Identification of candidate biomarkers and therapeutic agents for heart failure by bioinformatics analysis

Vijayakrishna Kolur1, Basavaraj Vastrad2, Chanabasayya Vastrad3*, Shivakumar Kotturshetti3 and Anandkumar Tengli4

Abstract

Introduction: Heart failure (HF) is a heterogeneous clinical syndrome and affects millions of people all over the world. HF occurs when the cardiac overload and injury, which is a worldwide complaint. The aim of this study was to screen and verify hub genes involved in developmental HF as well as to explore active drug molecules.

Methods: The expression profiling by high throughput sequencing of GSE141910 dataset was downloaded from the Gene Expression Omnibus (GEO) database, which contained 366 samples, including 200 heart failure samples and 166 non heart failure samples. The raw data was integrated to find differentially expressed genes (DEGs) and were further analyzed with bioinformatics analysis. Gene ontology (GO) and REACTOME enrichment analyses were performed via ToppGene; protein–protein interaction (PPI) networks of the DEGs was constructed based on data from the HiPPIE interactome database; modules analysis was performed; target gene—miRNA regulatory network and target gene—TF regulatory network were constructed and analyzed; hub genes were validated; molecular docking studies was performed.

Results: A total of 881 DEGs, including 442 up regulated genes and 439 down regulated genes were observed. Most of the DEGs were significantly enriched in biological adhesion, extracellular matrix, signaling receptor binding, secretion, intrinsic component of plasma membrane, signaling receptor activity, extracellular matrix organization and neutrophil degranulation. The top hub genes ESR1, PYHIN1, PPP2R2B, LCK, TP63, PCLAF, CFTR, TK1, ECT2 and FKBP5 were identified from the PPI network. Module analysis revealed that HF was associated with adaptive immune system and neutrophil degranulation. The target genes, miRNAs and TFs were identified from the target gene—miRNA regulatory network and target gene—TF regulatory network. Furthermore, receiver operating characteristic (ROC) curve analysis and RT-PCR analysis revealed that ESR1, PYHIN1, PPP2R2B, LCK, TP63, PCLAF, CFTR, TK1, ECT2 and FKBP5 might serve as prognostic, diagnostic biomarkers and therapeutic target for HF. The predicted targets of these active molecules were then confirmed.

Conclusion: The current investigation identified a series of key genes and pathways that might be involved in the progression of HF, providing a new understanding of the underlying molecular mechanisms of HF.

Keywords: Heart failure, Differentially expressed genes, Molecular docking, Enrichment analysis, Prognosis

© The Author(s) 2021. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
oedema and hepatomegaly [1]. Morbidity and mortality linked with HF is a prevalent worldwide health problem holding a universal position as the leading cause of death [2]. The numbers of cases of HF are rising globally and it has become a key health issue. According to a survey, the prevalence of HF is expected to exceed 50% of the global population [3]. Research suggests that modification in multiple genes and signaling pathways are associated in controlling the advancement of HF. However, a lack of investigation on the precise molecular mechanisms of HF development limits the treatment efficacy of the disease at present.

Previous study showed that HF was related to the expression of MECP2 [4] RBM20 [5], CaMKII [6], troponin I [7] and SERCA2a [8]. Toll-Like receptor signaling pathway [9], activin type II receptor signaling pathway [10], CaMKII signaling pathways [11], Drp1 signaling pathways [12] and JAK-STAT signaling pathway [13] were liable for progression of HF. More investigations are required to focus on treatments that enhance the outcome of patients with HF, to strictly make the diagnosis of the disease based on screening of biomarkers. These investigations can upgrade prognosis of patients by lowering the risk of advancement of HF and related complications. So it is essential to recognize the mechanism and find biomarkers with a good specificity and sensitivity.

The recent high-throughput RNA sequencing data has been widely employed to screen the differentially expressed genes (DEGs) between normal samples and HF samples in human beings, which makes it accessible for us to further explore the entire molecular alterations in HF at multiple levels involving DNA, RNA, proteins, epigenetic alterations, and metabolism [14]. However, there still exist obstacles to put these RNA seq data in application in clinic for the reason that the number of DEGs found by expression profiling by high throughput sequencing were massive and the statistical analyses were also too sophisticated [15–19].

In this study, first, we had chosen dataset GSE141910 from Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) [20]. Second, we applied for limma tool in R software to obtain the differentially expressed genes (DEGs) in this dataset. Third, the ToppGene was used to analyze these DEGs including biological process (BP), cellular component (CC) and molecular function (MF) REACTOME pathways. Fourth, we established protein–protein interaction (PPI) network and then applied Cytoype PEWCC1 for module analysis of the DEGs which would identify some hub genes. Fifth, we established target gene—miRNA regulatory network and target gene—TF regulatory network. In addition, we further validated the hub genes by receiver operating characteristic (ROC) curve analysis and RT-PCR analysis. Finally, we performed molecular docking studies for over expressed hub genes. Results from the present investigation might provide new vision into potential prognostic and therapeutic targets for HF.

Materials and methods

Data resource

Expression profiling by high throughput sequencing with series number GSE141910 based on platform GPL16791 was downloaded from the GEO database. The dataset of GSE141910 contained 200 heart failure samples and 166 non heart failure samples. It was downloaded from the GEO database in NCBI based on the platform of GPL16791 Illumina HiSeq 2500 (Homo sapiens).

Identification of DEGs in HF

DEGs of dataset GSE141910 between HF groups and non heart failure groups were respectively analyzed using the limma package in R [21]. Fold changes (FCs) in the expression of individual genes were calculated and DEGs with \( P < 0.05 \), \(|\log FC| > 1.158\) for up regulated genes and \(|\log FC| < −0.83\) for down regulated genes were considered to be significant. Hierarchical clustering and visualization were used by Heat-map package of R.

Functional enrichment analysis

Gene Ontology (GO) analysis and REACTOME pathway analysis were performed to determine the functions of DEGs using the ToppGene (ToppFun) (https://toppgene.chhmc.org/enrichment.jsp) [22] GO terms (http://geneontology.org/) [23] included biological processes (BP), cellular components (CC) and molecular functions (MF) of genomic products. REACTOME (https://reactome.org/) [24] analyzes pathways of important gene products. ToppGene is a bioinformatics database for analyzing the functional interpretation of lists of proteins and genes. The cutoff value was set to \( P < 0.05 \).

Protein–protein interaction network construction and module screening

PPI networks are used to establish all protein coding genes into a massive biological network that serves an advance compassionate of the functional system of the proteome [25]. The HiPPIE interactome (https://cbdm.uni-mainz.de/hippie/) [26] database furnish information regarding predicted and experimental interactions of proteins. In the current investigation, the DEGs were mapped into the HiPPIE interactome database to find significant protein pairs with a combined score of >0.4. The PPI network was subsequently constructed using Cytoscape software, version 3.8.2 (www.cytoscape.org) [27]. The nodes with a higher node degree [28], higher betweenness centrality [29], higher stress centrality [30]
and higher closeness centrality [31] were considered as hub genes. Additionally, cluster analysis for identifying significant function modules with a degree cutoff > 2 in the PPI network was performed using the PEWCC1 (http://apps.cytoscape.org/apps/PEWCC1) [32] in Cytoscape.

**Target gene—miRNA regulatory network construction**
The miRNet database (https://www.mirnet.ca/) [33] contains information on miRNA and the regulated genes. Using information collected from the miRNet database, hub genes were matched with their associated miRNA. The target gene—miRNA regulatory network then was constructed using Cytoscape software. MiRNAs and target are selected based on highest node degree.

**Target gene—TF regulatory network construction**
The NetworkAnalyst database (https://www.networkanalyst.ca/) [34] contains information on TF and the regulated genes. Using information collected from the NetworkAnalyst database, hub genes were matched with their associated TF. The target gene—TF regulatory network then was constructed using Cytoscape software. TFs and target genes are selected based on highest node degree.

**Receiver operating characteristic (ROC) curve analysis**
Then ROC curve analysis was implementing to classify the sensitivity and specificity of the hub genes for HF diagnosis and we investigated how large the area under the curve (AUC) was by using the statistical package pROC in R software [35].

**RT-PCR analysis**
H9C2 cells (ATCC) were cultured in Dulbecco’s minimal essential medium (DMEM) (Sigma-Aldrich) supplemented with 10% fetal calf serum (Sigma-Aldrich) and 1% streptomycin (Sigma-Aldrich) at 37 °C in 5% CO₂. HL-1 cells (ATCC) was culture in Claycomb medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Sigma-Aldrich), 1% streptomycin (Sigma-Aldrich), 1% glutamax (Sigma-Aldrich) and 0.1 mM nor-epinephrine (Sigma-Aldrich) at 37 °C in 5% CO₂. Total RNA was isolated from cell culture of H9C2 for HF and HL-1 for normal control using the TRI Reagent (Sigma, USA). cDNA was synthesized using 2.0 μg of total RNA with the Reverse transcription cDNA kit (Thermo Fisher Scientific, Waltham, MA, USA). The 7 Flex real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA) was employed to detect the relative mRNA expression. The relative expression levels were determined by the 2−ΔΔCt method and normalized to internal control beta-actin [36]. All RT-PCR reactions were performed in triplicate. The primers used to explore mRNA expression of ten hub genes were listed in Table 1.

**Identification of candidate small molecules**
SYBYL-X 2.0 perpetual drug design software has been used for surflex-docking studies of the designed novel molecules and the standard on over expressed genes of PDB protein. Using ChemDraw Software, all designed molecules and standards were sketched, imported and saved using open babel free software in sdf. template. The protein of over expressed genes of ESR1, LCK, PPP2R2B, TP63 and their co-crystallised protein of PDB code 4PXM, 1KSW, 2HV7, 3VD8 and 6RU6 were extracted from Protein Data Bank [37–40]. Optimizations of the designed molecules were performed by standard process by applying Gasteiger Huckel (GH) charges together with the TRIPOS force field. In addition, energy minimization was achieved using MMFF94s and MMFF94 algorithm methods. The preparation of the protein was done after protein incorporation. The co-crystallized ligand and all water molecules have been eliminated from the crystal structure; more hydrogen’s were added and the side chain was set, TRIPOS force field was used for the minimization of structure. The interaction efficiency of the compounds with the receptor was expressed in kcal/mol units by the Suflex-Dock score. The best location was integrated into the molecular region by the interaction between the protein and the ligand. Using Discovery Studio Visualizer, the visualisation of ligand interaction with receptor is performed.

**Results**
**Identification of DEGs in HF**
We identified 881 DEGs in the GSE141910 dataset using the limma package in R. Based on the limma analysis, using the adj P val<0.05, |log FC|>1.158 for up regulated genes and |log FC|<−0.83 for down regulated genes.

| Genes | Forward primers | Reverse primers |
|-------|----------------|-----------------|
| ESR1  | CCTCTGCTATTACCATATGAG | AGTCATTGTCCTCCTGAGTC |
| PYH1N1 | GCAGACGACCTACAGCAGAAG | AGATACTGACCAACCTCTG |
| PPP2R2B | ACCAGAGACTATCTCGACGG | GTACTGACCAACCTGTAAGTC |
| LCK   | CTAGTCCGCTTTATGCGG | AATATTACTAGGCTCCCCG |
| TP63  | ATTCATTTGAGGACAGATCCGG | GGGGCTCTTCTACATACTGCC |
| PCLAF | GACCATATAAATACGGGG | CCAAGGCAAAACAGAAG |
| FKBPS | GCTTGGGCTTTGTTTCTCG | CTTGACATGCTCTGACAA |
| CTRF  | CTGTCGCGGCTTTTCTCTC | CTGGTGACTGCTCTGACAA |
| TK1   | AGATCAGGCTAGCTCGG | ACTGGTGAGGGCGATCTG |
| ECT2  | GCTGATTTGAGGATATCGG | GCCAAGGACATGACAGC |

Table 1 The sequences of primers for quantitative RT-PCR
regulated genes, a total of 881 DEGs were identified, consisting of 442 genes were up regulated and 439 genes were down regulated. The DEGs are listed in Additional file 1: Table S1. The volcano plot for DEGs is illustrated in Fig. 1. Figure 2 is the hierarchical clustering heat-map.

**Functional enrichment analysis**

Results of GO analysis showed that the up regulated genes were significantly enriched in BP, CC, and MF, including biological adhesion, regulation of immune system process, extracellular matrix, cell surface, signaling receptor binding and molecular function regulator.
(Table 2); the down regulated genes were significantly enriched in BP, CC, and MF, including secretion, defense response, intrinsic component of plasma membrane, whole membrane, signaling receptor activity and molecular transducer activity (Table 2). Pathway analysis showed that the up regulated genes were significantly enriched in extracellular matrix organization and immunoregulatory interactions between a lymphoid and a non-lymphoid cell (Table 3); the down regulated genes were significantly enriched in neutrophil degranulation and SLC-mediated transmembrane transport (Table 3).

**Protein–protein interaction (PPI) network and module analysis**
Based on the HiPPIE interactome database, the PPI network for the DEGs (including 6541 nodes and 13,909 edges) was constructed (Fig. 3A). Up regulated gene with higher node degree, higher betweenness centrality, higher stress centrality and higher closeness centrality were as follows: ESR1, PYHIN1, PPP2R2B, LCK, TP63 and so on. Down regulated genes had higher node degree, higher betweenness centrality, higher stress centrality and higher closeness centrality were as follows PCLAF, CFTR, TK1, ECT2, FKBP5 and so on. The node degree, betweenness centrality, stress centrality and closeness centrality are listed in Table 4.

Additionally, two significant modules, including module 1 (10 nodes and 24 edges) and module 2 (5 nodes and 10 edges) (Fig. 3B) and module 3 (55 nodes and 115 edges), were acquired by PEWCC1 plug-in (Fig. 3C). Furthermore, GO terms and REACTOME pathways were significantly enriched by module 1, including adaptive immune system, immunoregulatory interactions between a lymphoid and a non-lymphoid cell, hemostasis, biological adhesion and regulation of immune system process. Meanwhile, the nodes in module 2 were significantly enriched in GO terms and REACTOME pathways, including neutrophil degranulation and secretion.

**Target gene—miRNA regulatory network construction**
Associations between 2063 miRNAs and their 319 target genes were collected from the target gene—miRNA regulatory network (Fig. 4). MiRNAs of hsa-mir-4533, hsa-mir-548ac, hsa-mir-548i, hsa-mir-5585-3p, hsa-mir-6750-3p, hsa-mir-200c-3p, hsa-mir-1273 g-3p, hsa-mir-1244, hsa-mir-4789-3p and hsa-mir-766-3p, which exhibited a high degree of interaction, were selected from this network. Furthermore, the results also showed that FSCN1 was the target of hsa-mir-4533, ESR1 was the target of hsa-mir-548ac, TMEM30B was the target of hsa-mir-548i, SCN2B was the target of hsa-mir-5585-3p, CENPA was the target of hsa-mir-6750-3p, FKBP5 was the target of hsa-mir-200c-3p, PCLAF was the target of hsa-mir-1273g-3p, CEP55 was the target of hsa-mir-1244, ATP2A2 was the target of hsa-mir-4789-3p and TK1 was the target of hsa-mir-766-3p, and are listed in Table 5.

**Target gene—TF regulatory network construction**
Associations between 330 TFs and their 247 target genes were collected from the target gene—TF regulatory network (Fig. 5). TFs of ESRR, RERE, HMG20B, THRAP3, ATF1, MXD3, ARID4B, CBF, TAF7 and CREM, which exhibited a high degree of interaction, were selected from this network. Furthermore, the results also showed that FSCN1 was the target of ESRR, APOA1 was the target of RERE, COL1A1 was the target of HMG20B, HBB was the target of THRAP3, LCK was the target of ATF1, SOCS3 was the target of MXD3, BCL6 was the target of ARID4B, FKBP5 was the target of CBF, ANLN was the target of TAF7 and ATP2A2 was the target of CREM, and are listed in Table 5.

**Receiver operating characteristic (ROC) curve analysis**
First of all, we performed the ROC curve analysis among 10 hub genes based on the GSE141910. The results showed that ESR1, PYHIN1, PPP2R2B, LCK, TP63, PCLAF, CFTR, TK1, ECT2 and FKBP5 achieved an AUC value of >0.7, demonstrating that these ten genes have high sensitivity and specificity for HF, suggesting they can be served as biomarkers for the diagnosis of HF (Fig. 6).

**RT-PCR analysis**
RT-PCR was used to validate the hub genes between normal and HF cell lines. The results suggested that the mRNA expression level of ESR1, PYHIN1, PPP2R2B, LCK and TP63 were significantly increased in HF compared with that in normal, while PCLAF, CFTR, TK1, ECT2 and FKBP5 were significantly decreased in HF compared with that in normal and are shown in Fig. 7.

**Identification of candidate small molecules**
In the present study docking simulations are performed to spot the active site and foremost interactions accountable for complex stability with the receptor binding sites. In heart failure recognized over expressed genes and their proteins of x-ray crystallographic structure are chosen from PDB for docking studies. Most generally, medications containing benzothiadiazine ring hydrochlorothiazide are used in heart failure either alone or in conjunction with other drugs, based on this the molecules containing heterocyclic ring of benzothiadiazine are designed and hydrochlorothiazide is uses as a reference standard. Docking experiments using Sybyl-X 2.1.1. drug design perpetual software were used on the designed molecules. Docking studies were performed in order to understand the biding interaction of standard
| GO ID   | CATEGORY | GO Name                                      | P Value  | FDR B&H | FDR B&Y | Bonferroni | Gene Count | Gene                                                                 |
|---------|----------|----------------------------------------------|----------|---------|---------|------------|------------|-----------------------------------------------------------------------|
| GO:0022610 | BP       | biological adhesion                          | 1.32E−13 | 3.37E−10| 3.08E−09| 6.75E−10   | 72         | HLA-DQA1, DACT2, CD83, MDK, UBASH3A, ITGBL1, FAP, MAFAP4, SERPINE2,   |
|          |          |                                               |          |         |         |            |            | NRXN2, COL14A1, CCR7, ALOX15, COL1A1, LAMB4, COL8A2, STAT2, COL16A1,|
|          |          |                                               |          |         |         |            |            | COMP, TXB21, FERTMT1, XG, CCDC280, APOA1, PDKD12, ZAP70, HAPLN1, TEMN4, |
|          |          |                                               |          |         |         |            |            | SKAP1, CNTNAP2, PDESA, CARD11, CNTNA2, SLAMF7, ATP1B2, CX3CR1, LRRC15,|
|          |          |                                               |          |         |         |            |            | IDO1, MYOC, SIGLEC8, ISLR, SMO2C2, ITGAL, ITGB7, FREM1, PTN, KIRREL3,|
|          |          |                                               |          |         |         |            |            | NTM, GLI2, FBLN7, DPT, NTSE, ECM2, LCK, OMG, OPCML, TGFB2, RASGRP1, CD2,|
|          |          |                                               |          |         |         |            |            | CD3E, THBS4, CD5, CD6, THY1, TGD1, CD27, CD40LG, ROBO2, GREM1, LYG, HBB,|
|          |          |                                               |          |         |         |            |            | LEF1                                                                 |
| GO:0002682 | BP       | regulation of immune system process          | 2.20E−10 | 2.25E−07| 2.05E−06| 1.12E−06   | 72         | IL34, HLA-DQA1, ESRI1, TL7, CD83, IL17D, MDK, UBASH3A, TNFRSF4F, PYHIH1,|
|          |          |                                               |          |         |         |            |            | ZBP1, FCER1A, MS4A2, FCR2, FCN1, CCR7, SMPD3, CCL24, SCARA3, ALOX15, |
|          |          |                                               |          |         |         |            |            | COL1A1, IL31RA, TXB21, XG, CXCL14, APOA1, ZAP70, SH2D1B, SKAP1, PDESA,|
|          |          |                                               |          |         |         |            |            | CARD11, SLAMF7, CTSG, CX3CR1, IDO1, CXCL10, ITGAL, ACE, STI1, ITGB7, |
|          |          |                                               |          |         |         |            |            | PTN, TB1D10C, FCRL3, BPI, GLU2, KLRB1, NPPA, CAMK4, LCK, TGFB2, RASGRP1,|
|          |          |                                               |          |         |         |            |            | CD1C, CD1E, CD2, CD3D, CD3E, THBS4, CD3G, CD247, CD5, CD6, THY1, TGIT,|
|          |          |                                               |          |         |         |            |            | MS4A1, CD27, GPR68, CD40LG, CD48, GREM1, SH2D1A, LEF1, LRRC17          |
| GO:0031012 | CC       | extracellular matrix                          | 1.09E−20 | 2.77E−18| 1.89E−17| 5.54E−18   | 52         | MATN2, COL22A1, MDK, COLQ, MAFAP4, SERPINE2, HMCN2, AEBP1, FCN1, |
|          |          |                                               |          |         |         |            |            | CMA1, CTHRC1, COL14A1, SCARA3, COL1A1, LAMB4, COL8A2, COL9A1, COL9A2,|
|          |          |                                               |          |         |         |            |            | COL10A1, MXRAS, FMOD, COL16A1, COMP, CCDC280, APOA1, HAPLN1, CTSG,|
|          |          |                                               |          |         |         |            |            | ADAMTS2, LRRC15, ASPN, MYOC, NDP, SMO2C2, FREM1, PTN, S55C5D, SULF1,|
|          |          |                                               |          |         |         |            |            | DPT, NPPA, ADAMTS1, ECM2, OGN, ITHS, TGFB2, LEFTY2, EYS, THBS4, P3H2,|
|          |          |                                               |          |         |         |            |            | LTB2, GREM1, LUM, LRRC17                                             |
| GO ID   | CATEGORY       | GO Name                  | P Value  | FDR B&H | FDR B&Y | Bonferroni | Gene Count | Gene                                                                 |
|---------|----------------|--------------------------|----------|---------|---------|------------|------------|-----------------------------------------------------------------------|
| GO:000986 CC cell surface | 5.07E−17 | 8.61E−15 | 5.86E−14 | 2.58E−14 | 63      | HYAL4, NRG1, HLA-DQA1, CD83, TNFRSF4, ITGB1, FAP, SERPINE2, Fcer1A, MSA2, Fcer2, FCN1, CXCL9, CCR7, IL31RA, SFRP4, STAB2, DUOX2, APOA1, ACKR4, FCR1L6, SCUBE2, CNTNAP2, SLAMF7, CTSG, IL2RB, CX3CR1, LRRCL5, CXCL10, NDP, ITGAL, ACE, ITGB7, GFRA3, PTN, PROM1, SSC5D, Fcrl3, SULF1, MR2, SNT, CLEC9A, NT5E, TGFB2, LHCGR, CD1C, HHRP, CD1E, CD2D, CD30, CD3B, CD3G, CD5, CD6, THY1, TIGIT, MSA41, CD27, CD40LG, CD48, ROBO2, GREM1, L9Y |
| GO:0005102 MF signaling receptor binding | 1.36E−09 | 5.99E−07 | 4.41E−06 | 1.20E−06 | 73      | IL34, NRG1, HLA-DQA1, ESR1, GDP6, PENK, TAC4, KDM5D, IL17D, MDK, ITGB1, FAP, SERPINE2, Fcer2, NRXN2, FCN1, CLEC11A, UCHL1, AGTR2, CXCL9, NGEF, CTRC1, C1QTNF2, CCL22, CCL24, CXCL11, COL16A1, COMP, WNT10B, WNT9A, CXCL14, APOA1, FCRL6, GNA14, OASL, RASL11B, LRRCL5, CXCL10, ADAM18, MYDC, SYT2, NDP, ACE, GDNF, ITGB7, GFRA3, PTN, LYPD1, SCG2, NPPA, NPPB, MCHR1, ECM2, CMTM2, ESM1, LCK, OGN, TGFβ2, LEFTY2, CD2, CD3E, THBS4, CD3G, THY1, TIGIT, MSA41, C1QTNF9, CD40LG, LTB, GREM1, SYT1, LEF1, LIG1 |
| GO:0098772 MF molecular function regulator | 3.79E−04 | 1.59E−02 | 1.17E−01 | 3.34E−01 | 58      | IL34, NRG1, ESR1, GDP6, PENK, TAC4, IL17D, MDK, KCNIP1, SERPINE2, MYOZ2, NRXN2, CLEC11A, SCN2B, AGTR2, CXCL9, NGEF, CCL22, CCL24, CXCL11, HTRA2, PI6, SCG5, WNT10B, WNT9A, CXCL14, APOA1, LRRCS5, PPP2R2B, ATP1B2, CXCL10, NDP, BIRC7, GDNF, PTN, TBC1D10C, LYPD1, SCG2, NPPA, NPPB, AZIN2, CMTM2, OGN, RGS4, ITH5, TGFβ2, LEFTY2, RASGRP1, THBS4, THY1, CD27, C1QTNF9, CD40LG, RGS17, LTB, GREM1, LEF1, INKA1 |
| GO ID     | CATEGORY | GO Name     | P Value | FDR B&H | FDR B&Y | Bonferroni | Gene Count | Gene                                                                 |
|-----------|-----------|-------------|---------|---------|---------|------------|------------|---------------------------------------------------------------------|
| GO:0046903| BP        | secretion   | 1.07E−11 | 5.64E−08 | 5.16E−07 | 5.64E−08   | 78         | SERPINA3, HK3, SYN3, ACP3, TRPC4, CFTIR, CD109, HMox2, CHI3L1, F5, F8, F13A1, S100A8, S100A9, SAA1, FCER1G, MGST1, Pik3caH, HP, AGTR1, PLA2G2A, CCR1, FGFR10, C1QTNF1, PLA2G4F, FGR, MERTK, SERPINF2, ALOX5, SYT13, IL17RB, CNR1, ALOX15B, FLI3, ANPEP, P2RY12, ANXA3, FPR1, CRI, SLCT1A1, SLCT2A1, ARQ1, ARNTL, SLCT1A1, SLCT2A1, LIG3, NSEG1, ATP2A2, IL10, SIGLEC9, GPR84, NHR2C, SS9R5, HP5E, KNCB1, IL1R2, PTX3, GLUL, SYN2, BANK1, WNK3, KNG1, CRISPLD2, CACNA1E, CD177, SIGLEC14, EDN1, EDN2, EDNRB, THBS1, RNASE2, CD38, TLK2, SERPINE1, ELANE, STEAP3, IL1RL1, MCEMP1 |
| GO:0006952| BP        | defense response | 1.04E−06 | 1.63E−04 | 1.49E−03 | 5.50E−03   | 65         | SERPINA3, EREG, VSIG4, TMIGD3, CLEC7A, RAET1E, CHI3L1, F8, CD163, S100A8, S100A9, SAA1, FCER1G, HP, HPR, AGTR1, PLA2G2A, CCR1, FGR, SERPINF2, ALOX5, ALOX5AP, IL17RB, CNR1, SELE, ADAMT54, ANXA3, FPR1, APOB, SAMHD1, CR1, FCN3, AQP4, ARG1, SLCT1A1, MARCO, IL10, BCL6, IL18R1, GGTS, IL1R2, PTX3, SIGLEC10, KNG1, CACNA1E, CD177, SOCS3, SIGLEC14, ADAMT55, LBP, S1PR3, EDN1, EDNRB, FOSS1, THBS1, RNASE2, NAMPT, TLK2, SERPINE1, ELANE, IRAK3, ELF3, IL1RL1, CALCRL, OSMR |
### Table 2 (continued)

| GO ID     | CATEGORY | GO Name                               | P Value  | FDR B&H | FDR B&Y | Bonferroni | Gene Count | Gene                                                                 |
|-----------|----------|---------------------------------------|----------|---------|---------|------------|------------|-----------------------------------------------------------------------|
| GO:0031226 CC    | intrinsic component of plasma membrane | 1.74E−10  | 4.55E−08 | 3.11E−07 | 9.10E−08 | 74         | TPO, EREG, OPN4, TRPC4, CFTR, TMIGD3, KCNIP2, CD163, FCER1G, SCN3A, AGTR1, CCR1, C1QTNF1, MERTK, SYT13, IL17RB, CNR1, TRHDE, SELE, LRRC8E, FLT3, SLCA47, P2RY12, SLC31A2, CR1, LGRI, AQP3, AQP4, SLC1A1, SLC2A1, SLC5A1, MSR1, SLC11A1, SIGLEC7, ART3, SLC2OA1, ATP2A2, MARCO, GABBR2, SIGLEC9, SLC4A1, GPR84, SSTR2, SSTR5, IL18R1, LAPTMS, GGT5, SLC52A3, LYEVE1, KCNA7, KCNB1, KCNQ3, NEC1, KCNQ1, KCNQ2, ADGRD1, CACNA1E, GPRA, GPR12, SLC38A4, GPR183, GPRC5A, RGR, S1PR3, RHAG, EDNRB, TGFB3, TLR2, LGRI, CALCRL, OSMR, HAS2, CDH16 |
| GO:0098805 CC    | whole membrane | 1.91E−03  | 4.99E−02 | 3.41E−01 | 9.97E−01 | 51         | EREG, SYN3, ACP3, TRPC4, CFTR, CD109, HMOX2, CD163, FCER1G, MGST1, PLA2G4F, GPAT2, MOG, CNR1, SELE, ANPEP, P2RY12, ANXA3, FPR1, APOB, SCGN, CR1, AQP4, SLC1A1, SLC2A1, ARGI, MSR1, SLC11A1, NSG1, RAB39A, MARCO, SIGLEC9, GPR84, HPSE, LAPTMS, KCNQ3, SYN2, SLC9A7, WASF1, CD177, SIGLEC14, GBR14, STEAP4, EDNRB, GRIP1, CD38, TLR2, STEAP3, HAS2, SERPINAS, MCEMP1 |
| GO:0038023 MF    | signaling receptor activity | 2.36E−04  | 1.97E−02 | 1.49E−01 | 2.53E−01 | 55         | EREG, OPN4, CLEC7A, FCER1G, FCGR3A, AGTR1, CCR1, ADGRF5, ADGRF4, MERTK, IL17RB, CNR1, SELE, FLT3, MYOT, ANPEP, P2RY12, ANXA3, FPR1, CR1, LGRI, SIGLEC7, MARCO, PALLD, IL10, GABBR2, IL15RA, GPR82, DNER, PAQR5, GPR84, IL20RA, SSTR2, SSTR5, IL18R1, LYEVE1, IL1R2, NEC1, ADGRD1, GPR4, GPR12, NPTX2, GPR183, GPRC5A, PKHD1L1, RGR, S1PR3, EDNRB, TGFB3, TLR2, SERPIN1, IL1R1, LGRI, CALCRL, OSMR |


hydrochlorothiazide and designed molecules on over expressed protein. The X- RAY crystallographic structure of one proteins from each over expressed genes of ESR1, LCK, PPP2R2B, PYHIN1, TP63 and their co-crystallised protein of PDB code 4PXM, 1KSW, 2HV7, 3VD8 and 6RU6 respectively were selected for the docking studies to identify and predict the potential molecule based on the binding score with the protein and successful in heart failure. For the docking tests, a total of 34 molecules were built and the molecule with binding score greater than 5 is believed to be good. The designed molecules obtained docking score of 5 to 7 were HIM10, HTZ5, HIM6, HIM2, HIM1, HIM17, HIM12, HIM11, HIM10, HTZ3, HIM12, HIM11, HIM10, HTZ3 and HIM11, HIM3, HTZ15, HTZ8, HTZ17, HTZ1, HTZ3 and HTZ8, HTM, HTZ16, HTZ15, HMT, HTZ1, HTZ1, HIM3, HTZ17, HTZ2 and HTZ7, HTZ4, HTZ2, HTZ17, HTZ15 with proteins 4PXM and, 1KSW and 2HV7 and 3VD8 and 6RU6 respectively. The molecules obtained very less binding score are HTZ1, HIM12, HTZ2, HTZ4 with protein 4PXM and the standard hydrochlorothiazide (HTZ) obtained less binding score with all proteins, the values are depicted in Table 6.

**Discussion**

HF is the most prevalent form of cardiovascular disease among the elderly. A complete studies of HF, comprising pathogenic factors, pathological processes, clinical manifestations, early clinical diagnosis, clinical prevention, and drug therapy targets urgency to be consistently analyzed. In the present investigation, bioinformatics analysis was engaged to explore HF biomarkers and the pathological processes in myocardial tissues, acquired from HF groups and non heart failure groups. We analyzed GSE141910 expression profiling by high throughput sequencing obtained 881 different genes between HF groups and non heart failure groups, 442 up regulated and 439 down regulated genes. HBA2 and HBA1 have a key role in hypertension [41], but these genes might be linked with development HF. SFRP4 was linked with progression of myocardial ischemia [42]. Emmens et al. [43] and Broch et al. [44] found that PENK (proenkephalin) and IL1RL1 were up regulated in HF. ALOX15B has lipid accumulation and inflammation activity and is highly expressed in atherosclerosis [45]. Studies have shown that expression of MYH6 was associated with hypertrophic cardiomyopathy [46].
### Table 3  
The enriched pathway terms of the up and down regulated differentially expressed genes

| Pathway ID | Pathway name                                                                 | P-value | FDR B&H | FDR B&H | Bonferroni | Gene count | Gene                                                                                           |
|------------|--------------------------------------------------------------------------------|---------|---------|---------|------------|------------|------------------------------------------------------------------------------------------------|
| 1270244    | Extracellular matrix organization                                              | 3.33E−08| 1.80E−05| 1.23E−04| 1.80E−05   | 24         | COL22A1, MFA4, CMA1, COL14A1, COL1A1, COL8A2, COL9A1, COL9A2, COL10A1, FMO2,                        |
|            |                                                                                 |         |         |         |            |            | COMP, HAPLN1, ADAMTS14, CTSG, ASPN, ITGAL, ITGB7, CAPN6, TGFβ2, P3H2, TLL2, LTBP2, LUM           |
| 1269201    | Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell        | 8.13E−06| 7.31E−04| 5.03E−03| 4.39E−03   | 13         | SH2D1B, SLAMF7, SIGLEC8, ITGAL, ITGB7, KLRB1, CD1C, CD3D, CD3E, CD3G, CD247, CD40LG, SH2D1A       |
| 1269544    | GPCR ligand binding                                                             | 3.92E−04| 1.51E−02| 1.04E−01| 2.12E−01   | 22         | GN9B, PENK, F2RL2, AGTR2, APLNR, CXCL9, CCR7, CXCL11, OXER1, HTR2A, HTR2B, WNT10B, WNT9A,        |
|            |                                                                                 |         |         |         |            |            | ACKR4, CRHBP, S1PR5, FZD2, CX3CR1, CXCL10, MCHR1, LHCGR, GPR68                                 |
| 1268749    | Metabolism of Angiotensinogen to Angiotensins                                   | 5.26E−04| 1.85E−02| 1.27E−01| 2.84E−01   | 4          | CMA1, CTSG, ACE, GZMH                                                                            |
| 1269868    | Muscle contraction                                                              | 3.44E−02| 4.03E−01| 1.00E+00| 1.00E+00   | 9          | KCNIP1, RYR3, SCN2B, ATP1A4, ATP1B2, MYH1, KCNKT7, NPPA, TNNT1                                 |
| 1269340    | Hemostasis                                                                      | 6.57E−02| 5.21E−01| 1.00E+00| 1.00E+00   | 20         | GN9B, CEACAM3, F2RL2, SERPINE2, APOA1, GNA14, PDE5A, ATPIB1, IL2RB, CTSW, JSLR, ITGAL, LCK,    |
|            |                                                                                 |         |         |         |            |            | TGFβ2, LEFTY2, RASGRP1, CD2, P2RX6, CD48, HBB                                                   |
| 1269171    | Adaptive Immune System                                                          | 1.32E−01| 6.87E−01| 1.00E+00| 1.00E+00   | 23         | NRG1, HLA-DQA1, ZAP70, SH2D1B, CARD11, SLAMF7, SIGLEC8, ITGAL, ITGB7, ASB18, IER3, MRC2,        |
|            |                                                                                 |         |         |         |            |            | KLRB1, LCK, RASGRP1, CD1C, CD3D, CD3E, CD3G, CD247, FBX16, CD40LG, SH2D1A                     |
| 1457780    | Neutrophil degranulation                                                        | 4.82E−06| 3.14E−03| 2.22E−02| 3.14E−03   | 28         | SERPINA3, HK3, ACP3, HMOX2, CH4LI, S100A8, S100A9, FCER1G, MGST1, HP, FGR, ALOX5, ANPEP,        |
|            |                                                                                 |         |         |         |            |            | FPR1, CR1, ARG1, SLC11A1, SIGLEC9, GPR84, HPSE, PTX3, CRISPLD2, CXCL17, SIGLEC14,                |
|            |                                                                                 |         |         |         |            |            | RNASE2, TLR2, ELANE, MCEMP1                                                                     |
| 1269907    | SLC-mediated transmembrane transport                                           | 6.91E−04| 4.74E−02| 3.35E−01| 4.51E−01   | 16         | HK3, SLC7A11, SLC4A7, SLC1A1, SLC2A1, SLC5A1, SLC11A1, SLC22A16, SLC32A1, SLC4A4,                |
|            |                                                                                 |         |         |         |            |            | GCKR, LCN15, SLC9A7, SLC25A18, SLC38A4, RHAG                                                   |
| 1269545    | Class A/V (Rhodopsin-like receptors)                                            | 8.72E−04| 4.74E−02| 3.35E−01| 5.69E−01   | 17         | OPN4, SAA1, AGTR1, CCR1, CRN1, P2RY12, FPR1, SSTR2, SSTR5, KNG1, GPR4, GPR183, RGR, S1PR3, EDN1, |
|            |                                                                                 |         |         |         |            |            | EDN2, EDNRB                                                                                     |
| 1269340    | Hemostasis                                                                      | 2.18E−03| 7.11E−02| 5.02E−01| 1.00E+00   | 26         | SERPINA3, CD109, SLC7A11, F5, F8, F13A1, SERPINB8, FGER1G, FGR, MERTK, SERPINF2, SELE, P2RY12, |
|            |                                                                                 |         |         |         |            |            | APOB, KIF188, ATP2A2, NH-1RC2, KNG1, PDE11A, CD177, DOCK9, GRB7, GRB14, THBS1, SERPINE1,        |
|            |                                                                                 |         |         |         |            |            | SERPIN5                                                                                         |
In functional enrichment analysis, some genes involved with regulation of cardiovascular system processes were enriched in HF. Liu et al. [47], Kosugi et al. [48], McMacken et al. [49], Pan and Zhang [50], Li et al. [51] and Jiang et al. [52] presented that expression of HLA-DQA1, KDM5D, UCHL1, SAA1, ARG1 and LYVE1 were associated with progression of cardiomyopathy. Hou et al. [53] and Olesen et al. [54] demonstrated that DACT2 and KCND3 were found to be substantially related to atrial fibrillation. Ge and Concannon [55], Ferjeni et al. [56], Anquetil et al. [57], Glawe et al. [58], Kawabata et al. [59], Li et al. [60], Buraczynska et al. [61], Amini et al. [62], Yang et al. [63], Du Töit et al. [64], Hirose et al. [65], Zhang et al. [66], Griffin et al. [67], Zouidi et al. [68], Trombetta et al. [69], Alharbi et al. [70], Ikarashi et al. [71], Dharmadhikari et al. [72], Sutton et al. [73] and Deng et al. [74] reported that UBASH3A, ZAP70, IDO1, ITGAL (integrin subunit alpha L), ITGB7, RASGRP1,
Table 4  Topology table for up and down regulated genes

| Regulation | Node   | Degree | Betweenness | Stress   | Closeness |
|------------|--------|--------|-------------|----------|-----------|
| Up         | ESR1   | 1094   | 0.250896    | 7.4E-08  | 0.392769  |
| Up         | PYHIN1 | 342    | 0.054258    | 1.1E+08  | 0.339882  |
| Up         | PPP2R2B | 199   | 0.023115    | 35,268,762 | 0.346839 |
| Up         | LCK    | 162    | 0.033618    | 18,589,354 | 0.353915 |
| Up         | TP63   | 142    | 0.018577    | 39,426,608 | 0.319664 |
| Up         | CD247  | 129    | 0.013832    | 18,625,274 | 0.317784 |
| Up         | PTN    | 105    | 0.016638    | 36,892,166 | 0.300662 |
| Up         | APLNR  | 103    | 0.016611    | 40,715,208 | 0.288093 |
| Up         | APOA1  | 100    | 0.018834    | 13,935,332 | 0.315866 |
| Up         | CENPA  | 98     | 0.009846    | 31,131,318 | 0.301786 |
| Up         | SKAP1  | 97     | 0.01599     | 10,109,482 | 0.313338 |
| Up         | FSCN1  | 88     | 0.010226    | 9,387,016  | 0.335367  |
| Up         | SCN2B  | 86     | 0.01089     | 20,233,242 | 0.284756  |
| Up         | TMEM30B| 79     | 0.016411    | 10,490,230 | 0.270807  |
| Up         | FOXS1  | 79     | 0.009408    | 26,398,078 | 0.323427  |
| Up         | COL1A1 | 76     | 0.010472    | 9,313,318  | 0.315866  |
| Up         | ZAP70  | 75     | 0.007096    | 9,313,318  | 0.315866  |
| Up         | UCHL1  | 74     | 0.007953    | 9,313,318  | 0.315866  |
| Up         | HBB    | 72     | 0.009984    | 9,313,318  | 0.315866  |
| Up         | NRG1   | 70     | 0.01151     | 9,313,318  | 0.315866  |
| Up         | LEF1   | 61     | 0.007998    | 9,313,318  | 0.315866  |
| Up         | NTSE   | 60     | 0.009599    | 9,313,318  | 0.315866  |
| Up         | MDK    | 59     | 0.006889    | 9,313,318  | 0.315866  |
| Up         | ISLR   | 58     | 0.010139    | 9,313,318  | 0.315866  |
| Up         | FATE1  | 57     | 0.011609    | 9,313,318  | 0.315866  |
| Up         | LRRC15 | 56     | 0.010069    | 9,313,318  | 0.315866  |
| Up         | MATN2  | 54     | 0.004491    | 9,313,318  | 0.315866  |
| Up         | LIPH   | 54     | 0.008197    | 9,313,318  | 0.315866  |
| Up         | MYOC   | 49     | 0.005138    | 9,313,318  | 0.315866  |
| Up         | SCARA3 | 49     | 0.006492    | 9,313,318  | 0.315866  |
| Up         | NPPA   | 46     | 0.009502    | 9,313,318  | 0.315866  |
| Up         | CD83   | 43     | 0.005149    | 9,313,318  | 0.315866  |
| Up         | COL14A1| 41     | 0.006129    | 9,313,318  | 0.315866  |
| Up         | CTS5   | 40     | 0.003821    | 9,313,318  | 0.315866  |
| Up         | SFRP4  | 40     | 0.004006    | 9,313,318  | 0.315866  |
| Up         | TRAF3IP3| 38   | 0.006447    | 9,313,318  | 0.315866  |
| Up         | CLEC11A| 38     | 0.005085    | 9,313,318  | 0.315866  |
| Up         | ATP1B4 | 38     | 0.005722    | 9,313,318  | 0.315866  |
| Up         | CD3E   | 37     | 0.003892    | 9,313,318  | 0.315866  |
| Up         | SH2D1A | 37     | 0.003969    | 9,313,318  | 0.315866  |
| Up         | DDX3Y  | 37     | 0.003754    | 9,313,318  | 0.315866  |
| Up         | PRPH   | 37     | 0.003871    | 9,313,318  | 0.315866  |
| Up         | BIRC7  | 35     | 0.004385    | 9,313,318  | 0.315866  |
| Up         | CARD11 | 35     | 0.002249    | 9,313,318  | 0.315866  |
| Up         | RXRG   | 35     | 0.002394    | 9,313,318  | 0.315866  |
| Up         | CCL22  | 34     | 0.005706    | 9,313,318  | 0.315866  |
| Up         | CD27   | 33     | 0.003915    | 9,313,318  | 0.315866  |
| Up         | GZMB   | 32     | 0.003277    | 9,313,318  | 0.315866  |
| Up         | THY1   | 32     | 0.003561    | 9,313,318  | 0.315866  |
Table 4 (continued)

| Regulation | Node   | Degree | Betweenness | Stress | Closeness |
|------------|--------|--------|-------------|--------|-----------|
| Up         | CHRNA3 | 32     | 0.004247    | 3,415,720 | 0.236631  |
| Up         | LSP1   | 32     | 0.003371    | 5,734,010 | 0.281836  |
| Up         | IL2RB  | 31     | 0.002351    | 1,938,172 | 0.305808  |
| Up         | HTR2B  | 30     | 0.004018    | 2,165,998 | 0.27434   |
| Up         | DLGAP1 | 29     | 0.003816    | 6,072,882 | 0.277754  |
| Up         | TRIM17 | 29     | 0.002943    | 5,543,810 | 0.275137  |
| Up         | CTNNA2 | 29     | 0.003554    | 3,511,510 | 0.30254   |
| Up         | SERPINE2 | 28 | 0.002577   | 3,031,536 | 0.27426   |
| Up         | CD1E   | 28     | 0.003282    | 3,419,392 | 0.255229  |
| Up         | MRC2   | 28     | 0.003395    | 2,950,548 | 0.296276  |
| Up         | C1QTNF2| 28     | 0.003203    | 2,505,388 | 0.270147  |
| Up         | SH2D1B | 27     | 0.001864    | 1,043,570 | 0.29028   |
| Up         | BRINP1 | 27     | 0.001516    | 4,402,948 | 0.27726   |
| Up         | Pdia2  | 27     | 0.001967    | 2,895,182 | 0.286453  |
| Up         | CHD5   | 27     | 0.001918    | 5,223,636 | 0.286503  |
| Up         | FAP    | 27     | 0.003531    | 5,820,086 | 0.268583  |
| Up         | IL31RA | 26     | 0.002073    | 1,606,954 | 0.263603  |
| Up         | GAP43  | 25     | 0.002745    | 2,793,268 | 0.279858  |
| Up         | CD5    | 25     | 0.001695    | 655,904   | 0.295861  |
| Up         | UBAH3B | 25     | 0.001652    | 1,347,264 | 0.290951  |
| Up         | ROBO2  | 25     | 0.002959    | 3,726,616 | 0.267048  |
| Up         | ITGB7  | 24     | 0.002686    | 3,351,660 | 0.276124  |
| Up         | HTR2A  | 24     | 0.002571    | 2,472,010 | 0.275833  |
| Up         | MOXD1  | 24     | 0.002391    | 2,492,756 | 0.259926  |
| Up         | ASB18  | 24     | 9.77E−04    | 3,202,162 | 0.273001  |
| Up         | CD2    | 23     | 0.002221    | 926,408   | 0.287954  |
| Up         | BCL11B | 23     | 8.18E−04    | 1,888,298 | 0.28836   |
| Up         | STAT4  | 23     | 0.001786    | 2,435,506 | 0.277166  |
| Up         | NGEF   | 23     | 0.001809    | 1,548,206 | 0.277636  |
| Up         | SMPD3  | 23     | 0.002518    | 2,175,774 | 0.281     |
| Up         | FZD2   | 22     | 0.003673    | 3,239,390 | 0.250335  |
| Up         | DJUSP1S| 22     | 0.001253    | 2,474,532 | 0.284472  |
| Up         | CD3D   | 21     | 0.001725    | 1,111,132 | 0.288589  |
| Up         | SYT17  | 21     | 0.002549    | 2,482,574 | 0.285802  |
| Up         | FCGRB3 | 21     | 0.002748    | 1,492,022 | 0.282286  |
| Up         | EGR2   | 21     | 0.002934    | 3,438,856 | 0.266406  |
| Up         | ZBP1   | 21     | 0.001876    | 2,664,938 | 0.26006   |
| Up         | CAMK4  | 21     | 0.001773    | 3,472,134 | 0.272716  |
| Up         | DMC1   | 20     | 0.002511    | 4,659,098 | 0.254277  |
| Up         | GDNF   | 20     | 0.002515    | 3,274,958 | 0.244751  |
| Up         | FCN1   | 20     | 0.002571    | 1,243,380 | 0.236742  |
| Up         | LUM    | 20     | 0.002276    | 1,515,870 | 0.283903  |
| Up         | GZMA   | 20     | 0.001051    | 3,258,230 | 0.276498  |
| Up         | TGFβ2  | 20     | 0.002259    | 1,632,566 | 0.277119  |
| Up         | SLAMF7 | 20     | 0.00211     | 1,035,482 | 0.271708  |
| Up         | MS4A1  | 20     | 0.002857    | 1,169,078 | 0.288398  |
| Up         | ETV4   | 20     | 0.001747    | 1,674,080 | 0.301883  |
| Up         | GLI2   | 20     | 0.001398    | 2,977,194 | 0.285902  |
| Up         | PHLD4A | 19     | 4.37E−04    | 818,256   | 0.298194  |
Table 4 (continued)

| Regulation | Node       | Degree | Betweenness | Stress     | Closeness |
|------------|------------|--------|-------------|------------|-----------|
| Up         | COL8A2     | 19     | 0.00147     | 1,089,762  | 0.273835  |
| Up         | GABRD      | 19     | 0.002826    | 2,629,544  | 0.25748   |
| Up         | LMF1       | 19     | 0.004342    | 2,024,228  | 0.265132  |
| Up         | F2RL2      | 19     | 0.001554    | 790,338    | 0.282933  |
| Up         | LYPD1      | 19     | 0.003123    | 3,995,458  | 0.266276  |
| Up         | CAPN6      | 19     | 0.001415    | 3,046,736  | 0.267802  |
| Up         | SOX8       | 19     | 0.003361    | 2,763,024  | 0.251306  |
| Up         | IER3       | 18     | 0.001921    | 3,613,164  | 0.282982  |
| Up         | BEX1       | 18     | 0.001034    | 1,286,206  | 0.273606  |
| Up         | COLQ       | 18     | 0.001173    | 1,414,826  | 0.261234  |
| Up         | NTM        | 18     | 0.00284     | 2,486,684  | 0.275102  |
| Up         | RPS4Y1     | 18     | 0.001013    | 1,200,768  | 0.287713  |
| Up         | FERMT1     | 18     | 0.001713    | 4,279,868  | 0.270315  |
| Up         | RGS17      | 18     | 0.002928    | 3,868,106  | 0.249895  |
| Up         | TNNI1      | 17     | 0.001349    | 1,550,004  | 0.266765  |
| Up         | MYOZ1      | 17     | 0.00128     | 2,111,156  | 0.283203  |
| Up         | KLHDC8A    | 17     | 0.001147    | 7,007,508  | 0.251036  |
| Up         | MYL1       | 17     | 7.90E-04    | 1,213,666  | 0.289945  |
| Up         | DIO2       | 16     | 0.001161    | 1,959,228  | 0.279416  |
| Up         | ITGAL      | 16     | 0.001182    | 1,521,116  | 0.271527  |
| Up         | CRABP2     | 16     | 4.13E-04    | 675,182    | 0.272171  |
| Up         | HSH2D      | 16     | 0.001425    | 889,856    | 0.26534  |
| Up         | CD48       | 3      | 0           | 0          | 0.265422  |
| Up         | CD3G       | 2      | 0           | 0          | 0.23833   |
| Up         | LY9        | 2      | 0           | 0          | 0.240141  |
| Up         | SIT1       | 2      | 0           | 0          | 0.264221  |
| Up         | ATP1A4     | 2      | 1.16E-04    | 79,140     | 0.235049  |
| Up         | FMOD       | 2      | 3.96E-05    | 20,526     | 0.240707  |
| Up         | CCDC80     | 2      | 3.58E-05    | 499,704    | 0.288908  |
| Up         | CCR7       | 2      | 0           | 0          | 0.244312  |
| Up         | KCNIP1     | 1      | 0           | 0          | 0.219995  |
| Up         | CD6        | 1      | 0           | 0          | 0.22832   |
| Up         | FCRL3      | 1      | 0           | 0          | 0.241062  |
| Up         | SERTAD4    | 1      | 0           | 0          | 0.257531  |
| Up         | PRF1       | 1      | 0           | 0          | 0.222162  |
| Up         | C1QTNF9    | 1      | 0           | 0          | 0.226548  |
| Up         | OPCML      | 1      | 0           | 0          | 0.215756  |
| Up         | ESM1       | 1      | 0           | 0          | 0.213551  |
| Up         | CD40LG     | 1      | 0           | 0          | 0.240053  |
| Up         | S1PR5      | 1      | 0           | 0          | 0.24224   |
| Up         | AGTR2      | 1      | 0           | 0          | 0.259256  |
| Up         | NPPB       | 1      | 0           | 0          | 0.211726  |
| Up         | SCG5       | 1      | 0           | 0          | 0.238721  |
| Up         | PDE5A      | 1      | 0           | 0          | 0.243548  |
| Up         | RYR3       | 1      | 0           | 0          | 0.274755  |
| Up         | RASEF      | 1      | 0           | 0          | 0.274755  |
| Up         | PODXL2     | 1      | 0           | 0          | 0.213106  |
| Up         | OGN        | 1      | 0           | 0          | 0.226548  |
| Up         | FLCH2      | 1      | 0           | 0          | 0.238721  |
| Regulation | Node      | Degree | Betweenness | Stress  | Closeness |
|------------|-----------|--------|-------------|---------|-----------|
| Up         | SCG2      | 1      | 0           | 0.267704| 0.326713  |
| Up         | P3H2      | 1      | 0           | 0.207132| 0.320713  |
| Up         | C12orf75  | 1      | 0           | 0.217608| 0.3217608 |
| Up         | ACE       | 1      | 0           | 0.241159| 0.3241159 |
| Up         | GNA14     | 1      | 0           | 0.217608| 0.3217608 |
| Up         | HDC       | 1      | 0           | 0.216614| 0.3216614 |
| Up         | CMA1      | 1      | 0           | 0.226713| 0.3226713 |
| Up         | CEACAM3   | 1      | 0           | 0.265519| 0.3265519 |
| Down       | PCLAF     | 817    | 0.135529    | 4.95E+08| 0.365547  |
| Down       | CFTR      | 800    | 0.168404    | 4.5E+08  | 0.378823  |
| Down       | TK1       | 188    | 0.034997    | 43,663,230 | 0.331089 |
| Down       | ECT2      | 164    | 0.020509    | 39,431,940 | 0.325989 |
| Down       | FKBP5     | 157    | 0.028064    | 15,963,868 | 0.346288 |
| Down       | ANLN      | 153    | 0.021564    | 38,168,832 | 0.325066 |
| Down       | ATP2A2    | 148    | 0.027131    | 19,656,040 | 0.363859 |
| Down       | BCL6      | 142    | 0.022279    | 29,459,916 | 0.314181 |
| Down       | TOP2A     | 132    | 0.018571    | 16,838,266 | 0.361426 |
| Down       | ZBTB16    | 132    | 0.025165    | 14,500,206 | 0.349976 |
| Down       | S100A9    | 124    | 0.01355     | 11,186,464 | 0.352219 |
| Down       | CEP55     | 123    | 0.019583    | 21,505,878 | 0.316891 |
| Down       | BLM       | 108    | 0.014259    | 18,458,556 | 0.321945 |
| Down       | AGTR1     | 100    | 0.019518    | 14,083,216 | 0.313564 |
| Down       | SAMHD1    | 94     | 0.011463    | 12,340,270 | 0.337357 |
| Down       | S100A8    | 88     | 0.011637    | 8,662,548  | 0.361486 |
| Down       | GRAP2     | 86     | 0.011721    | 16,819,438 | 0.305936 |
| Down       | CBS       | 83     | 0.011248    | 20,466,334 | 0.301591 |
| Down       | SOCS3     | 83     | 0.011071    | 9,067,820  | 0.324888 |
| Down       | GF11B     | 80     | 0.011791    | 21,469,034 | 0.299012 |
| Down       | APOB      | 78     | 0.014102    | 9,290,092  | 0.319133 |
| Down       | PKC1      | 77     | 0.004102    | 12,732,476 | 0.305408 |
| Down       | FMN3      | 76     | 0.008497    | 19,265,788 | 0.304512 |
| Down       | HMOX2     | 75     | 0.011098    | 15,258,770 | 0.312053 |
| Down       | PCNT      | 74     | 0.011297    | 9,190,430  | 0.312261 |
| Down       | PIK3C2A   | 69     | 0.005568    | 8,787,122  | 0.313053 |
| Down       | KIF14     | 69     | 0.01035     | 12,506,564 | 0.304668 |
| Down       | WASF1     | 67     | 0.009478    | 18,219,554 | 0.29633  |
| Down       | ARNT1     | 65     | 0.00974     | 19,494,854 | 0.295526 |
| Down       | ALOX5     | 65     | 0.010921    | 7,343,824  | 0.306424 |
| Down       | MCM10     | 64     | 0.006773    | 8,807,396  | 0.306438 |
| Down       | THBS1     | 64     | 0.008915    | 6,203,038  | 0.312694 |
| Down       | VSG14     | 64     | 0.01033     | 10,534,518 | 0.302037 |
| Down       | WWC1      | 64     | 0.007241    | 14,604,594 | 0.301647 |
| Down       | MELK      | 63     | 0.008554    | 18,629,232 | 0.283953 |
| Down       | P2RY12    | 63     | 0.008962    | 9,063,088  | 0.286641 |
| Down       | PPL       | 62     | 0.007851    | 16,137,388 | 0.297313 |
| Down       | MYBL2     | 59     | 0.006189    | 16,766,208 | 0.291964 |
| Down       | FAM107A   | 59     | 0.006172    | 12,510,302 | 0.289688 |
| Down       | GRIP1     | 58     | 0.008069    | 3,384,760  | 0.320055 |
| Down       | ELF3      | 56     | 0.004945    | 7,205,142  | 0.309118 |
Table 4 (continued)

| Regulation | Node  | Degree | Betweenness | Stress  | Closeness |
|------------|-------|--------|-------------|---------|-----------|
| Down       | PALLD | 55     | 0.00509     | 15,467,012 | 0.293274  |
| Down       | CTH   | 54     | 0.007754    | 5,179,060  | 0.296908  |
| Down       | EIF4EBP1 | 53   | 0.005367    | 9,748,852  | 0.303946  |
| Down       | KNG1  | 53     | 0.007017    | 4,204,766  | 0.304243  |
| Down       | GLUL  | 51     | 0.00728     | 10,616,752 | 0.30116   |
| Down       | SLC2A1| 51     | 0.004557    | 8,307,646  | 0.303974  |
| Down       | HP    | 51     | 0.006741    | 3,398,298  | 0.314741  |
| Down       | RPGR  | 50     | 0.004441    | 10,097,488 | 0.29384   |
| Down       | TLR2  | 50     | 0.00754     | 8,322,330  | 0.294595  |
| Down       | GRB7  | 49     | 0.004147    | 4,832,846  | 0.30113   |
| Down       | PPEF1 | 49     | 0.001983    | 3,327,232  | 0.298276  |
| Down       | TXNRD1| 49     | 0.00627     | 2,860,122  | 0.328561  |
| Down       | NAMPT | 48     | 0.005035    | 10,420,710 | 0.290538  |
| Down       | BMP7  | 47     | 0.007622    | 4,442,306  | 0.286981  |
| Down       | CA14  | 47     | 0.005218    | 4,884,858  | 0.279189  |
| Down       | CCR1  | 46     | 0.008305    | 11,217,842 | 0.27812   |
| Down       | CDC45 | 45     | 0.004479    | 3,231,162  | 0.30618   |
| Down       | ARG1  | 45     | 0.004931    | 2,347,856  | 0.32381   |
| Down       | SPC24 | 43     | 0.005356    | 6,589,710  | 0.294834  |
| Down       | FGR   | 43     | 0.003434    | 3,020,950  | 0.303452  |
| Down       | KIF5C | 42     | 0.004876    | 2,365,086  | 0.319586  |
| Down       | IL1R2 | 42     | 0.006825    | 9,489,620  | 0.289265  |
| Down       | SERPINA3 | 42  | 0.005518    | 4,047,110  | 0.293168  |
| Down       | DEPD1B| 42     | 0.002978    | 9,632,346  | 0.260838  |
| Down       | SLC4A7| 41     | 0.006314    | 2,752,106  | 0.316232  |
| Down       | SERPINA5| 41   | 0.003604    | 13,750,404 | 0.273206  |
| Down       | MMP3  | 40     | 0.008262    | 9,328,402  | 0.297003  |
| Down       | NCEH1 | 40     | 0.009405    | 3,554,728  | 0.304214  |
| Down       | SLC1A1| 38     | 0.008678    | 2,278,154  | 0.320573  |
| Down       | CLSPN | 38     | 0.003668    | 3,801,300  | 0.294343  |
| Down       | BCAT1 | 38     | 0.0052      | 9,066,238  | 0.269657  |
| Down       | MYH6  | 38     | 0.005049    | 1,731,786  | 0.308578  |
| Down       | IL20RA| 37     | 0.005252    | 8,769,348  | 0.267901  |
| Down       | HOOK1 | 37     | 0.005558    | 7,195,380  | 0.279177  |
| Down       | FLT3  | 37     | 0.002948    | 1,938,440  | 0.292408  |
| Down       | ADAMTS4| 37    | 0.005524    | 2,338,476  | 0.307519  |
| Down       | CAMSAP3| 36    | 0.003339    | 4,795,892  | 0.29578   |
| Down       | PLA2G2A| 35    | 0.003637    | 1,686,594  | 0.300565  |
| Down       | FOSL1 | 34     | 0.004151    | 10,955,318 | 0.269402  |
| Down       | NQO1  | 34     | 0.001945    | 5,351,260  | 0.289201  |
| Down       | ELANE | 34     | 0.005024    | 2,289,646  | 0.302834  |
| Down       | KCNQ3 | 34     | 0.002555    | 9,153,178  | 0.28203   |
| Down       | EPN3  | 34     | 0.005073    | 7,423,282  | 0.280302  |
| Down       | GPR183| 34     | 0.003642    | 4,243,792  | 0.256783  |
| Down       | CD109 | 34     | 0.006381    | 3,655,940  | 0.303565  |
| Down       | TUBA3E| 34     | 0.003459    | 6,711,886  | 0.289048  |
| Down       | TGFBR3| 33     | 0.005143    | 1,983,082  | 0.267386  |
| Down       | NID1  | 33     | 0.004536    | 1,702,236  | 0.311503  |
| Down       | STEAP3| 33     | 0.004665    | 2,788,716  | 0.285365  |
Table 4 (continued)

| Regulation | Node     | Degree | Betweenness | Stress  | Closeness |
|------------|----------|--------|-------------|---------|-----------|
| Down       | AMD1     | 32     | 0.005714    | 3,154,782 | 0.29099   |
| Down       | EDN1B    | 31     | 0.003092    | 7,273,156  | 0.265368  |
| Down       | IL17RB   | 31     | 0.004227    | 6,381,040  | 0.261527  |
| Down       | SLC19A2  | 30     | 0.004653    | 2,505,974  | 0.281302  |
| Down       | SLC22A16 | 30     | 0.004545    | 3,831,872  | 0.240618  |
| Down       | PHACTR3  | 29     | 0.002193    | 6,417,862  | 0.280976  |
| Down       | LAPTM5   | 29     | 0.003298    | 2,735,158  | 0.274317  |
| Down       | ANGPTL4  | 29     | 0.003467    | 1,447,446  | 0.325163  |
| Down       | PPM1E    | 29     | 0.002894    | 5,733,032  | 0.270427  |
| Down       | E2F2     | 28     | 0.002816    | 5,508,320  | 0.28041   |
| Down       | SERPINE1 | 28     | 0.001474    | 2,497,574  | 0.271302  |
| Down       | ACP        | 28     | 0.003084    | 2,749,550  | 0.291223  |
| Down       | KRT7      | 28     | 0.002861    | 1,288,592  | 0.315774  |
| Down       | SERPINB8 | 28     | 0.002944    | 3,167,812  | 0.28186   |
| Down       | FREM2     | 28     | 0.003954    | 3,395,758  | 0.276661  |
| Down       | RNF157    | 28     | 0.002172    | 6,196,626  | 0.265551  |
| Down       | PPIPSK2   | 28     | 0.003886    | 8,572,108  | 0.270014  |
| Down       | F8        | 27     | 0.002839    | 4,879,016  | 0.274836  |
| Down       | TUBAL3    | 27     | 0.002055    | 1,052,840  | 0.318915  |
| Down       | ELL2      | 26     | 0.003971    | 6,281,508  | 0.255859  |
| Down       | GRB14     | 25     | 0.002326    | 3,092,024  | 0.28378   |
| Down       | IRAK3     | 25     | 0.00257     | 6,090,170  | 0.265897  |
| Down       | MANEA     | 25     | 0.004508    | 5,075,608  | 0.263869  |
| Down       | CLEC7A    | 25     | 0.004246    | 4,293,212  | 0.277095  |
| Down       | KLF10     | 24     | 0.001607    | 3,013,994  | 0.281339  |
| Down       | GNMT      | 24     | 0.00165     | 3,015,768  | 0.269136  |
| Down       | ART3      | 24     | 0.002904    | 2,401,360  | 0.255748  |
| Down       | LRRCA8E   | 24     | 0.003739    | 4,188,308  | 0.288665  |
| Down       | SLA       | 23     | 0.001714    | 1,003,510  | 0.289329  |
| Down       | CLEC4G    | 23     | 0.002667    | 2,376,260  | 0.277495  |
| Down       | TUBB4A    | 25     | 1.28E−04    | 181,440    | 0.250652  |
| Down       | CD38      | 4      | 0.001607    | 3,013,994  | 0.281339  |
| Down       | FCGR3A    | 4      | 1.01E−04    | 73,756     | 0.268385  |
| Down       | F5        | 3      | 8.10E−07    | 708        | 0.248641  |
| Down       | EHF       | 2      | 7.11E−06    | 4180       | 0.254842  |
| Down       | KIAA1549  | 2      | 4.16E−04    | 193,604    | 0.261391  |
| Down       | S100A3    | 2      | 1.84E−05    | 45,822     | 0.254376  |
| Down       | ADH1B     | 2      | 3.40E−05    | 28,184     | 0.233546  |
| Down       | PAPSS2    | 2      | 1.05E−05    | 8726       | 0.251868  |
| Down       | PTX3      | 1      | 0.001607    | 3,013,994  | 0.281339  |
| Down       | IL15RA    | 1      | 0.00165     | 3,015,768  | 0.269136  |
| Down       | EDN1      | 1      | 0.002904    | 2,401,360  | 0.255748  |
| Down       | SERPINF2  | 1      | 0.003739    | 4,188,308  | 0.288665  |
| Down       | ZNF366    | 1      | 0.001714    | 1,003,510  | 0.289329  |
| Down       | ACR       | 1      | 0.002326    | 3,092,024  | 0.28378   |
| Down       | MATN3     | 1      | 0.00257     | 6,090,170  | 0.265897  |
| Down       | CNR1      | 1      | 0.002667    | 2,376,260  | 0.277495  |
| Down       | LBP       | 1      | 0.002667    | 2,376,260  | 0.277495  |
| Down       | ALOXSAP   | 1      | 0.002667    | 2,376,260  | 0.277495  |
CNR1, SLC2A1, SLC11A1, GPR84, SSTR5, KCNB1, GLUL (glutamate-ammonia ligase), BANK1, CACNA1E, LGR5, AQP3, SIGLEC7, SSTR2 and DNER (delta/notch like EGF repeat containing) could be an index for diabetes, but these genes might be responsible for progression of HF. Experiments show that expression of FAP (fibroblast activation protein alpha) [75], THBS4 [76], CD27 [77], LEF1 [78], CTHRC1 [79], ESR1 [80], CXCL9 [81], SERPINA3 [82], TRPC4 [83], F13A1 [84], PIK3C2A [85], KCNIP2 [86] and GPR4 [87] contributed to myocardial infarction. MFAP4 [88], ALOX15 [89], COL1A1 [90], APOA1 [91], PDE5A [92], CX3CR1 [93], THY1 [94], GREM1 [95], FMOD (fibromodulin) [96], NPPA (natriuretic peptide A) [97], LTBP2 [98], LUM (lumican) [99], IL34 [100], NRG1 [101], CXCL14 [102], CXCL10 [103], ACE (angiotensin I converting enzyme) [104], CFTR (cystic fibrosis transmembrane conductance regulator) [105], S100A8 [106], S100A9 [106], HP (haptoglobin) [107], AGTR1 [108], ATP2A2 [109], IL10 [110], EDN1 [111], TLR2 [112], MCEMP1 [113], TPO (thyroid oxidase) [114], CD163 [115], IL18R1 [116], KCNA7 [117] and CALCRL (calcitonin receptor like receptor) [118] have an important role in HF. Li et al. [119], Deckx et al. [120], Ichihara et al. [121] and Paik et al. [122] showed that the SERPINE2, OGN (osteoglycin), AGTR2 and WNT10B promote cardiac interstitial fibrosis. Cai et al. [123], Mo et al. [124], Sun et al. [125], Martinelli et al. [126], Zhao et al. [127], Assimes et al. [128] and Piechota et al. [129] showed that CCR7, FCN1, ESM1, F8 (coagulation factor VIII), C1QTNF1, ALOX5 and MSR1 were an important target gene for coronary artery disease. STAB2 have been suggested to be associated with venous thromboembolic disease [130]. Genes such as COMP (cartilage oligomeric matrix protein) [131], CH3L1 [132], PLA2G2A [133], P2RY12 [134], CR1 [135], HPSE (heparanase) [136], PTX3 [137] and SERPINE1 [138] were...
related to atherosclerosis. CCDC80 [139], CMA1 [140], MDK (midkine) [141], GNA14 [142], SCG2 [143], NPPB (natriuretic peptide B) [144], FGF10 [145], ARNTL (aryl hydrocarbon receptor nuclear translocator like) [146], WNK3 [147], EDNRB (endothelin receptor type B) [148], THBS1 [149], SELE (selectin E) [150], SLC4A7 [151], AQP4 [152] and KCNK3 [153] are thought to be responsible for progression of hypertension, but these genes

Fig. 4 Target gene—miRNA regulatory network between target genes. The light orange color diamond nodes represent the key miRNAs; up regulated genes are marked in green; down regulated genes are marked in red

Fig. 5 Target gene—TF regulatory network between target genes. The sky blue color triangle nodes represent the key TFs; up regulated genes are marked in green; down regulated genes are marked in red
might to be associated with progression of HF. CNT-NAP2 [154], GLI2 [155], DPT (dermatopontin) [156], AEBP1 [157], ITIH5 [158], CXCL11 [159], GDNF (glial cell derived neurotrophic factor) [160], MCHR1 [161], FLT3 [162], ELANE (elastase, neutrophil expressed) [163], OSMR (oncostatin M receptor) [164] and IL15RA [165] are involved in development of obesity, but these genes might be key for progression of HF. CTSG (cathepsin G) is a protein coding gene plays important roles in aortic aneurysms [166]. Evidence from Safa et al. [167], Chen et al. [168], Zhou et al. [169], Hu et al. [170], Lou et al. [171], Zhang et al. [172] and Chen et al. [173] study indicated that the expression of CCL22, CCR1, FPR1, KNG1, CRISPLD2, CD38 and GPRC5A were linked with progression of ischemic heart disease. Li et al. [174] showed that STEAP3 expression can be associated with cardiac hypertrophy progression.

The HiPPIE interactome database was used to construct the PPI network, and modules analysis was performed. We finally screened out up regulated hub genes and down regulated hub genes, including ESR1, PYHIN1, PPP2R2B, LCK, TP63, PCLAF, CD247, CD2, CD5, CD48, CFTR, TK1, ECT2, FKBP5, S100A9 and S100A8 from the PPI network and its modules. TP63 might serve as a potential prognostic factor in cardiomyopathy [175]. The expression of FKBP5 is related to the progression of coronary artery disease [176]. CD247 plays a central role in hypertension [177], but this gene might be involved in the HF. PYHIN1, PPP2R2B, LCK (LCK proto-oncogene, Src family tyrosine kinase), PCLAF (PCNA clamp associated factor), TK1, ECT2, CD2, CD5 and CD48 might be the novel biomarker for HF.

### Table 5  miRNA—target gene and TF—target gene interaction

| Regulation | Target Genes | Degree | MicroRNA | Regulation | Target Genes | Degree | TF |
|------------|--------------|--------|----------|------------|--------------|--------|----|
| Up         | FSCN1        | 99     | hsa-mir-4533 | Up         | FSCN1        | 62     | ESRRA |
| Up         | ESR1         | 72     | hsa-mir-548ac | Up         | APOA1        | 48     | RERE |
| Up         | TMEM30B      | 64     | hsa-mir-548i | Up         | COL1A1       | 21     | HMG20B |
| Up         | SCN2B        | 46     | hsa-mir-5585-3p | Up         | HBB          | 16     | THRAP3 |
| Up         | CENPA        | 35     | hsa-mir-6750-3p | Up         | LCK          | 15     | ATF1 |
| Up         | APOA1        | 22     | hsa-mir-6722-5p | Up         | FOX51        | 14     | YBX1 |
| Up         | PPP2R2B      | 14     | hsa-mir-149-3p | Up         | CENPA        | 10     | SAP30 |
| Up         | TP63         | 12     | hsa-mir-1178-3p | Up         | SCN2B        | 5      | RCOR2 |
| Up         | PYHIN1       | 5      | hsa-mir-205-3p | Up         | TMEM30B      | 5      | ZNF24 |
| Up         | APLNR        | 2      | hsa-mir-10b-5p | Up         | APOA1        | 4      | FOXJ2 |
| Up         | PTN          | 1      | hsa-mir-155-5p | Up         | NRG1         | 2      | SUZ12 |
| Up         | LCK          | 1      | hsa-mir-335-3p | Up         | PTN          | 2      | L3MBT2 |
| Up         | CD247        | 1      | hsa-mir-346 | Up         | UCH1         | 2      | MAZ |
| Down       | FKBPs        | 88     | hsa-mir-200c-3p | Up         | ESR1         | 1      | EZH2 |
| Down       | PCLAF        | 62     | hsa-mir-1273g-3p | Up         | ZAP70        | 1      | ZFX |
| Down       | CEPS5        | 57     | hsa-mir-1244 | Down       | SOCS3        | 48     | MXD3 |
| Down       | ATP2A2       | 55     | hsa-mir-4789-3p | Down       | BCL6         | 44     | ARID4B |
| Down       | TK1          | 45     | hsa-mir-766-3p | Down       | FKBPs        | 43     | CBFB |
| Down       | ZBTB16       | 43     | hsa-mir-1976 | Down       | ANLN         | 38     | TAF7 |
| Down       | SAMHD1       | 26     | hsa-mir-3124-3p | Down       | ATP2A2       | 35     | CREM |
| Down       | TOP2A        | 17     | hsa-mir-186-5p | Down       | CBS          | 31     | IKZF1 |
| Down       | BCL6         | 13     | hsa-mir-339-5p | Down       | BLM          | 19     | ZNF501 |
| Down       | ECT2         | 13     | hsa-mir-132-3p | Down       | ECT2         | 15     | KLF16 |
| Down       | CFTR         | 9      | hsa-mir-145-5p | Down       | CEPS5        | 10     | FOSL2 |
| Down       | S100A9       | 7      | hsa-mir-4679 | Down       | GRAP2        | 10     | CEBPD |
| Down       | AGTR1        | 5      | hsa-mir-410-3p | Down       | ZBTB16       | 4      | TRIM28 |
| Down       | ANLN         | 5      | hsa-mir-503-5p | Down       | S100A8       | 3      | STAT3 |
| Down       | BLM          | 3      | hsa-mir-193b-3p | Down       | S100A9       | 2      | CEBPG |
|            |              |        |           |            |              |        |     |

*Table 5* miRNA—target gene and TF—target gene interaction

*Regulation* indicates whether the gene is upregulated or downregulated. *Target Genes* are the genes that are regulated by the miRNA or TF. *Degree* represents the number of interactions for each gene. *MicroRNA* and *Target Genes* are listed for each regulation. *TF* indicates the transcription factor associated with the gene.
The miRNet database and NetworkAnalyst database were used to construct the target gene—miRNA regulatory network and target gene—TF regulatory network. We finally screened out target genes, miRNA, TFs, including FSCN1, ESR1, TMEM30B, SCN2B, CENPA, FKBP5, PCLAF, P2R2B, ATP2A2, TK1, APOA1, COL1A1, HBB, LCK, SOCS3, BCL6, ANLN, hsa-mir-4533, hsa-mir-548ac, hsa-mir-548i, hsa-mir-5585-3p, hsa-mir-6750-3p, hsa-mir-1273 g-3p, hsa-mir-1244, hsa-mir-4789-3p, hsa-mir-766-3p, ESRRA, RERE, HMG20B, THRAP3, ATF1, MXD3, ARID4B, CBFB, TAF7 and CREM from the target gene—miRNA regulatory network and target gene—TF regulatory network. SCN2B [178] and SOCS3 [179] are considered as markers for HF and might be a new therapeutic target. BCL6 levels are correlated with disease severity in patients with atherosclerosis [180]. A previous study showed that hsa-mir-1273 g-3p [181], hsa-mir-4789-3p
[182] and ATF1 [183] could be involved in hypertension, but these markers might be responsible for progression of HF. hsa-miR-518f, was demonstrated to be associated with cardiomyopathy [184]. An evidence demonstrating a role for ESRRA (estrogen related receptor alpha) [185] and THRAP3 [186] in diabetes, but these genes might be liable for development of HF. FSCN1, TMEM30B, CENPA (centromere protein A), CEP55, HBB (hemoglobin subunit beta), ANLN (anillin actin binding protein), hsa-mir-4533, hsa-mir-548ac, hsa-mir-5585-3p, hsa-mir-6750-3p, hsa-mir-200c-3p, hsa-mir-1244, RERE (arginine-glutamic acid dipeptide repeats), HMG20B, MXD3, ARID4B, CBFB (core-binding factor subunit beta), TAF7 and CREM (cAMP response element modulator) might be the novel biomarker for HF.

The molecules HIM6, HIM10 obtained good binding score of more 5 to 6.999 with all proteins and the molecules HIM11, HIM12, HIM14, HTZ9, HTZ10 and HTZ12 obtained binding score above 5 and less than 9 with PDB protein code of 2HV7, 3VD8 and 6RUR respectively. The molecule HIM11 obtained highest binding score of 8.678 with 2HV7 and its interaction with amino acids are molecule HIM11 (Fig. 9) has obtained with a high binding score with PDB protein 2HV7, the interactions of molecule is the C6 side chin acyl carbonyl C=O formed hydrogen bond interaction with amino acid GLN-207 with bond length 1.92 Å and 3’ N–H group of imidazole ring formed hydrogen bond interaction with VAL-305 with bond length 2.36 Å respectively. It also formed other interactions of carbon hydrogen bond of –CH₃ group of carboxylate at C6 with PRO-304 and amide-pi stacked and pi–pi stacked interaction of electrons of aromatic ring A with ALA-204 and ring C with HIS-155 and HIS-308. Molecule formed pi-alkyl interaction of ring B with PRO-304 and all interactions with amino acids and bond length are depicted by 3D and 2D figures (Fig. 10 and Fig. 11).
| Sl. No/ Code | Over expressed gene: LCK | TPR6 | Over expressed gene: ESR1 | Code | Total Score Crash (Polar) |
|-------------|-------------------------|------|--------------------------|------|--------------------------|
| PDB: 3VD8   |                         |      |                         |      |                          |
| 1           | −0.807                  | 0.004| 5.794                    | 0.004|                          |
|             | −1.450                  | 0.221|                          |      |                          |
| PDB: 6066   |                         |      |                         |      |                          |
| 1           | −0.514                  | 3.828| 5.770                    | 0.315|                          |
|             | −1.440                  | 2.231|                          |      |                          |
| PDB: 3V08   |                         |      |                         |      |                          |
| 1           | −1.465                  | 5.063| 5.414                    | 5.063|                          |
|             | −1.432                  | 5.227|                          |      |                          |

**Table 6** Docking results of Designed Molecules on Over Expressed Proteins

| Sl. No/ Code | Over expressed gene: ESR1 | Code | Total Score Crash (Polar) |
|-------------|--------------------------|------|--------------------------|
| PDB: K5QW   |                         |      |                          |
| 1           | −1.770                  |      |                          |
|             | −1.481                  |      |                          |
| PDB: 4PM7   |                         |      |                          |
| 1           | −2.814                  |      |                          |
|             | −1.408                  |      |                          |
| PDB: 3V18   |                         |      |                          |
| 1           | −0.870                  |      |                          |
|             | −1.415                  |      |                          |
| Sl. No/Code | Over expressed gene: ESR1 | Over expressed gene: LCK | Over expressed gene: PPP2R2B | Over expressed gene: PYHIN 1 | Over expressed gene: TP63 |
|------------|---------------------------|--------------------------|-------------------------------|-----------------------------|-------------------------|
|            | Sl. No/Code               | PDB: 4PXM                | PDB: 1KSW                     | PDB: 2HV7                   | PDB: 3VD8                | PDB: 6RU6                |
|            |                           | Total Score              | Crash (-Ve)                  | Polar                       | Total Score              | Crash (-Ve)                  | Polar                       | Total Score              | Crash (-Ve)                  | Polar                       | Total Score              | Crash (-Ve)                  | Polar                       |
| HTZ16      | 4.227                     | -4.787                   | 0.298                        |                             | 4.654                    | -1.534                   | 2.096                        | 6.143                    | -1.204                   | 2.879                        | 4.854                    | -0.994                   | 1.564                        | 7.102                    | -0.917                   | 3.001                        |
| HTZ17      | 3.784                     | -5.018                   | 0.380                        |                             | 3.661                    | -0.897                   | 1.676                        | 4.016                    | -1.179                   | 1.384                        | 4.039                    | -0.569                   | 2.706                        | 4.256                    | -1.040                   | 1.236                        |
| HTZ STD    | 4.722                     | -1.084                   | 1.063                        |                             | 3.319                    | -0.890                   | 3.033                        | 3.564                    | -0.272                   | 2.367                        | 3.394                    | -0.882                   | 1.169                        | 4.237                    | -0.801                   | 1.855                        |
Conclusions

The present investigation aimed at characterizing the expression profiling by high throughput sequencing of the HF patients. Our bioinformatics analyses revealed key gene signatures as candidate biomarkers in HF. Hub genes (ESR1, PYHIN1, PPP2R2B, LCK, TP63, PCLAF, CFTR, TK1, ECT2 and FKBP5) were diagnosed as an essential genetic factors in HF. In general, DEGs linked with HF genes, including already known markers of HF and other HF related diseases, and novel biomarkers, were diagnosed. Potential implicated miRNAs and TFs were also diagnosed. The diagnosed hub genes might
represent candidate diagnostic and prognostic biomarkers, and therapeutic targets. The current investigation reported novel genes and signaling pathways in HF, and further investigation is required.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12872-021-02146-8.

**Additional file 1: Table S1.** The statistical metrics for key differentially expressed genes (DEGs).

**Acknowledgements**

I thank Michael Patrick Morley, Perelman School of Medicine at the University of Pennsylvania, Penn Cardiovascular Institute, Philadelphia, USA, very much.

**Authors’ contributions**

VK—Methodology and validation. BY—Writing original draft, and review and editing. CV—Software and investigation. SK—Supervision and resources.

**Availability of data and materials**

The datasets supporting the conclusions of this article are available in the GEO repository. (https://www.ncbi.nlm.nih.gov/geo/) The datasets supporting the conclusions of this article are available in the GEO repository. (https://www.ncbi.nlm.nih.gov/geo/)

**Declarations**

**Ethical approval**

This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed consent**

No informed consent because this study does not contain human or animals participants.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

1. Whaen Heart Care & Super Specialty Centre, Vivekananda General Hospital, Deshpande Nagar, Hubli, Karnataka 580029, India. 2. Department of Biochemistry, Basaveshwar College of Pharmacy, Gadag, Karnataka 582103, India. 3. Biostatistics and Bioinformatics, Chananasa娃 Nilaya, Bharratjinaigail, Dharwad 580001, Karnataka, India. 4. Department of Pharmaceutical Chemistry, JSS College of Pharmacy, Mysuru and JSS Academy of Higher Education & Research, Mysuru, Karnataka 570015, India.

**Received:** 24 March 2021   **Accepted:** 14 June 2021

**Published online:** 04 July 2021

**References**

1. Xanthopoulos A, Tripodoskias F, Starling RC. Heart failure with preserved ejection fraction: classification based upon phenotype is essential for diagnosis and treatment. Trends Cardiovasc Med. 2018;28(6):392–400. https://doi.org/10.1016/j.tcm.2018.01.001.

2. Mosterd A, Hoes AW. Clinical epidemiology of heart failure. Heart. 2007;93(9):1137–46. https://doi.org/10.1136/hrt.2003.025270.

3. Roger VL, Weston SA, Redfield MM, Hellieman-Homan JP, Killian J, Yawn BP, Jacobsen SJ. Trends in heart failure incidence and survival in a community-based population. JAMA. 2004;292(3):344–50. https://doi.org/10.1001/jama.292.3.344.

4. Wang, C, Wang F, Cao Q, Li Z, Huang L, Chen S. The effect of MeCP2 on heart failure. Cell Physiol Biochem. 2018;47(6):2380–7. https://doi.org/10.1159/000491610.

5. Ma J, Lu L, Guo W, Ren J, Yang J. Emerging role for RBM20 and its splicing substrates in cardiac function and heart failure. Curr Pharm Des. 2016;22(31):4744–51. https://doi.org/10.2174/13816126167010145322.

6. Zhang, T, Brown. JH. Role of Ca2+/calmodulin-dependent protein kinase II in cardiac hypertrophy and heart failure. Cardiovasc Res. 2004;63(3):476–86. https://doi.org/10.1016/j.cardiores.2004.04.026.

7. Missose E, Calzolari C, Pau B. Circulating cardiac troponin I in severe congestive heart failure. Circulation. 1997;96(9):2953–8. https://doi.org/10.1161/01.cir.96.9.2953.

8. Kho C, Lee A, Jeong D, Oh JG, Chianine AH, Kizana E, Park WJ, Hajar RJ. SUMO1-dependent modification of SERCA2a in heart failure. Nature. 2011;477(7366):601–5. https://doi.org/10.1038/nature10407.

9. Roh JD, Hobson R, Chaudhari V, Quintero P, Yeri A, Benson M, Xiao C, Zlotoff D, Bezerides V, Houctis N, et al. Activin type II receptor signaling in cardiac aging and heart failure. Sci Transl Med. 2019;11(482):eaau8680. https://doi.org/10.1126/scitranslmed.aau8680.

10. Yu L, Feng Z. The role of toll-like receptor signaling in the progression of heart failure. Mediators Inflamm. 2018;2018:9874109. https://doi.org/10.1155/2018/9874109.

11. Zhang Y, Zhang L, Zhang Y, Fan X, Yang W, Yu B, Kou J, Li FY. QiFupMai powder injection attenuates coronary artery ligation-induced heart failure through improving mitochondrial function via regulating ROS generation and CaMII signaling pathways. Front Pharmacol. 2019;10:381. https://doi.org/10.3389/fphar.2019.00381.

12. Yang Y, Tian Y, Hu S, Bi S, Li S, Hu Y, Kou J, Jing J, Yu B. Extract of Sheng-Mai–San ameliorates myocardial ischemia-induced heart failure by modulating Ca2+/calmodulin-mediated Drp1 signaling pathways. Int J Mol Sci. 2017;18(9):1825. https://doi.org/10.3390/ijms18091825.

13. Booz GW, Day JD, Baker KM. Interplay between the cardiac renin-angiotensin system and JAK-STAT-signaling role in cardiac hypertrophy, ischemia/reperfusion dysfunction, and heart failure. J Mol Cell Cardiol. 2002;34(11):1443–53. https://doi.org/10.1016/S0022-5193(02)02076.

14. Li X, Li B, Jiang H. Identification of time-series differentially expressed genes and pathways associated with heart failure post-myocardial infarction using integrated bioinformatics analysis. Mol Med Rep. 2019;19(6):5281–90. https://doi.org/10.3892/mmr.2019.10190.

15. Liu Y, Morley M, Brandimarto J, Hennahalili S, Hu Y, Ashley EA, Tang WH, Moravec CS, Margulies KB, Cappola TP, et al. RNA-Seq identifies novel myocardial gene expression signatures of heart failure. Genomics. 2015;105(2):83–9. https://doi.org/10.1016/j.jgeno.2014.12.002.

16. Schiano C, Costa V, Aprile M, Grimaldi V, Maiello C, Esposito R, Sorcelli A, Colantuoni V, Donatelli F, Ciccidoccola A, et al. Heart failure: Pilot transcriptomic analysis of cardiac tissue by RNA-sequencing. Cardiol J. 2017;24(5):539–53. https://doi.org/10.5603/CJ.a2017.0052.

17. Rahman MR, Islam T, Zaman T, Shahjaman M, Karim MR, Huq F, Quinn JMW, Holsinger RMQ, Gov E, Moni MA. Identification of molecular signatures and pathways to identify novel therapeutic targets in Alzheimer’s disease: Insights from a systems biomedicine perspective. Genomics. 2020;112(2):1290–9. https://doi.org/10.1016/j.jgeno.2019.07.018.

18. Rahman MR, Islam T, Turanli B, Zaman T, Faruquee HM, Rahman MM, Mollah MNH, Nanda RK, Arga KY, Gov E, et al. Network-based approach to identify molecular signatures and therapeutic agents in Alzheimer’s disease. Comput Biol Chem. 2019;78:431–9. https://doi.org/10.1016/j.compbiolchem.2018.12.011.

19. Rahman MR, Petralia MC, Curieko R, Bramanti A, Fagone P, Shahjaman M, Wu L, Sun Y, Turanli B, Arga KY, et al. Comprehensive analysis of RNA-Seq gene expression profiling of brain transcriptomes reveals novel genes, regulators, and pathways in autism spectrum disorder. Brain Sci. 2020;10(10):747. https://doi.org/10.3390/brainsci10100747.
20. Clough E, Barrett T. The gene expression omnibus database. Methods Mol Biol. 2016;1418:89–110. https://doi.org/10.1007/978-1-4939-3578-9_5.

21. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. limma powers differential expression analyses for RNA-seq and microarray studies. Nucleic Acids Res. 2015;43(7):e47. https://doi.org/10.1093/nar/gkv007.

22. Thomas PD. The gene ontology and the meaning of biological function. Methods Mol Biol. 2017;1446:15–24. https://doi.org/10.1007/978-1-4939-3743-1_2.

23. Chen J, Bardees EE, Aronov BJ, Jegga AG. TopGene Suite for gene list enrichment analysis and candidate gene prioritization. Nucleic Acids Res. 2009;37(Web Server issue):W305–11. https://doi.org/10.1093/nk/gkp427.

24. Fabregat A, Jupe S, Matthews L, Sidiro poucois K, Gillespie M, Garapati P, Heva R, Jassal B, Kör neringer F, May B, et al. The reactome pathway knowledgebase. Nucleic Acids Res. 2018;46(D1):D649–55. https://doi.org/10.1093/nar/gkw1132.

25. Kong J, Li L, Zhimin L, Yan J, Ji D, Chen Y, Yuan yuan W, Chen X, Sha o H, Wang J, et al. Potential protein biomarkers for systemic lupus erythematosus determined by bioinformatics analysis. Comput Biol Chem. 2019;83:107155. https://doi.org/10.1016/j.compbiochem.2019.107135.

26. Alunis-Lobato G, Andrade-Navarro MA, Schaeffer MH. HIPPIE v2.0: enhancing meaningfulness and reliability of protein-protein interaction networks. Nucleic Acids Res. 2017;45(D1):D408–14. https://doi.org/10.1093/nar/gkw985.

27. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498–504. https://doi.org/10.1101/gr.1239303.

28. Przyul N, Wic gle DA, Jurisica I. Functional topology in a network of protein interactions. Bioinformatics. 2004;20(3):340–8. https://doi.org/10.1093/bioinformatics/btg415.

29. Nguyen TP, Liu WC, Jordán F. Inferring pleiotropy by network analysis: linked diseases in the human PPI network. BMC Syst Biol. 2011;5:179. https://doi.org/10.1186/1752-0509-5-179.

30. Shi Z, Zhang B. Fast network centrality analysis using GPUs. BMC Bioinformatics. 2017;18:325. https://doi.org/10.1186/s12859-017-1611-3.

31. Al-Fawwaz M, Benjouayem M, El-Chami M, Goubran H, Peltier A, Marik P. Network analysis enhances meaningfulness and reliability of protein-protein interaction enrichment. Nucleic Acids Res. 2018;46(D1):D649–55. https://doi.org/10.1093/nar/gkw427.

32. Zhou G, Soufan O, Ewald J, Hancock REW, Basu N, Xia J. NetworkAnalyzer 3.0: a visual analytics platform for comprehensive gene expression profiling and meta-analysis. Nucleic Acids Res. 2019;47:W234–41. https://doi.org/10.1093/nar/gkz474.

33. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, Müller M. pROC, an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 2011;12:77. https://doi.org/10.1186/1471-2105-12-77.

34. Fabregat A, Jupe S, Matthews L, Sidiropolous K, Gillespie M, Garapati P, Hav R, Jassal B, Körning F, May B, et al. The reactome pathway knowledgebase. Nucleic Acids Res. 2018;46(D1):D649–55. https://doi.org/10.1093/nar/gkx1132.

35. Fabregat A, Jupe S, Matthews L, Sidiropolous K, Gillespie M, Garapati P, Hav R, Jassal B, Körning F, May B, et al. The reactome pathway knowledgebase. Nucleic Acids Res. 2018;46(D1):D649–55. https://doi.org/10.1093/nar/gkw1132.

36. Shi Z, Zhang B. Fast network centrality analysis using GPUs. BMC Bioinformatics. 2017;18:325. https://doi.org/10.1186/s12859-017-1611-3.

37. Al-Fawwaz M, Benjouayem M, El-Chami M, Goubran H, Peltier A, Marik P. Network analysis enhances meaningfulness and reliability of protein-protein interaction enrichment. Nucleic Acids Res. 2018;46(D1):D649–55. https://doi.org/10.1093/nar/gkw427.

38. Zhou G, Soufan O, Ewald J, Hancock REW, Basu N, Xia J. NetworkAnalyzer 3.0: a visual analytics platform for comprehensive gene expression profiling and meta-analysis. Nucleic Acids Res. 2019;47:W234–41. https://doi.org/10.1093/nar/gkz474.

39. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, Müller M. pROC, an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 2011;12:77. https://doi.org/10.1186/1471-2105-12-77.
56. Ferjeni Z, Bouzid D, Fourati H, Stayoussif M, Abida O, Kammoun T, Hashichia M, Penha-Gonçalves C, Masmoudi H. Association of TCR/CD3, PTPN22, CD28 and ZAP70 gene polymorphisms with type 1 diabetes risk in Tunisian population: family based association study. Immunol Lett. 2015;163(1-2):7–12. https://doi.org/10.1016/j.imlet.2014.11.006.

57. Anquetil F, Mondanneli G, Gonzalez N, Rodriguez Calvo T, Zapardiel Gonzalez J, Krogvold L, Dahl-Jorgensen K, Van den Eynde B, Crabona C, Grohmann U, et al. Loss of IDO1 expression from human pancreatic β-cells precedes their destruction during the development of type 1 diabetes. Diabetes. 2018;67(9):1858–66. https://doi.org/10.2337/db17-1288.

58. Glawe JD, Patrick DR, Huang M, Sharp CD, Barlow SC, Kevil CG. Genetic deficiency of ITGAM or ITGAV prevents autoimmune diabetes through distinctly different mechanisms in NOD/LtJ mice. Diabetes. 2009;58(6):1292–301. https://doi.org/10.23736/S0012-1878.09.15495-2.

59. Kawabata Y, Nishida N, Awata T, Kawasaki E, Imagawa A, Shimada A, Li JY, Tao F, Wu XX, Tan YZ, He L, Lu H. Common RASGRP1 gene variants affect CD4+ T cell differentiation. Nat Immunol. 2015;16(7):780–8. https://doi.org/10.1038/ni.3384.

60. Buraczynska M, Wacinski P, Zukowski P, Dragan M, Ksiazek A. Common and rare polymorphisms in the cannabinoid type 1 receptor gene (CNR1) is associated with susceptibility to obesity in Pima Indians. Obesity (Silver Spring). 2016;24(12):2701–9. https://doi.org/10.1002/oby.21728.

61. Fan Y, Wang J, Zhang W, Li J, Yang Y, Zhao Y, Ouyang Z, Liang C, Wang J, Guo Q, et al. Loss of ITGB1 expression in autoimmune diabetic mouse model. J Mol Neurosci. 2018;64(3):385–96. https://doi.org/10.1007/s12031-018-0691-y.

62. Yang JH, Downes K, Howson JM, Nutland S, Stevens HE, Walker NM, Du Toit E, Browne L, Irving-Rodgers H, Massa HM, Fozzard N, Jennings MP, Peak IR. Effect of GPR84 deletion on obesity and diabetes development of type 2 diabetes mellitus. Bioorg Med Chem. 2016;24(11):3998–4005. https://doi.org/10.1016/j.bmc.2016.07.002.

63. Alharbi KK, Ali Khan I, Syed R, Alhajri FK, Mohammed AK, Vinodson B, Al-Daghri NM. Association of JAZF1 and TSPAN8/LGR5 variants in relation to type 2 diabetes mellitus in a Saudi population. Diabetes Metab Syndr. 2015;7:92. https://doi.org/10.1016/j.dsx.20130908-015-0091-7.

64. Ikarashi N, Mizukami N, Kon R, Kaneko M, Uchino R, Fujisawa I, Fukuda N, Sakai H, Kamei J. Study of the mechanism underlying the onset of diabetic xeroderma focusing on an aquaporin-3 in a streptozotocin-induced diabetic mouse model. Int J Mol Sci. 2019;20(15):3782. https://doi.org/10.3390/ijms20153782.

65. Dharmadhikari G, Stolz B, Hauke M, Morgan NG, Varki A, de Koning E, Kelm S, Maedler K. Siglec-7 restores β-cell function and survival and reduces inflammation in pancreatic islets from patients with diabetes. Sci Rep. 2017;7:45319. https://doi.org/10.1038/srep45319.

66. Sutton BS, Palmer ND, Langefeld CD, Xue B, Proctor A, Ziegler JT, Haffner SM, Norris JM, Bowden DW. Association of SSTR2 polymorphisms and glucose homeostasis phenotypes: the Insulin Resistance Atherosclerosis Family Study. Diabetes. 2009;58(6):1457–62. https://doi.org/10.2337/db08-0640.

67. Deng Z, Shen J, Ye J, Shu Q, Zhao J, Fang M, Zhang T. Association between single nucleotide polymorphisms of delta/notch-like epidermal growth factor (EGF)-related receptor (DNER) and Delta-like 1 (DLL1) with the risk of type 2 diabetes mellitus in a Chinese Han population. Cell Biochem Biophys. 2015;71(1):331–5. https://doi.org/10.1007/s10564-015-9851-4.

68. Liu Y, Tao F, Wu XX, Tan YZ, He L, Lu H. Common RASGRP1 gene variants that confer risk of type 2 diabetes. Genet Test Mol Biomarkers. 2015;19(8):439–43. https://doi.org/10.1089/gtmb.2015.0005.

69. Osawa H, Tanaka S, Takahashi K, Nagata M, et al. Genome-wide association study of the single nucleotide polymorphism in the CSAD/lnc-ITGB7-1 on chromosome 12q13.13 associated with susceptibility to type 2 diabetes. Diabetes. 2019;68(3):665–75. https://doi.org/10.2337/db18-0134.

70. Li JY, Tao F, Wu XX, Tan YZ, He L, Lu H. Common RASGRP1 gene variants that confer risk of type 2 diabetes. Genet Test Mol Biomarkers. 2015;19(8):439–43. https://doi.org/10.1089/gtmb.2015.0005.
lead for the treatment of myocardial infarction. ACS Med Chem Lett. 2016;7(5):493–7. https://doi.org/10.1016/j.acsmedchemlett.2016.04.004.

88. Wang HB, Yang J, Shuai W, Yang J, Liu LB, Xu M, Tang QZ. Deletion of microfibrillar-associated protein 4 attenuates left ventricular remodeling and dysfunction in heart failure. J Am Heart Assoc. 2020;9(17):e015307. https://doi.org/10.1161/JAHA.119.015307.

89. Kayama Y, Minamino T, Toko H, Sakamoto M, Shimizu I, Takahashi H, Okada S, Tateno K, Moriya J, Yokoyama M, et al. Cardiac 12/15 lipoxygenase-induced inflammation is involved in heart failure. J Exp Med. 2009;206(7):1565–74. https://doi.org/10.1084/jem.20082596.

90. Hua X, Wang YY, Pu X, Yang Q, Hu Y, Jiang Y, Lai S, Xu Y, Zhao Z, Song J. Multi-level transcriptome sequencing identifies COL1A1 as a candidate marker in human heart failure progression. BMC Med. 2020;20(1):2. https://doi.org/10.1186/s12916-019-1469-4.

91. Gombos T, Forhécz Z, Pozsonyi Z, Jánoskuti L, Prohászkó Z, Karádi I. Long-term survival and apolipoprotein A1 level in chronic heart failure: interaction with tumor necrosis factor-α -308 G/A polymorphism. J Card Fail. 2017;23(9):113–20. https://doi.org/10.1016/j.cardfail.2016.06.004.

92. Westermann D, Becker PM, Lindner D, Savvatis K, Xia Y, Fröhlich M, Koller L, Blum S, Korpak M, Richter B, Goliasch G, Zorn G, Brekalo M, Li Y, Song D, Mao L, Abraham DM, Bursac N. Lack of Thy1 defines a receptor CX3CR1 on CD4 T cells in patients with chronic heart failure. Int J Cardiol. 2014;171(1):96–7. https://doi.org/10.1016/j.ijcard.2013.11.082.

93. Li Y, Song D, Mao L, Abraham DM, Bursac N. Lack of Thy1 defines a pathogenic fraction of cardiac fibroblasts in heart failure. Biomaterials. 2020;236:119824. https://doi.org/10.1016/j.biomaterials.2020.119824.

94. Mueller KA, Tavliaki E, Schneider M, Joronen D, Reisler T, Kardoff R, Gavaz M, Mueller II, Zueen CS. Gremlin-1 identifies fibrosis and predicts adverse outcome in patients with heart failure undergoing endomyocardial biopsy. J Card Fail. 2013;19(10):678–84. https://doi.org/10.1016/j.cardfail.2013.09.001.

95. Andenæs K, Lunde IG, Mohammadzadeh N, Dahl CP, Aronsen JM, Strand ME, Palmoso S, Jaastad I, Christensen G, Engbretsen KV, et al. The extracellular matrix proteoglycan fibromodulin is upregulated in clinical and experimental heart failure and affects cardiac remodeling. PLoS ONE. 2018;13(7):e0201422. https://doi.org/10.1371/journal.pone.0201422.

96. Abuzaanona A, Lanfear D. Pharmacogenomics of the natriuretic peptide system in heart failure. Curr Heart Fail Rep. 2017;14(6):536–42. https://doi.org/10.1007/s11897-017-0365-5.

97. Bai Y, Zhang F, Zhang X, Huang J, Hu S, Wei Y. LTBP-2 acts as a novel marker in human heart failure progression. BMC Med. 2020;18(1):2. https://doi.org/10.1186/s12916-019-1469-4.

98. Angrisano T, Schiattarella GG, Keller S, Pironti G, Florio E, Maglifiu F, Bottino R, Pero R, Lembi F, Avvedimento EV, et al. Epigenetic switch at the p53a2a and myh7 gene promoters in pressure overload-induced heart failure. PLoS ONE. 2014;9(9):e106024. https://doi.org/10.1371/journal.pone.0106024.

99. Amir O, Rogowski O, David M, Lahat N, Wolf R, Lewis B. Circulating interleukin-10 association with higher mortality in systolic heart failure patients with elevated tumor necrosis factor-alpha. Isr Med Assoc J. 2010;12(3):158–62.

100. Taylor MR, Slavov D, Humphrey K, Zhao L, Cockcroft J, Zhu X, Lavoir P, Bristow MR, Mestroni L, Lazzeroni LC. Pharmacogenetic effect of an endothelin-1 haplotype on response to bucindolol therapy in chronic heart failure. Pharmacogenet Genom. 2009;19(1):35–43. https://doi.org/10.1097/FCG.0b013e328317ec57.

101. Taylor MR, Slavov D, Humphrey K, Zhao L, Cockcroft J, Zhu X, Lavoir P, Bristow MR, Mestroni L, Lazzeroni LC. Pharmacogenetic effect of an endothelin-1 haplotype on response to bucindolol therapy in chronic heart failure. Pharmacogenet Genom. 2009;19(1):35–43. https://doi.org/10.1097/FCG.0b013e328317ec57.

102. Liu S, Xinyong C, Hongmin Z, Jing W, Lang H, Ping Z. TLR2 and TLR3 expression as a biomarker for the risk of doxorubicin-induced heart failure. Toxicol Lett. 2018;295:205–11. https://doi.org/10.1016/j.toxlet.2018.06.1219.

103. Raman K, O’Donnell MJ, Członkowska A, Duarte YC, Lopez-Jaramillo P, Peñaherrera E, Sharma M, Shoamanesh A, Skowronska M, Yusuf S, et al. Peripheral plasma MCEMP1 gene expression as a biomarker for stroke prognosis. Stroke. 2016;47(3):652–8. https://doi.org/10.1161/STROKEAHA.115.011854.

104. Wells BJ, Hueston WJ. Are thyroid peroxidase antibodies associated with cardiovascular disease risk in patients with subclinical hypothyroidism? Clin Endocrinol (Oxf). 2005;62(5):580–4. https://doi.org/10.1111/j.1365-2265.2003.02262.x.

105. Piaszynska-Kopczynska K, Markincikiewicz-Siemon M, Lisowska A, Wazikiewicz E, Witkowski M, Jasiewicz M, Miklasz P, Iakim P, Galar B, Musial WJ, et al. Alterations of soluble TWEAK and CD163 concentrations in patients with chronic heart failure. Cytokine. 2016;80:7–12. https://doi.org/10.1016/j.cyto.2016.02.005.

106. Grisoni ML, Prout C, Alanne M, Desuremain M, Salomaa V, Kuulasmaa K, Cambien F, Nicaud V, Wiklund PG, Virtamo J, et al. Lack of association between polymorphisms of the IL18R1 and IL18RAP genes and cardiovascular risk: the INTERSALT study. Circulation. 2002;105(11):1101–7. https://doi.org/10.1161/01.CIR.0000023220.01735.60.

107. Bari M, Khatib K, Mackenzie HS, Murakami O, Anhara Z, Sone M, Mouri T, Brenner BM, Isos T. Increased expression of
adrenomedullin and adrenomedullin-receptor complexes, receptor-activity modifying protein (RAMP)2 and calcitonin-receptor-like receptor (CRRLR) in the hearts of rats with congestive heart failure. Clin Sci (London). 2000;99(6):541–6.

119. Li X, Wang G, Qiu M, Jiang H, Li T, E X, Feng Y, Zhang Y, Liu X, Qian M, et al. Aspirin Reduces Cardiac Intrastitial Fibrosis by Inhibiting Erk1/2-Serpine2 and P-Akt Signalling Pathways. Cell Physiol Biochem. 2018;45(5):1955–66. https://doi.org/10.1159/000487972.

120. Deck S, Høgermont W, Carai P, Rienks M, Dresselaers T, Himmelreich U, van Leeuwen R, Lommen W, van der Velden J, Gonzalez A, et al. Osteoglycin prevents the development of age-related diastolic dysfunction during pressure overload by reducing cardiac fibrosis and inflammation. Matrix Biol. 2018;66:110–24. https://doi.org/10.1016/j.matri.2017.09.002.

121. Ichihara S, Senbonmatsu T, Price E Jr, Ichiiki T, Gaffney FA, Inagami T. Angiotension II type 2 receptor is essential for left ventricular hypertrophy and cardiac fibrosis in chronic angiotension II-induced hypertension. Circulation. 2001;104(3):346–51. https://doi.org/10.1161/01.cir.104.3.346.

122. Park DT, Rai M, Ryzhov S, Sanders LN, Aisagbonhi O, Funke MJ, Feoktistov I, Hatzopoulos AK. Wnt10b gain-of-function improves cardiac repair by arteriole formation and attenuation of fibrosis. Circ Res. 2015;117(9):804–16. https://doi.org/10.1161/CIRCRESAHA.115.308886.

123. Cai W, Tao J, Xiong J, Tian X, Liu T, Feng X, Bai J, Yan C, Han Y, Chong W. Contribution of homeostatic chemokines CCL19 and CCL21 and their receptor CCR7 to coronary artery disease. Arterioscler Thromb Vasc Biol. 2014;34(9):493–3 https://doi.org/10.1161/ATVBAHA.113.303081.

124. Mo XG, Liu W, Yang Y, Imani S, Lu S, Dan G, Nie X, Yan J, Zhan R, Li X, et al. NCF2, MYO1F, S1PR4, and FCN1 as potential noninvasive diagnostic biomarkers in patients with obstructive coronary artery: a weighted gene co-expression network analysis. J Cell Biochem. 2019;120(18):19215–33. https://doi.org/10.1002/jcb.29128.

125. Sun H, Fang F, Li K, Zhang H, Zhang L, Li J, Qin Y, Wei Y, et al. Circulating ESM-1 levels are correlated with the presence of coronary artery disease in patients with obstructive sleep apnoea. Respir Res. 2019;20(1):188. https://doi.org/10.1186/s12931-019-1143-6.

126. Martinei N, Grelli O, Lunghi B, Pinotti M, Marchetti G, Malerba G, Pigatto PF, Corscher R, Oliervi O, Bernardi F. Polymorphisms at ALOX5 and ALOX5AP and risk of coronary artery disease. Hum Genet. 2017;139(5):511–8. https://doi.org/10.1007/s00439-016-1808-5.

127. Sasagawa S, Nishimura Y, Sawada H, Zhang E, Okabe S, Murakami S, Ashikawa Y, Yuge M, Kawaguchi K, Kawase R, et al. Comperative transcriptome analysis identifies CCDC88 as a novel gene associated with pulmonary arterial hypertension. Front Pharmacol. 2016;7:142. https://doi.org/10.3389/fphar.2016.00142.

128. Wu Y, Li Q, Yang K, Xiao C. Association of CMA1 gene tag single nucleotide polymorphisms with essential hypertension in Yi population from Yunnan. Chinese. 2013;41(4):494–59. https://doi.org/10.3766/cmja.issn.1309-9046.2014.04.008.

129. Sato W, Sato Y. Middline in nephogenesis, hypertension and kidney diseases. Br J Pharmacol. 2014;171(4):879–87. https://doi.org/10.1111/j.1476-5381.2013.04385.x.

130. Kohara K, Tabara Y, Nakura J, Imai Y, Ohkubo T, Hata A, Soma M, Nakayama T, Umemura S, Hirawa N, et al. Identification of hypertension-susceptibility genes and pathways by a systemic multiple candidate gene approach: the millennium genome project for hypertension. Hypertens Res. 2008;31(2):203–12. https://doi.org/10.1007/s10992-008-9413-8.

131. Wen G, Wessel J, Zhou W, Ehret GB, Rao F, Stridsberg M, Mahata SK, Gent PM, Das M, Cooper RS, et al. An ancestral variant of Secrecoglin II confers regulation by PHOX2 transcription factors and association with hypertension. Hum Mol Genet. 2017;16(14):1752–64. https://doi.org/10.1093/hmg/ddm123.

132. Seidelmann SB, Vardany O, Claggett B, Yu B, Shen AM, Ballantyne CM, Selvin E, MacRae CA, Boerwinkle E, Solomon SD. An NPB promoter polymorphism associated with elevated N-terminal pro-B-type natriuretic peptide and lower blood pressure, hypertension, and mortality. J Am Heart Assoc. 2017;6(4):e005257. https://doi.org/10.1161/JAHA.116.005257.

133. Shi L, Tian C, Sun L, Cao F, Meng Z. The IndRNA TUG1/miR-145-5p/FGF10 regulates proliferation and migration in VSMCs of hypertension. Biochem Biophys Res Commun. 2018;501(3):688–95. https://doi.org/10.1016/j.bbrc.2018.05.049.

134. Woon PY, Kaisaki PJ, Bragança J, Bihoreau MT, Levy JC, Farrall M, Gauvrit E, et al. The EDNRB polymorphisms are associated with metabolic syndrome and type 2 diabetes mellitus. Results from the genetics of atherosclerotic disease Mexican study. Immunobiology. 2017;222(10):967–72. https://doi.org/10.1016/j.imb.2016.08.014.

135. Pi S, Mao L, Chen J, Shi H, Liu Y, Guo X, Li Y, Zhou L, He H, Yu C, et al. The P2RY12 receptor promotes VSMC-derived foam cell formation by inhibiting autophagy in advanced atherosclerosis. Autophagy. 2020. https://doi.org/10.1080/15548627.2020.1741202.

136. de Vries MA, Trompet S, Mooijaart SP, Smit RA, Böhringer S, Castro Cabezas M, Jukema JW. Complement receptor 1 gene polymorphisms are associated with cardiovascular risk. Atherosclerosis. 2017;257:16–21. https://doi.org/10.1016/j.atherosclerosis.2016.12.017.

137. Osterholm C, Folkersen L, Lengquist M, Ponten F, Renné T, Li J, Hedlin U. Increased expression of heparanase in symptomatic carotid atherosclerosis. Atherosclerosis. 2013;226(1):67–73. https://doi.org/10.1016/j.atherosclerosis.2012.09.030.

138. Norata GD, Garlanda C, Catapano AL. The long pentraxin PTX3 a modulator of the immunoinflammatory response in atherosclerosis and cardiovascular diseases. Trends Cardiovasc Med. 2010;20(2):35–40. https://doi.org/10.1016/j.tcm.2010.03.005.

139. Koch W, Schimpf M, Ehl A, Mueller JC, Hoppmann R, Schömig A, Kasatati A. 4Gs/5Gs polymorphism and haplotypes of SERPINE1 in atherosclerosis, cardiovascular diseases of coronary arteries. Thromb Haemost. 2010;103(6):1710–80. https://doi.org/10.1111/j.1538-7836.2010.04702.x.

140. Sasagawa S, Nishimura Y, Sawada H, Zhang E, Okabe S, Murakami S, Ashikawa Y, Yuge M, Kawaguchi K, Kawase R, et al. Comparative transcriptome analysis identifies CCDC80 as a novel gene associated with pulmonary arterial hypertension. Front Pharmacol. 2016;7:142. https://doi.org/10.3389/fphar.2016.00142.

141. Zicha J, Dolebová Z, Zedník V, Šilhavý J, Simková M, MILENJK, Vaněčková I, Kunej Z, Pravec M. Pharmacogenetic analysis of captopril effects on blood pressure: possible role of the Ednrb (endothelin...
149. Maloney JP, Stearman RS, Bull TM, Calabrese DW, Tripp-Addison ML, Wick MJ, Broekel U, Robbins IM, Wheeler LA, Cogan JD, et al. Loss-of-function thrombospondin-1 mutations in familial pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol. 2012;302(6):L541–54. https://doi.org/10.1152/ajplung.00282.2011.

150. Ouyang Y, Wu H, Tan A, Yang H, Gao Y, Li H, Lu S, Huang J, Gu D. The hypertension risk variant Rs208430 functions as an enhancer of SLC4A7. Am J Hypertens. 2017;30(2):202–8. https://doi.org/10.1093/ajh/hpw127.

151. Kerty E, Heuser K, Indahl UG, Berg PR, Nakken S, Lien OM, Owstens ØP, Nagelhus EA. Is the brain water channel aquaporin-4 a pathogenic factor in idiopathic intracranial hypertension? Results from a combined clinical and genetic study in a Norwegian cohort. Acta Ophthalmol. 2013;91(1):88–91. https://doi.org/10.1111/j.1755-3786.2011.02231.x.

155. Shi Y, Long F. Hedgehog signaling via Gli2 prevents obesity induced hypertension. Mamm Genome. 2012;23(7–8):431–42. https://doi.org/10.1007/s00099-012-9490-8.

157. Zhang L, Reidy SP, Nicholson TE, Lee HJ, Majdalawieh A, Webber C, Anveden Å, Sjöholm K, Jacobson P, Palsdottir V, Walley AJ, Froguel P, Kochumon S, Madhoun AA, Al-Rashed F, Azim R, Al-Ozairi E, Al-Mulla Mwangi SM, Nezami BG, Obukwelu B, Anitha M, Marri S, Fu P, Epperson Mazzarella L, Botteri E, Matthews A, Gatti E, Di Salvatore D, Bagnardi V, Brandt J, Warnke K, Jörgens S, Arolt V, Beer K, Domschke K, Haverkamp 2011;301(4):H1596–605. https://doi.org/10.1152/ajpheart.00948.2009.

161. Hogberg TF, Frimmer TM, Saxal PK. Melanin concentrating hormone receptor 1 (MCHR1) antagonists-Still a viable approach for obesity treatment? Bioorg Med Chem Lett. 2012;22(19):6039–47. https://doi.org/10.1016/j.bmccl.2012.08.025.

164. Piquer-Garcia I, Campderros L, Taxerès SD, Gavaldà-Navarro A, Pardo R, Vila M, Pellietero S, Martínez E, Tarasco J, Moreno P, et al. A role for oncostatin M in the impairment of glucose homeostasis in obesity. J Clin Endocrinol Metab. 2020;105(3):e337–48. https://doi.org/10.1210/clinem/dgpa290.

165. Quinlan LS, Anderson BG. Interleukin-15, IL-1 receptor-alpha, and obesity: concordance of laboratory animal and human genetic studies. J Obes. 2011;2011:456347. https://doi.org/10.1155/2011/456347.

166. Wang J, Sukhova GK, Liu J, Ozaiki K, Lesner A, Libby P, Kovaren PT, Shi GP. Catepsin G deficiency reduces periaortic calcium chloride injury-induced abdominal aortic aneurysms in mice. J Vasc Surg. 2015;62(6):1615–24. https://doi.org/10.1016/j.jvs.2014.06.004.

168. Chen C, Peng H, Zeng Y, Dong G, CD14, CD163, and CR1R are involved in heart and blood communication in ischemic cardiac disease. J Int Med Res. 2020;48(9):30006520951649. https://doi.org/10.1177/030003720.001649.
180. Wei Y, Zhu M, Corbalán-Campos J, Heyll K, Weber C, Schober A. Regulation of Csf1r and Bcl6 in macrophages mediates the stage-specific effects of microRNA-155 on atherosclerosis. Arterioscler Thromb Vasc Biol. 2015;35(4):796–803. https://doi.org/10.1161/ATVBAHA.114.304723.

181. Wu N, Jin L, Cai J. Profiling and bioinformatics analyses reveal differential circular RNA expression in hypertensive patients. Clin Exp Hypertens. 2017;39(5):454–9. https://doi.org/10.1080/10641963.2016.1273944.

182. Li Z, Chyr J, Jia Z, Wang L, Hu X, Wu X, Song C. Identification of hub genes associated with hypertension and their interaction with miRNA based on weighted gene coexpression network analysis (WGCNA) analysis. Med Sci Monit. 2020;26: e923514. https://doi.org/10.12659/MSM.923514.

183. Yang S, Gao Y, Liu G, Li J, Shi K, Du B, Si D, Yang P. The human ATF1 rs11169571 polymorphism increases essential hypertension risk through modifying miRNA binding. FEBS Lett. 2015;589(16):2087–93. https://doi.org/10.1089/febs.2015.06.029.

184. Sun Q, Li C, Liu J, Wang Z, Liu Y, Lu C, Chen Y, Wen S. Expression profile of microRNAs in hypertrophic cardiomyopathy and effects of microRNA-20 in inducing cardiomyocyte hypertrophy through regulating gene MFN2. DNA Cell Biol. 2019;38(8):796–807. https://doi.org/10.1089/dna.2019.4731.

185. Larsen LH, Rose CS, Sparsø T, Overgaard J, Torekov SS, Grarup N, Jensen DP, Albrechtsen A, Andersen G, Eik J, et al. Genetic analysis of the estrogen-related receptor alpha and studies of association with obesity and type 2 diabetes. Int J Obes (Lond). 2007;31(2):365–70. https://doi.org/10.1038/sj.ijo.0803408.

186. Choi JH, Choi SS, Kim ES, Jedrychowski MP, Yang YR, Jang HJ, Suh PG, Banks AS, Gygi SP, Spiegelman BM. Thraps3 docks on phosphoserine 273 of PPARγ and controls diabetic gene programming. Genes Dev. 2014;28(21):2361–9. https://doi.org/10.1101/gad.249367.114.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.