Identification of nondiabetic heart failure-associated genes by bioinformatics approaches in patients with dilated ischemic cardiomyopathy

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Abstract. Heart failure (HF) is a common pathological condition affecting 4% of the worldwide population. However, approaches for predicting or treating nondiabetic HF (ND-HF) progression are insufficient. In the current study, the gene expression profile GSE26887 was analyzed, which contained samples from 5 healthy controls, 7 diabetes mellitus-HF patients and 12 ND-HF patients with dilated ischemic cardiomyopathy. The dataset of 5 healthy controls and 12 ND-HF patients was normalized with robust multichip average analysis and the differentially expressed genes (DEGs) were screened by unequal variance t-test and multiple-testing correction. In addition, the protein-protein interaction (PPI) network of the upregulated and downregulated genes was constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins database and the Cytoscape software platform. Subsequently, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed. A total of 122 upregulated and 133 downregulated genes were detected. The most significantly up- and downregulated genes were *EIF1AY* and *SERPINE1*, respectively. In addition, 38 and 77 nodes were obtained in the up- and downregulated PPI network. DEGs that owned the highest connectivity degree were *USP9Y* and *UTY* in the upregulated network, and *CD44* in the downregulated networks, respectively. *NPPA* and *SERPINE1* were also found to be hub genes in the PPI network. Several GO terms and pathways that were enriched by DEGs were identified, and the most significantly enriched KEGG pathways were drug metabolism and extracellular matrix-receptor interaction. In conclusion, the two DEGs, *NPPA* and *SERPINE1*, may be important in the pathogenesis of HF and may be used for the diagnosis and treatment of HF.

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Introduction

Heart failure (HF), also known as chronic HF, is one of the major causes of mortality affecting approximately 4% of the world's population, while the prevalence of this condition is currently increasing (1,2). In addition to the widely known risk factor of glucose abnormalities (observed in ~43% of HF patients) (3), HF can also result from certain other factors, which is classified as nondiabetic HF (ND-HF) and is based on a complicated pathological mechanism (4,5). Since HF greatly affects human health and has an unclear pathogenesis, numerous studies have investigated this condition.

Previous studies have proposed certain markers associated with HF. For instance, hyperuricemia and elevated levels of circulating markers of inflammation are common in HF (6,7), and thus the inflammatory biomarker YKL-40 was investigated and found to be significantly associated with all-cause mortality in patients with HF (8). In addition, as a marker of cardiomyocyte injury, cardiac troponin T is a predictor of adverse outcomes for patients with chronic HF (9). Troughton *et al* (10) observed that patients with impaired systolic function or symptomatic HF could be treated under N-terminal brain natriuretic peptide (N-BNP) guidance to partly reduce the total number of cardiovascular events. Despite vast efforts to predict and prevent HF in order to decrease the morbidity and mortality associated with this condition, there is no clear division between ND-HF and diabetic HF. Furthermore, simple and reliable measurements to diagnose this disease earlier and to effectively predict the prognosis remain insufficient.

In the current study, the gene expression profiles generated from healthy controls and ND-HF patients were analyzed. Biopsy tissues were collected during the surgical ventricular restoration in patients with dilated hypokinetic ischemic cardiomyopathy. Differentially expressed genes (DEGs) were screened and their possible roles in the pathogenesis of HF were explored using multiple bioinformatics methods. The

main aim of the present study was to identify better markers for the diagnosis and treatment of ND-HF.

Materials and methods

Microarray dataset. The microarray dataset under the accession number GSE26887 (11) were obtained from the Gene Expression Omnibus (12) database (http://www.ncbi.nlm.nih.gov/geo/) of the National Center for Biotechnology Information (Bethesda, MD, USA). The gene expression profile was generated based on the platform GPL6244 (Affymetrix Human Gene 1.0 ST Array; Affymetrix, Inc., Santa Clara, CA, USA). This dataset was derived from RNA samples extracted from 12 ND-HF patients (12 males) and 5 healthy controls (2 males, 3 females). Myocardial biopsy samples were collected from the vital, non-infarcted zone of left ventricular of patients with dilated ischemic hypokinetic cardiomyopathy during surgical ventricular restoration procedures (11). In addition, left ventricle cardiac biopsy samples were collected by Greco *et al* (11) from the vital, non-infarcted zone of control patients who had succumbed to mortality (as a result of non-cardiac associated causes), within <24 h.

Data preparation and DEGs screening. Robust multichip average (RMA) (13), which contained three steps including background adjustment, quantile normalization and summarization, was used as a probe set algorithm. The original dataset and the annotation file of the platform were preprocessed using the RMA method of the BioConductor Oligo package (version 2.12; www.bioconductor.org). Probe set IDs were transformed into gene symbols, and the gene expression matrix was constructed.

Statistically significant differences in the expression levels of the various genes were first calculated by the unequal variance t-test, and was then adjusted for multiple testing using the Benjamini and Hochberg procedure (14). After comparing the expression of these genes in the control and HF tissues, the adjusted P-value was obtained, and DEGs with an adjusted P-value of <0.05 and a $|\log_2$ fold change (FC)| of >1 were screened and were considered as potential HF-associated genes.

Protein-protein interaction (PPI) network. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (15) is a widely used database that includes the known and predicted protein interactions. PPI network analysis of the upregulated and downregulated DEGs was performed utilizing the STRING online tool. A confidence (combined) score of >0.4 was selected as the threshold of PPIs.

The PPI network was constructed using the Cytoscape software platform (16) based on the PPI associations identified. Since the vast majority of biological networks are subject to the scale-free (without scale) properties of the network, the connectivity degree was used for the analysis of important nodes (hub proteins) in the PPI network (17,18).

Gene ontology (GO) and pathway analysis. The Kyoto Encyclopedia of Genes and Genomes (KEGG) (19) is an authoritative database containing a variety of biochemical pathways. In addition, the Database for Annotation, Visualization and Integration Discovery (DAVID) (20) is a gene functional classification tool that organizes and condenses abundant heterogeneous annotation content. Functional enrichment analysis was conducted in order to recognize the DEG enriched biochemical pathways using KEGG database and GO-associated biological functions. Furthermore, DAVID online tools were applied for the GO and KEGG pathway enrichment analyses with a P-value set to <0.05.

Table I. Top 10 upregulated and downregulated genes.

Gene	$\log_2$FC	Adjusted P-value
Upregulated		
EIF1AY	3.412	0.00281
NPPA	3.115	0.00038
DSC1	2.445	0.00031
NEB	2.418	0.00545
MYL4	2.346	0.00204
UTY	2.254	0.00414
FRZB	2.171	0.00165
USP9Y	2.095	0.00550
SLN	2.092	0.00712
RBMY1A1	1.993	0.00038
Downregulated		
SERPINE1	-3.182	0.00301
SERPINA3	-2.904	0.00037
TNC	-2.223	0.01926
SPP1	-2.129	0.01003
CYP1B1	-2.027	0.00858
S100A8	-1.955	0.00130
ANKRD2	-1.903	0.00079
GFPT2	-1.824	0.00020
MYC	-1.821	0.00117
CD163	-1.805	0.00022

$\log_2$FC, $\log_2$-transformed fold change of gene expression.

Results

DEG screening. A significant gender difference of the sample source existed between the control and ND-HF subjects; thus, gender-correlated results were carefully considered or abandoned. A total of 255 DEGs were obtained in the ND-HF patients, including 122 upregulated and 133 downregulated genes. As shown in Table I, the *EIF1AY*, *NPPA* and *DSC1* were the three most upregulated genes. Similarly, the three most downregulated genes were S*ERPINE1*, *SERPINA3* and *TNC*, and their respective $\log_2$ FC values were -3.182, -2.904 and -2.223 (Table I).

PPI network. In total, 38 nodes and 53 node-pairs were identified in the PPI network of the upregulated DEGs. Furthermore, 77 nodes and 149 node-pairs were obtained in the PPI network of downregulated DEGs. Subsequent to filtering out the low-degree nodes and nodes without connections, the up- and downregulated PPI networks were constructed, as shown in Figs. 1 and 2, respectively.

Tables II and III exhibited the connectivity degree of the top 30% nodes in the PPI network of upregulated and

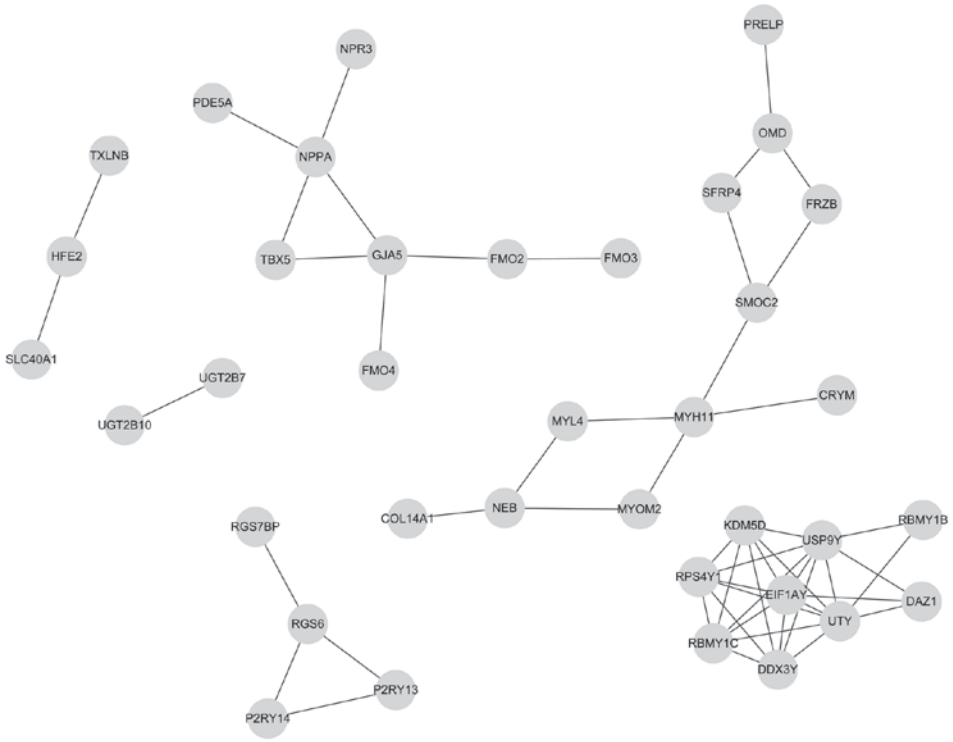

Figure 1. Upregulated differentially expressed genes in the protein-protein interaction network.

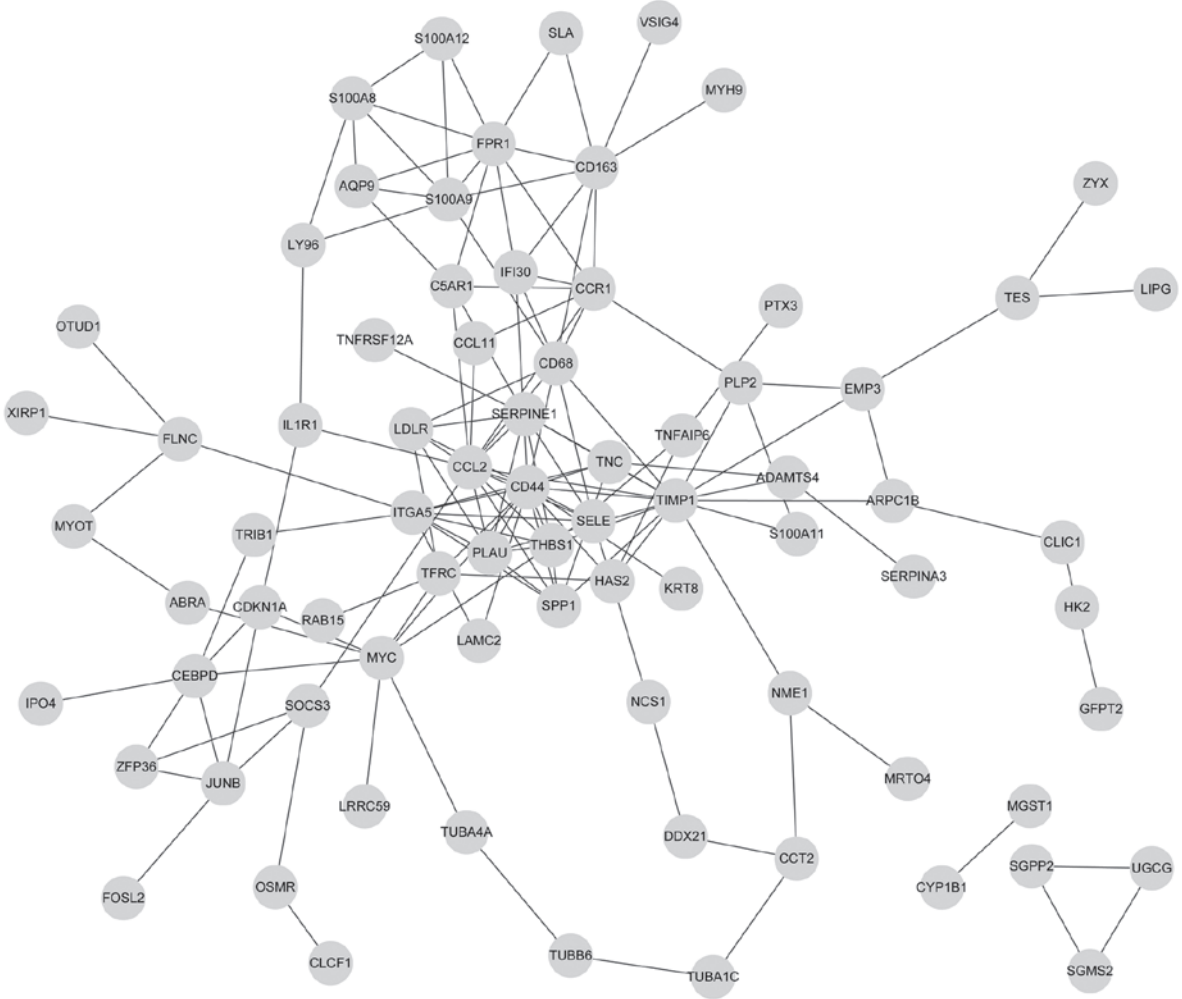

Figure 2. Downregulated differentially expressed genes in the protein-protein interaction network.

Table II. Top 30% of the node connections in the upregulated protein-protein interaction network.

Gene	Degree
USP9Y	8
UTY	8
EIF1AY	7
DDX3Y	6
KDM5D	6
RBMY1C	6
RPS4Y1	6
GJA5	4
MYH11	4
NPPA	4

Degree refers to the number of gene connections within the network.

Table III. Top 30% of the node connections in the downregulated protein-protein interaction network.

Gene	Degree
CD44	16
TIMP1	15
CCL2	14
THBS1	9
SERPINE1	9
FPR1	9
CD68	9
ITGA5	9
CCR1	8
CD163	8
MYC	8
PLAU	8
SPP1	7
S100A9	7
CEBPD	6
SELE	6
TNC	6
LDLR	6
C5AR1	5
IFI30	5
TFRC	5
S100A8	5
JUNB	5

Degree refers to the number of gene connections within the network.

downregulated DEGs, respectively. According to the calculation results, the connectivity degree of each node was >4 in the upregulated and downregulated networks. The connectivity degree of *NPPA* was 4, without any connections with the *USP9Y*, *UTY* and *EIF1AY* genes. In the downregulated network, the top five nodes with a high connectivity degree were *CD44*, *TIMP1*, *CCL2*, *THBS1* and *SERPINE1*.

GO and KEGG pathway analyses of DEGs. GO analysis revealed that the significantly-enriched GO terms of upregulated DEGs included muscle contraction, muscle system process, circulatory system process (involving *NPPA*), blood circulation, muscular organ development, male gamete generation, spermatogenesis, cGMP metabolic process and skeletal system development. In addition, the significantly enriched GO terms of downregulated DEGs were mainly associated with the stimulus response, response to bacterium and response to nutrient. *NPPA* was also involved in the GO term of regulation of cell growth (Table IV).

KEGG pathway analysis revealed that the significantly enriched pathways of upregulated DEGs were drug metabolism, ascorbate and aldarate metabolism, and pentose and glucuronate interconversions (Table V). By contrast, the significantly enriched pathways of the downregulated DEGs were extracellular matrix (ECM)-receptor interactions (involving the genes *THBS1*, *CD44* and *TNC*), pathogenic *Escherichia coli* infection, focal adhesion (involving *TNC* and *THBS1*), cytokine-cytokine receptor interaction (involving *CCL11* and *CCL2*), hematopoietic cell lineage (involving *CD44*), sphingolipid metabolism, and bladder cancer (involving *THBS1*; Table V).

Discussion

HF with fairly high morbidity and mortality (21), is increasing in prevalence with the aging of the worldwide population (22). In order to improve the understanding on the underlying mechanisms and identify molecular markers of HF, particularly in dilated ischemic cardiomyopathy-associated HF, the present study screened the DEGs between control and ND-HF patients. In addition, these DEGs were used for PPI network construction, while GO and KEGG pathway analyses were also performed. A total of 122 upregulated and 133 downregulated genes were detected. The most significantly upregulated and downregulated genes were *NPPA* and *SERPINE1*, respectively. Furthermore, *NPPA* and *SERPINE1* were not only differentially expressed in ND-HF patients, but were also found to be hub nodes in the PPI network. Certain GO terms and KEGG pathways enriched by DEGs were obtained. Therefore, these hub genes and functional terms may be closely associated with ND-HF.

The protein encoded by the upregulated *NPPA* gene is the atrial natriuretic peptide (ANP), which is a member of the natriuretic peptide family that is involved in the control of the extracellular fluid volume and electrolyte homeostasis (23,24). The GO term of circulatory system process, in which *NPPA* is involved, is vital for homeostasis. In addition, mutations in *NPPA* gene are linked to atrial fibrillation (25). In 1998, Maeda *et al* (26) stated that brain natriuretic peptide (BNP) levels were correlated with the left ventricular end-diastolic pressure. However, a more recent study by Seronde *et al* (27) found that the mid-regional sequence of pro-ANP (MR-proANP) has a more long term prognostic value when compared with BNP in patients with acute HF. Furthermore, Potocki *et al* (28) suggested that MR-proANP appears to provide incremental information superior to BNP in certain subgroups of patients. Notably, GO terms and KEGG pathways enriched by *NPPA* or other genes are essential in cardiac failure. Therefore, due to these advantages of ANP when compared with BNP, *NPPA*

Table IV. Gene ontology term enrichment analyses of the differentially expressed genes.

Category	GO-BP Term	Count	P-value
Upregulated			
GO:0006936	Muscle contraction	4	0.004604
GO:0003012	Muscle system process	4	0.005971
GO:0003013	Circulatory system process	4	0.007904
GO:0008015	Blood circulation	4	0.007904
GO:0007517	Muscle organ development	4	0.011139
GO:0048232	Male gamete generation	4	0.030149
GO:0007283	Spermatogenesis	4	0.030149
GO:0046068	cGMP metabolic process	2	0.030618
GO:0001501	Skeletal system development	4	0.032968
Downregulated			
GO:0009611	Response to wounding	21	5.31×10^{-13}
GO:0006954	Inflammatory response	17	3.16×10^{-12}
GO:0006952	Defense response	20	7.41×10^{-11}
GO:0032496	Response to lipopolysaccharide	6	3.84×10^{-5}
GO:0002237	Response to molecule of bacterial origin	6	6.54×10^{-5}
GO:0009617	Response to bacterium	7	3.78×10^{-4}
GO:0009991	Response to extracellular stimulus	8	1.04×10^{-4}
GO:0031667	Response to nutrient levels	7	4.22×10^{-4}
GO:0007584	Response to nutrients	6	6.38×10^{-4}
GO:0033273	Response to vitamins	4	0.004253

GO, gene ontology; BP, biological process.

Table V. Kyoto Encyclopedia of Genes and Genomes pathway analysis of the differentially expressed genes.

Pathway term	Pathway description	Count	P-value	Associated genes
Upregulated				
hsa00982	Drug metabolism	5	6.20×10^{-6}	*FMO4*, *FMO2*, *FMO3*, *UGT2B10*, *UGT2B7*
hsa00053	Ascorbate and aldarate metabolism	2	0.036201	*UGT2B10*, *UGT2B7*
hsa00040	Pentose and glucuronate interconversions	2	0.038293	*UGT2B10*, *UGT2B7*
Downregulated				
hsa04512	Extracellular matrix-receptor interaction	6	5.13×10^{-4}	*CD44*, *ITGA5*, *TNC*, *LAMC2*, *THBS1*, *SPP1*
hsa05130	Pathogenic *Escherichia coli* infection	5	0.001056	*ARPC1B*, *LY96*, *TUBB6*, *TUBA4A*, *TUBA1C*
hsa04510	Focal adhesion	7	0.005006	*ITGA5*, *TNC*, *LAMC2*, *ZYX*, *FLNC*, *THBS1*, *SPP1*
hsa04060	Cytokine-cytokine receptor interaction	7	0.017379	*CCL11*, *IL1R1*, *CCL2*, *TNFRSF12A*, *OSMR*, *CLCF1*, *CCR1*
hsa04640	Hematopoietic cell lineage	4	0.031377	*IL1R1*, *TFRC*, *CD44*, *ITGA5*
hsa00600	Sphingolipid metabolism	3	0.038951	*SGMS2*, *SGPP2*, *UGCG*
hsa05219	Bladder cancer	3	0.044581	*CDKN1A*, *THBS1*, MYC

may also be an essential gene associated with ND-HF and may be used as a potential therapeutic target in ND-HF.

In addition to the poor contractility and low cardiac output, patients with HF also present with abnormal manifestations of platelets and endothelial dysfunction (29), while HF patients in sinus rhythm still present a higher thromboembolic risk (30). *SERPINE1*, also known as plasminogen activator inhibitor 1 (*PAI-1*) precursor, which was downregulated and pertained to

the serine proteinase inhibitor superfamily, has a core effect in the regulation of fibrinolysis, coagulation, inflammation and neuromuscular patterning (31). Askari *et al* (32) hypothesized that genetic disruption of PAI-1 is essential in order to suppress ventricular remodeling in null mice with myocardial infarction; furthermore, PAI-1 is essential in microvascular integrity and cardiac homeostasis (33). Based on the results of the present and previous studies (31,33), the plasma level of *SERPINE1* is associated with thrombophilia and an increased risk of coronary artery disease (34). Therefore, *SERPINE1* may be a useful marker for the diagnosis and treatment of ND-HF.

HF is accompanied by degradation of the collagen network of the ECM (35), and may subsequently cause heart dysfunction (36). Zheng *et al* (37) reported an overall decrease in the ECM-associated genes which are indispensable to the overall ECM structure and collagen assembly. Therefore, it is no surprise that *CD44*, which is involved in the ECM-receptor interaction, was found to be downregulated in the current study. Chatila *et al* (38) also found that certain compositions of the infarct border zone may slow down left ventricular remodeling by suppressing inflammation. In the current study, GO terms enriched by downregulated genes were mainly associated with the stimulus and immune response. Considering these findings, the connections between ECM and inflammation participating in HF require further investigation, particularly in ND-HF.

In conclusion, based on the bioinformatics methods used in the current study, a number of DEGs were highlighted, particularly *NPPA* and *SERPINE1*, although the results were interfered by certain Y-linked genes to some extent. These two genes may be potential therapeutic targets and molecular markers contributing to improved prevention and treatment of cardiogenic disease. Additionally, the complicated correlation between ECM-protein expression and inflammation was further investigated. However, further comparison of these genes and those obtained from diabetic HF patients with dilated ischemic cardiomyopathy and controls is required to verify these results. Furthermore, gender-matched studies are needed, with a sufficiently large sample size. Future research should focus on these areas and verify these DEGs based on serum sample analysis.

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