

Identification of aberrantly expressed genes during aging in the mouse heart via integrated bioinformatics analysis

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Abstract

Cardiovascular disease (CVD) represents a global problem and is associated with high levels of morbidity/mortality in the elderly (>65 years old). The present study aimed to identify the key candidate genes and pathways in cardiac aging via integrated bioinformatics analysis. The GSE43556 and GSE8146 gene expression datasets were obtained from the Gene Expression Omnibus (GEO) database, and differentially expressed genes (DEGs), defined as $P < .05$ and $|\log \text{fold-change (FC)}| > 0.5$, were identified. Functional enrichment and protein-protein interaction network construction were subsequently performed. First, 142 DEGs shared between the two GEO datasets were identified. Second, biological functional enrichment analysis illustrated that these DEGs mainly participate in “inflammatory response” and “monocarboxylic acid metabolic process.” Moreover, Kyoto Encyclopedia of Genes and Genomes pathway analysis revealed that the DEGs were mainly enriched in the PI3K-Akt signaling pathway. Subsequently, the association between the expression of DEGs in the aged heart was evaluated using the Search Tool for the Retrieval of Interacting Genes database and Cytoscape software. The present study elucidated the key genes and signaling pathways associated with cardiac aging, thus improving the understanding of the molecular mechanisms underlying cardiac aging. These identified genes may be used as molecular biomarkers for the diagnosis and treatment of cardiac aging.

Abbreviations: CP = ceruloplasmin, CVD = cardiovascular disease, CX3CL1 = C-X3-C motif chemokine ligand 1, DEGs = differentially expressed genes, FC = fold change, FGG = fibrinogen γ , GEO = gene expression omnibus, PENK = proenkephalin, PI3K = phosphoinositide 3-kinase, PNPLA2 = patatin like phospholipase domain containing 2, SCG2 = secretogranin II.

Keywords: bioinformatics, biomarkers, cardiovascular disease, Gene Expression Omnibus, heart aging

1. Introduction

Cardiovascular disease (CVD) accounts for > 30% of global deaths and is increasingly prevalent in the aging population.^[1] Notably, aging is considered a major independent risk factor for common heart disease, including heart failure (HF).^[2] During aging, the heart undergoes significant physiological and molecular changes, including cardiomyocyte hypertrophy and increased fibrosis, resulting in increased ventricular stiffness and a reduction in diastolic cardiac function, but no change in systolic function.^[3] Despite the growing demand for interventions, effective treatments for age-related cardiac remodeling and dysfunction have yet to be determined.^[4] Therefore, identifying the molecular mechanisms underlying cardiac aging is very important for diagnosis, prevention and individualized treatment.

Cardiac transcriptome analysis is a powerful approach for the analysis of gene function. Gene expression profiling techniques, alongside statistical analysis and publicly available bioinformatics tools, can be used to identify pathways, regulatory sequences and candidate genes that are implicated in human aging.^[5] Gene chips, as a gene detection technology, have been widely used and the corresponding data have been stored in public databases.^[6] The integration and reanalysis of these genomic data provides the possibility to identify biomarkers related to certain diseases. A number of studies based on a single cohort have elucidated the pathogenesis of cardiac aging based on microarray data profiles,^[7,8] which leads to poor reproducibility and consistency. To overcome these shortcomings, comprehensive bioinformatics methods should be combined with expression profiling technology.

PH, JF, and JH contributed equally to this study.

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The datasets generated during and/or analyzed during the current study are publicly available.

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In the present study, the differentially expressed genes (DEGs) between aged and young heart tissue samples were analyzed to achieve a better understanding of heart senescence. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of the DEGs were performed, and the protein-protein interaction (PPI) network generation and module analysis of the DEGs was conducted. The aim of the present study was to identify key genes and pathways in cardiac aging using bioinformatics analysis, to subsequently explore the intrinsic mechanisms of cardiac aging, and to distinguish novel potential diagnostic and therapeutic biomarkers. The findings of the present study may provide further insight into the pathogenesis and development of cardiac aging at the molecular level.

2. Materials and methods

2.1. Datasets

The Gene Expression Omnibus (GEO; <https://www.ncbi.nlm.nih.gov/geo>) is a public functional genomics data repository that includes array- and sequence-based data, which is free for all users. For the present study, a data search was conducted in the GEO database. The search terms “mouse,” “aging,” “heart” and “mRNA” provided the datasets, GSE43556^[9] and GSE8146,^[10] which were found to meet the conditions. GSE43556 was based on the GPL1261 platform [Mouse430.2] Affymetrix Mouse Genome 430 2.0 Array. The GSE43556 dataset has 8 samples, including 4 aging heart samples and 4 matched young heart samples. GSE8146 was based on the GPL81 [MG_U74Av2] Affymetrix Murine Genome U74A Version 2 Array. The GSE8146 dataset consists of 20 samples, 10 of which were included in the present analysis, including 5 aged heart samples and 5 matched young heart samples.

2.2. Analysis of gene chip data and DEGs

Gene chip data analysis of the GEO datasets was performed in R 3.6.3 software (<http://www.r-project.org>). The sample size of a single data set is small and is thus prone to bias; therefore, instead of analyzing the DEGs that appear in only one dataset, a joint analysis of the two GEO datasets was performed. To make the data comparable, we first standardized the data using the surrogate variable analysis (SVA) R package for batch correction (<https://www.bioconductor.org/packages/release/bioc/html/sva.html>). The combined dataset was normalized performed using Limma software package (<https://www.bioconductor.org/packages/release/bioc/html/limma.html>). The DEGs between the aged and young hearts were detected using unpaired Student *t* test. Multiple verification was executed using the Normalize Between Arrays method. Based on factors such as the distribution of the data in our dataset, experimental design, and sample size, $\log_2\text{FC} > 0.5$ and adjusted $P < .05$ were selected as thresholds to obtain true differentially expressed genes while ensuring repeatability and robustness of the analysis. We also used FDR correction was used to ensure significance of results and reduce false positives. The ggplot2 package of the R software program was used to produce volcano plots of the DEGs. The pheatmap package of the R software program was used to produce heatmaps of the DEGs.

2.3. GO and KEGG pathway enrichment analyses of DEGs

The Metascape online database (<https://metascape.org>) is a tool for gene function classification, and includes a set of functional annotation tools for investigators to analyze the biological roles of genes, and to perform GO and KEGG pathway enrichment

analyses of DEGs. A count of > 2 and $P < .05$ was considered the cutoff criteria.

2.4. PPI network construction and module analysis

Functional PPI analysis can be used to explain the molecular mechanism of key cell activities. The Search Tool for the Retrieval of Interacting Genes (STRING) database (<https://string-db.org/>) was used to obtain the PPI relationships for DEGs. Briefly, DEGs were uploaded to the STRING database and the result was visualized using Cytoscape software (<http://cytoscape.org/>). Furthermore, significant modules were detected through the Molecular Complex Detection (MCODE) plugin in Cytoscape (<https://apps.cytoscape.org/apps/mcode>) based on the constructed PPI networks. The Metascape database was adopted to analyze the GO function and KEGG pathway enrichment of the two modules with the highest scores.

2.5. Identification of hub genes

Hub genes are important nodes with a number of interaction partners in PPI networks, which were analyzed by Cytoscape software. In addition, the Degree, Edge Percolated Component (EPC), Eccentricity, Maximal Clique Centrality (MCC), Bottleneck and Maximum Neighborhood Component (MNC) algorithms were useful methods for selecting hub genes from PPI networks.^[11] The CytoHubba plug-in was installed in Cytoscape, and the Degree, EPC, Eccentricity, MCC, Bottleneck and MNC scores of all nodes in the PPI network were determined. The 20 nodes with the highest score of the 6 algorithms were selected, which were intersected. In order to increase the reliability of hub genes, overlapping genes were considered to be hub genes related to heart aging. The hub genes were further analyzed by GO and KEGG pathway enrichment analyses using the Metascape database.

3. Results

3.1. Identification of DEGs

Gene expression profiles of heart samples from aged mice and young control mice were obtained from the GSE43556 and GSE8146 datasets and DEGs were analyzed using R software. GSE43556 and GSE8146 were combined after batch correction. After combination, a total of 8458 genes were obtained. we conducted normalized expression analysis using limma software package, data after normalization were shown in Figure 1. Setting the cutoff criteria as $P < .05$ and $\log_2\text{FC} > 0.5$, 142 DEGs were identified, accounting for 1.68% of the total number of genes, of which 54 were down-regulated and 88 were up-regulated. A volcano plot was generated to show the DEGs between the two groups on the basis of the gene expression values (Fig. 2). The 142 DEGs were also used to generate a heatmap (Fig. 3). These genes were well clustered between aged mouse hearts and young control mouse hearts. Boxplots of the top 10 upregulated genes and top 10 downregulated genes are shown in Figure 4. The most upregulated gene was phenylalanine hydroxylase (PAH), followed by potassium channel, subfamily K, member 1 (KCNK1); small nucleolar RNA host gene 11 (SNHG11); carboxypeptidase X 2 (CPXM2); C-C motif chemokine ligand 8 (CCL8); fibrinogen γ (FGG); amylase 1 (AMY1); tachykinin 1 (TAC1); major facilitator superfamily domain containing 4A (MFSD4A); and endothelin 3 (EDN3). The most downregulated gene was dopa decarboxylase (DDC), followed by mucin-like 2 (MUC2); acyl-CoA thioesterase 1 (ACOT1); pyruvate dehydrogenase kinase 4 (PDK4); mitogen-activated protein kinase kinase 6 (MAP3K6); osteomodulin (OMD); uncoupling protein 3 (UCP3); 3-hydroxy-3-methylglutaryl-coenzyme A

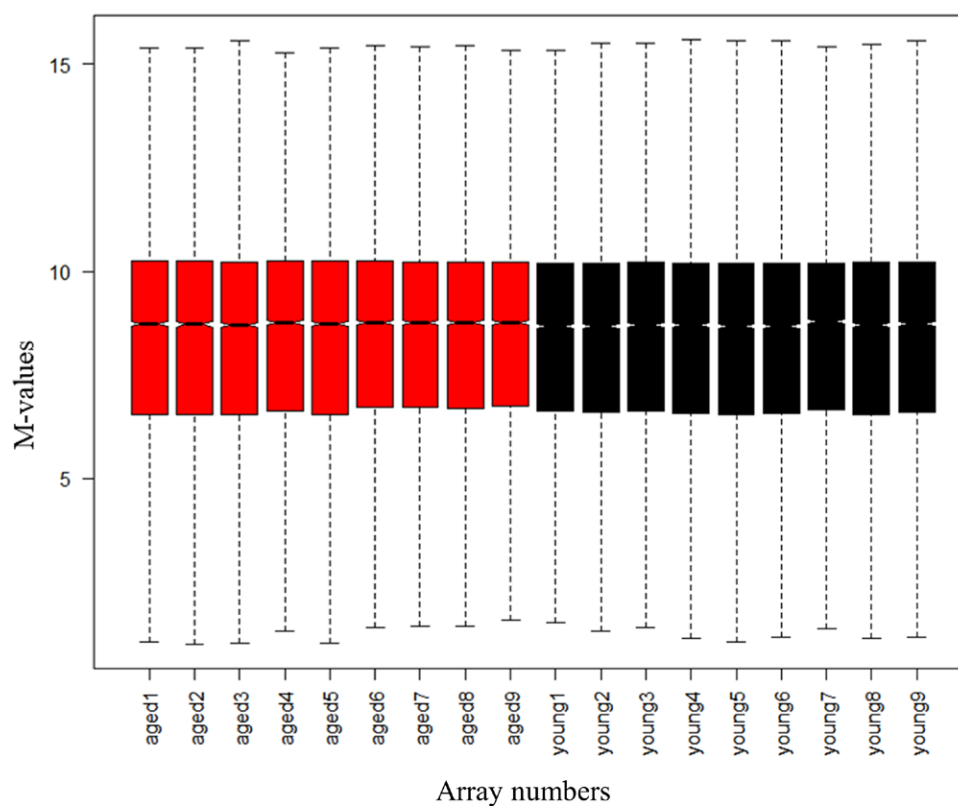


Figure 1. Post-batch effect correction plots. The gene expression profiles of the samples from both datasets were standardized, merged, and subjected to batch effect correction. The x-axis label represents sample symbols and the y-axis label represents the gene expression values. The horizontal lines in each column represent the mean value of gene expression.

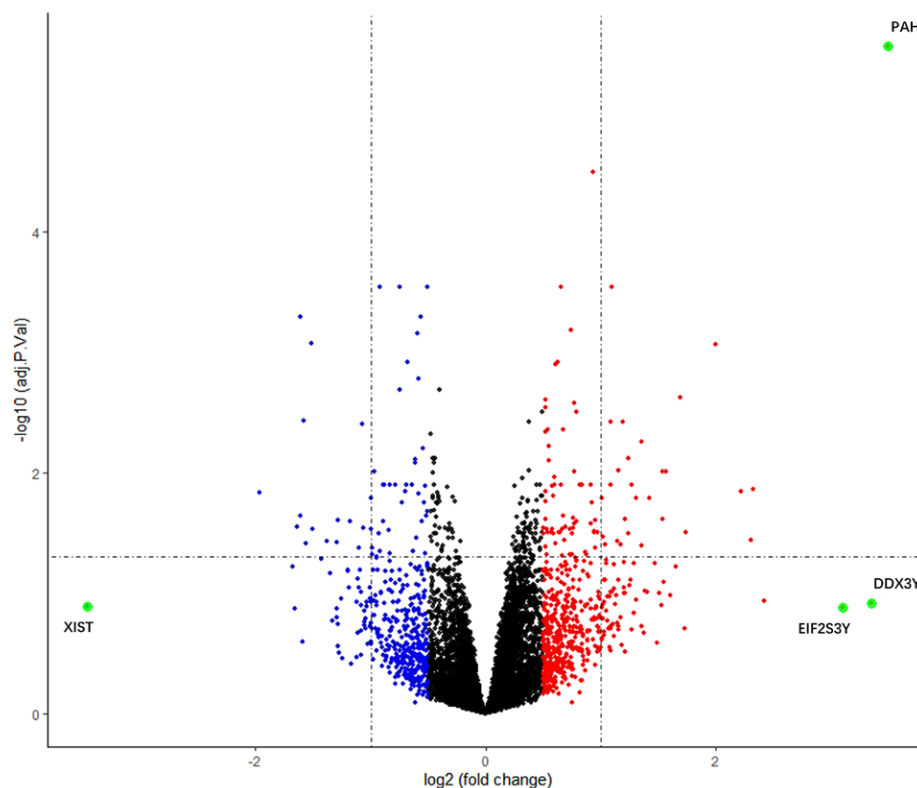


Figure 2. Volcano plot of the combined datasets. On the left is the volcano plot of DEGs. Red plots represent genes with $|\log FC| > 0.5$ and $P < .05$. Blue plots represent genes with $|\log FC| < 0.5$ and $P < .05$. Green plots represent genes with $|\log FC| > 3$ and $P < .05$. Black plots represent the remaining genes with no significant difference. FC = fold change.

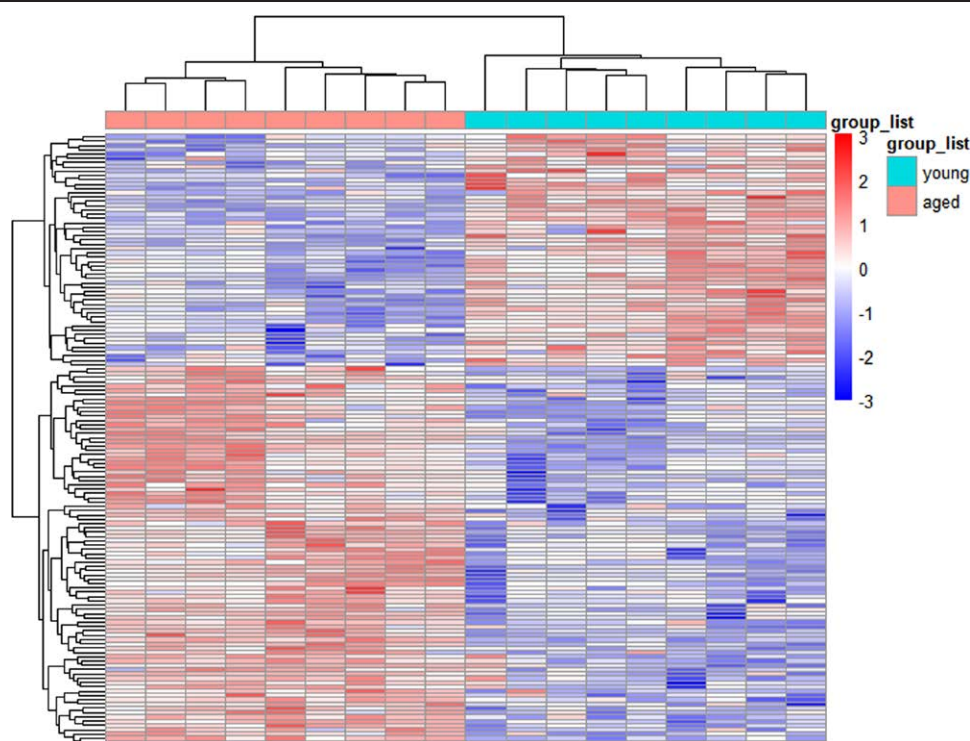


Figure 3. Heatmap of DEGs. Heatmap of the Upregulated and Downregulated DEGs. The heatmap showcases the expression patterns of the 88 upregulated and 54 downregulated DEGs. The color red signifies upregulation, whereas the color blue denotes downregulation. Each row represents a single gene and each column represents a sample. DEGs = differentially expressed genes.

synthase 2 (HMGCS2); angiotensin-like 4 (ANGPTL4); and testicular haploid expressed gene (THEG).

3.2. GO functional and KEGG pathway enrichment analysis of DEGs

The functions of DEGs were analyzed using Metascape. The GO analysis demonstrated that, in terms of biological processes, DEGs were mainly enriched in “inflammatory response” and “monocarboxylic acid metabolic process” (Fig. 5A; Table 1). In terms of cellular components, DEGs were mainly enriched in the “mitochondrial inner membrane” and “cytoplasmic vesicle membrane” (Fig. 5A; Table 1). In terms of molecular function, DEGs were mainly enriched in “protein kinase activity” and “protein homodimerization activity” (Fig. 5A; Table 1). The KEGG pathway analysis showed that the DEGs were enriched in eight pathways; the most significant pathway was the “PI3K-Akt signaling pathway” (Fig. 5B; Table 2).

3.3. PPI analysis

Based on information from the STRING database, a PPI network comprising 142 nodes and 128 edges was constructed using Cytoscape software (Fig. 6A). Subsequently, the networks were analyzed using the plugin MCODE with them following criteria: Node score > 4 and number of nodes > 4. Finally, two significant modules were selected (Fig. 6B and C). The genes in the two modules were enriched in the KEGG pathway “PPAR signaling pathway”; and were enriched in the GO biological processes “regulation of lipid metabolic process,” “fatty acid metabolic process,” “negative regulation of endothelial cell apoptotic process” and “response to inorganic substance” (Table 3).

3.4. Hub gene screening

Hub genes were detected in the network using the CytoHubba plugin, and six hub genes were identified using the six calculation

methods (Degree, EPC, Bottleneck, Eccentricity, MCC and MNC). The results are shown in Table 4. The 6 most significant genes were patatin like phospholipase domain containing 2 (PNPLA2), proenkephalin (PENK), ceruloplasmin (CP), secretogranin II (SCG2), FGG and C-X3-C motif chemokine ligand 1 (CX3CL1).

4. Discussion

Despite advances in current therapeutics, CVD has remained an intractable disease in recent decades. Aging is regarded as an essential disease-causing determinant of CVD.^[3] Therefore, identifying the etiological and molecular mechanisms underlying age-related cardiac remodeling and dysfunction is of great importance for prevention and therapy. With the rapid development of DNA microarray and high-throughput sequencing technologies, it is possible to research diseases at the gene level. DNA microarray gene expression profiling has been widely applied to explore DEGs involved in cardiac aging.^[7,8] The present study extracted data from the GSE43556 and GSE8146 datasets. A total of 142 DEGs were identified between aged heart tissue samples and young heart tissue samples using bioinformatics analysis. Subsequently, bioinformatics methods were utilized to explore these DEGs, including GO term enrichment, KEGG signaling pathway enrichment and PPI network construction.

The DEGs were mostly involved in inflammatory response, monocarboxylic acid metabolic process, hormone secretion and angiogenesis. This conforms to the knowledge that inflammatory response, metabolic process, hormone secretion and angiogenesis are all important mechanisms of cardiac aging.^[12–15] Numerous studies have demonstrated that the inflammatory response and hormone secretion serve essential roles in the prevention of heart disease.^[16,17] Martin et al^[18] reported that inhibiting the inflammatory response may reverse maladaptive remodeling in aging and HF. DEGs were also found to be involved in monocarboxylic acid metabolic process and angiogenesis. It has previously been reported that myocardial energy metabolism has

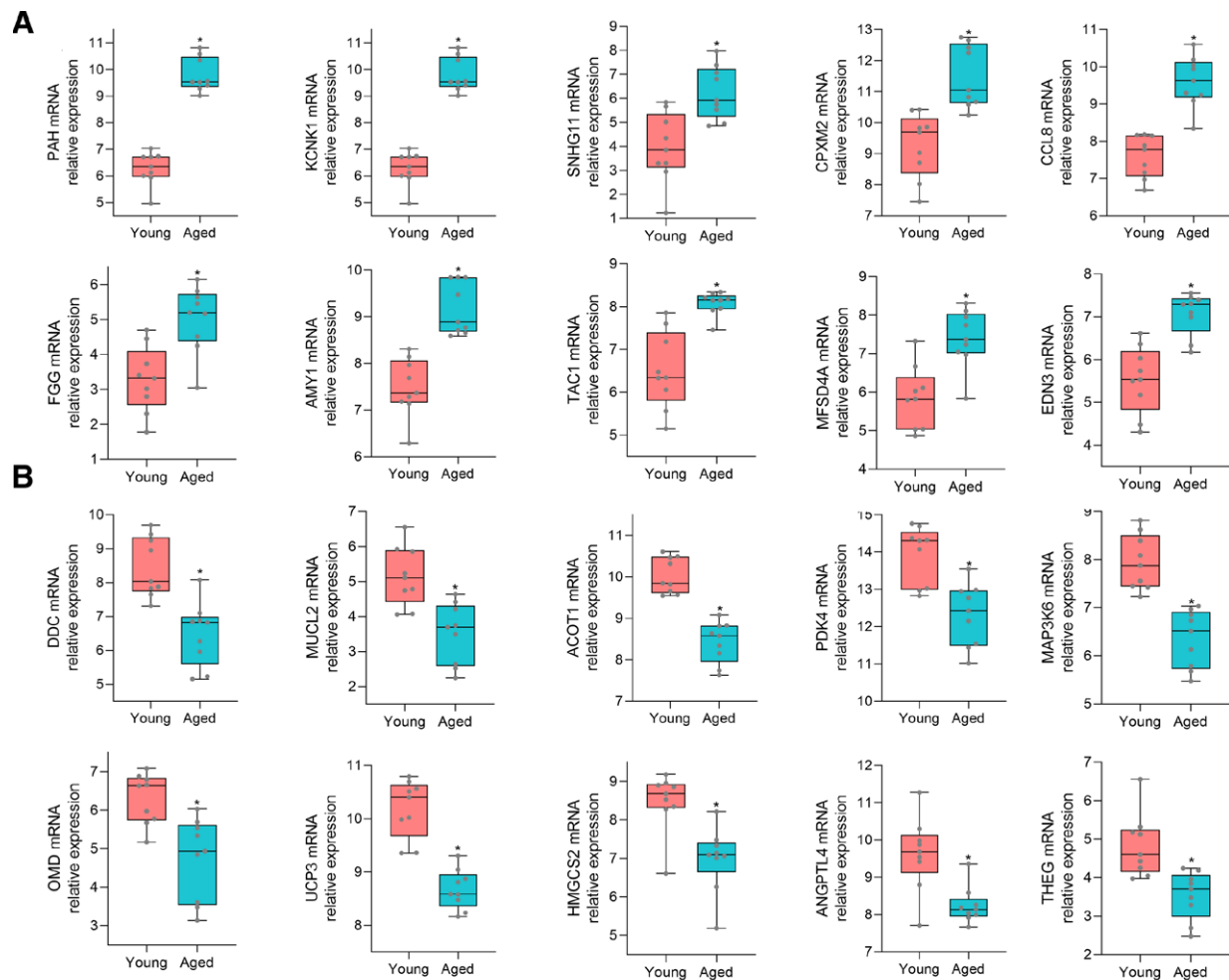


Figure 4. Boxplots of the top 10 upregulated genes and top 10 downregulated genes. (A) PAH, KCNK1, SNHG11, CPXM2, CCL8, FGG, AMY1, TAC1, MFSD4A, and EDN3 were significantly upregulated in aged heart tissues compared with in young heart tissues. $*P < .05$ vs young. (B) DDC, followed by MUCL2, ACOT1, PDK4, MAP3K6, OMD, UCP3, HMGCS2, ANGPTL4, and THEG were significantly downregulated in aged heart tissues compared with in young heart tissues. $*P < .05$ vs young.

an important role in maintaining the normal function of the heart, and disturbances of myocardial energy metabolism often lead to HF.^[19] In addition, disturbances of monocarboxylic acid, including lactate acid and pyruvate, can also lead to HF.^[20–22] Furthermore, aging impairs VEGF-mediated and androgen-dependent regulation of angiogenesis,^[23] and angiogenesis has been reported to contribute to CVD.^[24,25] Thus, exploring these biological functions could assist in the prediction of age-related cardiac remodeling. Subsequently, pathway analysis was performed using KEGG processes. The results indicated that all DEGs were mainly related to the PI3K-Akt signaling pathway. Numerous studies have shown that activating the PI3K-Akt signaling pathway can inhibit cardiac aging and HF,^[26–28] thus suggesting that the PI3K-Akt signaling pathway is an important target for the prevention and treatment of cardiac aging.

A PPI network of the DEGs with 142 nodes and 128 edges was constructed. In this network, 2 significant modules were screened depending on the degree of importance. The 2 most significant submodules of DEGs were extracted from the PPI network with MCODE scores of ≥ 4 . After GO functional and KEGG pathway enrichment analyses of the DEGs in the two modules, the genes in these modules were revealed to be mainly enriched in regulation of lipid metabolic process. Metabolic alterations caused by senescence occurred at different function levels.^[29–31] It has been shown that in the aging heart, mitochondrial respiratory functions are affected, especially the oxidative

phosphorylation enzymes and the citric acid cycle.^[29] A decrease in age-related fatty acid oxidation has also been observed in normal hearts.^[30] Furthermore, decreased lipid fluidity is associated with aging. When the lipid metabolism process is disturbed, the mitochondria in elderly hearts exhibit higher levels of lipid peroxidation and injury.^[31] Thus, regulating cardiac lipid metabolism may be an important approach to delay the aging of the heart. The DEGs were also enriched in the PPAR signaling pathway, which are also mainly involved in regulating glucose metabolism and lipid metabolism.^[32] Previous studies have revealed that PPAR α delays the development of some spontaneous lesions associated with aging in the hearts of SV129 mice,^[33] proving the importance of this signaling pathway during cardiac aging.

The present study further analyzed the hub genes in the PPI network through six calculation methods and obtained six hub genes (PNPLA2, PENK, CP, SCG2, FGG, and CX3CL1), which may serve as potential targets for diagnosis and treatment of cardiac aging. These genes have been rarely studied in cardiac aging, and only a small number of studies have confirmed changes in the expression of CP and penk in aging tissues.^[34,35] PNPLA2 has been recognized as a vital triglyceride hydrolase in lipid droplets/adiposome turnover in mammalian cells.^[36] As previously mentioned, lipid metabolism disorders are closely related to cardiac aging, therefore PNPLA2 should be considered as an important gene associated with

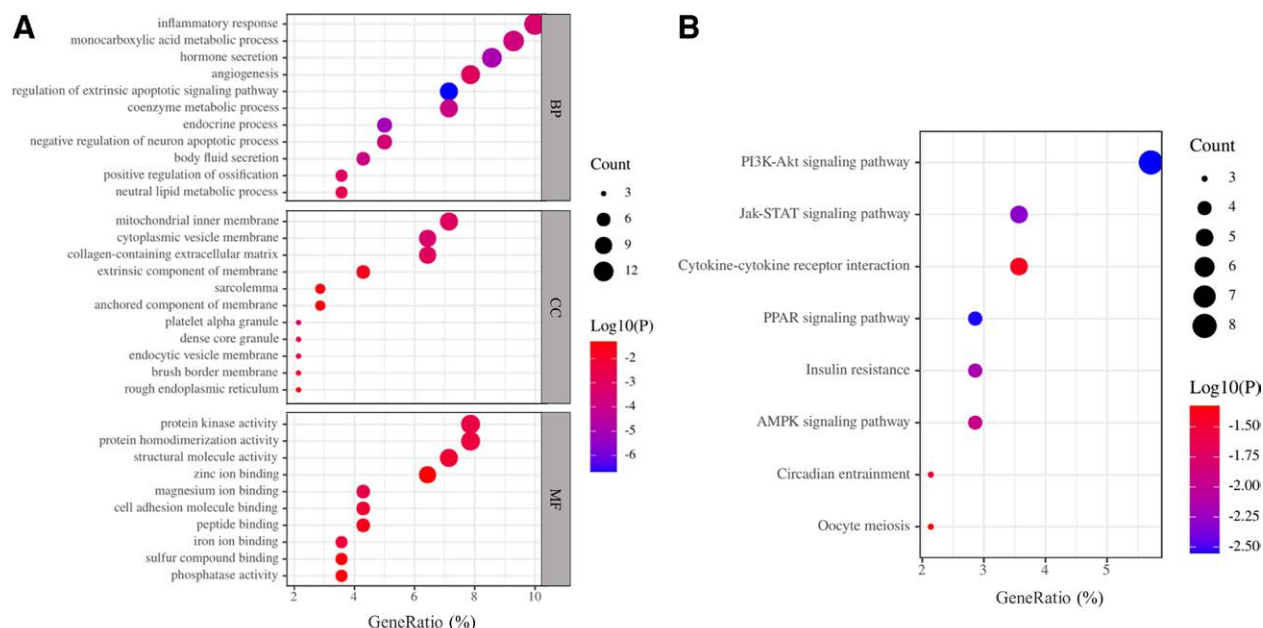


Figure 5. GO function and KEGG pathway enrichment analyses of DEGs. The most significantly enriched (A) GO terms and (B) KEGG pathways of DEGs. GeneRatio represents the ratio of the number of enriched DEGs and the number of annotated background genes in this pathway. The x-axis shows GeneRatio of each pathway. The y-axis refers to GO terms and KEGG pathways, respectively. BP = biological process, CC = cellular component, DEG = differentially expressed gene, GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function.

Table 1

GO analysis of differentially expressed genes during heart aging

| Category | GO | Description | Count | % | Log10(P) | Log10 (q value) |
|-------------------------|------------|---|-------|------|----------|-----------------|
| GO biological processes | GO:0006954 | inflammatory response | 14 | 10 | -3.35 | -0.7 |
| | GO:0032787 | monocarboxylic acid metabolic process | 13 | 9.29 | -3.71 | -0.84 |
| | GO:0046879 | hormone secretion | 12 | 8.57 | -4.96 | -1.36 |
| | GO:0001525 | angiogenesis | 11 | 7.86 | -3.00 | -0.48 |
| | GO:2001236 | regulation of extrinsic apoptotic signaling pathway | 10 | 7.14 | -6.67 | -2.47 |
| | GO:0006732 | coenzyme metabolic process | 10 | 7.14 | -3.91 | -0.92 |
| | GO:0050886 | endocrine process | 7 | 5 | -5.10 | -1.38 |
| | GO:0043524 | negative regulation of neuron apoptotic process | 7 | 5 | -3.54 | -0.78 |
| | GO:0007589 | body fluid secretion | 6 | 4.29 | -3.87 | -0.92 |
| | GO:0045778 | positive regulation of ossification | 5 | 3.57 | -3.15 | -0.57 |
| GO cellular components | GO:0006638 | neutral lipid metabolic process | 5 | 3.57 | -2.74 | -0.32 |
| | GO:0005743 | mitochondrial inner membrane | 10 | 7.14 | -3.02 | -0.48 |
| | GO:0030659 | cytoplasmic vesicle membrane | 9 | 6.43 | -3.29 | -0.48 |
| | GO:0062023 | collagen-containing extracellular matrix | 9 | 6.43 | -2.99 | -0.48 |
| | GO:0019898 | extrinsic component of membrane | 6 | 4.29 | -1.69 | 0 |
| | GO:0042383 | sarcolemma | 4 | 2.86 | -1.58 | 0 |
| | GO:0031225 | anchored component of membrane | 4 | 2.86 | -1.36 | 0 |
| | GO:0031091 | platelet alpha granule | 3 | 2.14 | -3.54 | -0.48 |
| | GO:0031045 | dense core granule | 3 | 2.14 | -2.86 | -0.42 |
| | GO:0030666 | endocytic vesicle membrane | 3 | 2.14 | -2.49 | -0.15 |
| GO molecular functions | GO:0031526 | brush border membrane | 3 | 2.14 | -1.82 | 0 |
| | GO:0005791 | rough endoplasmic reticulum | 3 | 2.14 | -1.57 | 0 |
| | GO:0004672 | protein kinase activity | 11 | 7.86 | -2.47 | 0 |
| | GO:0042803 | protein homodimerization activity | 11 | 7.86 | -2.27 | 0 |
| | GO:0005198 | structural molecule activity | 10 | 7.14 | -2.04 | 0 |
| | GO:0008270 | zinc ion binding | 9 | 6.43 | -1.33 | 0 |
| | GO:0000287 | magnesium ion binding | 6 | 4.29 | -2.60 | 0 |
| | GO:0050839 | cell adhesion molecule binding | 6 | 4.29 | -2.14 | 0 |
| | GO:0042277 | peptide binding | 6 | 4.29 | -1.62 | 0 |
| | GO:0005506 | iron ion binding | 5 | 3.57 | -2.21 | 0 |

q values are calculated using the Benjamini-Hochberg procedure to account for multiple testings.

GO = gene ontology.

Table 2
KEGG pathway analysis of differentially expressed genes during heart aging

| Category | GO | Description | Count | % | Log10(P) | Log10 (q values) |
|--------------|----------|--|-------|------|----------|--------------------|
| KEGG Pathway | mmu04151 | PI3K-Akt signaling pathway | 8 | 5.71 | -2.55 | -0.153109128 |
| | mmu04630 | Jak-STAT signaling pathway | 5 | 3.57 | -2.30 | -0.153109128 |
| | mmu04060 | Cytokine-cytokine receptor interaction | 5 | 3.57 | -1.40 | -0.089052639 |
| | mmu03320 | PPAR signaling pathway | 4 | 2.86 | -2.54 | -0.079164969 |
| | mmu04931 | Insulin resistance | 4 | 2.86 | -2.16 | -0.079164969 |
| | mmu04152 | AMPK signaling pathway | 4 | 2.86 | -1.94 | 0 |
| | mmu04713 | Circadian entrainment | 3 | 2.14 | -1.51 | -0.079164969 |
| | mmu04114 | Oocyte meiosis | 3 | 2.14 | -1.33 | -0.079164969 |

q values are calculated using the Benjamini-Hochberg procedure to account for multiple testings.
KEGG = Kyoto Encyclopedia of Genes and Genomes.

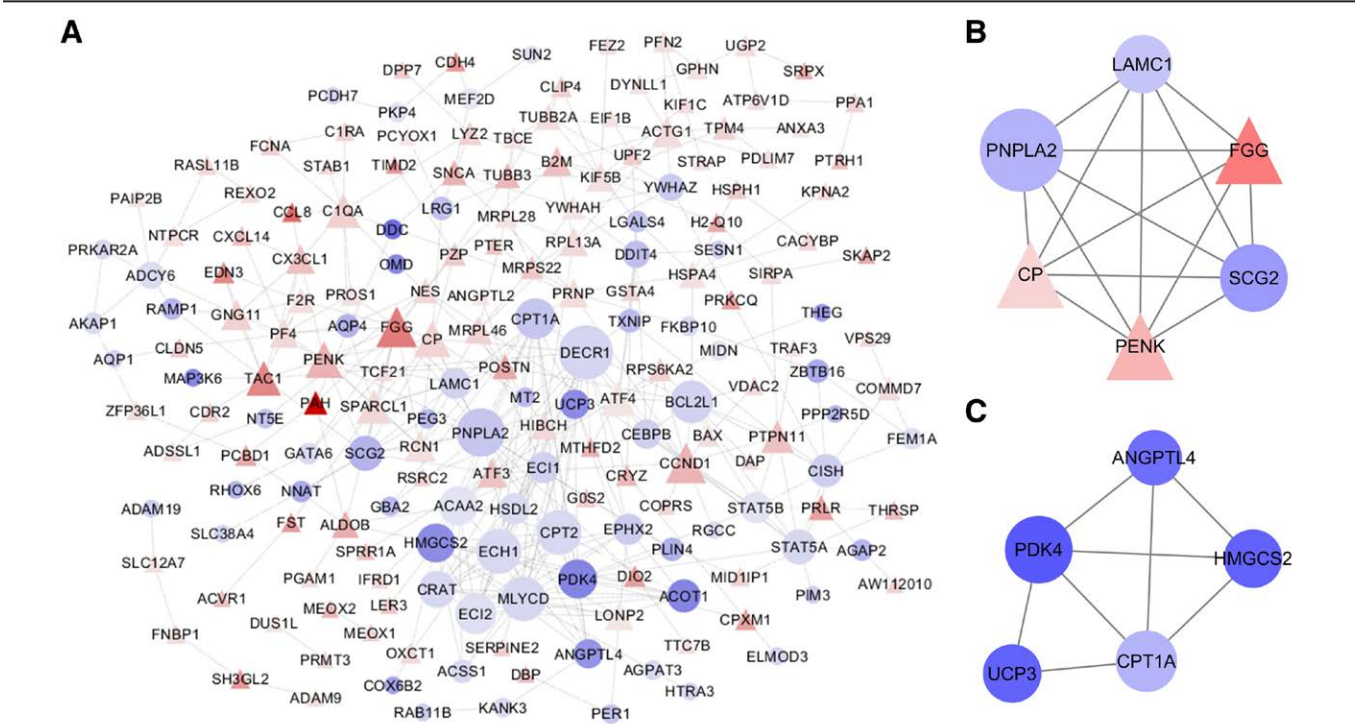


Figure 6. PPI network and significant modules of differentially expressed genes. (A) PPI network. Triangles indicate significantly upregulated genes; circles indicate significantly downregulated genes. Node size is positively related to combined score and node color is related to fold change. Significant modules in the PPI network with a Molecular Complex Detection score ≥ 4 : (B) Module 1 (score = 6); (C) module 2 (score = 4). PPI = protein-protein interaction.

Table 3
GO and KEGG pathway analyses of genes in the selected modules

| Category | GO | Description | Count | % | Log10(P) | Log10 (q values) |
|-------------------------|------------|---|-------|-------|----------|--------------------|
| GO Biological Processes | GO:0019216 | regulation of lipid metabolic process | 4 | 36.36 | -4.60 | -1.19 |
| | GO:2000352 | negative regulation of endothelial cell apoptotic process | 3 | 27.27 | -6.27 | -2.08 |
| | GO:0006631 | fatty acid metabolic process | 3 | 27.27 | -3.01 | 0 |
| | GO:0010035 | response to inorganic substance | 3 | 27.27 | -2.68 | 0 |
| KEGG Pathway | mmu03320 | PPAR signaling pathway | 3 | 27.27 | -4.94 | -2.25 |

q values are calculated using the Benjamini-Hochberg procedure to account for multiple testings.
GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes.

cardiac aging. CP is a major systemic NO oxidase activated by hypoxia, which seems to be able to exert both antioxidant and prooxidative effects.^[37] As we all known that NO regulates cardiac mitochondrial function in health and disease.^[38,39] Recent researches has reported that mitochondrial regulation of oxidative stress is a potential target for cardiac aging.^[40] Thus, we speculate that PENK may be involved in the process

of cardiac aging by regulating mitochondrial function. SCG2 is a neuroendocrine secretory protein belonging to the chromogranin/secretogranin family.^[41] Cleavage of SCG2 produces the active peptide neurotrophin, which induces coronary angiogenesis after myocardial infarction by enhancing VEGF signaling in endothelial cells.^[42] Impaired angiogenesis is an important mechanism in the remodeling of the aging heart.^[43]

Table 4**Hub genes analyzed by different topological algorithms in the protein-protein interaction network**

| Topological algorithm | Top 20 genes were ranked by score |
|--|---|
| Maximal Clique Centrality (MCC) | PNPLA2, PENK, CP, SCG2, FGG, LAMC1, PDK4, CPT1A, TAC1, ANGPTL4, GNG11, CX3CL1, BCL2L1, UCP3, HMGCS2, PTPN11, C1QA, ALDOB, B2M, PAH |
| Degree | PNPLA2, PENK, SCG2, CP, FGG, TAC1, PDK4, CX3CL1, BCL2L1, C1QA, CPT1A, B2M, PTPN11, CISH, LAMC1, GNG11, ATF3, UCP3, HMGCS2, ALDOB |
| Edge Percolated Component | PNPLA2, FGG, PENK, SCG2, CP, LAMC1, PDK4, CPT1A, TAC1, ANGPTL4, UCP3, HMGCS2, CX3CL1, GNG11, PZP, PLIN4, ALDOB, MT2, ACOT1, PAH |
| Maximum Neighborhood Component | PENK, CP, PNPLA2, PDK4, SCG2, FGG, TAC1, LAMC1, GNG11, BCL2L1, CPT1A, UCP3, HMGCS2, ANGPTL4, ALDOB, PAH, PTPN11, CX3CL1, CCND1, PLIN4 |
| EcCentricity | PNPLA2, LAMC1, LRG1, GOS2, FGG, CP, SCG2, PENK, B2M, MT2, DIO2, PZP, ANGPTL4, CPT1A, PLIN4, PDK4, UCP3, ATF3, GNG11, CX3CL1 |
| BottleNeck | SCG2, PNPLA2, PENK, ATF3, CP, CX3CL1, PTPN11, CCND1, FGG, B2M, C1QA, CISH, LRG1, PAH, NNAT, PLIN4, TAC1, MID1P1, PRLR, THRSP |
| Common Genes of 6 Topological Algorithms | PNPLA2, PENK, CP, SCG2, FGG, CX3CL1 |

Therefore, SCG2 was predicted to be an aging-related gene. FGG has a vital role in coagulation, hemostasis and inflammation.^[44] There is no doubt that chronic inflammation is one of the important mechanisms of aging. Inflammatory aging plays an important role in the occurrence and development of age-related cardiovascular diseases. In addition, the link between cardiac aging and chronic inflammation has also been supported by research, which mentions that the inflammatory response caused by cardiac cell aging can trigger tissue remodeling and is a key factor in promoting the progression of aging-related heart diseases.^[45] From this, we speculate that FGG may be involved in cardiac aging by regulating inflammatory responses. CX3CL1 is a unique member of the CX3C chemokine subfamily. It has been reported that CX3CL1 is coupled to and acts through G protein.^[46] CX3CL1 is coupled to and acts through G protein,^[46] participating in the regulation of biological processes such as inflammation^[17] and angiogenesis,^[47] suggesting that it may be closely related to cardiac aging. These findings indicated that there is a close relationship between hub genes and cardiovascular function. Thus, it was hypothesized that they may participate in aging-related heart dysfunction. Taken together, the six genes may be involved in the progression of aging-related cardiac remodeling and dysfunction.

For shortcomings, the findings of this study are primarily based on bioinformatics analysis, and the hub genes and key signaling pathways during cardiac aging have not yet been experimentally validated in aging mouse models. In future work, we will conduct experiments in aging mouse models to verify the the hub genes and key signaling pathways identified in this study during cardiac aging. More importantly, further genetic intervention research needs to be implemented to confirm their exact biological functions to determine whether they are key regulators of cardiac aging and whether they could serve as potential therapeutic targets.

5. Conclusion

In conclusion, through bioinformatics analysis, a series of molecular pathways related to cardiac aging were explored. In addition, six hub genes that may be related to cardiac aging, namely PNPLA2, PENK, CP, SCG2, FGG and

CX3CL1, were identified from the GEO dataset. Further research is needed to elucidate the downstream mechanisms of potential targets. Understanding the mechanisms of these aging processes may result in novel breakthroughs in the prevention and treatment of cardiac aging and aging-related dysfunction.

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