,9].

Once diagnosed with the end stage of liver cirrhosis, liver transplantation is regarded as the final treatment scheme [10]. There is an urgent need to identify new biomarkers in patients with AIH to better detect people at early stage of AIH, so that effective therapies can be used to curb disease progression. The bioinformatic analysis of miRNA/mRNA sequences draws increasing attention in non-tumor disease research recently [11]. A series of online databases and tools have contributed a lot to understanding and predicting disease development. The Gene Expression Omnibus (GEO) database freely distribute high-throughput molecular abundance data [12]. The miRbase database is a searchable database of published miRNA sequences and annotation [13]. And the miRWalk database stores experimentally verified miRNA-target interactions [14]. These databases provide access to publicly available data for researchers. Weighted Gene Co-expression Network Analysis (WGCNA), as a method to screen disease-related modules, is the most common and useful method for discovering the link between key genes and clinical characteristics [15]. The least absolute shrinkage and selection operator (LASSO) is another algorithm to obtain a more refined model by constructing a penalty function [16]. However, there are still little researches on AIH. Here, the key genes are screened by differential expression miRNAs analysis, and WGCNA combined with LASSO, in order to explore the key genes associated with AIH.

These miRNA target mRNAs are intersected with the differentially expression mRNA in another dataset. For the obtained mRNAs, it is further validated the key genes in the rat AIH model, so as to explore the critical genes and provide candidate markers for the diagnosis of AIH.

Materials and Methods

Datasets

GSE140249 (platform: GPL21263) [17] and GSE159676 (platform: GPL6244) [18] were downloaded by "GEOquery" R package from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). The dataset GSE140249 contains 49 AIH human serum samples and 27 normal human serum samples, which tested the microarray through non-coding RNA profiling. The dataset GSE159676 contains 3 AIH liver tissue samples and 6 normal human liver tissue samples, which contains the mRNA array using the Affymetrix Human Gene 1.0 st. It is worth mentioning that we followed the methods of Jianbo Qing, et al. 2022.

Differentially Expressed Genes Analysis

Differentially expressed genes (DEGs) were identified by "edgeR" R package in R Studio software (Version: R 3.5.1). It is considered DEGs as FDR <0.05 and |log2Fold Change (FC)| > 1.5, in which log-2FC > 1.5 thinks that the expression of gene is up-regulated, and log-2FC<-1.5 thinks that the expression of gene is down-regulated. DEGs were visualized as volcano and heatmap by the "ggplot2" package in R Studio software.

Weighted Gene Co-Expression Network Analysis

Weighted Gene Co-Expression Network Analysis (WGCNA) is a well-established method for analyzing the relationship between diseases phenotype and genes. The dataset GSE140249, which had a large sample size, was chosen to find key genes related AIH phenotype by WGCNA in R studio. The cluster diagram was drawn to select the appropriate threshold to eliminate outliers. A scale-free network was constructed by calculating different power value (range from 1 to 20), and the suitable power value was chosen when scale free R2 was 0.8. Subsequently, we performed the cluster analysis to recognize the highly similar modules and the min module size set as 30. The connection between modules and traits were calculated to identify the key trait-module genes. In the module-trait analysis, the module genes were shown with module membership (MM) and gene significance (GS), so as to analysis the significance of high connectivity genes in modules.

miRNA Prediction

miRNA-mRNA target regulation network was predicted by miRbase (https://www.mirbase.org/) and miRwalk (http://mirwalk.umm.uni-heidelberg.de/) database. The miRNAs of interation in databases above were as the predicted miRNA targeted mRNA. The miRNA-gene network was visualized by Cytoscape software (Version: 3.9.1).

Functional Enrichment Analysis

Online tool DAVID (https://david.ncifcrf.gov/) was used to perform the Gene Ontology (GO) with the threshold of P-value <0.05 [19]. GO analysis including molecular function (MF), biological process (BP) and Cellular Component (CC). The records of GO analysis were visualized as chord chart by "ggplot2" R package.

Animal Model of Autoimmune Hepatitis

SD Rats were acquired by the Spelfor (Beijing) biotechnology company. 5-6 rats were group housed in a temperature- and humidity-controlled, specific-pathogen-free animal facility at 25℃ under a 12 h–12 h light–dark cycle with free access to food and water. For the AIH study, rats (aged 6-8 weeks) were injected with ConA (16mg/kg) through tail vein injection to establish AIH model. Rat in the control group received an equal amount of 0.9% normal saline. Rats anesthetized with pentobarbital sodium were sacrificed after 16-24h treatment, the blood and liver tissues were collected. Serum alanine aminotransferase (ALT) and aspartate transaminase (AST) levels were measured using ALT/AST detection kit (Applygen, E2021/E2023). The liver tissues were obtained for hematoxylin and eosin (H&E) staining and RT-qPCR analysis.

RNA Extraction and RT-qPCR

RNA was extracted from the liver tissues using TRIzol reagent (Dakewe Biotech Co., 8034011) and reverse-transcribed to cDNA using the Takara PrimeScriptTM RT reagent Kit (Takara, RR037A). Primer sequences were obtained from PrimerBank (https://pga.mgh.harvard.edu/ primerbank/) and synthesized at SYNBIO Technologies. Ltd. (Suzhou, China) (Table 1). RT-qPCR was performed using the Applied Biosystems™ PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific, A25776) on an ABI Applied Biosystems. RT-qPCR amplification was conducted in triplicate for each sample, and the expression of target genes was normalized to GAPDH. Relative expression was determined using the 2-△△ Ct method. Each experiment was repeated more than three times.

Table 1: Primers for RT-qPCR.

Gene	Primer	Sequence (5'- >3')
DYNC1H1	Forward	GGGAAGGAGATCGTGCTGAG
	Reverse	CAGCCAAATGCCAAACTGCT
ALG1	Forward	CACGACATGTGGTCGTTGTG
	Reverse	CCCCGCAGATCTGTCAACTT
PTPRB	Forward	TGGCCTCGGGACTACAAAAC
	Reverse	TTGGTGAGACAGGGTGATGC

TRAM2	Forward	GTGCTGACCGACAGACAAGA
	Reverse	CAAGTGGGAAGGCACATCCT
SPN	Forward	GTCCTGACAAAGCCTCAGGAA
	Reverse	GGCTTCAGAGGTACTTGGGG
SMAD2	Forward	GCCGCCCGAAGGGTAGAT
	Reverse	TTCTGTTCTCCACCACCTGC
AP3M2	Forward	AGAAAGCGCATCGTGTATGTT
	Reverse	CTCTGGATTTGGCTAGACCCATTA
GP2	Forward	GCCCATCGTAAGTTCCCTGA
	Reverse	GGAATCACGTTGGTTTGGGC
MICA/Mill1	Forward	CACTTGACACAGGTTCCCCG
	Reverse	GCACGCTTCTCTATGTTGCC
HOXA3	Forward	CTGCGGTCTGAAGGCTACG
	Reverse	AAGGGCTTGACACCCGTAAG
GAPDH	Forward	GTATCGGACGCCTGGTTAC
	Reverse	CTTGCCGTGGGTAGAGTCAT

Statistical Analysis

The R Studio (version 3.5.1) was used to generate images. GraphPad Prism 8.0 was used to perform statistical analysis and drawing. Unpair t-test was selected as the statistical method. P-value < 0.05 was considered as statistically significant. Data was presented as mean ± standard deviation (SD).

Results

Identification of Differentially Expressed miRNA Between AIH and Healthy Control

The study whole analysis process is presented in Figure 1. GSE140249 was analyzed by "edgeR" package in R with FDR <0.05 and |log2FC| > 1.5 as the threshold. In dataset GSE140249, 470 differentially expressed miRNAs (DE miRNAs) were identified, which contained 249 up-regulated genes and 221 down-regulated genes (Figure 2A & 2B). All the DE miRNAs in the datasets were respectively performed clustering and shown in the form of heatmaps (Figure 2A). The top 10 DE miRNAs in the dataset were shown in Table 2.
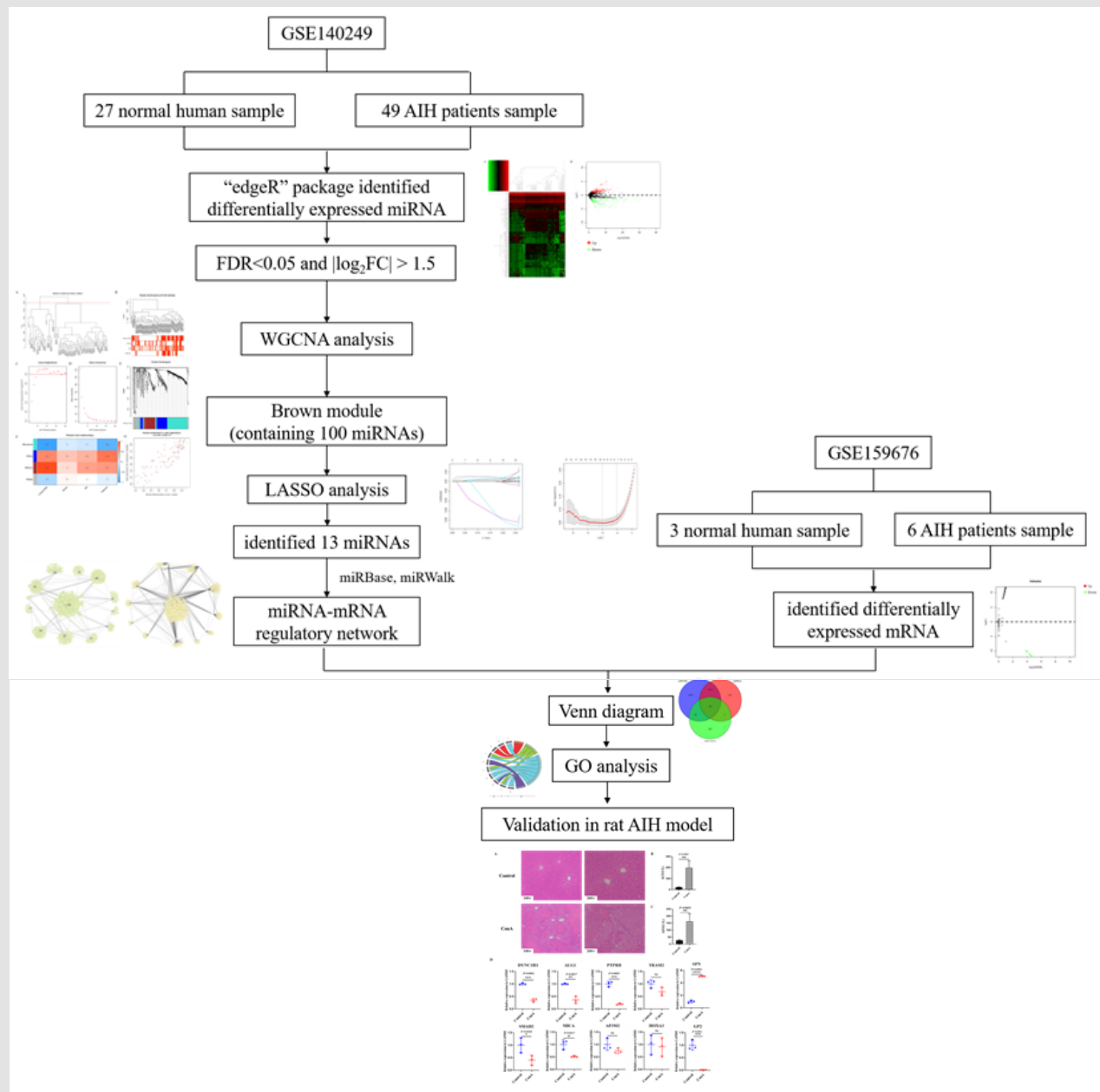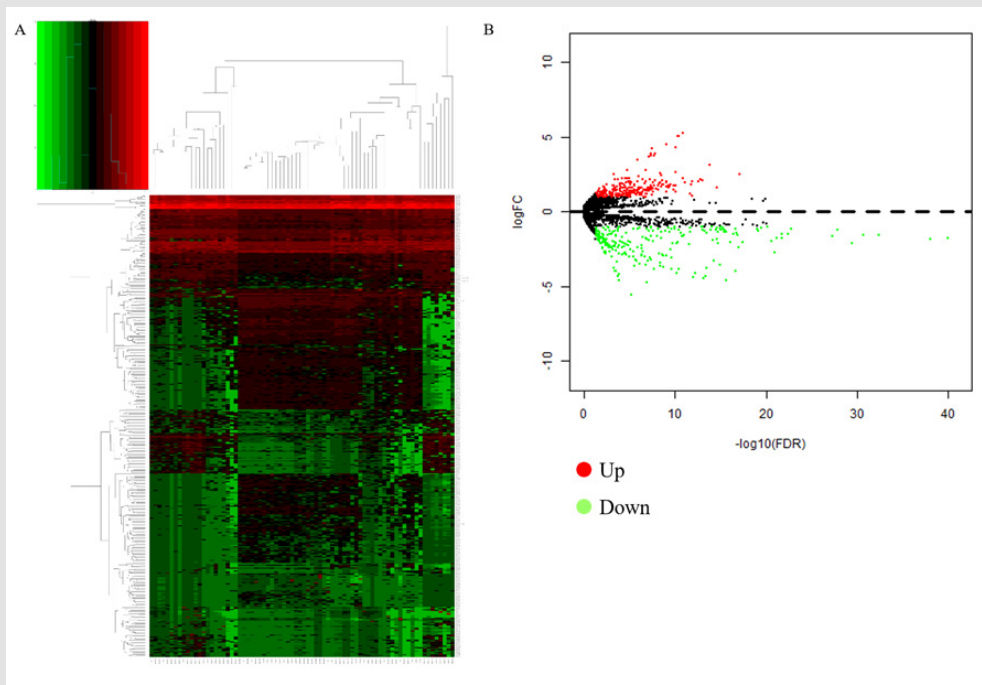
Figure 1: Flow chart of the study.

Figure 2: The miRNA differential expression in GSE140249.
(A)   The heatmap of DEGs in GSE140249.
(B)   The volcano plot of DEGs in GSE140249.

Table 2: The top 10 DE miRNAs in GSE140249.

miRNA_ID	log FC	log CPM	*P*-Value	FDR
hsa-miR-3158-5p	-1.74158	6.34132	1.08E-40	2.77E-37
hsa-miR-193a-5p	-1.84582	7.048599	8.84E-39	1.13E-35
hsa-miR-6836-3p	-1.54197	9.35137	4.39E-33	3.75E-30
hsa-miR-1343-3p	-1.60199	8.552899	2.29E-31	1.47E-28
hsa-miR-188-5p	-2.14498	5.612885	3.33E-30	1.71E-27
hsa-miR-4746-5p	-1.21447	6.791652	1.26E-28	5.37E-26
hsa-miR-602	-1.64823	6.829339	5.03E-28	1.84E-25
hsa-miR-3131	-1.17633	8.338945	1.54E-23	4.93E-21
hsa-miR-5190	-1.47569	5.056688	3.53E-23	1.01E-20
hsa-miR-920	-1.77062	7.953802	1.95E-22	4.74E-20

Construction of Co-expression Module and Identification of Key Trait Module miRNAs

WGCNA was performed to excavate key genes significantly related to traits by combining with DEGs. 25 sample was regarded as outlier removed in dataset GSE140249 under the cut height of 12000 (Figure 3A). Sample clustering tree and phenotypic heatmap were shown in Figure 3B. The darker the color, the higher the expression in phenotypic heatmap, and the grey module represented missing values. The top 75% genes of the median absolute deviation in this dataset were screened for WGCNA. When the soft-thresholding power (β) was equal to 5, the evaluation parameter R2 was greater than 0.85 (Figures 3C & D). A scale-free network was constructed with the soft-thresholding power of β = 5 and the genes were classified into different modules according to expression profiles. A total of 4 consensus co-expression modules were identified via the hierarchical clustering (Figure 3E). The genes which could not been classified into modules belong to grey module. 100 miRNAs were extracted in brown module, which had the most significant gene significance (GS) in AIH (Figure 3F). The scatter diagram was drawn to reveal the relationship between GS and module membership (MM) (Figures 3G, cor = 0.87, P = 6.2e-31). Above results show that brown module was highly correlated with the occurrence of AIH, and 100 miRNAs contained in which were considered as the trait-module key miRNAs.
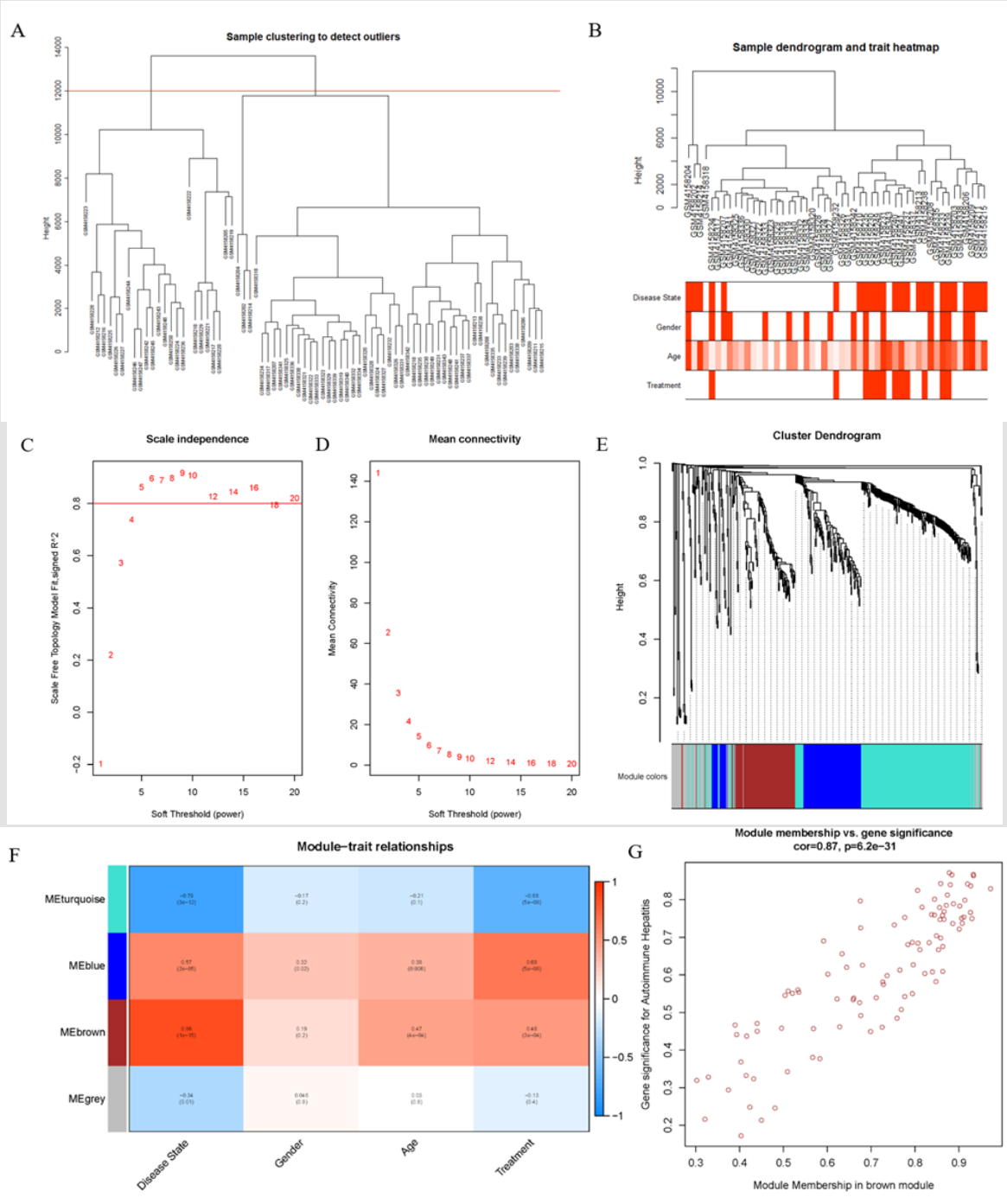
Figure 3: Screening of key trait-module miRNAs using WGCNA.

(A)   Sample clustering to detect outliers.

(B)   Sample clustering tree and phenotypic heatmap.

(C)   The analysis of scale-free fit index and various soft-threshold powers.

(D)   The analysis of mean connectivity and soft-threshold powers.

(E)   Dendrogram clustered using dynamic tree cut algorithm.

(F)   Correlation graph between gene modules and sample traits.

(G)   Scatter plot between GS and MM in the brown module.

## Construction of the miRNA Signature Based on LASSO Regression

Among the total of 100 miRNAs, LASSO regression analysis was performed for further filtration. The regression coefficient diagrams were present including Lasso coefficient profiles and cross-validation parameters. Coefficient profiles decreased as the lambda value decreased (Figure 4A & B). The best lambda was 0.049. In total, 13 miRNAs were retained including hsa-miR-122-5p, hsa-miR-154-3p, hsa-miR-188-5p, hsa-miR-302c-5p, hsa-miR-515-3p, hsa-miR-602, hsa-miR-675-3p, hsa-miR-4772-5p, hsa-miR-5587-5p, hsa-miR-6836-5p, hsa-miR-7152-3p, hsa-miR-7854-3p, hsa-miR-513c-5p. The miRNA–gene interaction table was downloaded using the miRbase and miRwalk database. Two miRNA–gene interaction networks were constructed by Cytoscape software (Figure 4C & D). GSE159676 was analyzed by "edgeR" package in R with FDR <0.05 and |log2FC| > 1.5 as the threshold. In dataset GSE159676, 383 DEGs were identified, which contained 1 up-regulated genes and 382 down-regulated genes (Figure 5A). Based on the miRbase and miRwalk database, the miRNA–mRNA interaction was constructed. It was found that a total of 14 DEGs were screened out in miRbase, miRwalk and GSE159676 dataset, which was shown by Venn diagram in Figure 5B. In order to further learn the function of AIH-related genes, 10 differential mRNAs (DE mRNAs) were found to be enriched in gene ontology (GO) database, mainly in biological process (BP) and cell components (CC), including axon cytoplasm, cell surface, integral component of membrane and anterior/posterior pattern specification (Figure 5C).
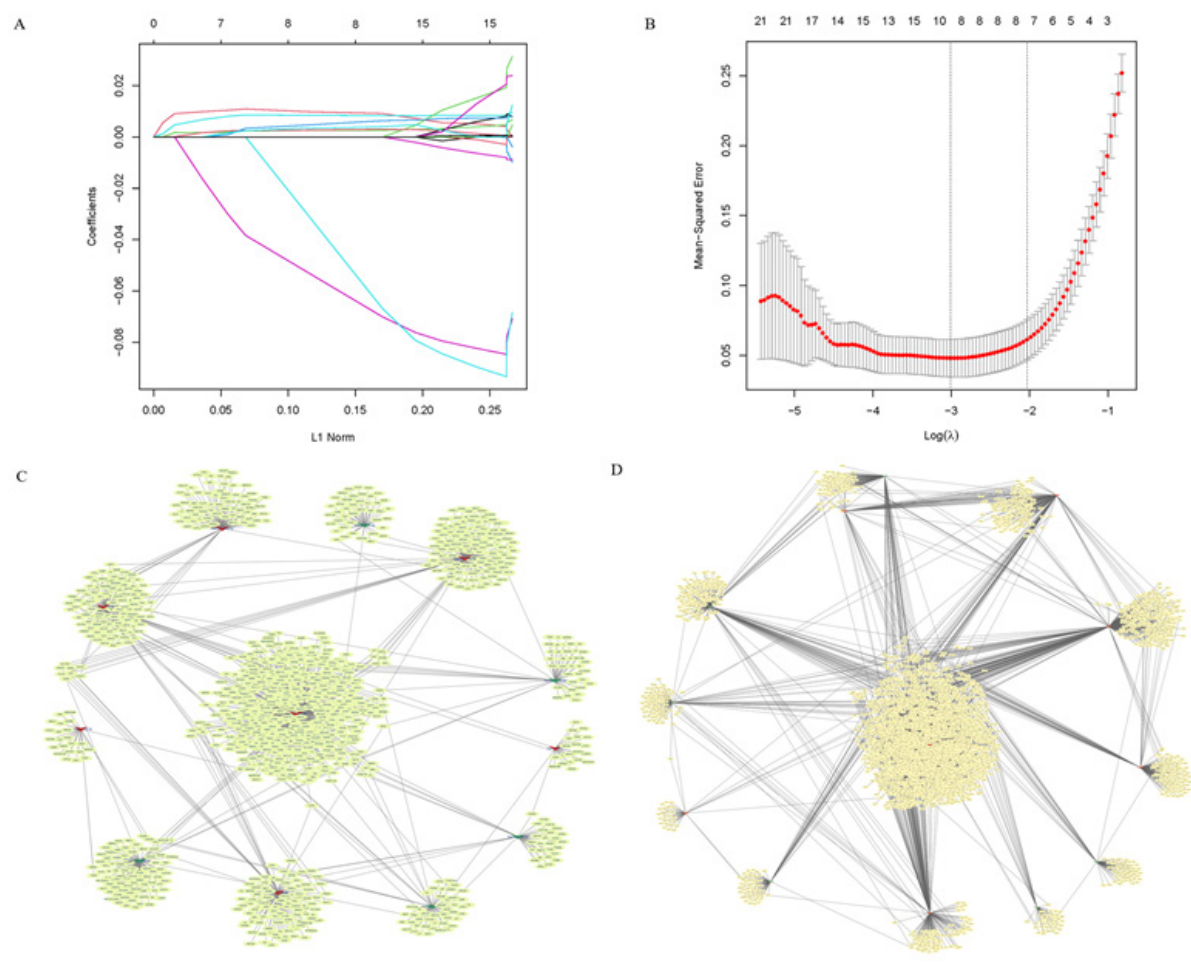
Figure 4: LASSO regression analysis and the prediction of miRNA targeted mRNA.
(A) LASSO coefficient profiles of the miRNAs.
(B) Cross-validation to tune parameter selection in LASSO regression analysis.
(C) miRNA–gene interaction networks in miRBase database.
(D) miRNA–gene interaction networks in miRWalk database.
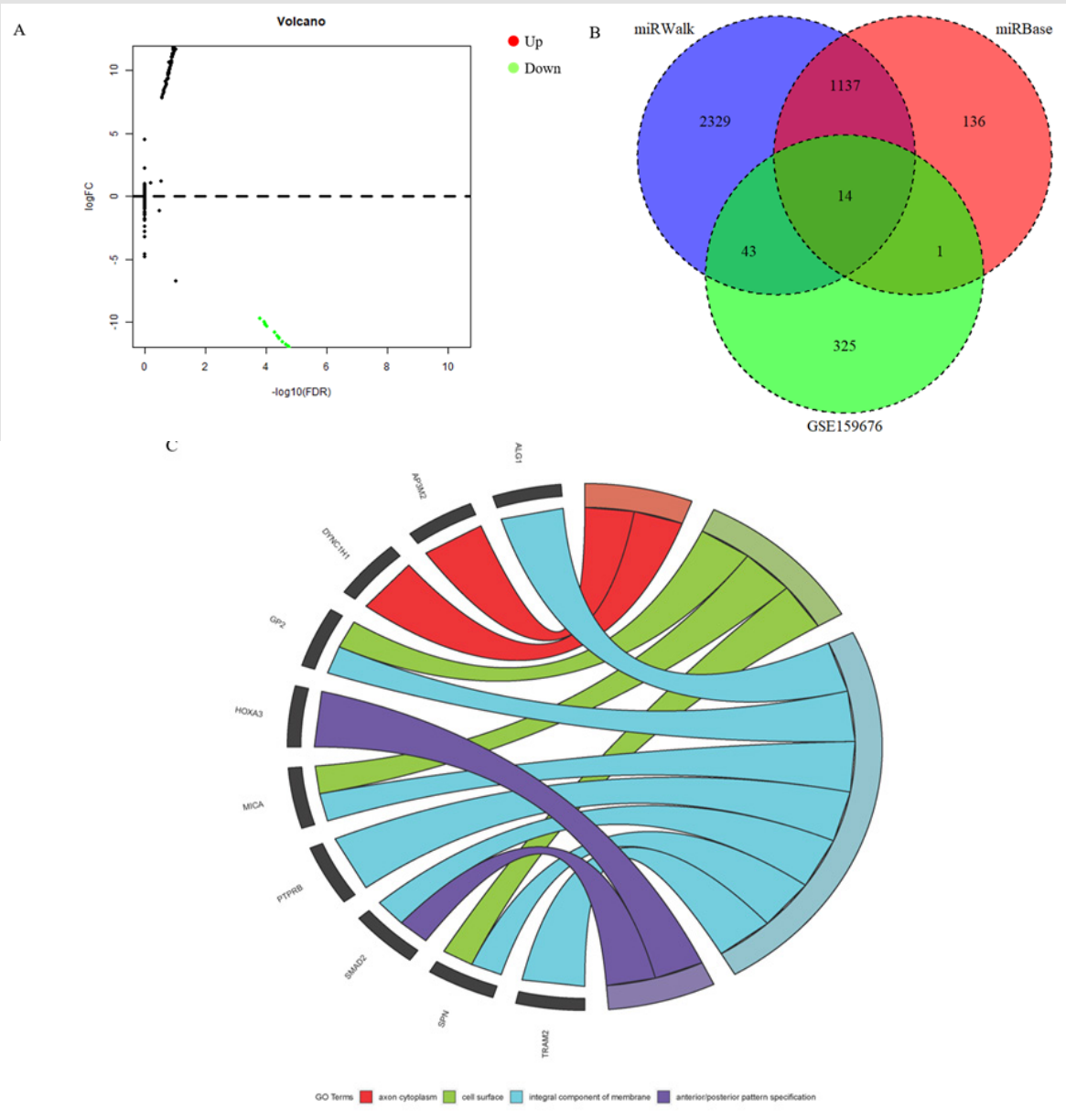
Figure 5: Identification of DE mRNAs and Functional Enrichment Analysis.
(A)   The volcano plot of DE mRNAs in GSE159676.
(B)   The Venn diagram of the mRNAs in miRbase, miRwalk and GSE159676 database.
(C)   Biological Process (BP) and Cell Components (CC) in 10 DE mRNAs.

Validation of the Key mRNA in Rat AIH Model

To further validate the role of the 10 DE mRNAs in AIH, we pre-pared the Con A-induced rat AIH model. Some studies have report-ed that Con A could induce AIH by activating immune cells, mainly T lymphocyte. H&E staining analysis revealed that Con A (16mg/kg) induced rat displayed infiltration of inflammatory cells and interface necrosis (Figure 6A). Compared to the control group, the Con A group had the higher level of ALT and AST (Figure 6B & C). As expected, qPCR showed that DYNC1H1, ALG1, PTPRB, SMAD2, MICA and GP2 were lowly expressed in Con A induced rat AIH model, SPN was high-ly expressed, while there was no significance in TRAM2, AP3M2 and HOXA3 (Figure 6D).
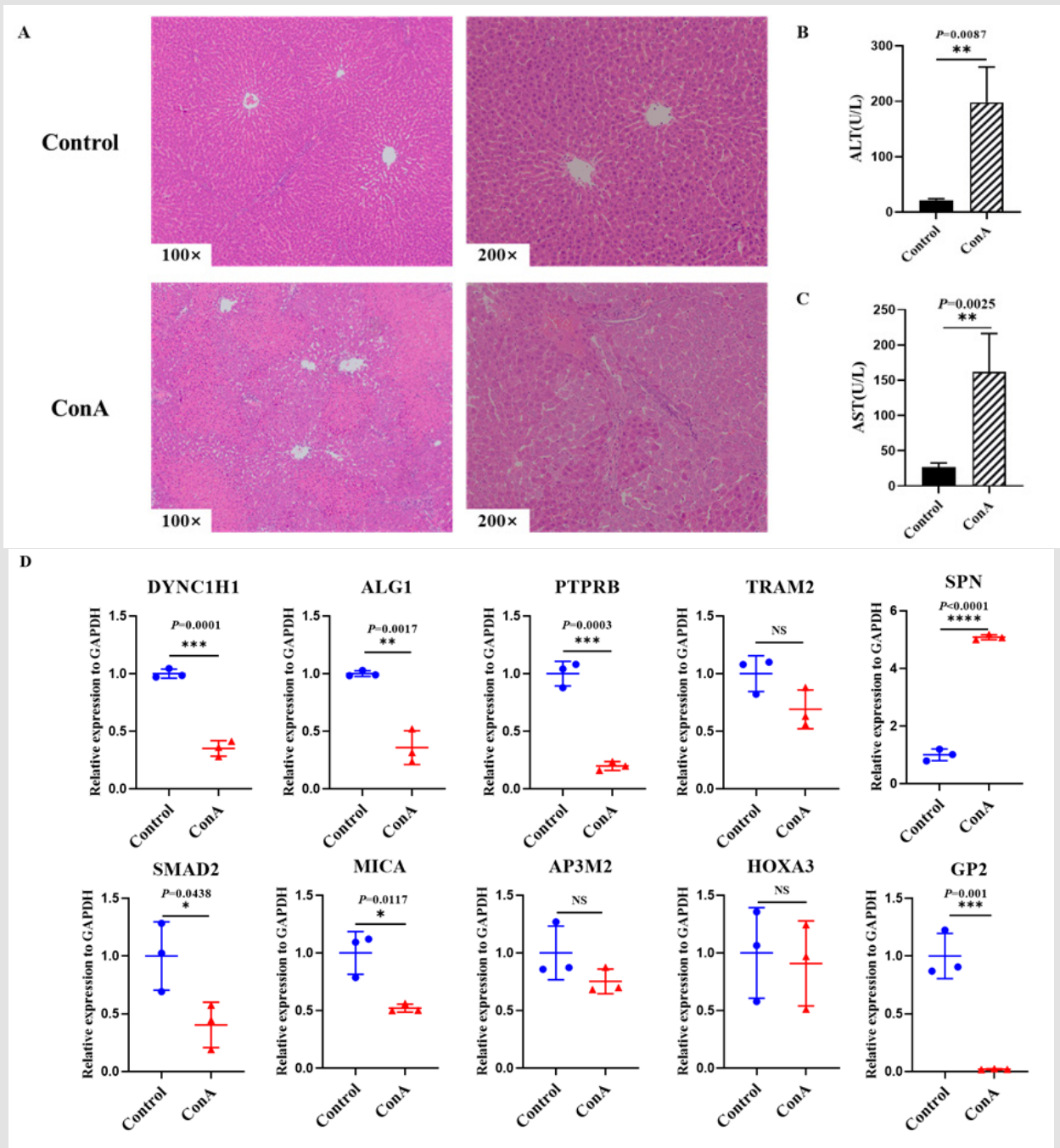
Figure 6: Validation of the key mRNA in rat AIH model (n=3).

(A)   H&E staining of the liver section in rat model.

(B)   Serum ALT and AST level in rat model.

(C)   RT-qPCR analysis. NS, no significance (Student's t-test).

Discussion

There are few biomarkers related to AIH, and the diagnosis of AIH mainly depends on the change of autoantibodies and invasive tests. In this study, comprehensive bioinformatics methods were used to mine AIH-related databases, including WGCNA, LASSO algorithm and the prediction of target genes. A lot of mRNAs related to AIH were screened by the bioinformatics analysis, and which was further validated in the rat AIH model. DYNC1H1, ALG1, PTPRB, SMAD2, MICA and GP2 were lowly expressed in AIH, while SPN was highly expressed in AIH. It could provide a solid foundation for the following researches. There are some studies on the identified mRNAs. DYNC1H1 (Dynein cytoplasmic 1 heavy chain 1, DYNC1H1) was located in several cellular components, including manchette, neuronal cell body and nuclear envelope [20]. Studies reported that DYNC1H1 was associated with liver hepatocellular carcinoma (LIHC), and which might play an important role in the progression of LIHC through epithelial-mesenchymal transition (EMT) and immune response [21]. ALG1 (chitobiosyldiphosphodolichol beta-mannosyltransferase, ALG1) is an essential mannosyltransferase involved in N-glycosylation [22,23]. Many studies have found that new glycosylation-related gene mutations could lead to diseases. Glycoprotein and glycolipid synthesis are one of the main functions of the liver. Congenital disorders of glycosylation (CDG) could affect the structure and function of liver and gallbladder, which can lead to fatty liver, liver fibrosis and abnormal bile duct development [24].

PTPRB (protein tyrosine phosphatase, receptor type, B, PTPRB) was predicted to be involved in angiogenesis and inactivation of protein kinase activity [25]. It was reported that miR-665 could targeted PTPRB promoting lung cancer and tumor cell migration, invasion, and proliferation [26]. SMAD2(SMAD family member 2, SMAD2) was involved in regulation of gene expression, and transforming growth factor beta receptor signaling pathway [27]. It has been reported that SMAD2 was closely related to a variety of liver diseases, such as liver fibrosis, liver cancer, primary biliary cholangitis (PBC), etc [28]. Another study constructed the adult mice of hepatocyte-specific deletion of Smad2, and showed that SMAD2 could inhibit the growth and dedifferentiation of hepatocytes [29]. MICA (Major Histocompatibility Complex Class I-Related Chain A) was associated with various liver disease, such as nonalcoholic fatty liver disease, liver fibrosis and hepatocellular carcinoma [30-31]. Glycoprotein 2 (GP2) is a sensitive marker in primary sclerosing cholangitis, which could provide strong evidence for AIH [32]. SPN (Sialophorin, SPN) was closely related to the cellular immunity, including T cell receptor signaling pathway, monocyte activation, and thymocyte aggregation [33]. The above analysis results show that AIH-related mRNA predicted by bioinformatics has been studied in various liver diseases, but there is no AIH-related research. It is worthy to be further verified the role of these mRNAs in the occurrence and development of AIH.

Immune-mediated hepatocyte injury is the main pathogenesis of AIH. Recent studies reported that abnormal immune tolerance was closely related to AIH, especially in T lymphocyte [34]. At present, the therapeutic targets for AIH mainly focus on immunity, such as glucocorticoid and azathioprine [35], but new targets are urgently needed for patients who are respond or tolerate. Therefore, future research needs to reveal more genetic susceptibility sites of AIH from three different perspectives: genetics, immunity and environment, so as to provide more effective means for accurate clinical intervention [36]. Bioinformatics is a multi-disciplinary frontier cross-discipline, which has the advantages of massive and diversified data acquisition and analysis. It has greatly impacted the traditional mode of medical research activities, and can provide a direction for developing new therapeutic targets [37,38].

Conclusion

In conclusion, this integrative analysis indicated that comprehensive bioinformatic analysis could provide a recommendable way to identify key genes related to nontumor disease [39]. Seven key genes correlated with AIH were identified, which also was validated in rat AIH model. DYNC1H1, ALG1, PTPRB, SMAD2, MICA, GP2 and SPN may play key roles in the occurrence and development of AIH.

Acknowledgments

Ethics Approval and Consent to Participate

Consent for Publication

Conflicts Of Interest

Data Availability

References

1. Trivedi PJ, Hirschfield GM (2021) Recent advances in clinical practice: epidemiology of autoimmune liver diseases[J]. Gut 70(10): 1989-2003.

2. Terziroli Beretta-Piccoli B, Mieli-Vergani G, Vergani D (2022) Autoimmmune hepatitis[J]. Cell Mol Immunol 19(2):158-176.

3. Katsumi T, Ueno Y (2022) Epidemiology and surveillance of autoimmune hepatitis in Asia[J]. Liver international: official journal of the International Association for the Study of the Liver 42(9): 2015-2022.

4. Vuerich M, Wang N, Kalbasi A, Graham JJ, Longhi MS, et al. (2021) Dysfunctional Immune Regulation in Autoimmune Hepatitis: From Pathogenesis to Novel Therapies[J]. Front Immunol 12: 746436.

5. Li Y, Sun Y, Liu Y, Wang B, Li J, et al. (2022) Genome-wide meta-analysis identifies susceptibility loci for autoimmune hepatitis type 1[J]. Hepatology 76(3): 564-575.

6. Ellinghaus D (2022) How genetic risk contributes to autoimmune liver disease[J]. Semin Immunopathol 44(4): 397-410.

7. Harrington C, Krishnan S, Mack CL, Cravedi P, Assis DN, et al. (2022) Non-invasive biomarkers for the diagnosis and management of autoimmune hepatitis[J]. Hepatology 76(6): 1862-1879.

8. Hubener S, Oo YH, Than NN, Hubener P, Weiler-Normann C, et al. (2016) Efficacy of 6-Mercaptopurine as Second-Line Treatment for Patients With Autoimmune Hepatitis and Azathioprine Intolerance[J]. Clinical gastroenterology and hepatology: the official clinical practice journal of the American Gastroenterological Association 14(3): 445-453.

9. Pape S, Snijders R, Gevers TJG, Chazouilleres O, Dalekos GN, et al. (2022) Systematic review of response criteria and endpoints in autoimmune hepatitis by the International Autoimmune Hepatitis Group[J]. Journal of hepatology 76(4): 841-849.

10. Zhong CP, Xi ZF, Xia Q (2018) Clinical analysis of liver transplantation in autoimmune liver diseases[J]. Hepatobiliary & pancreatic diseases international: HBPD INT 17(1): 27-31.

11. Yao M, Zhang C, Gao C, Wang Q, Dai M, et al. (2021) Exploration of the Shared Gene Signatures and Molecular Mechanisms Between Systemic Lupus Erythematosus and Pulmonary Arterial Hypertension: Evidence from Transcriptome Data[J]. Front Immunol 12: 658341.

12. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, et al. (2013) NCBI GEO: archive for functional genomics data sets--update[J]. Nucleic Acids Res 41(Database issue): D991-D995.

13. Kozomara A, Birgaoanu M, Griffiths-Jones S (2019) miRBase: from microRNA sequences to function[J]. Nucleic Acids Res 47(D1): D155-D62.

14. Dweep H, Sticht C, Pandey P, Gretz N (2011) miRWalk--database: prediction of possible miRNA binding sites by "walking" the genes of three genomes[J]. J Biomed Inform 44(5): 839-847.

15. Langfelder P, Horvath S (2008) WGCNA: an R package for weighted correlation network analysis[J]. BMC Bioinformatics 9: 559.

16. Teppola P, Taavitsainen VM (2013) Parsimonious and robust multivariate calibration with rational function Least Absolute Shrinkage and Selection Operator and rational function Elastic Net[J]. Anal Chim Acta 768: 57-68.

17. Saeki C, Matsuzaki J, Kuroda M, Fujita K, Ichikawa M, et al. (2022) Identification of circulating microRNAs as potential biomarkers for hepatic necroinflammation in patients with autoimmune hepatitis[J]. BMJ Open Gastroenterology. 9(1).

18. Lei L, Bruneau A, El Mourabit H, Guégan J, Folseraas T, et al. (2022) Portal fibroblasts with mesenchymal stem cell features form a reservoir of proliferative myofibroblasts in liver fibrosis[J]. Hepatology 76(5): 1360-1375.

19. Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, et al. (2022) DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update) [J]. Nucleic Acids Res 50(W1): W216-W221.

20. Strickland AV, Schabhuttl M, Offenbacher H, Synofzik M, Hauser NS, et al. (2015) Mutation screen reveals novel variants and expands the phenotypes associated with DYNC1H1[J]. Journal of neurology 262(9): 2124-2134.

21. Wang Y, Han J, Zhou H, Ai S, Wan D, et al. (2022) A Prognosis Marker Dynein Cytoplasmic 1 Heavy Chain 1 Correlates with EMT and Immune Signature in Liver Hepatocellular Carcinoma by Bioinformatics and Experimental Analysis[J]. Disease markers 2022: 6304859.

22. Jaeken J, Lefeber D, Matthijs G (2015) Clinical utility gene card for: ALG1 defective congenital disorder of glycosylation[J]. Eur J Hum Genet 23(10):1431.

23. Albright CF, Robbins RW (1990) The sequence and transcript heterogeneity of the yeast gene ALG1, an essential mannosyltransferase involved in N-glycosylation[J]. Journal of Biological Chemistry 265(12): 7042-7049.

24. Lipinski P, Bogdanska A, Socha P, Tylki-Szymanska (2021) A Liver Involvement in Congenital Disorders of Glycosylation and Deglycosylation[J]. Front Pediatr 9: 696918.

25. Soady KJ, Tornillo G, Kendrick H, Meniel V, Olijnyk-Dallis D, et al. (2017) The receptor protein tyrosine phosphatase PTPRB negatively regulates FGF2-dependent branching morphogenesis[J]. Development 144(20): 3777-3788.

26. Xia J, Li D, Zhu X, Xia W, Qi Z, et al. (2020) Upregulated miR-665 expression independently predicts poor prognosis of lung cancer and facilitates tumor cell proliferation, migration and invasion. Oncol Lett 19(5): 3578-3586.

27. Buwaneka P, Ralko A, Gorai S, Pham H, Cho W, et al. (2021) Phosphoinositide-binding activity of Smad2 is essential for its function in TGF-beta signaling[J]. J Biol Chem 297(5):101303.

28. Kamato D, Little PJ (2020) Smad2 linker region phosphorylation is an autonomous cell signalling pathway: Implications for multiple disease pathologies[J]. Biomed Pharmacother 124: 109854.

29. Ju W, Ogawa A, Heyer J, Nierhof D, Yu L, et al. (2006) Deletion of Smad2 in mouse liver reveals novel functions in hepatocyte growth and differentiation[J]. Mol Cell Biol 26(2): 654-667.

30. Karrar A, Rajput B, Hariharan S, Abdelatif D, Houry M, et al. (2021) Major Histocompatibility Complex Class I-Related Chain A Alleles and Histology of Nonalcoholic Fatty Liver Disease[J]. Hepatology communications 5(1): 63-73.

31. Mantovani S, Varchetta S, Mele D, Donadon M, Torzilli G, et al. (2020) An Anti-MICA/B Antibody and IL-15 Rescue Altered NKG2D-Dependent NK Cell Responses in Hepatocellular Carcinoma[J]. Cancers (Basel) 12(12).

32. Tornai D, Ven PL, Lakatos PL, Papp M (2022) Serological biomarkers for management of primary sclerosing cholangitis[J]. World journal of gastroenterology 28(21): 2291-2301.

33. Sialophorin, a surface sialoglycoprotein defective in the Wiskott-Aldrich syndrome, is involved in human T lymphocyte proliferation. pdf[J].

34. Renand A, Cervera-Marzal I, Gil L, Dong C, Garcia A, et al. (2020) Integrative molecular profiling of autoreactive CD4 T cells in autoimmune hepatitis[J]. Journal of hepatology 73(6): 1379-1390.

35. Doycheva I, Watt KD, Gulamhusein AF (2019) Autoimmune hepatitis: Current and future therapeutic options[J]. Liver international: official journal of the International Association for the Study of the Liver 39(6): 1002-1013.

36. Manns MP, Vogel A (2006) Autoimmune hepatitis, from mechanisms to therapy[J]. Hepatology 43(2 Suppl 1): S132-S144.

37. Chen C, Hou J, Tanner JJ, Cheng J (2020) Bioinformatics Methods for Mass Spectrometry-Based Proteomics Data Analysis[J]. Int J Mol Sci 21(8).

38. Karim MR, Beyan O, Zappa A, Costa IG, Rebholz-Schuhmann D, et al. (2021) Deep learning-based clustering approaches for bioinformatics[J]. Brief Bioinform 22(1): 393-415.

39. Sharkawy RE, Bayoumi A, Metwally M, Mangia A, Berg T, et al. (2019) A variant in the MICA gene is associated with liver fibrosis progression in chronic hepatitis C through TGF-beta1 dependent mechanisms[J]. Sci Rep 9(1): 1439.

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