Identification of Shared Gene Signatures in Different Stages of Non-Alcoholic Fatty Liver Disease Using Integrated Microarray Dataset

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Abstract

Purpose: Non-alcoholic fatty liver disease (NAFLD) is the most preventable type of chronic liver disease worldwide and a risk factor for developing cirrhosis or hepatocellular carcinoma (HCC) if untreated. Although experts have performed lots of efforts to find the underlying mechanisms of NAFLD, it remains a challenge to recognize them. The aim of this study is to distinguish common gene signature and pathways in the human liver during NAFLD progression through the systems biology method.

Methods: In this study, the three microarray datasets, GSE48452, GSE63067 and GSE89632, were selected from the NCBI GEO database to explore differentially expressed genes (DEGs) among healthy controls, simple steatosis and non-alcoholic steatohepatitis (NASH) patients. Furthermore, protein-protein interaction (PPI) networks and pathway enrichment analysis were used to detect common genes and biological pathways in different stages of NAFLD.

Results: The current study was included 47 healthy subjects, 36 patients with simple steatosis and 46 NASH patients. Common high degree genes among all three sets were CHI3L1, GFBP2, NRG1, PEG10 and FADS2. The top five genes in the hepatic PPI networks of three datasets were STAT3, JUN, CANX, FN1 and MYC. Signal transduction, immune response, and anti-apoptosis were the most important biological pathways between healthy *vs*. NASH, while cell communication, signal transduction and immune response were three top biological pathways between healthy *vs*. simple steatosis. Also, the most eminent biological pathways between NASH *vs*. simple steatosis were metabolism and energy pathways.

Conclusion: The present study represented the unique and shared key genes and pathways between different stages of NAFLD, which may facilitate the understanding of the NAFLD mechanism and identify potential therapeutic targets in this disease.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease in the world population which is associated with lifestyle modification and obesity and may progress to severe hepatic disorders including simple steatosis, non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [1]. NAFLD affects around 90 and 25% of obese patients and people worldwide, respectively [2]. NASH might lead to liver cirrhosis and, subsequently, to HCC. It is the second most common risk factor for HCC in patients who underwent liver transplantation in the USA [3, 4]. Moreover, NAFLD is associated with other comorbidities, including metabolic syndrome, type 2 diabetes, cardiovascular disorders, and extrahepatic cancers [5].

The pathogenesis of NAFLD is associated with genetic and environmental factors. Multiple organs, including the adipose tissue, hypothalamus, the gut, and the liver, are involved in NAFLD's pathogenesis. This multifactorial disease is related to the altered gut microbiota, increased oxidative stress, and dysregulated adipokines and hormones [2, 6]. Given the complexities of this disease and limited information about the underlying molecular mechanisms implicated in the progression of the illness,

despite lots of efforts that have been devoted to detecting pharmacological therapies, there are no approved remedies for the treatment of NAFLD [7, 8]. Although this disorder is produced by lifestyle modification, diet, and physical activity, alterations may be useful, but it is not easy to achieve. Consequently, novel and effective treatments are difficult because the diagnosis and staging are typically established by hepatic biopsy. Hence, non-invasive biomarkers that might recognize patients at risk of progression to the advanced stage are immediately necessary and helpful for drug development.

Identifying gene-specific expression panels could improve our understanding of the disease's pathogenesis and progression mechanisms or drug assessment. To the best of our knowledge, there was one study that performed protein-protein interaction (PPI) networks and pathway enrichment analysis to screen differentially expressed genes (DEGs) in different stages of NAFLD compared with control [9, 10]. Wang *et al.* used one microarray dataset, GST48452, to detect DEGs among control, healthy obese, steatosis and NASH samples [9]. Furthermore, another study used 6 datasets (GSE48452, GSE66676, GSE72756, GSE63067, GSE89632, and GSE107231) to find DEGs between NAFLD patients and controls. However, GSE72756 has been used in their study in which normal liver tissues from NAFLD patients were as controls and in another dataset, GSE66676, liver tissues from adolescents (13.4–19.8 yrs.) compared with other datasets that analyzed adults [10]. As we know, NAFLD in adolescents is different from that in adults [11]. In this study, the three microarray datasets including GSE48452, GSE63067 and GSE89632 were further analyzed to explore DEGs among healthy controls, simple steatosis and NASH patients. Furthermore, PPI networks and pathway enrichment analysis were performed to find genes/proteins and biological pathways shared in different stages of NAFLD.

2. Materials And Methods

2.1. Data processing

Three datasets, GSE63067, GSE89632, and GSE48452, were selected from the NCBI GEO database for processing related to the differential detection of genes [12–14]. These three datasets included microarray data for the study, which after downloading the data, were processed by the LIMMA package in R, which in the processing steps included normalization and clustering and remove noisy data and then the calculation of DEG by eBays Method. Finally, for all three data groups, DEG with FC ≥ 2 and p-value ≤ 0.05 was selected as the final DEG list. Data were in three groups: Healthy, Steatosis, and non-alcoholic steatohepatitis (NASH). The details of the data are shown in Table 1.

Table 1
Characteristics of the included GEO datasets.

GSE_ID	Participants	Tissues	Platform	Year
GSE63067	9 NASH,2 Steatosis, 9 Healthy	Liver	GPL570	2014
GSE89632	19 NASH, 20 Steatosis, 24 Healthy	Liver	GPL14951	2016
GSE48452	18 NASH, 14 Steatosis, 14 Healthy	Liver	GPL11532	2013

For both groups in each data set, a differential list was obtained. The lists were DEGs related to two healthy and steatosis groups named SH, healthy and NASH named NH, and finally steatosis and NASH named NS, respectively.

2.2. Physical Protein Interaction Network

To plot the physical interaction network, all groups SH, NS, NH were combined in the dataset, and then String-db.org was used. All physical and experimental interactions in each list were examined, and the interaction network was extracted. To study the deeper connections between gene interactions in liver tissue, all gene lists were given as input to the HIPPIE tool(http://cbdm-01.zdv.uni-mainz.de/~mschaefer/hippie/information.php), and interaction data were extracted by filtering interactions related to liver tissue. Finally, all the extracted networks were entered into the Cytoscape (https://cytoscape.org/), and the hub genes (with the highest degree) were identified by the Network Analyzer tool.

2.3. Similarity Analysis

To investigate the similarities between the three processing sets, identical groups were selected in pairs, and overlapping genes were selected. Also, considering that the enrichment analysis was performed for each group, each analysis's overlaps were examined.

2.4. Enrichment Analysis

To find the biological pathways involved and the gene ontology in the gene list, they were extracted by processing by the FunRich (http://www.funrich.org/). These data were used to investigate the biological similarities of the gene sets in the gene list.

3. Results

3.1. Identification of DEGs in NAFLD

All three of these microarray data sets were first analyzed separately. The results are shown in Supplementary 1. According to the Data processing section criteria, the DEGs were screened out by using the "LIMMA" package in R software. The Result table of the three microarrays was shown in Table 2. Also, the overlap genes in this step shown in Fig. 1. No genes common to the three groups were found in the GSE48452 dataset (a). In the GSE63067dataset (b), two common genes were found between the three groups: Intercellular Adhesion Molecule 1 (ICAM1) and paternally expressed gene-10 (PEG10). In the GSE89632 dataset (c), three common genes were found between the three groups, which are flavin-containing monooxygenases 1 (FMO1), PEG10, and Metallothionein 1A (MT1A).

Table 2
Result of DEGs calculation.

GSE_ID	GSE63067		GSE89632		GSE48452	
	UPs	DOWNs	UPs	DOWNs	UPs	DOWNs
SH	283	66	186	366	11	5
NS	229	59	17	10	16	5
NH	120	19	246	320	25	10

By examining three data sets to find the list of shared genes between all three sets, Five genes were found, which can be seen in Fig. 2. These genes are CHI3L1 (Chitinase 3 Like 1), GFBP2 (Insulin-like growth factor (IGF) binding protein 2), NRG1 (Neuregulin 1), PEG10 and FADS2 (Fatty Acid Desaturase 2).

3.2. Gene overlap in matched groups

The DEGs of each processed group used to identify the similarities in the two sets. We plot the Venn diagram for shown overlap genes in the three groups (Fig. 3). IGFBP2 (insulin-like growth factor-binding proteins) and PEG10 are the common genes in the NH's list (Figure. 3a). There are no common genes in the NSs list (Figure. 3b). There was only the PEG10 gene in common between the SHs list (Figure. 3c).

3.3. PPI networks

Gene interactions for each group indicate a significant number of interactions in that group so that for each group, the genes with the highest degree of interaction can be considered significant points of the network. Table 3 shows the top three lists of genes with the highest degree in all three groups' interaction networks.

Table 3
Three genes with the highest degree in each group.

	Gene name	Degree	Description
NH	IL6	129	Interleukin 6
	MYC	92	MYC Proto-Oncogene, BHLH Transcription Factor
	JUN	88	Jun Proto-Oncogene, AP-1 Transcription Factor Subunit
NS	RPS27A	48	Ribosomal Protein S27a
	STAT3	39	Signal Transducer And Activator Of Transcription 3
	FN1	39	Fibronectin 1
SH	IL6	158	Interleukin 6
	STAT3	113	Signal Transducer And Activator Of Transcription 3
	CXCL8	109	C-X-C Motif Chemokine Ligand 8

For example, the IL6 gene is known in the SH group network with an interactive degree of 158 in liver disease, so it is a significant focal point. On the other hand, STAT3, CXCL8 genes are also significant due to the high degree of interaction in the same network. Three global interaction networks were extracted from three gene lists shown in Figs. 4, 5, and 6.

Besides, to study the genetic connections in a more detailed way, these connections were examined specifically for the tissue in the liver tissue, which can be seen in Fig. 7.

These liver tissue connections suggest that hub genes can play a crucial role in disease due to their location and role in the interaction network. As shown in Fig. 7, the STAT3 and JUN genes, as the highest gene associated with other genes, plays a crucial role in liver tissue.

3.4. Enrichment analysis

By examining the overlap between the ontologies of genes and the common biological pathways in each group, we arrive at three enrichment lists. Tables 4, 5, and 6 show all enrichment list subscriptions.

Table 4
Top 10 biological pathways for NH's group.

Biological pathway	P-value (Hypergeometric test)	Bonferroni method
Cytokine signaling in Immune system	2.21E-06	0.003694
IL6-mediated signaling events	6.52E-06	0.010881
Syndecan-1-mediated signaling events	4.95E-07	0.000825
Regulation of CDC42 activity	1.87E-05	0.031212
Glypican pathway	1.01E-06	0.001683
GMCSF-mediated signaling events	4.28E-08	7.15E-05
Insulin Pathway	3.15E-07	0.000526
Nectin adhesion pathway	1E-07	0.000167
TRAIL signaling pathway	4.22E-08	7.05E-05
IGF1 pathway	3.53E-07	0.000589

Table 5
Top 10 biological pathways for the NS's group.

Biological pathway	P-value (Hypergeometric test)	Bonferroni method
Syndecan-1-mediated signaling events	*1.33E-06*	*0.002225*
Regulation of CDC42 activity	2.05E-07	0.000341
Glypican pathway	*7.38E-07*	*0.001231*
GMCSF-mediated signaling events	1.07E-06	0.001778
Insulin Pathway	*9.52E-07*	*0.001588*
Nectin adhesion pathway	1.16E-06	0.001934
TRAIL signaling pathway	*2.86E-06*	*0.004771*
IGF1 pathway	1.04E-06	0.001728
CDC42 signaling events	*1.22E-07*	*0.000204*
Signaling events mediated by Hepatocyte Growth Factor Receptor (c-Met)	1.1E-06	0.001829

Table 6
Top 10 biological pathways for the SH's group.

Biological pathway	P-value (Hypergeometric test)	Bonferroni method
Growth hormone receptor signaling	2.69E-05	0.044938
Regulation of cytoplasmic and nuclear SMAD2/3 signaling	2.22E-05	0.036968
IL6-mediated signaling events	8.63E-07	0.001439
Syndecan-1-mediated signaling events	3.38E-07	0.000564
Regulation of CDC42 activity	2.21E-08	3.69E-05
Glypican pathway	1.23E-07	0.000205
GMCSF-mediated signaling events	6.42E-08	0.000107
Insulin Pathway	2.05E-07	0.000341
Nectin adhesion pathway	1.43E-07	0.000238
TRAIL signaling pathway	4.08E-08	6.8E-05

As the most important term of gene ontology in the biological process, we can mention Signal transduction, Immune response, and Anti-apoptosis in the NH's group, Metabolism and Energy pathways in the NS's group and Cell communication, Signal transduction and Immune response in the SH's group play a key role. They are also part of the pathways that have significant overlap between the study groups. There are all enrichment analyzes in Supplementary 2, 3, and 4.

4. Discussion

In the present study, PPI networks were produced based on differential gene expression provided from tissue liver in simple steatosis and NASH patients compared with healthy controls. Network analysis of PPI networks helped us detect common genes and pathways implicated in these diseases.

Nodes Analysis in the PPI network for DEGs of simple steatosis *vs.* healthy controls demonstrated that interleukin-6 (IL-6), and signal transducer and activator of transcription 3 (STAT3) and C-X-C Motif Chemokine Ligand 8 (CXCL8) had the top three highest degrees. Interleukin-6 is a family of cytokines synthesized and secreted by monocytes, macrophages, and neutrophils after stimulation of toll-like receptors (TLRs) by lipopolysaccharides (LPS). IL-6 is bounded into its receptor (IL-6R), then the IL-6/IL-6R complex was bounded to gp130, which activates STAT1 and STAT3. Subsequently, activated STAT3 up-regulates inhibitor of cytokine signaling [15]. There are controversial reports regarding the level of IL-6, its receptors and the progression of liver injuries. For instance, Kroy *et al.* reported mice with IL-6 and gp130 knockout promoted hepatic steatosis [16]. However, Hong and coworkers showed IL-6 reduced liver steatosis in NAFLD mice models and proposed remedial effects of IL-6 administration in the treatment of

fatty liver patients [17]. The second hub gene is STAT3. STATs are transcription factors present in the cytoplasm as inactive form (dephosphorylated STATs) that tyrosine kinases activate STATs. Phosphorylated STATs produce dimers; thus, dimerized STATs translocate to the nucleus and bind to target genes. Therefore, their effects including response to ILs and growth factors and regulation of cell growth, differentiation, and motility were performed [18]. Studies demonstrate that STATs are dephosphorylated by T-Cell Protein Tyrosine Phosphatase (TCPTP). Since TCPTP was oxidized and inactivated in the obese mice model of NASH, it has been observed to increase STATs signaling [19]. Besides, mice with TCPTP-deficient elevated phosphorylated STAT1 and STAT3 and correlated with worsening NASH [19]. Consistent with these findings, one of the transcriptional targets of STAT-1 or STAT-3 is Cxcl9 that is up-regulated in NASH patients [19]. The up-regulation of Cxcl9 gene expression in NAFLD patients accompanied by the exacerbation of fibrosis [20].

The top three genes in the PPI networks of NASH *vs.* healthy controls were IL-6, c-MYC (MYC), and JUN. MYC, a proto-oncogene, is a transcription factor that regulates several biological processes such as cell growth, proliferation, and apoptosis. Mice with transgenic *c-myc* expression showed increased levels of *c-myc* are correlated with ROS accumulation, p53 inhibition, and hepatic disease progression after feeding ethanol. Additionally, patients with alcoholic liver disease (ALD) showed up-regulation of c-myc expression that may lead to acyl-CoA oxidase (AKT) activation and p53 inhibition [21]. Another study reported that enhanced protein expression of MYC accompanying elevated level of copper contributes to the development of cirrhosis to hepatocellular carcinoma (HCC) [22]. SIRT7 is a kind of decarboxylases that inhibit endoplasmic reticulum (ER) stress, accumulation of unfolded proteins via stabilizing at the ribosomal proteins promoters, and interacting with MYC to suppress some gene expression. Mice with SIRT7 -deficient progress to fatty liver disease; subsequently, MYC's inactivation prevents the progression of fatty liver disease because of SIRT7 deficiency [23]. Another hub gene was JUN, a proto-oncogene that is an eminent member of the activator protein 1 (AP1) family. The expression of c-Jun elevated in liver biopsy of simple steatosis and NASH compared with healthy controls, and hepatic expression of c-Jun increased in the NASH model of mice [24, 25]. Besides, c-Jun diminished hepatic expression of the type II inositol 1,4,5-trisphosphate receptor (ITPR2), a calcium channel leading to liver regeneration [25]. Another study showed that c-Jun expression could progress simple steatosis and NASH by regulating Osteopontin's expression [26]. The AP-1 transcription factor c-Jun is a putative β-Catenin target gene and promotes hepatocyte survival, proliferation, and liver tumorigenesis, suggesting that c-Jun may be a key target β-Catenin signaling in the liver. c-Jun is also frequently expressed in human HCCs and acts as an oncogene in the liver [27].

The most degree three genes in the PPI networks of NASH *vs.* simple steatosis were ribosomal protein S27a (RPS27a), STAT3, and fibronectin1 (FN1). RPS27a is one ribosomal protein synthesized as a fusion protein (at the C terminus) along with ubiquitin (Ub) at the N terminus. This precursor protein quickly hydrolyzed to an Ub and RPS27a protein [28, 29]. There is evidence reporting ribosomal proteins related to apoptosis, which is one of NAFLD features and can promote the conversion of simple steatosis to NASH [30, 31]. Moreover, the expression of other ribosomal proteins, including RPL36A and RPL14, were down-regulated in obese, steatosis, and NASH patients compared with healthy controls [32]. The down-

regulated level of RPL36A and RPL14 can elevate apoptosis. FN is another hub gene that a glycoprotein presented in bio-fluids such as plasma and cell surface and the extracellular matrix implicated in cell adhesion and migration, tissue repair, and blood coagulation. A 5-year follow-up study from obese patients at an early stage of NASH has shown that parenchymal FN might be a marker for anticipating fibrosis progression [33]. Furthermore, elevated FN levels from liver biopsy were associated with fibrosis progression in different liver diseases (alcoholic fatty liver, alcoholic hepatitis, alcoholic cirrhosis, chronic active hepatitis) compared with the control group [34].

The top five genes in the hepatic PPI networks of three data sets were STAT3, JUN, calnexin (CANX), FN1, and MYC. All these hub genes have been discussed above, except CANX. CANX is a transmembrane ER protein that is implicated in the folding of new glycoproteins [35]. Growing evidence indicates that this chaperone is related to ER stress and apoptosis. The levels of CANX were reduced in liver tissue and its microsomes in NASH patients compared to healthy controls [36]. Furthermore, some TFs relevant to ER stress enhanced, while some apoptosis markers reduced in liver NASH patients [36].

The shared genes among the three groups (SH, NH, and SN) for each dataset have been shown in Fig. 1a-c. There was no common gene for dataset GSE48452. However, dataset GSE63067 and dataset GSE89632 had two and three shared genes, respectively. These genes for GSE63067 were ICAM1 and PEG10. ICAM1 is a glycoprotein located in the cell surface and expressed in some cells such as liver, endothelial, epithelial hematopoietic cells. The Overexpression of ICAM1 may occur by some inflammatory cytokines, including IL-1 and TNF-α [37]. The study of Ito *et al*. suggested that serum ICAM1 might be a diagnostic marker for NASH because its concentration increases in NASH patients compared with healthy individuals.

Moreover, there was a positive correlation between serum ICAM1 level and the severity of liver fibrosis and inflammation [37]. Another study showed that hepatic expression of ICAM1 is significantly higher in NASH patients than in the simple steatosis and normal controls. A significant correlation was observed between the steatosis degree and hepatic ICAM1 expression [38]. Another common gene, PEG10, which prohibits apoptosis via interacting with SIAH1, is a tumor suppressor protein. Overexpression of PEG10 is linked to some malignancies, such as HCC and chronic lymphocytic leukemia [39, 40]. Besides, PEG10 expression increased in simple steatosis and NASH patients, as well as its concentration is associated with severity of liver disease [41]. Therefore, this protein might serve as promising therapies to prevent HCC in NAFLD patients. The shared genes among the three groups for dataset GSE89632 were FMO1, PEG10, and MT1A. Six FMO genes have been detected in mammalians that are important for the metabolism of various compounds, such as toxic and therapeutic substances [42]. A prior study displayed that human liver FMO1 is expressed in the fetus, while FMO3 is the main isoform in adult [42]. FMO3 and in smaller quantities, FMO1 metabolize trimethylamine (TMA) into trimethylamine- *N*-oxide (TMAO) [43]. Methylamines (TMA and TMAO) are a type of metabolite produced by the gut microbiota through the degradation of choline into TMA and adsorbed into circulation and metabolized into TMAO in the liver [43]. TMAO promotes inflammation and insulin resistance via elevating inflammatory cytokines.

Furthermore, TMAO inhibits bile acid production and impairs the main pathway to remove cholesterol from the body and causes TMAO-mediated atherosclerosis, and is associated with NAFLD's severity [44, 45]. Intestinal dysbiosis in cirrhotic patients is related to the low level of bile acids [46, 47]. Methylamines were known as markers of NAFLD, and metabolic syndrome and increased levels of TMAO are related to the severity of hepatic steatosis [48–50]. Another hub gene, MT1A, is a metalloproteins family with a high affinity for binding essential and toxic metals such as zinc and cadmium, respectively [51, 52]. MT1A's biological functions are protection against toxic metals, involvement in apoptosis, defense against oxidative stress (OS), proliferation and differentiation of cells, and protection against cytotoxicity as well as genotoxicity [53, 54, 52]. Additionally, MT1A has an indispensable role in the regeneration of hepatocytes [55]. The down-regulation of MT1A gene expression has been demonstrated in simple steatosis versus healthy controls, and a decrease was observed in NASH patients compared with the control group [13]. Also, it seems metallothioneins play a leading role in HCC pathogenesis, as down-regulated gene expression of MT1A isoform, MT1G, was observed in more than 60% of tumors relevant to the adjacent nonmalignant liver [56].

Venn diagrams (Fig. 3a-c) illustrate the number of shared DEGs that were found in the three datasets for SH, NH and NS groups. There are no shared DEGs for NS; however, one (PEG10) and two (IGFBP2 and PEG10) shared genes were observed in SH and NH groups, respectively. IGFBPs are a family of secreted proteins binding to insulin-like growth factors (IGFs), regulating IGFs bioavailability and its function [57]. Recently evidence introduced this protein as a non-invasive biomarker for the development and pathogenesis of fatty liver disease [58, 59]. The IGFBP2 promoter was hypermethylated, and its mRNA expression downregulated in a mouse model of fatty liver; therefore, secretion of hepatic IGFBP2 and plasma level of IGFBP2 reduced [58]. This observation is in line with human studies, as serum/plasma levels of IGFBP2 decreased in obese males and females and obese men with NAFLD and NASH compared with the non-obese control group [60, 58]. Reduced level of IGFBP2 is along with decreased oxidation of fatty acids and enhanced de novo lipogenesis in hepatocytes [58].

Nevertheless, IGFBP2 concentrations increased in fibrosis caused by chronic hepatitis C compared with healthy individuals. The levels of IGFBP2 were positively associated with fibrosis stages. This contradiction might be attributed to the participation of IGFBP2 in the modulation of the parenchymal structure in advanced liver fibrosis [61].

Shared genes among the three datasets were extracted after nodes analysis in the PPI network; these genes included CHI3L1, NRG1, FADS2, IGFBP2, and PEG10 (Fig. 2). CHI3L1, also called YKL40, belongs to a chitinase family member lacking chitinase activity and enriches in the liver. Some functions of CHI3L1 are the regulation of cell proliferation, differentiation, apoptosis, and cancer metastasis. It is also related to inflammation and infection [62]. Many studies reported that CHI3L1 is a useful liver fibrosis marker in NAFLD and hepatitis B virus infection [63, 64, 62]. Liver fibrosis may be produced by accumulation and activation of liver macrophages [65]. Besides, CHI3L1 suppresses hepatic macrophage apoptosis by inhibiting Fas expression and activation of phosphoinositide 3-kinase/protein kinase B signaling and increases hepatic fibrosis [66]. Another gene was NRG1 that is a member of the epidermal growth factor

(EGF) family and binds to a class of receptor tyrosine kinases, ErbB receptors. NRG1 is implicated in the differentiation, proliferation, and survival of cells [67]. NRGs are local growth factors, while NRG1 has been found in plasma as a diagnostic marker for various maladies such as chronic heart failure (67) and acute lung injury associated with inflammation [68]. NRG-1 treatment enhanced the fibrotic region in the liver from the animal model of liver fibrosis.

Furthermore, elevated expression of TGF- β, a mediator of fibrogenesis, was observed in isolated hepatocytes after NRG1 treatment compared with untreated cells. The fibrotic impact of NRG1 might be because of the phosphorylated ErbB3 receptor and activated the NRG-1/ErbB3 pathway [69]. The other common gene was FADS2, the rate-limiting enzyme in the biosynthesis of unsaturated fatty acids and catalyzed linoleic conversion to γ-linolenic α-linolenic acid stearidonic acid [70]. Increased activity of delta-6 desaturase was observed in NASH patients and was related to DNA hypomethylation of two CpG sites in FADS2 [71]. Also, Walle *et al.* [72] showed similar findings. They found a reduced level of liver DNA methylation in the FADS2 loci is associated with both more excellent serum activity of delta-6 desaturase and higher hepatic mRNA expression of FADS2. Furthermore, they suggested that FADS2 contributes to NAFLD's early stages and not in an advanced stage of hepatic fibrosis.

5. Conclusion

This study revealed unique and shared key genes in simple steatosis and NASH. After further verification, the shared genes (for example, the top five genes with the highest degree among three datasets in three groups: STAT3, JUN, CANX, FN1 and MYC) might be a putative candidate for therapeutic targets to treat NAFLD. As mentioned above, some of the high degree genes, including IL-6, TLRs, FMO3, STAT3 and STAT1 are directly/indirectly related to gut microbiota. The previous studies showed that gut dysbiosis might be implicated in NASH and NAFLD pathogenesis. Therefore, further investigations are needed to clarify the role of gut microflora and involved genes. Moreover, through pathway analysis, DEGs were significantly enriched in several pathways such as signal transduction, immune response, anti-apoptosis, cell communication and metabolism and energy pathways. By considering the important biological pathways that have been reported in this study, it is suggested that in future studies, researchers study targeted on the function of these pathways in NAFLD disease.

Abbreviations

NAFLD**,** Non-alcoholic fatty liver disease; NASH, Non**-**alcoholic steatohepatitis; PPI, Protein-protein interaction; DEGs, Differentially expressed genes; ICAM1, Intercellular Adhesion Molecule 1; PEG10, Paternally expressed gene-10; FMO1, Flavin Containing Dimethylaniline Monoxygenase 1; CHI3L1, Chitinase 3 Like 1; NRG1, Neuregulin 1; FADS2, Fatty Acid Desaturase 2; IGFBP2, Insulin-like growth factor-binding proteins; IGFs, Insulin-like growth factors; IL-6, Interleukin-6; STAT3, Signal transducer and activator of transcription 3; CXCL8, C-X-C Motif Chemokine Ligand 8; TLRs, Toll-like receptors; LPS, Lipopolysaccharides; TCPTP, T-Cell Protein Tyrosine Phosphatase; ALD, Alcoholic liver disease; AKT, Acyl-CoA oxidase; HCC, Hepatocellular carcinoma; TMA, Trimethylamine; TMAO, Trimethylamine- *N*-oxide; OS,

Oxidative stress; ER, Endoplasmic reticulum; RPS27a, Ribosomal protein S27a; FN1, Fibronectin1; Ub, Ubiquitin; CANX, Calnexin; MT1A, Metallothionein 1A.

Declarations

Authors' Contributions

MA designed the project, carried out the bioinformatics studies, and performed data analysis. BFNMG participated in designing the project and contributed to supervision and data analysis, and wrote the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

Not applicable

Informed consent

Not applicable

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Figures

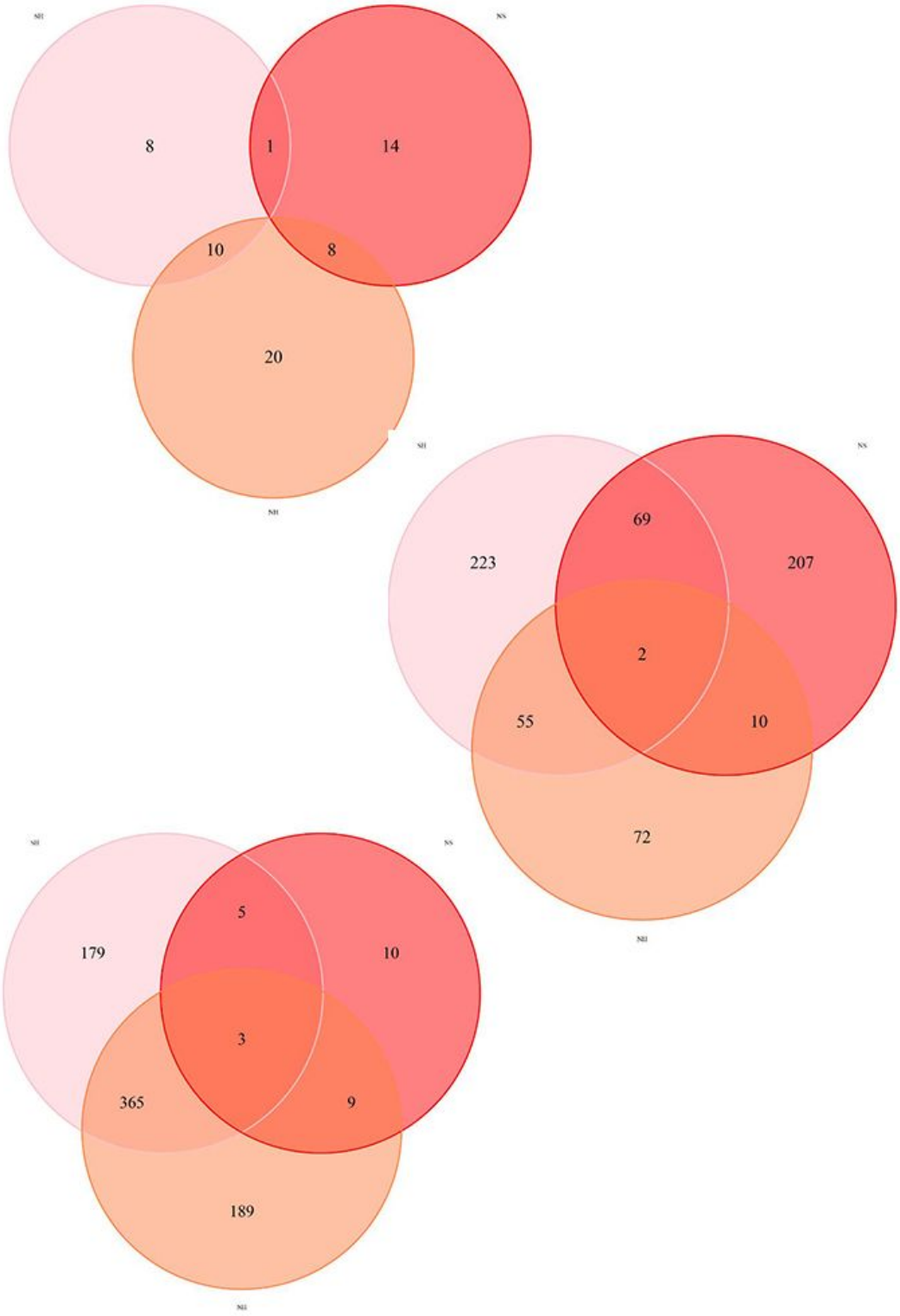


Figure 1

The shared genes among the three groups (SH, NH, and SN) for each dataset have been shown in Figure 1 a-c. There was no common gene for dataset GSE48452. However, dataset GSE63067 and dataset GSE89632 had two and three shared genes, respectively.

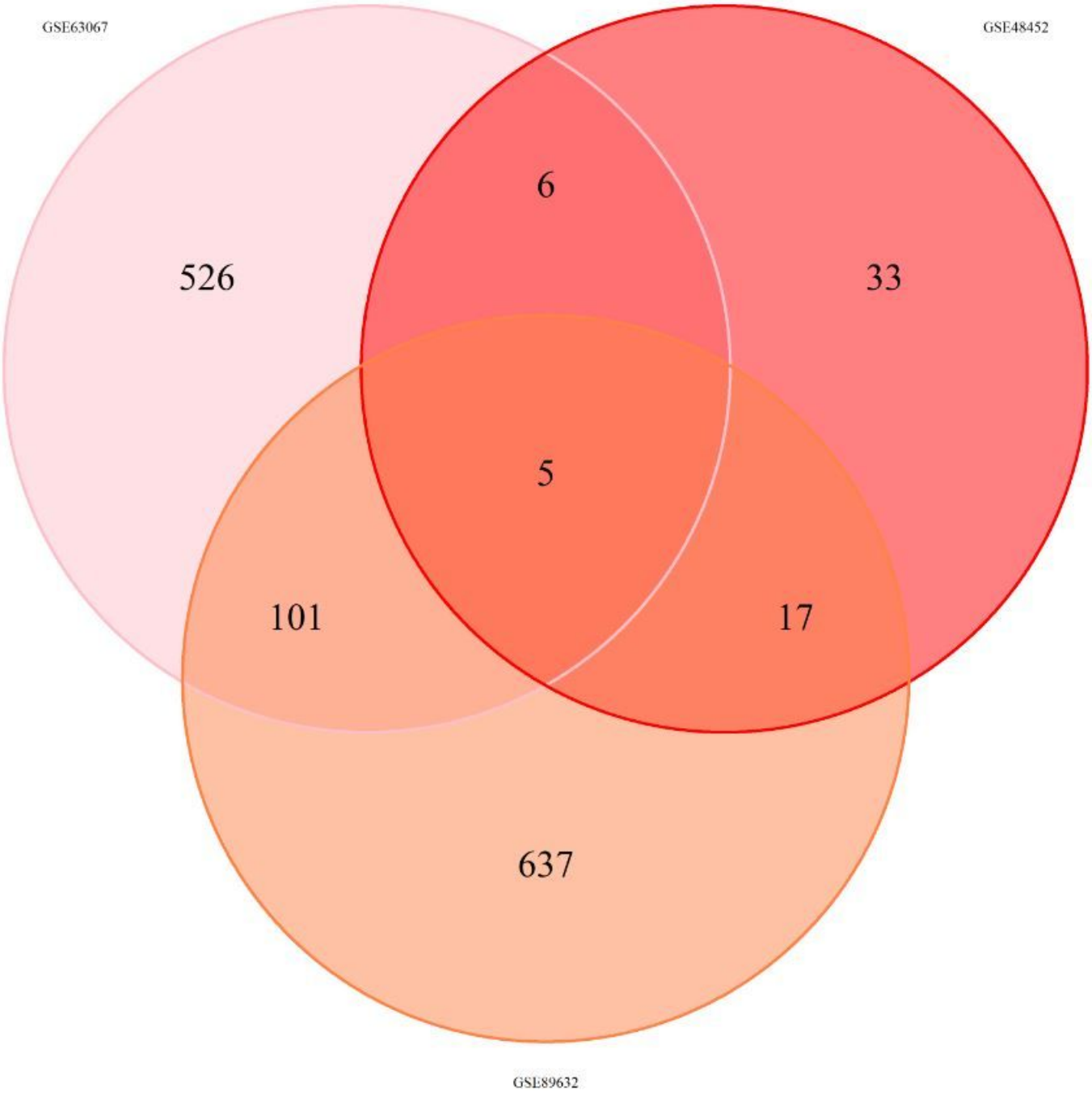
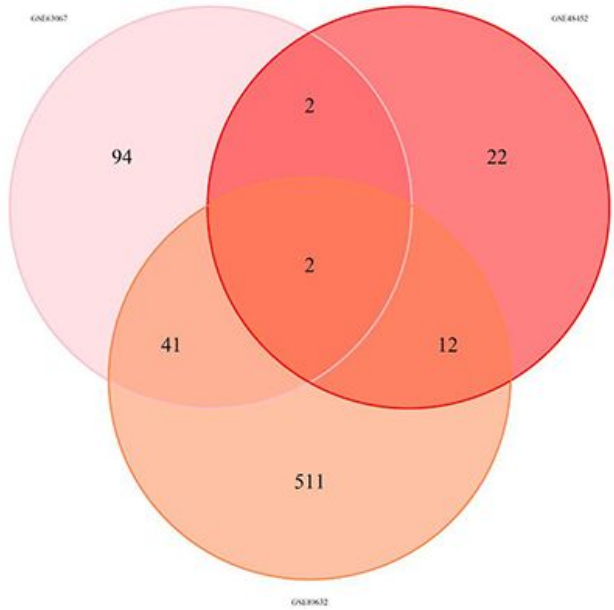


Figure 2

By examining three data sets to find the list of shared genes between all three sets, Five genes were found, which can be seen in Figure 2. These genes are CHI3L1 (Chitinase 3 Like 1), GFBP2 (Insulin-like growth factor (IGF) binding protein 2), NRG1 (Neuregulin 1), PEG10 and FADS2 (Fatty Acid Desaturase 2).



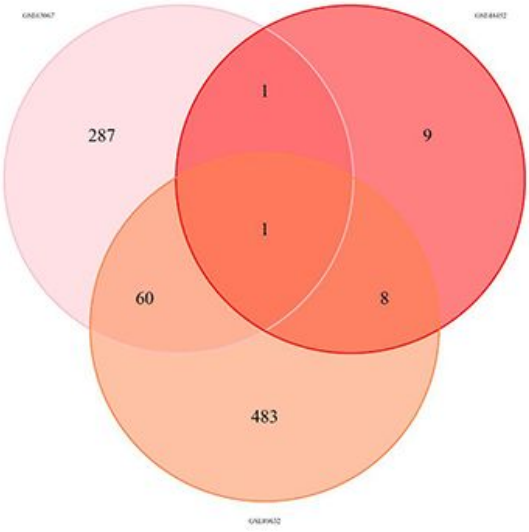


Figure 3

The DEGs of each processed group used to identify the similarities in the two sets. We plot the Venn diagram for shown overlap genes in the three groups (Figure 3). IGFBP2 (insulin-like growth factor-binding proteins) and PEG10 are the common genes in the NH's list (Figure. 3 a). There are no common genes in the NSs list (Figure. 3 b). There was only the PEG10 gene in common between the SHs list (Figure. 3 c).

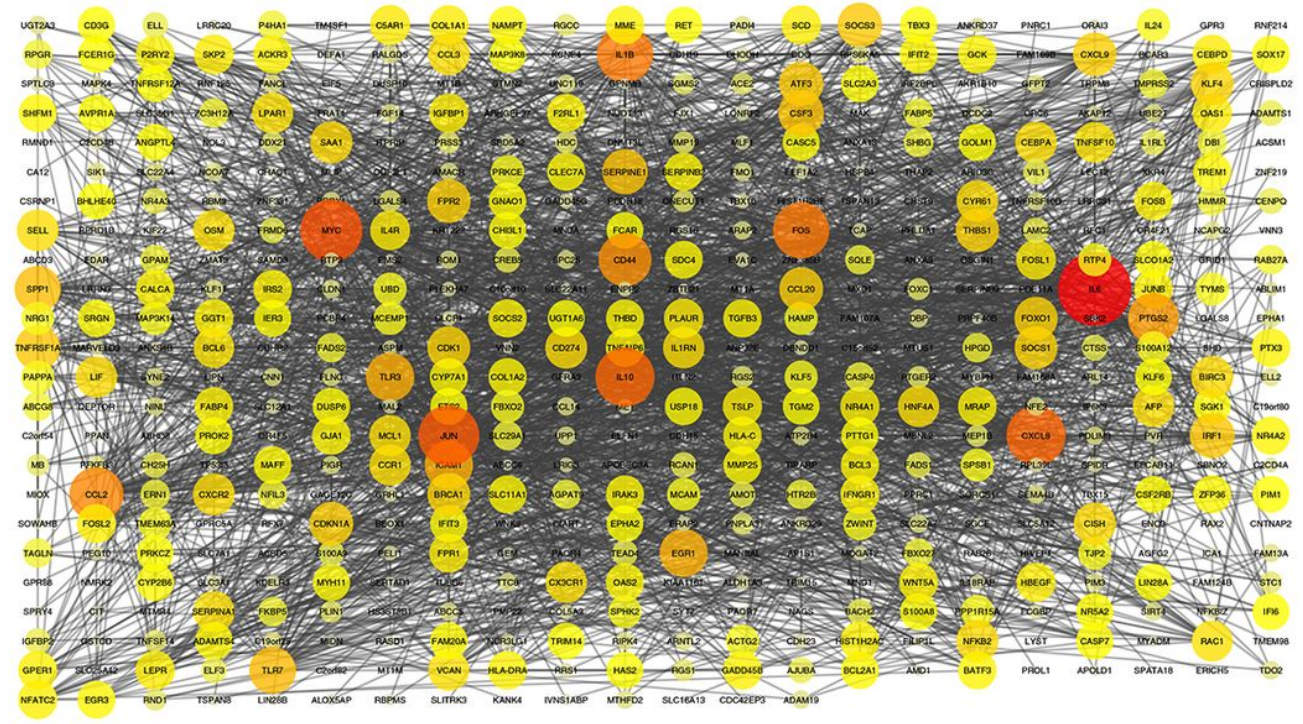


Figure 4

For example, the IL6 gene is known in the SH group network with an interactive degree of 158 in liver disease, so it is a significant focal point. On the other hand, STAT3, CXCL8 genes are also significant due to the high degree of interaction in the same network. Three global interaction networks were extracted from three gene lists shown in Figures 4

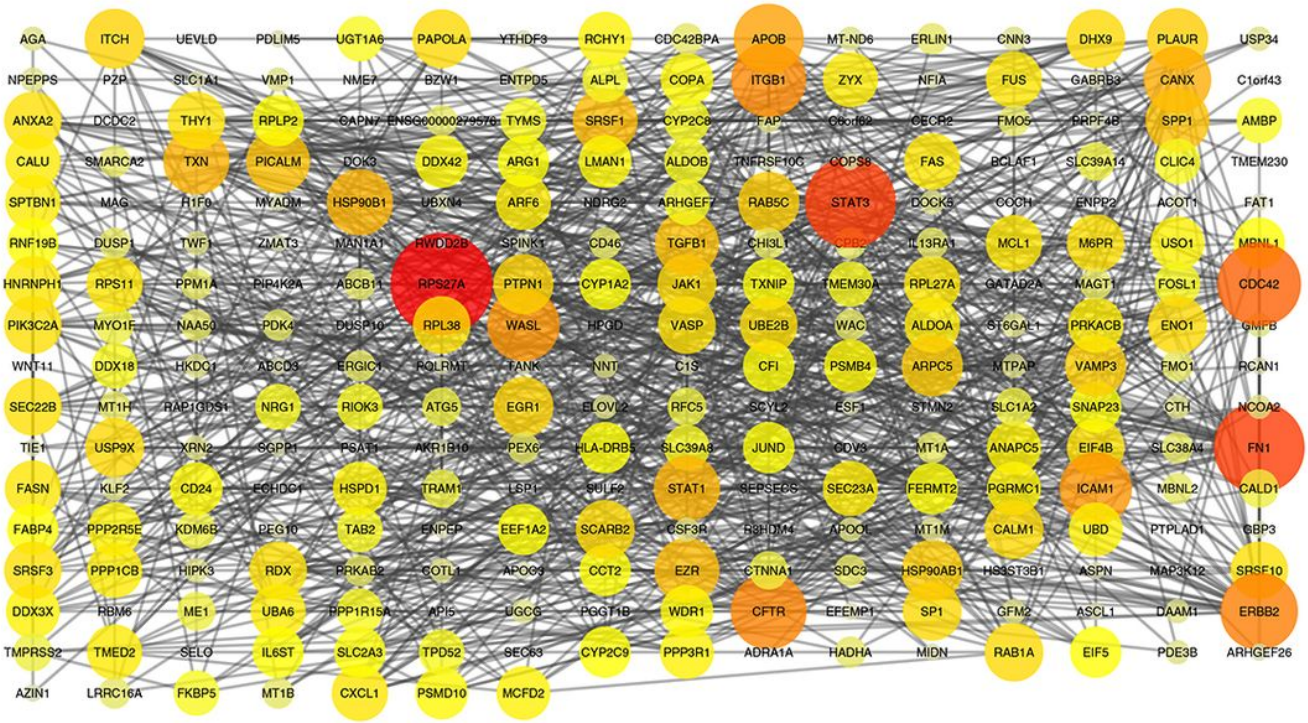


Figure 5

For example, the IL6 gene is known in the SH group network with an interactive degree of 158 in liver disease, so it is a significant focal point. On the other hand, STAT3, CXCL8 genes are also significant due to the high degree of interaction in the same network. Three global interaction networks were extracted from three gene lists shown in Figures 5.

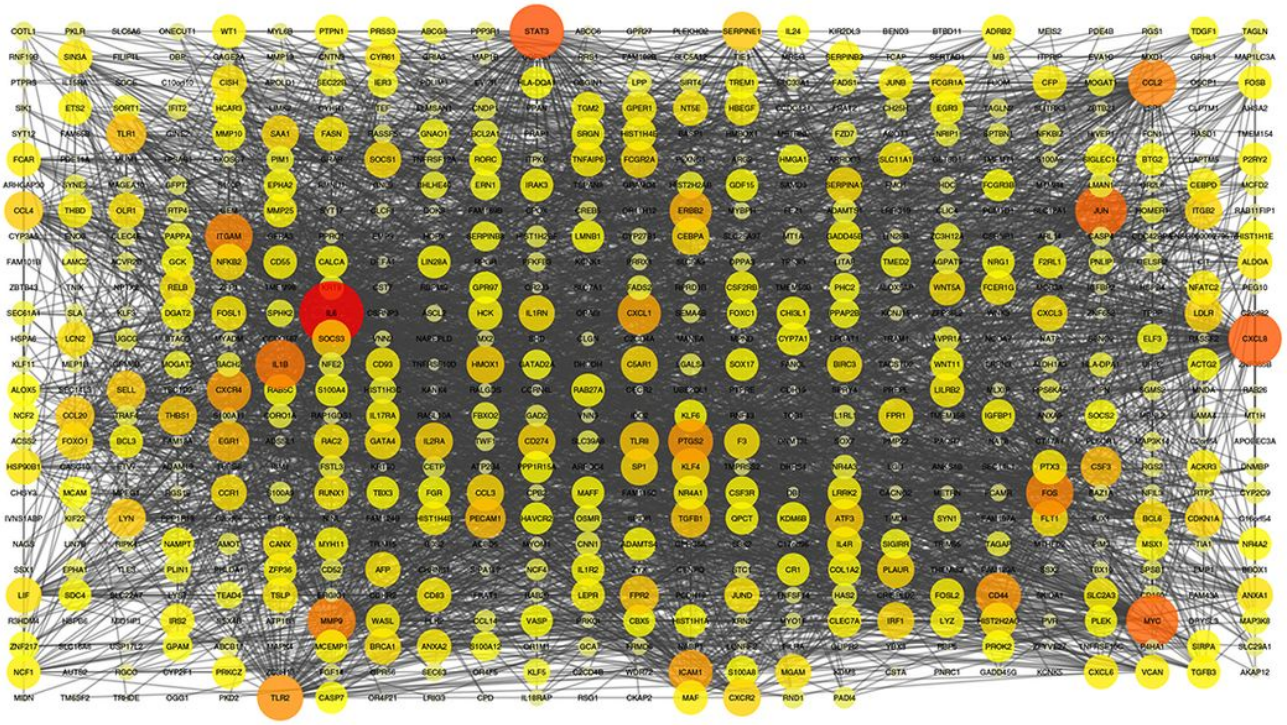


Figure 6

For example, the IL6 gene is known in the SH group network with an interactive degree of 158 in liver disease, so it is a significant focal point. On the other hand, STAT3, CXCL8 genes are also significant due to the high degree of interaction in the same network. Three global interaction networks were extracted from three gene lists shown in Figures 6.

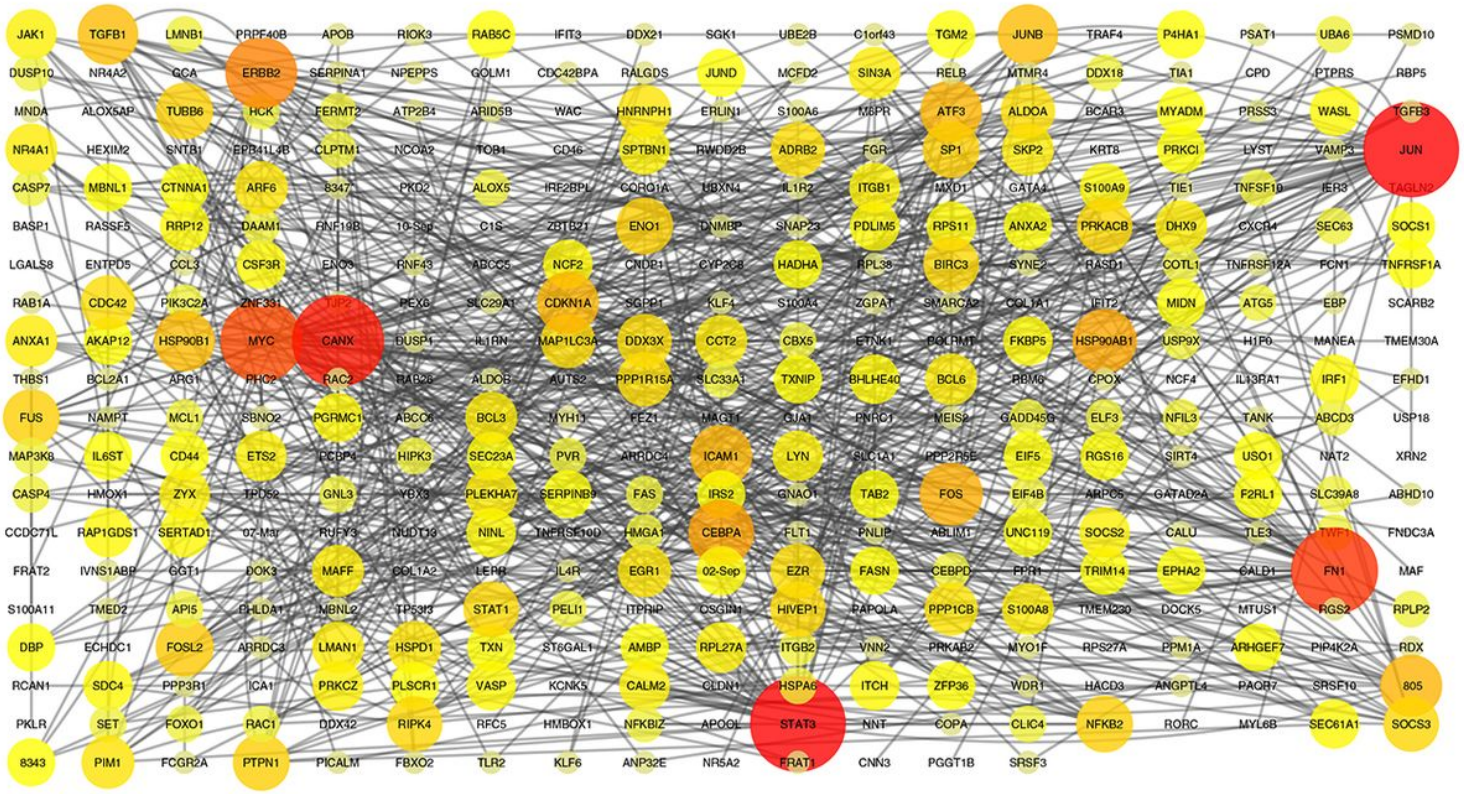


Figure 7

Besides, to study the genetic connections in a more detailed way, these connections were examined specifically for the tissue in the liver tissue, which can be seen in Figure 7. These liver tissue connections suggest that hub genes can play a crucial role in disease due to their location and role in the interaction network. As shown in Fig. 7, the STAT3 and JUN genes, as the highest gene associated with other genes, plays a crucial role in liver tissue.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary1.xlsx
- Supplementary2.xlsx
- Supplementary3.xlsx
- Supplementary4.xlsx