A ferroptosis-related gene signature for lung function and quality of life in patients with idiopathic pulmonary fibrosis

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Abstract

Background: Rapid advances in genetic and genomic technologies have begun to reshape our understanding of idiopathic pulmonary fibrosis (IPF). Ferroptosis, an iron-dependent form of regulated cell death, play an important role in the development of IPF. Therefore, our study aimed to explore the role of ferroptosis-related genes (FRGs) and their correlation with lung dysfunction and quality of life in patients with IPF.

Methods: Datasets were acquired by researching the Gene Expression Omnibus. FRGs were acquired by researching GeneCard database and PubMed. Ferroptosis-related differentially expressed genes (FRDEGs) were identified according to integrating FRGs and the DEGs identified in the GSE110147 dataset. Candidate key genes were identified from the miRNA-target FRDEGs network and protein-protein interactions (PPI) network. The relationship between key genes and lung function or quality of life was calculated using the GSE32537 datasets.

Results: 293 FRGs were obtained, and 71 FRDEGs were identified. According to enrichment analysis, cell growth and death and pathways associated cancer were the important pathways, and significant biological processes were mainly consisted of cellular responses to stimulus and various situations. In addition, this study constructed an PPI network and a miRNA-target network based on the 71 FRDEGs, determined 19 candidate key genes. Furthermore, acyl-CoA synthetase long chain family member 1 (ACSL1), integrin subunit beta 8 (ITGB8) and ceruloplasmin (CP) were identified as the key genes. The expression level of ACSL1 was the strongest predictor for lung function (negatively) including percent predicted forced vital capacity (FVC% predicted) and percent predicted diffusion capacity of the lung for carbon monoxide (DIco% predicted) and quality of life (negatively). In addition, ITGB8 and CP were negatively associated with FVC% predicted. According to DrugBank and PubMed, 4 drugs and 16 drugs have been found to act on ACSL1 and CP, respectively.

Conclusion: These results imply that FRGs may shed new understanding on disease mechanism and provide potential biomarkers and therapy target to predict IPF progression.

Introduction

Idiopathic pulmonary fibrosis (IPF), a common interstitial lung disease (ILD) of unknown etiology with repeated acute lung injury, causes worsening dyspnea and deteriorating lung function [1]. The incidence of IPF among people aged 18–64 years between 2005 and 2010 according to a study in the United States was 6.1 new cases per 100000 person-years [2]. Currently, two drugs (Pirfenidone and Nintedanib) have been identified to be moderately effective in treating IPF [3, 4]. However, the prognosis of IPF remains severe, with death usually occurring within 2–3 years after diagnosis [5, 6], and the 5-year survival rate is only 20% [7]. Through the past decades, rapid advances in genetic and genomic technologies have begun to reshape our understanding of IPF. Studies have uncovered some genes that are linked to IPF, including telomerase reverse transcriptase, TERT [8, 9]; transforming growth factor beta 1, TGFB1 [10]; and mucin 5B, MUC5B [11] et al. However, the pathophysiologic mechanisms of IPF are complex and remain incompletely understood.

Ferroptosis is a new type of regulated cell death (RCD) which is dependent on iron, and different from apoptosis, cell necrosis and autophagy [12]. Previous study had confirmed that iron overload may cause lung

fibrosis according to increased lipid peroxidation and decreased glutathione peroxidase 4 (GPX4) activity in lung tissues [13]. Furthermore, studies have verified that ferroptosis plays an important role in the development of pulmonary fibrosis, and ferroptosis inhibitor may attenuate pulmonary fibrosis progression [14, 15]. Many genes such as GPX4, solute carrier family 7 member 11 (SLC7A11), transforming growth factor beta receptor 1 (TGFBR1) and so on have also been identified as regulators or markers of ferroptosis, and were associated with the development of pulmonary fibrosis [14–16]. However, the systematic exploration of role of ferroptosis-related genes (FRGs) as well as their values for lung function and quality of life are absent in patients with IPF.

Therefore, the purposes of the study are to analyzed the characteristics of ferroptosis-related differentially expressed genes (FRDEGs) in IPF based on the Gene Expression Omnibus (GEO) or other databases, such as miRDB, GeneCards etc., and construct miRNA-target interactions network to explore a novel approach for the determination of gene functions and the pathogenesis of IPF. Furthermore, we summarized the information derived from miRNA-target interactions, gene oncology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, protein-protein interaction (PPI) data, and then screened out useful potential biomarkers for lung function and quality of life and therapeutic targets for IPF.

Materials And Methods

Acquisition of datasets.

Figure 1 shows the workflow of our study. On the GEO database (http://www.ncbi.nlm.nih.gov/geo/), we selected datasets must meet the following items: (1) the gene expression profile was measured using same platform; (2) the detected samples came from the lung tissues of patients with IPF or healthy donors; (3) raw data or a gene expression matrix should be provided. Finally, two datasets were identified, GSE110147 and GSE32537 (platform: GPL6244). Approval of the Ethics Committee was not required because the information of patients was obtained from the GEO.

Human miRNA-target interactions data were downloaded from miRDB [17]. FRGs were obtained from GeneCards database (https://www.genecards.org/) by searching the terms “ferroptosis” and PubMed by searching the terms “Ferroptosis [MeSH] OR Ferroptosis* [tiab]”. Consequently, 103 FRGs and 190 FRGs were respectively collected from GeneCards and PubMed in the study as shown in **Supplementary Table 1**.

Datasets preprocessing

The raw data (CEL format) of GSE110147 and GSE32537 were downloaded from GEO. “Affy” package (http://bioconductor.org/packages/release/bioc/html/affy.html, v.1.68.0) was used to normalize the array data according to the robust multi-array average (RMA) method. We defined IPF differentially expressed genes (DEGs) as expression levels of genes were significantly diverse in IPF patients compared with the controls (|log Fold Change|>1 and adjusted p-value < 0.05). “Limma” package (v.3.46.0) [18] was used for the analysis of DEGs. In addition, St. George’s Respiratory Questionnaire (SGRQ) score and lung function [percent predicted forced vital capacity (FVC% predicted) and percent predicted diffusion capacity of the lung for carbon monoxide (Dlco% predicted)] were extracted from the GSE32537 dataset (Table 1).

Table 1
Demographic data for subjects used in this study.

Characters	GSE32537 (119 IPF)
Age (years)	62 ± 8
Sex (%)	
Male	77 (64.7)
Female	42 (35.3)
Smoking history (%)	
Yes	70 (58.8)
No	41 (34.5)
NA	8 (6.7)
FVC% predicted	61.25 ± 17.02
Dlco% predicted	45.13 ± 20.297
SGRQ score	47.43 ± 21.45
Data are presented as mean ± SD or n(%).	

Analysis of data

GO and KEGG enrichment analyses of the FRDEGs of IPF were analyzed and visualized by R package “clusterProfiler” [19]. Heatmap was constructed according to R packages “gplots” (v.3.1.1) and “RColorBrewer (v.1.1-2)”. STRING (http://www.string.embl.de/, version: 11.0b) was used to analyze the protein-protein interactions (PPI) [20]. Cytoscape (version 3.7.1) [21] was used to visualize miRNA-target network and PPI network, and its MCODE were used to make the visualization of PPI network and identify the modules in the network [parameters: Degree cutoff ≥ 2 (degrees of each nodes in module were larger than 2 at least), K-core ≥ 2 (subgraphs of each node in module were more than 2 at least)].

Drug discovery

DrugBank [22] and PubMed were used to screen drugs associated with related gene that was predicted to be an important gene in this study.

Statistical analysis

Continuous variables were compared between two groups by applying the non-parametric t test. Associations between the expression levels of genes and lung function and SGRQ score were determined by Spearman correlation coefficient. All statistical analyses were carried out with GraphPad Prism 7.0, and $P < 0.05$ was considered statistically significant.

Results

FRDEGs of IPF

After integrating 293 FRGs and the DEGs identified in the GSE110147, 47 up-regulated and 24 down-regulated FRDEGs were identified (Fig. 2A-2B).

PPI network

The PPI network was constructed based on the 71 FRDEGs according to the STRING database (average node degree: 5.6, PPI enrichment p-value: < 1.0e-16), which was visualized by Cytoscape [20, 21]. We removed the nodes with no connections, Therefore, the final network contained 66 nodes and 196 edges (Fig. 2C). Ceruloplasmin (CP) was the highest up-regulated gene, and angiopoietin like 4 (ANGPTL4) was the highest down-regulated gene in the PPI network. We calculated the connectivity degree of each node, and selected those with degrees ≥ 15, as follows: mitogen-activated protein kinase 3 (MAPK3, down-regulated), heme oxygenase 1 (HMOX1, down-regulated), KRAS proto-oncogene, GTPase (KRAS, up-regulated), heat shock protein family A member 5 (HSPA5, up-regulated) and ATM serine/threonine kinase (ATM, up-regulated). In addition, one module (**Figure S1**) were selected after MCODE analysis of the whole network, and the results of enrichment analysis of FRDEGs within the module were showed in **Figure S2** by R package “clusterProfiler” [19], which revealed the important pathways: cell growth and death, and pathways associated cancer.

Key gene ontology and pathways enriched in IPF

In order to reveal the biological significance of 71 FRDEGs regulating IPF at a single level, we used R package “clusterProfiler” [19] to conduct biological pathway enrichment and biological process annotation for the 71 genes mentioned above. The 20 most significantly KEGG pathways were selected (**Supplementary Table 2**, Fig. 3A, 3F). More importantly, cell growth and death, pathways associated cancer and signal transduction were the main pathways, implying that FRDGEs may participate in the process of IPF according to these pathways (**Figure S3A**). Hsa04216 (Ferroptosis, including 11 FRDEGs) was the first significantly enriched pathway (**Figure S3B**). FRDEGs-related top 20 biological processes (BP), cellular component (CC) and molecular function (MF) were showed in Fig. 3B-3D respectively. The top 20 GOs were showed in **Supplementary Table 3** and Fig. 3E, which were consisted of cellular responses to stimulus and various situations.

Some potential biomarkers had been found in IPF

A total of 1638 miRNA-target interactions associated with 68 of 71 FRDEGs and 463 related miRNAs were derived from miRDB [17] and visualized by Cytoscape (Fig. 4). The related nodes with degrees ≥ 25 were shown in Table 2. The more interactions with miRNAs, the more degree is. Therefore, integrin subunit beta 8 (ITGB8) was considered the hub node. In addition, for miRNA, the related nodes with degrees ≥ 9 were shown in Table 3. The top 5 hub nodes with higher degrees were hsa-miR-513a-3p, hsa-miR-513c-3p, hsa-miR-19a-3p, hsa-miR-19b-3p, hsa-miR-3065-5p.

Table 2
The node with degrees ≥ 25 were shown according to the miRNA-target network

Target Gene	Degrees	Target Gene	Degrees	Target Gene	Degrees
ITGB8	99	**HIF1A**	36	**DLD**	27
ACSL4	87	**TFRC**	36	**RNF20**	27
PIK3CA	81	**ACSL3**	35	**TFAM**	27
TGFBR1	77	**G3BP1**	34	**NCOA4**	26
KRAS	69	**CAV1**	33	**HMGB1**	25
FBXW7	65	**IREB2**	33	**NFE2L2**	25
PRKAA1	60	**SNX4**	32		
ITGA6	51	**ATM**	31		
MYB	45	**GCLC**	31		
ACSL1	40	**MAP3K5**	31		
TP63	40	**HMGCR**	30		

Table 3
The node with degrees ≥ 9 were shown according to miRNA-target network.

miRNA	Degrees	miRNA	Degrees	miRNA	Degrees
hsa-miR-513a-3p	17	**hsa-miR-374b-5p**	9	**hsa-miR-548e-3p**	9
hsa-miR-513c-3p	17	**hsa-miR-493-5p**	9	**hsa-miR-548f-3p**	9
hsa-miR-19a-3p	12	**hsa-miR-506-3p**	9	**hsa-miR-548h-5p**	9
hsa-miR-19b-3p	12	**hsa-miR-548a-3p**	9	**hsa-miR-548j-5p**	9
hsa-miR-3065-5p	10	**hsa-miR-548am-5p**	9	**hsa-miR-548o-5p**	9
hsa-miR-124-3p	9	**hsa-miR-548b-5p**	9	**hsa-miR-582-5p**	9
hsa-miR-1297	9	**hsa-miR-548c-5p**	9	**hsa-miR-664b-3p**	9
hsa-miR-374a-5p	9	**hsa-miR-548d-5p**	9	**hsa-miR-450b-5p**	9

Identify of key genes

The top 12 genes with high degrees in Table 2 and the top 7 genes with high degrees or high DEG level as mentioned above in the PPI network were selected as the candidate key genes. Subsequently, the expression levels of 19 candidate key genes were compared between IPF patients and healthy control in the GSE32537 dataset. Finally, acyl-CoA synthetase long chain family member 1 (ACSL1, down-regulated), CP (up-regulated),

tumor protein p63 (TP63, up-regulated), ITGB8 (up-regulated) and MYB proto-oncogene, and transcription factor (MYB, up-regulated) were selected as the key genes (Fig. 5A-5E). According to linear regression, ASCL1 was negatively associated with FVC% predicted, DIco% predicted, and positively associated with SGRQ score, and the Spearman correlation coefficients were calculated as -0.4132, -0.3609 and 0.2964, respectively (Fig. 6A-6C). In addition, CP and ITGB8 were only negatively associated with FVC% predicted, the Spearman correlation coefficients were calculated as -0.2095 and – 0.2345, respectively (Fig. 6D, 6G). However, the correlations between CP and DIco% predicted or SGRQ score were not significant (Fig. 6E-6F), ITGB8 showed the same result (Fig. 6H-6I)

Drug discovery

According to DrugBank [22], 16 drugs have been found to be acted on CP, and 2 drugs have been found to be acted on ACSL1. According to searching from PubMed, 2 drugs [Benzimidazole series (compound 13) [23] and Aspirin [24]] were found to be acted on ACSL1, however, no additional drugs associated with CP or ITGB8 were found. These drugs and related papers were listed in Table 4.

Table 4
The drugs acting on ACSL1 and CP in DrugBank and PubMed.

Targeted gene	Drug	DrugBank ID	Drug group	Pharmacological action?	Action	PubMed IDs
ACSL1	Adenosine phosphate	DB00131	Approved, investigational, nutraceutical	Unknown	Product of	16981708 17350930
	ATP	DB00171	Investigational, nutraceutical	Unknown		17139284 17016423
	Benzimidazole series (compound 13)	NA	Investigational	Unknown	Inhibitor	33285268
	Aspirin	NA	Investigational	Unknown	Inhibitor	28359761
CP	Copper	DB09130	Approved, investigational	No	Binder	14652164
	Calcium	DB01373	Nutraceutical	Unknown		17242517
	Iron	DB01592	Approved	Unknown		21049900
	Zinc	DB01593	Approved, investigational	Unknown		23896426
	Cupric sulfate	DB06778	Approved	Unknown		NA
	Ferrous sulfate anhydrous	DB13257	Approved	Yes	Substrate	775938
	Cupric oxide	DB11134	Approved	Unknown	Binder	NA
	Silver	DB12965	Approved, investigational	Unknown	Binder	NA
	Zinc acetate	DB14487	Approved, investigational	Unknown		23896426
	Ferrous gluconate	DB14488	Approved	Unknown		21049900
	Ferrous succinate	DB14489	Approved	Unknown		21049900
	Ferrous ascorbate	DB14490	Approved	Unknown		21049900
	Ferrous fumarate	DB14491	Approved	Unknown		21049900
	Ferrous glycine sulfate	DB14501	Approved	Unknown		21049900

Targeted gene	Drug	DrugBank ID	Drug group	Pharmacological action?	Action	PubMed IDs
	Zinc chloride	DB14533	Approved, investigational	Unknown	Ligand	23896426
	Zinc sulfate, unspecified form	DB14548	Approved, experimental	Unknown	Ligand	23896426

Discussion

IPF is a serious lung disease, and until today, there is no effective way to treat it. In this study, 71 FRDEGs from 293 FRGs were identified in disease samples compared to normal control in the GSE110147 dataset. The bioprocess enrichment analysis showed that the 71 FRDEGs mentioned above were significantly correlated with a series of biological processes: cellular responses to stimulus and various situations. Persistent alveolar epithelial injury and the abnormal repair are the important causes of lung fibrosis [25]. Therefore, cellular responses to the persistent injury are important in the development of IPF. Abnormal cellular responses may lead to epithelial-mesenchymal transition (EMT), which may promote the development of lung fibrosis [15]. Therefore, FRGs may participate in the development of IPF according to these biological processes.

Furthermore, KEGG pathways analysis of 71 FRDEGs and the module identified from the PPI network showed that cell growth and death, pathways associated cancer and signal transduction were significant enriched pathways. Similar to cancer, IPF affects susceptible individuals and shares common risk factors for cancer such as smoking, environmental or professional exposure, viral infections, and chronic tissue injury [26]. The incidence of cancer in IPF patients is higher compared with matched controls, especially for lung cancer [27]. Ferroptosis, FoxO signaling pathway, HIF-1 signaling pathway and so on play key roles in the development and prognosis of cancer [28–31]. In addition, the programmed death ligand-1/programmed cell death 1 (PD-L1/PD-1) axis can promote cancer cells to escape the surveillance of the immune system. And studies showed that PD-L1 was overexpressed in the lung tissues [32], lung fibroblasts [33] and CD4 T cells [34] in IPF. Therefore, we speculated that FRDEGs may participate in the development of cancer in patients with IPF according to these pathways.

MicroRNAs (miRNAs), a kind of small non-coding regulatory rna, are composed of 18–25 nucleotides that inhibit the translation or degradation of RNA transcripts in a sequence-specific manner, thus controlling the expression of protein-coding/non-protein-coding genes [35, 36]. To date, several studies have suggested that differently expressed miRNAs, DEGs, and microRNA-controlled differential gene expression represent key topics in the field of biomedical research into pulmonary fibrosis [37–39]. In this study, we constructed a miRNA-target FRDEGs network, and found that ITGB8 has the highest degree in the network, followed by ACSL4 and PIK3CA, which may be important biomarkers for regulating IPF. According to searching in the ILDGDB database [40] (a manually curated database of genomics, transcriptomics, proteomics and drug information for interstitial lung diseases), no related study was found for the three genes in patients with IPF.

However, studies have verified the important role of ITGB8 in renal fibrosis [41], ACSL4 in liver fibrosis [42] and PIK3CA in myocardial fibrosis [43]. Therefore, further study is needed.

Subsequently, we verified the expression of 19 candidate key genes derived from the miRNA-target network and the PPI network in the GSE32537 dataset, then, 5 key genes were found. According to linear regression, ACSL1 was the strongest predictor for lung function and quality of life. ACSL1 plays a key role in fatty acid metabolism. Studies have found that lipid metabolism dysregulation play an important role in the pathogenesis of IPF [44, 45]. In addition, the levels of stearic acid (the one of fatty acid) is down-regulated in IPF lung tissues than in control lung tissues, and further study found that stearic acid had antifibrotic activity [45]. Therefore, ACSL1 may play a key role in the development of IPF according to regulating the fatty acid metabolism. Interestingly, ACSL1 is up-regulated in the GSE110147 dataset, however, it is down-regulated in the GSE32537 dataset. The expression level of ACSL1 may need further study to confirm.

The drugs were also screened in DrugBank and PubMed for ACSL1, ITGB8 and CP. Four drugs and sixteen drugs have been found to act on ACSL1 and CP, respectively. For example, representative compound 13 was remarkable inhibitor against not only ACSL1 (IC50 = 0.042 μM) but also other ACSL isoforms [23]. However, more experimental verifications are still needed to prove this hypothesis.

Conclusion

These results suggest that FRDEGs may provide new clues to potential biomarkers and therapeutic targets for predicting the lung function and quality of life of patients with IPF. However, the results need further study to verify.

Abbreviations

idiopathic pulmonary fibrosis, IPF; gene ontology, GO; interstitial lung disease, ILD; telomerase reverse transcriptase, TERT; transforming growth factor beta 1, TGFB1; mucin 5B, MUC5B; regulated cell death, RCD; glutathione peroxidase 4, GPX4; ferroptosis-related genes, FRGs; solute carrier family 7 member 11, SLC7A11; transforming growth factor beta receptor 1, TGFBR1; ferroptosis-related differentially expressed genes, FRDEGs; Gene Expression Omnibus, GEO; protein-protein interaction, PPI; differentially expressed genes, DEGs; Kyoto Encyclopedia of Genes and Genomes, KEGG; St. George's Respiratory Questionnaire, SGRQ; percent predicted forced vital capacity, FVC% predicted; percent predicted diffusion capacity of the lung for carbon monoxide, Dlco% predicted; Ceruloplasmin, CP; angiopoietin like 4, ANGPTL4; mitogen-activated protein kinase 3, MAPK3; heme oxygenase 1, HMOX1; KRAS proto-oncogene, GTPase, KRAS; heat shock protein family A member 5, HSPA5; ATM serine/threonine kinase, ATM; biological processes, BP; cellular component, CC; molecular function, MF; integrin subunit beta 8, ITGB8; acyl-CoA synthetase long chain family member 1, ACSL1; tumor protein p63, TP63; MYB proto-oncogene, transcription factor, MYB; death ligand-1/programmed cell death 1, PD-L1/PD-1; MicroRNAs, miRNAs.

Declarations

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Author contributions

Yang Y performed data collection. Li YP and Ning SW performed data collection, prepared the first manuscript draft, validated data collection, refined the research idea, performed data analysis and edited manuscripts. designed the study and wrote the manuscript. Wang C and Chen H developed the research idea, refined the research idea, validated data collection and edited manuscripts. All authors read and approved the final manuscript. Wang C and Chen H are the guarantor of the manuscript.

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Conflicts of Interest

The authors have no conflicts of interest.

Ethics approval and consent to participate

Not applicable.

Competing interests

Not applicable.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available in the GEO repository, https://doi.org/10.1186/s12931-018-0857-1 [46]and https://thorax.bmj.com/content/68/12/1114 [47].

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Figures

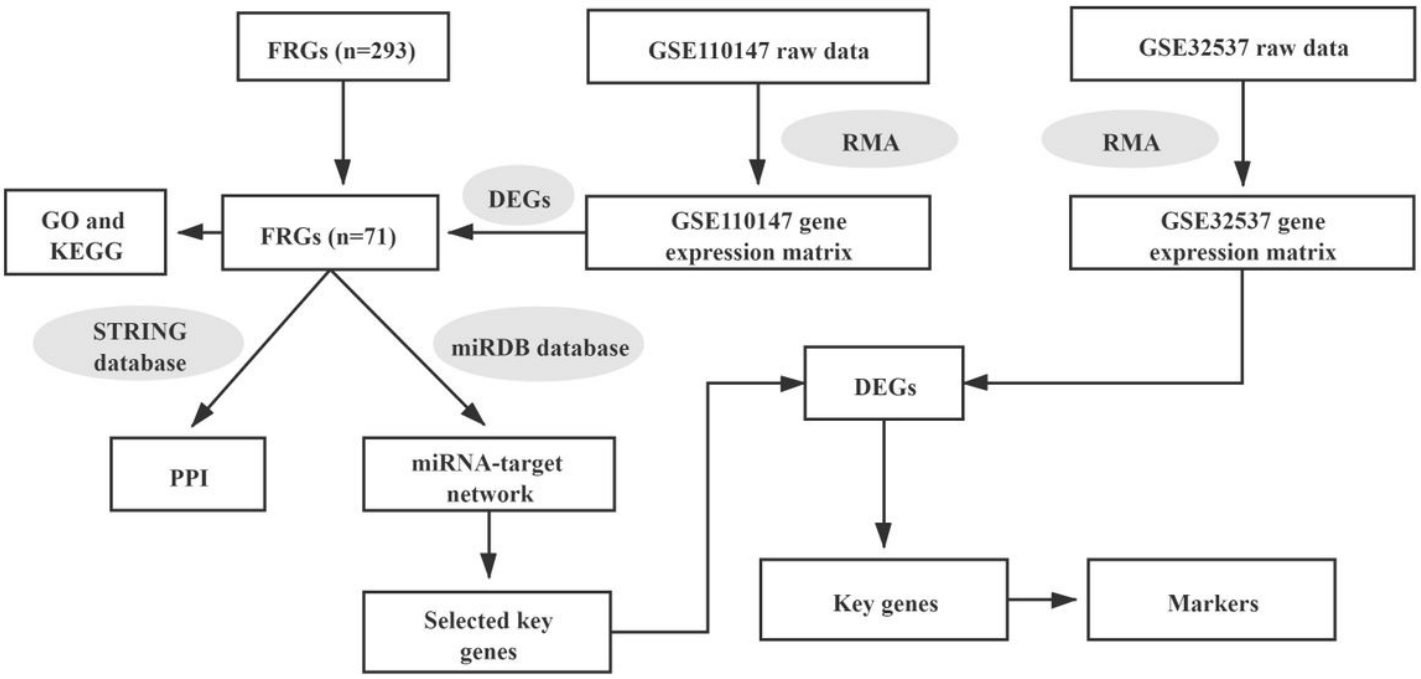

Figure 1

Workflow of this study.

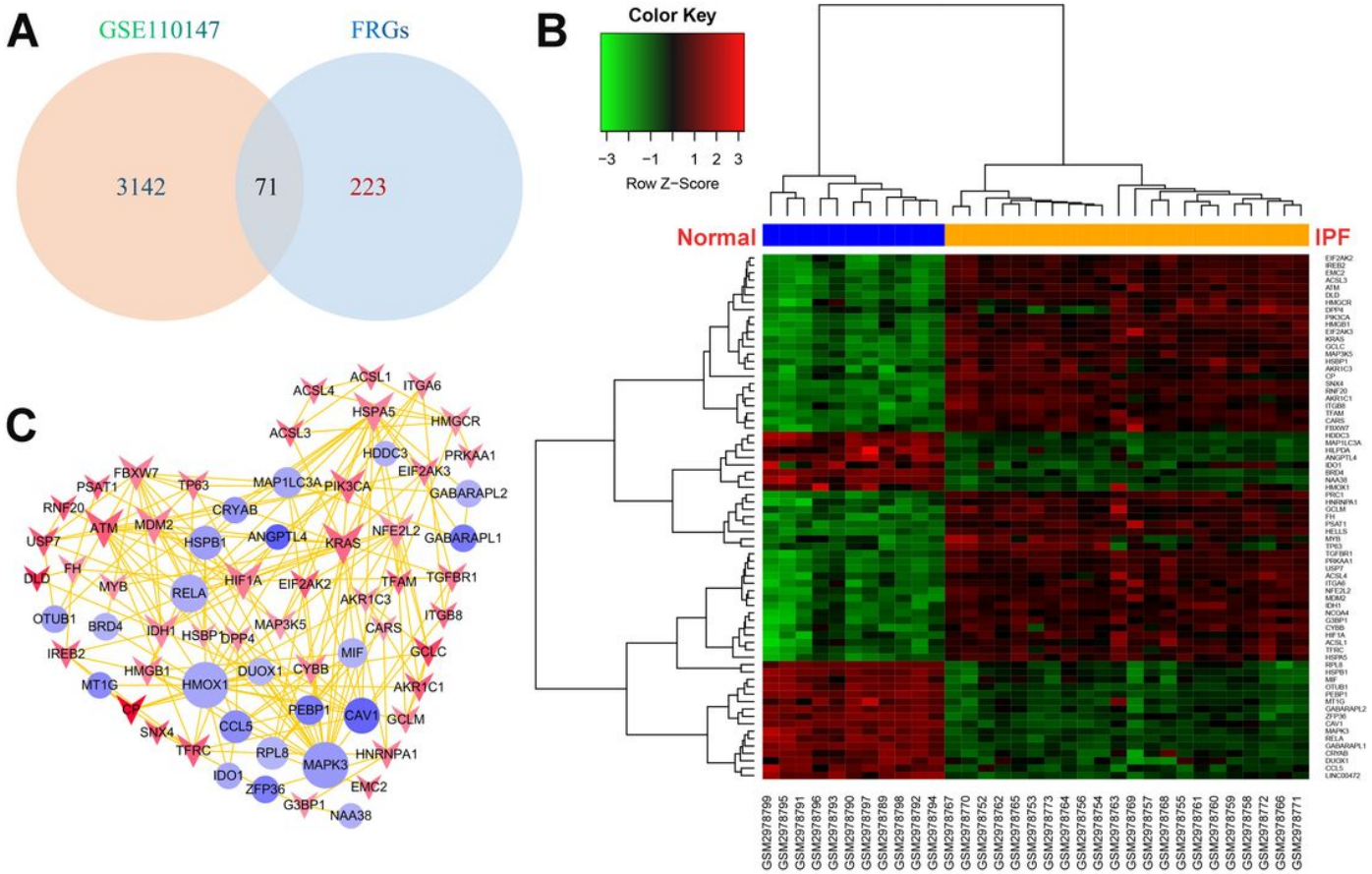

Figure 2

Protein-protein interaction network of FRDEGs. (A) The intersection of genes for FRGs and DEGs of GSE110147. (B) Network heatmap plot of 71 FRDEGs between IPF patients and normal controls. Red represents expression level of genes is up-regulated, green represents expression level of genes is down-regulated. A relative color scheme uses the minimum and maximum values in each row to convert values to colors. (C) The PPI network visualized by Cytoscape software. We removed the 5 nodes which had no connections with others. The triangle represented downregulation and the circular represented upregulation in IPF. Degree of connectivity was showed by different size of the two shapes; the bigger shape represents higher degree. The darker shade of red or blue represent the higher differentially expressed level.

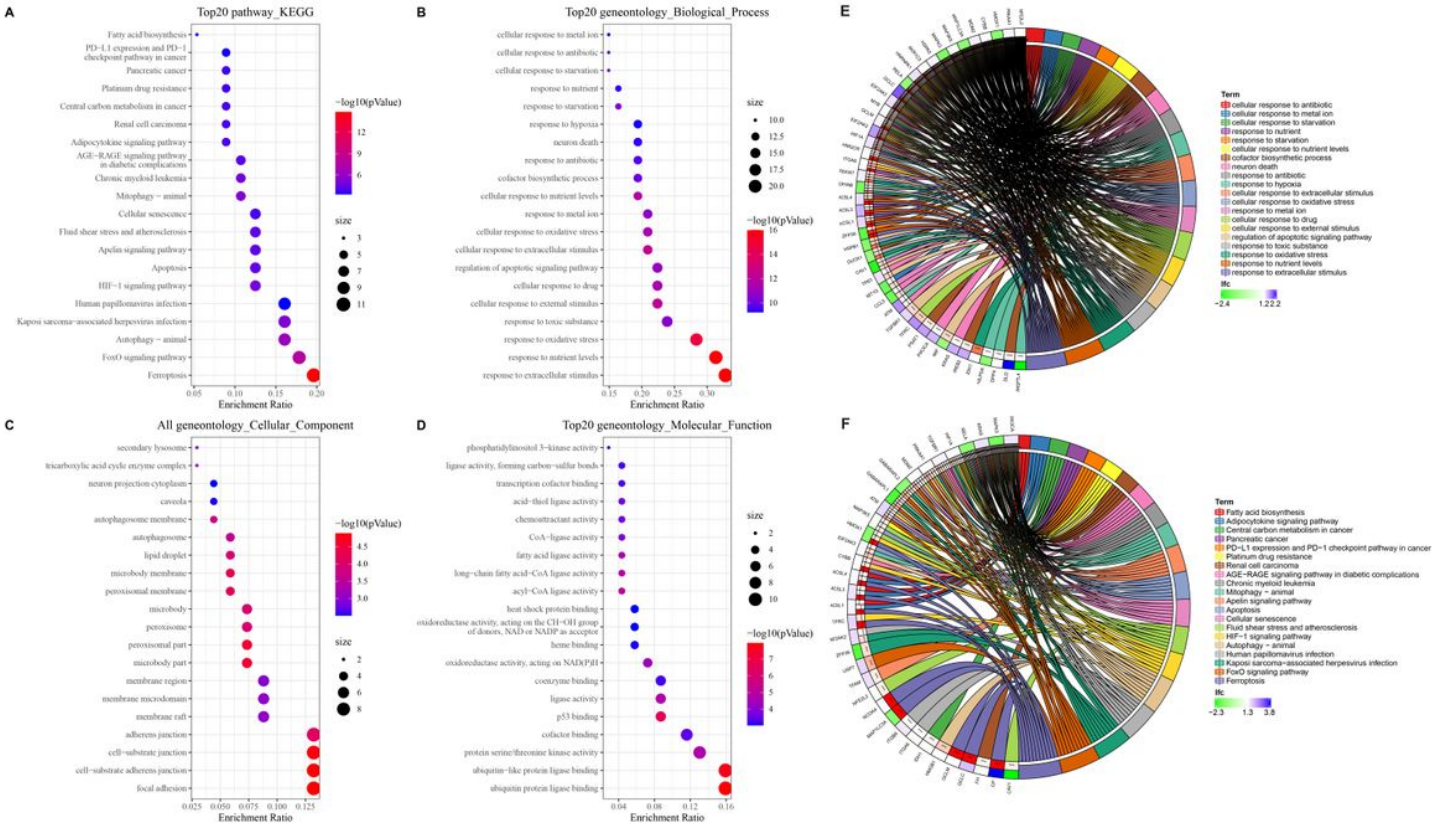

Figure 3

Significant GO terms and KEGG pathway analysis for the 71 FRDEGs. (A) The top 20 KEGG pathways terms. (B) The top 20 terms for biological processes. (C) The top 20 terms for cellular component. (D) The top 20 terms for molecular function. (E) The circle plot of GO enrichment. (F) The circle plot of KEGG pathways enrichment. Lfc represent the differentially expressed level.

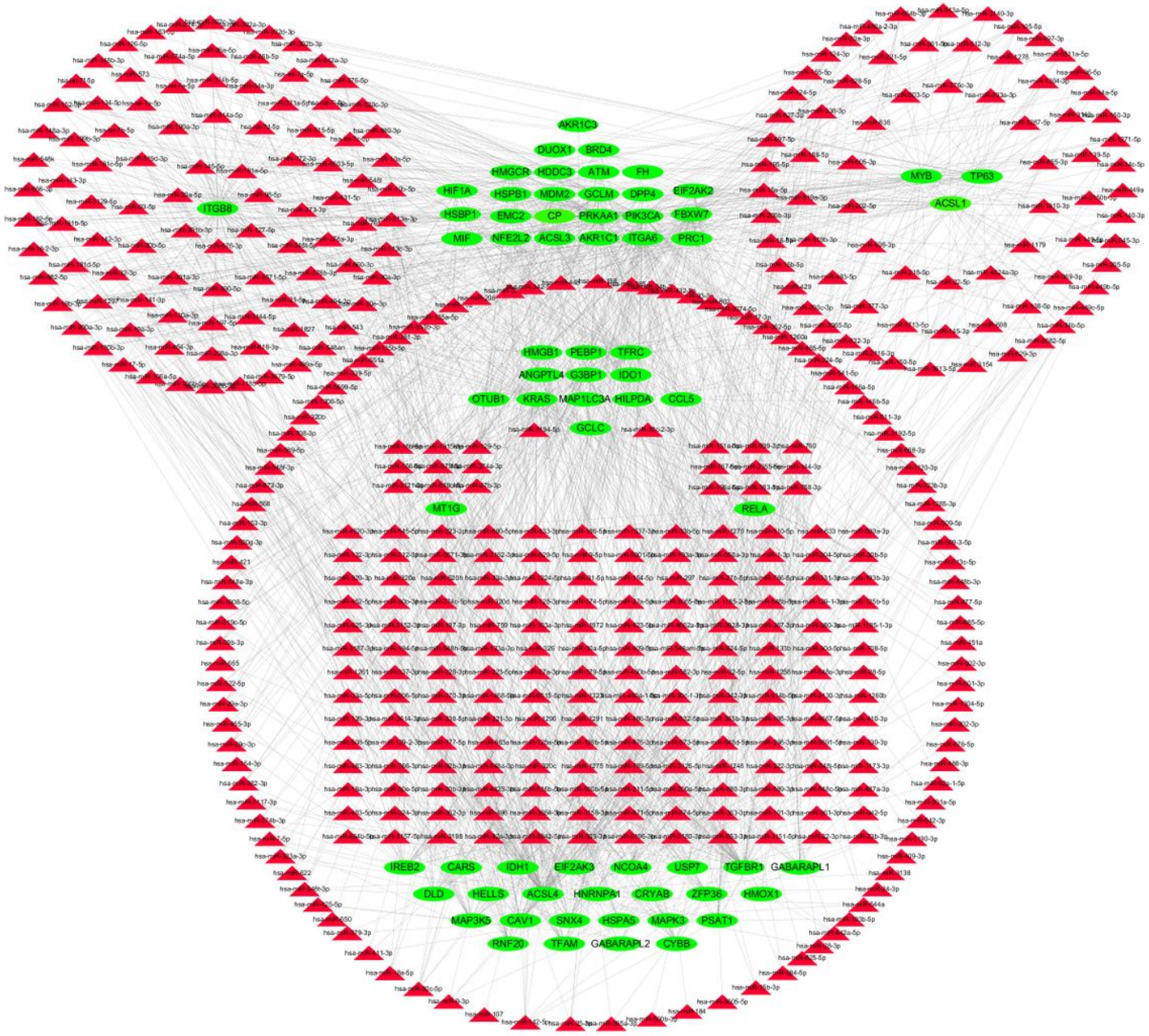

Figure 4

miRNA-target network in IPF. Red triangles represent miRNAs, and green ellipses indicate genes.

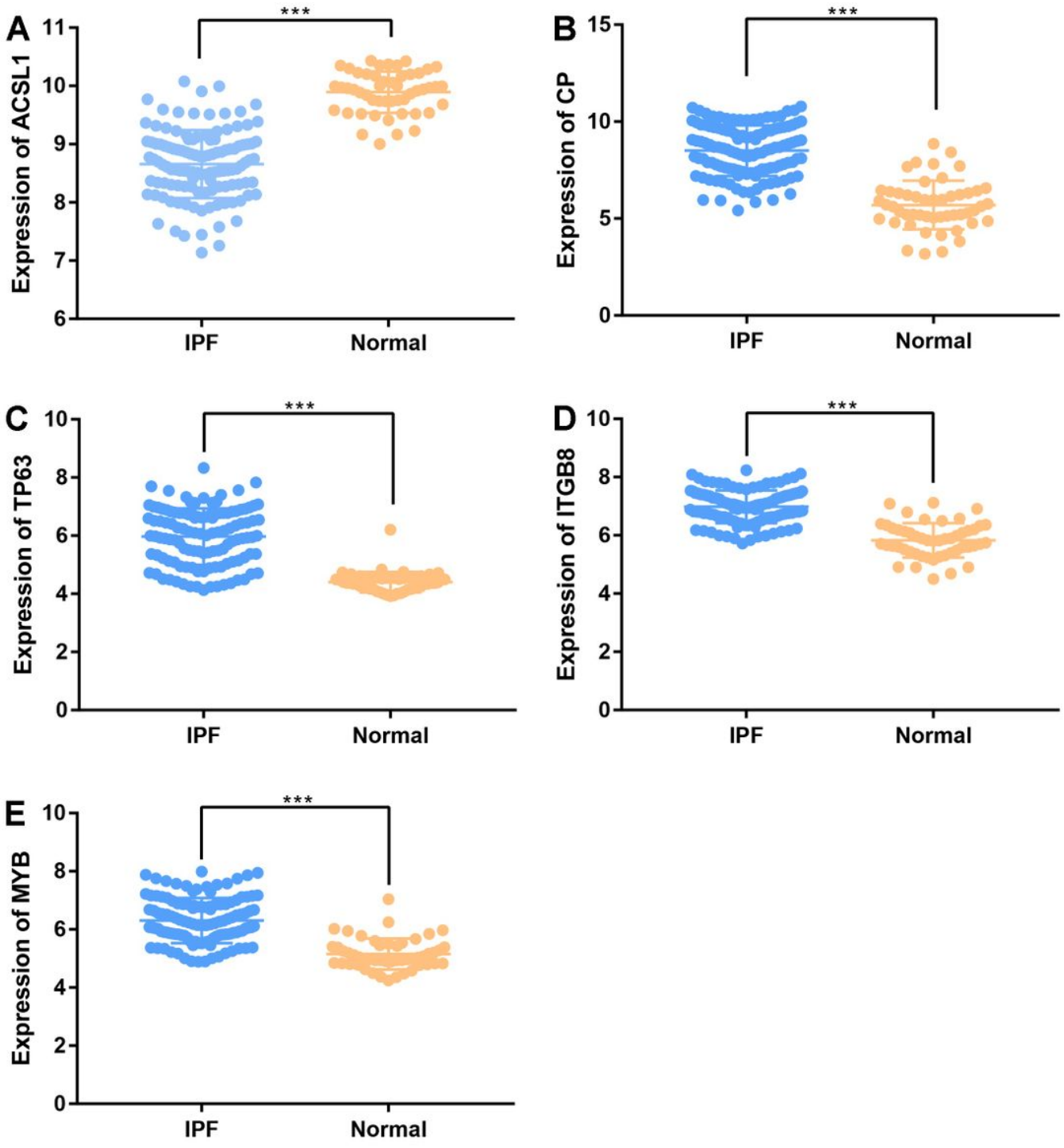

Figure 5

The expression of key genes in the GSE32537 dataset. (A) ACSL1; (B) CP; (C) TP63; (D) ITGB8; (E) MYB.

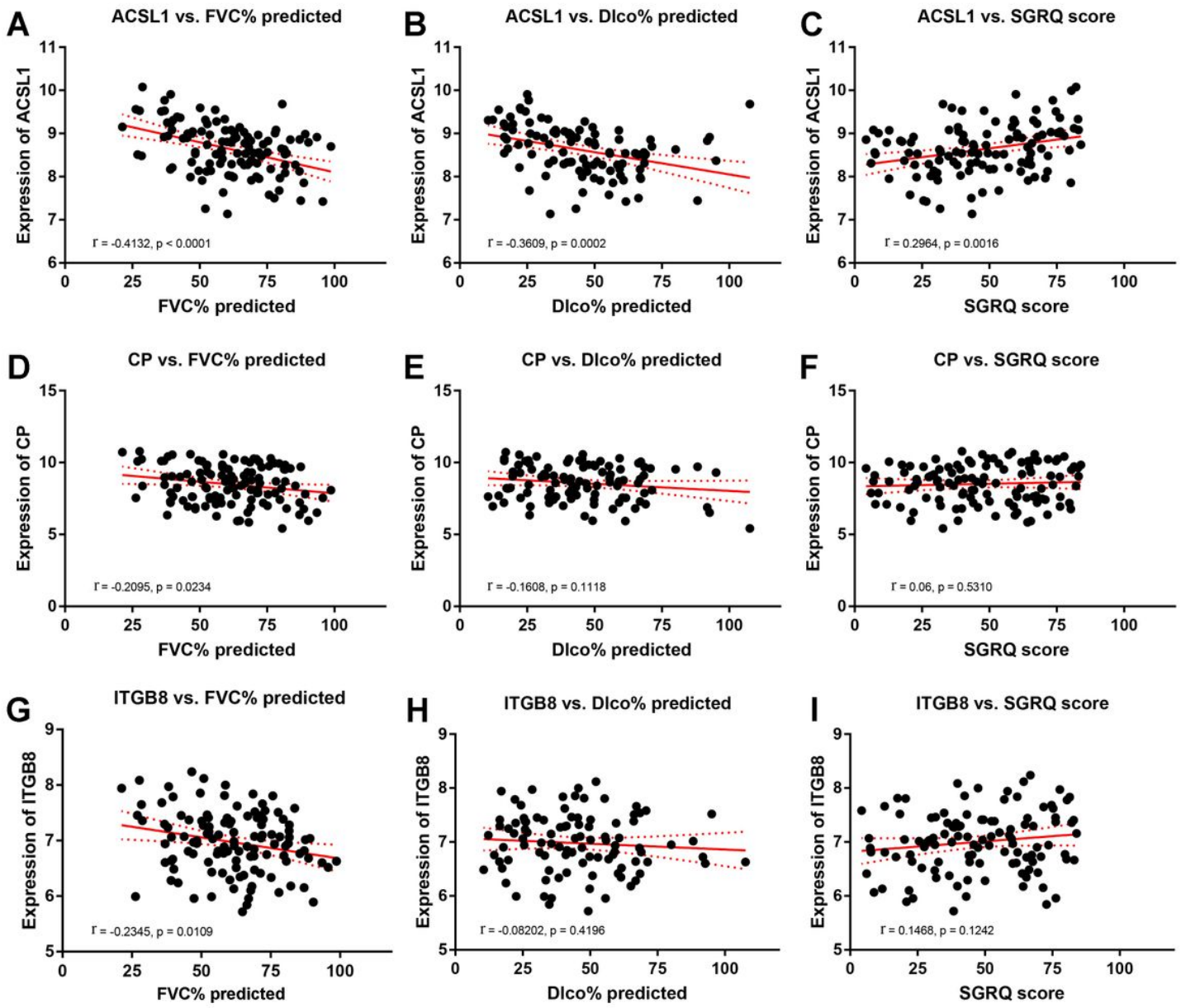

Figure 6

The relationship between key genes and lung function or quality of life in GSE32537 dataset. (A-C) The relationship between expression levels of ACSL1 and FVC% predicted or Dlco% predicted or SGRQ score. (D-F) The relationship between expression levels of CP and FVC% predicted or Dlco% predicted or SGRQ score. (H-J) The relationship between expression levels of ITGB8 and FVC% predicted or Dlco% predicted or SGRQ score.

Supplementary Files

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