Transcriptome Signatures Reveal Candidate Key Genes in the Peripheral Blood Mononuclear Cells of Patients With Coronary Artery Disease

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Research Article

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Abstract

Background

Coronary artery disease (CAD) is one of the most common disorders in the cardiovascular system. This study aims to explore potential signaling pathways and important biomarkers that drive CAD development.

Methods

The CAD GEO Dataset GSE113079 was featured to screen differentially expressed genes (DEGs). The pathway and Gene Ontology (GO) enrichment analysis of DEGs were analyzed using the ToppGene. We screened hub and target genes from protein-protein interaction (PPI) networks, target gene - miRNA regulatory network and target gene - TF regulatory network, and Cytoscape software. Validations of hub genes were performed to evaluate their potential prognostic and diagnostic value for CAD.

Results

1,036 DEGs were captured according to screening criteria (525 upregulated genes and 511 downregulated genes). Pathway and Gene Ontology (GO) enrichment analysis of DEGs revealed that these up and down regulated genes are mainly enriched in thyronamine and iodothyronamine metabolism, cytokine-cytokine receptor interaction, nervous system process, cell cycle and nuclear membrane. Hub genes were validated to find out potential prognostic biomarkers, diagnostic biomarkers and novel therapeutic target for CAD.

Conclusions

In summary, our findings discovered pivotal gene expression signatures and signaling pathways in the progression of CAD. CAPN13, ACTBL2, ERBB3, GATA4, GNB4, NOTCH2, EXOSC10, RNF2, PSMA1 and PRKAA1 might contribute to the progression of CAD, which could have potential as biomarkers or therapeutic targets for CAD.

Introduction

Coronary artery disease (CAD) remains the one of leading healthy issues worldwide and 23.3 million people will die of CAD by 2030 [1]. The risk factors for CAD mainly smoking, high blood pressure, high blood cholesterol levels, diabetes, overweight or obesity, physical inactivity, high stress and unhealthy diet [2]. At present, surgery has been applied to improve survival of CAD patients [3]. However, the molecular pathogenesis of CAD advancement is still largely unknown.

As an inventive and high-throughput investigation facilitate the concurrent analysis of expression changes in thousands of genes in CAD samples and contributes to diagnosis, prognosis and new drug discovery [4]. In current years, there have been huge research on the molecular pathogenesis of CAD
occurrence and progression by finding and analyzing differentially expressed genes (DEGs) with microarray technologies. Genes such as human paraoxonase/arylesterase (HUMPONA) [5], apolipoprotein E (apo E) [6], MMP-2, MMP-3, MMP-9 and MMP-12 [7], endothelial nitric oxide synthase (eNOS) [8] and angiotensin II type 1 (AT1) receptor [9] were associated with CAD progression. Signaling pathway such TLR4 signaling pathway [10], mTOR signaling pathway [11], CXCR4 signaling pathway [12], eNOS-activating pathways [13] and Akt pathway [14] were involved in development of CAD. However, certain essential genes and pathways of CAD have not been completely investigated. More investigations are necessary to enlighten these hub genes and pathways to add available therapeutic targets for the treatment of CAD.

In the current investigation, gene expression profile GSE113079 dataset was downloaded from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) [15]. The current investigation finds the differentially expressed genes (DEGs) at CAD based on the expression profile data of GSE113079. Subsequently, Pathway and gene ontology (GO) enrichment analysis for DEGs were carried out. A protein-protein interaction (PPI) network, target gene - miRNA regulatory network and target gene - TF regulatory network were constructed and analyzed. Finally hub genes were validated by immunohistochemical analysis and receiver operating characteristic (ROC) curve analysis. The current investigation aimed to find certain other essential genes and pathways in CAD and study potential therapeutic targets of the disease.

Materials And Methods

Microarray data and data preprocessing

Raw expression profile data from the Agilent microarray file GSE113079 [16] were downloaded from the public NCBI GEO database and executed on the GPL20115 platform. GSE113079 contains 93 CAD patients and 48 healthy controls. The raw expression files of microarray dataset was pre-processed according to the loess and quantile method [17] and probe identification numbers were converted to gene symbols using as a reference the Agilent-067406 Human CBC IncRNA + mRNA microarray V4.0 (Probe name version). When multiple probes compare to the same gene, the probe with the high p value from the downstream differential analysis was picked to resolve the differential gene expression value.

Identification of DEGs

The linear models for microarray data Limma package in Bioconductor [18] was used to identify DEGs by comparing the expression values between peripheral blood mononuclear cells from CAD patients and peripheral blood mononuclear cells from healthy control. The corresponding P value of the gene symbols after t test were used, and adjusted P < 0.05 and |logFC| > 0.97 for up regulated genes, and |logFC| < -0.963 for down regulated genes were used as the selection criteria.

Pathway enrichment analyses of DEGs
BIOCYC (https://biocyc.org/) [19], Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) [20], Pathway Interaction Database (PID, http://pid.nci.nih.gov/) [21], Reactome (https://reactome.org/PathwayBrowser/) [22], Molecular signatures database (MSigDB, http://software.broadinstitute.org/gsea/msigdb/) [23], GenMAPP (http://www.genmapp.org/) [24], Pathway Ontology (https://bioportal.bioontology.org/ontologies/PW) [25], PantherDB (http://www.pantherdb.org/) [26] and Small Molecule Pathway Database (SMPDB) (http://smpdb.ca/) [27] are databases resource for understanding high-level functions and biological systems from large-scale molecular datasets generated by high-throughput experimental technologies. The ToppGene (ToppFun) (https://toppgene.cchmc.org/enrichment.jsp) [28] in online tool was used to perform the pathway enrichment analyses of the DEGs. P < 0.05 was considered statistically significant.

**Gene ontology (GO) enrichment analysis of DEGs**

The GO (http://www.geneontology.org/) [29] is a represented terminology of terms defines gene products according to the biological process (BP), molecular function (MF), and cellular component (CC). We used ToppGene (ToppFun) (https://toppgene.cchmc.org/enrichment.jsp) [28], a web-accessible program that integrates functional genomic annotations, to view the GO enrichment of DEGs; a p value <0.05 was considered statistically significant.

**PPI network construction and module analysis**

STRING (https://string-db.org/) [30] is a protein-protein interaction (PPI) network analysis online tool. The current version of the STRING PPI database is 11.0, which screen more than 5,090 species and 24.6 million proteins and holds the upload of genome level data sets. To resolve which proteins encoded by the DEGs acts a dominant role in CAD, the DEGs were applied to STRING v.11.0 with medium confidence scores of 0.4. To find the hub genes, we visualized the PPI network using Cytoscape v.3.7.2 software (http://www.cytoscape.org/) [31] and analyzed the topological properties of these nodes using the Network Analyzer tool. Then we selected the nodes with high degrees centrality [32], high betweenness centrality [33], high stress centrality [34], high closeness centrality [35] and low clustering coefficient [36] values as hub genes. The PEWCC1 [37] built in Cytoscape is an automated method that was used to evaluate highly interconnected modules as molecular complexes or clusters. The analysis parameters were establish by default. The pathway and GO enrichment analysis was executed for DEGs, from which four significant modules of genes were diagnosed with p < 0.05 set as the threshold.

**Construction of target gene - miRNA regulatory network**

The NetworkAnalyst (https://www.networkanalyst.ca/) [38] online platform was used combine the results of mRNA (DEGs) with known miRNAs of human to construct the target gene - miRNA network and to predict target genes with differential expression miRNAs. In addition, we predicted the target genes for miRNAs using two online software: DIANA-TarBase (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index) [39] and miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/php/download.php) [40]. All 3 procedural predicted genes were
selected as targets for DEGs to construct differentially expressed miRNA. Target genes were arranged into the miRNA regulatory network separately to access each miRNA regulatory network which were visualized using Cytoscape ([http://www.cytoscape.org/](http://www.cytoscape.org/)) [31]. DEGs (up and down regulated) interaction with more number of miRNAs consider as target genes.

**Construction of target gene - TF regulatory network**

Transcription factor gene data of the NetworkAnalyst ([https://www.networkanalyst.ca/](https://www.networkanalyst.ca/)) [38] was used to identify the transcription factor regulatory networks linked with the target genes. The NetworkAnalyst describes transcription factor (TF) to genes from the perspective of ChEA database ([http://amp.pharm.mssm.edu/lib/chea.jsp](http://amp.pharm.mssm.edu/lib/chea.jsp)) [41] database resource. The NetworkAnalyst illustrate a more extensive transcription factor regulation network. Target genes were arranged into the TF regulatory network separately to access each transcription factor regulatory network which were visualized using Cytoscape ([http://www.cytoscape.org/](http://www.cytoscape.org/)) [31]. DEGs (up and down regulated) interaction with more number of TFs consider as target genes.

**Validations of hub genes**

The human protein atlas database (HPA) ([www.proteinatlas.org](http://www.proteinatlas.org)) [42] was used to analyze protein expression of hub genes in peripheral blood mononuclear cells in bone marrow. A receiver operating characteristic (ROC) curve was produce using the pROC package of the R software [43], and the area under the curve (AUC) was determined using generalized linear model (GLM) in machine learning algorithms to assess the predictive accuracy of hub genes.

**Results**

**Data preprocessing and identification of DEGs**

The gene expression profile with accession numbers GSE113079 was downloaded from GEO database. The results of before and after normalizing the microarray gene expression are shown in Fig. 1A and Fig. 1B. DEGs between peripheral blood mononuclear cells from CAD patients and peripheral blood mononuclear cells from healthy control were screened using limma package in R bioconductor (P-value <0.05, |logFC| > 0.97 for up regulated genes, and |logFC| < - 0.963 for down regulated genes). In this study, 1,036 total DEGs (525 up regulated genes and 511 down regulated genes, respectively) in GSE113079 was screened. The total number of DEGs collected for each subject in the differential gene expression analysis is given in Table 1. A volcano diagram was constructed for the DEGs and is shown in Fig. 2. The DEGs (up and down regulated genes) are presented by a cluster heatmap in Fig. 3 and Fig. 4.

**Pathway enrichment analyses of DEGs**

Pathway enrichment analyses were performed using ToppGene, analyzing the pathway classification of DEGs (up and down regulated genes). Pathways of up regulated were mainly enriched in thyronamine and iodothyronamine metabolism, trehalose degradation, cytokine-cytokine receptor interaction, Olfactory
transduction, FOXA1 transcription factor network, lissencephaly gene (LIS1) in neuronal migration and
development, signaling by GPCR, GPCR downstream signaling, C21 seroid hormone metabolism,
androgen and estrogen metabolism, ensemble of genes encoding extracellular matrix and extracellular
matrix-associated proteins, ensemble of genes encoding ECM-associated proteins including ECM-
affiliated proteins, ECM regulators and secreted factors, corticotropin releasing factor receptor
signaling pathway, 5HT4 type receptor mediated signaling pathway, corticotropin-releasing hormone
signaling, G protein signaling via galphaq family, ornithine transcarbamylase deficiency (OTC deficiency
and intracellular signaling through PGD2 receptor and prostaglandin D2 according to the BIOCYC, KEGG,
PID, Reactome, MSigDB, GenMAPP, Pathway Ontology, PantherDB and SMPDB pathway analysis results
(Table 2), whereas pathways of down regulated were mainly enriched in inosine-5'-phosphate
biosynthesis, sulfate activation for sulfonation, antigen processing and presentation, graft-versus-host
disease, Fc-epsilon receptor I signaling in mast cells, TGF-beta receptor signaling, signaling by
interleukins, generic transcription pathway, glycosaminoglycan degradation, sterol biosynthesis, ras-
independent pathway in NK cell-mediated cytotoxicity, hypoxia and p53 in the cardiovascular system, FAS
signaling pathway, angiogenesis, pathway of folate cycle/metabolism, vascular endothelial growth factor
signaling, sarcosinemia and purine metabolism according to the BIOCYC, KEGG, PID, Reactome, MSigDB,
GenMAPP, Pathway Ontology, PantherDB and SMPDB pathway analysis results (Table 3).

Gene ontology (GO) enrichment analysis of DEGs

The Gene Ontology (GO) enrichment analyses were conducted using online tool ToppGene. GO terms of
the up regulated and down regulated genes were listed in Table 4 and Table 5, respectively. Gene
Ontology (GO) enrichment analysis showed that the up regulated genes were mainly associated with
nervous system process, G protein-coupled receptor signaling pathway, intrinsic component of plasma
membrane, extracellular matrix, transmembrane signaling receptor activity and receptor regulator activity.
The down regulated genes were mainly associated with cell cycle, regulation of immune system process,
nuclear membrane, nuclear chromatin, DNA-binding transcription factor activity, RNA polymerase II-
specific and signaling receptor binding.

PPI network construction and module analysis

The PPI network of the up and down regulated genes was analyzed by using online STRING database.
The result of PPI network of up regulated was illustrated in Fig 5. A total of 3715 nodes with 6518 edges
were reflected in this well-established network system. The statistical results and scatter plot for node
degree distribution, betweenness centrality, stress centrality, closeness centrality and clustring coefficient
are shown in Fig. 6A - 6E and indicated that CAPN13, EGFR, ACTBL2, ACTL8, ERBB3, PRMT5, GATA4,
RHOV, CHD5, MAGEL2, THNSL2, SLC38A8, THPO and SPTSSB were the hub genes with high node degree
distribution, betweenness centrality, stress centrality, closeness centrality and low clustring coefficient in
the network are listed in Table 6. The top hub genes in this PPI network were selected for further pathway
and GO enrichment analyses using the ToppGene database. The results indicated that the hub genes
were mainly enriched in developmental biology, cytokine-cytokine receptor interaction, huntington
disease, epithelial cell differentiation, calcium signaling pathway, E2F transcription factor network, notch-mediated HES/HEY network, receptor regulator activity, cation transport and Jak-STAT signaling pathway. The result of PPI network of down regulated was illustrated in Fig 7. A total of 5135 nodes with 10628 edges were reflected in this well-established network system. The statistical results and scatter plot for node degree distribution, betweenness centrality, stress centrality, closeness centrality and clustering coefficient are shown in Fig. 8A - 8E and indicated that FYN, PAK2, CUL3, RPS6, NOTCH2, PDE4D, SPATA21, MYBL1, SMURF1, PDGFRB, DLG3, ADHFE1, NMB, SLC25A36, MLLT1 and RNF2 were the hub genes with high node degree distribution, betweenness centrality, stress centrality, closeness centrality and low clustering coefficient in the network are listed in Table 6. The top hub genes in this PPI network were selected for further pathway and GO enrichment analyses using the ToppGene database. The results indicated that the hub genes were mainly enriched in natural killer cell mediated cytotoxicity, Fc-epsilon receptor I signaling in mast cells, signaling by interleukins, mTOR signaling pathway, notch signaling pathway, purine metabolism, gene expression, endocytosis, cytokine-cytokine receptor interaction, regulation of hydrolase activity, signaling receptor binding, organelle envelope, nuclear chromatin and post-translational protein modification.

Four significant modules were selected for each up and down regulated genes using the PEWCC1E plug-in. The top four modules for up regulated genes were selected (module 13, 105 nodes and 235 edges; module 20, 77 nodes and 97 edges; module 21, 73 nodes and 81 edges; module 34, 53 nodes and 58 edges) are shown in Fig. 9. The results revealed that hub genes (ACTG2, GATA4, EGFR, TP73, ACTBL2, FOXJ1, BMP7 and CDK5R2) in these significant modules were mostly enriched in the muscle contraction, notch-mediated HES/HEY network, cytokine-cytokine receptor interaction, E2F transcription factor network, actin cytoskeleton, epithelial cell differentiation, biological adhesion and neuron projection. Similarly, top four modules for down regulated genes were selected (module 1, 92 nodes and 186 edges; module 2, 56 nodes and 187 edges; module 5, 49 nodes and 144 edges; module 11, 29 nodes and 57 edges) are shown in Fig. 10. The results revealed that hub genes (RPS6, PAK2, PODN, LMNA, EIF1AX, RPS27, HSPA8, FYN and LMNB1) in these significant modules were mostly enriched in the mTOR signaling pathway, Fc-epsilon receptor I signaling in mast cells, ensemble of genes encoding extracellular matrix and extracellular matrix-associated proteins, caspase cascade in apoptosis, postsynapse, cell cycle, regulation of immune system process and positive regulation of signal transduction.

**Construction of target gene - miRNA regulatory network**

NetworkAnalyst was applied to screen the miRNAs of the up and down regulated genes. The miRNAs predicted by at least two databases (among the following databases: DIANA-TarBase and miRTarBase) were diagnosed as the miRNAs of the target genes. Then, Cytoscape software was used to draw the target gene - miRNA regulatory network. The target gene - miRNA regulatory network for up regulated genes included 1867 nodes and 3735 edges (Fig. 11). As shown in Table 7, TRIM72 regulates 123 miRNAs (ex,hsa-mir-4537), TET3 regulates 105 miRNAs (ex,hsa-mir-3148), NFIB regulates 89 miRNAs (ex,hsa-mir-4517), SLC19A3 regulates 80 miRNAs (ex,hsa-mir-4500) and SMOC1 regulates 123 miRNAs (ex,hsa-mir-6133) were considered as target gene. We performed pathway and GO enrichment analysis of
these predicted target genes, which mainly enriched in muscle contraction, FOXA1 transcription factor network, intrinsic component of plasma membrane and biological adhesion. The target gene - miRNA regulatory network for down regulated genes included 2529 nodes and 10243 edges (Fig.12). As shown in Table 7, PPP1R15B regulates 168 miRNAs (ex, hsa-mir-7150), WEE1 regulates 167 miRNAs (ex, hsa-mir-3926), RPRD2 regulates 152 miRNAs (ex, hsa-mir-4452), LCOR regulates 146 miRNAs (ex, hsa-mir-4310) and SAR1A regulates 145 miRNAs (ex, hsa-mir-5698) were considered as target gene. We performed pathway and GO enrichment analysis of these predicted target genes, which mainly enriched in regulation of hydrolase activity, cell cycle, gene expression, nuclear chromatin and protein processing in endoplasmic reticulum.

**Construction of target gene - TF regulatory network**

NetworkAnalyst was applied to screen the TFs of the up and down regulated genes. The TFs predicted by database (ChEA database) was diagnosed as the TFs of the target genes. Then, Cytoscape software was used to draw the target gene - TF regulatory network. The target gene - TF regulatory network for up regulated genes included 539 nodes and 5790 edges (Fig. 13). As shown in Table 8, ACTL8 regulates 145 TFs (ex, EGR1), LHFPL3 regulates 132 TFs (ex, SOX2), CXCL12 regulates 119 TFs (ex, SUZ12), GLI2 regulates 117 TFs (ex, AR) and C7 regulates 114 TFs (ex, TP53) were considered as target gene. We performed pathway and GO enrichment analysis of these predicted target genes, which mainly enriched in epithelial cell differentiation, cytokine-cytokine receptor interaction, pathways in cancer and innate immune system. The target gene - TF regulatory network for down regulated genes included 608 nodes and 10262 edges (Fig. 14). As shown in Table 8, PRIM2 regulates 218 TFs (ex, SOX2), regulates 211 TFs (ex, MYC), GMDS regulates 210 TFs (ex, SPI1), C5ORF58 regulates 190 TFs (ex, RUNX1) and C10orf88 regulates 180 TFs (ex, FLI1) were considered as target gene. We performed pathway and GO enrichment analysis of these predicted target genes, which mainly enriched in metabolic pathways, gene expression, asparagine N-linked glycosylation and cell cycle.

**Validations of hub genes**

The ten hub genes (up and down regulated) were selected for further validation of their potential prognostic value. Upon comparing the expression of hub genes in the human protein atlas database (Fig. 15), it showed that CAPN13, ACTBL2, ERBB3, GATA4 and GNB4 were highly expressed in peripheral blood mononuclear cells of bone marrow, while NOTCH2, EXOSC10, RNF2, PSMA1 and PRKAA1 were less expressed in peripheral blood mononuclear cells of bone marrow. ROC analysis was performed from the 10 hub genes from GSE113079. The ROC curves of these ten hub genes all indicated favorable prognostic values for CAD. In addition, the area under ROC curve (AUC) of CAPN13, ACTBL2, ERBB3, GATA4, GNB4, NOTCH2, EXOSC10, RNF2, PSMA1 and PRKAA1 were 0.855 (p = 2.406664e-08), 0.923 (p = 5.413565e-10), 0.829 (p = 2.857413e-08), 0.903 (p = 4.513268e-09), 0.918 (p = 6.358925e-09), 0.891 (p = 4.367291e-09), 0.927 (p = 1.344048e-10), 0.911 (p = 5.076899e-10), 0.892 (3.057148e-08) and 0.904 (p = 1.902203e-08), respectively (Fig. 16).
Discussion

Currently, genetic and genomics related researches progress rapidly and provide new viewpoint to illuminate the molecular pathogenesis of CAD. And bioinformatics analysis also has show and devotes to search for candidate biomarkers to provide more precise screening, prompt diagnosis, sophisticated prognostic and new therapeutic targets for CAD based on massive genetic and genomics data [44]. In the present study, 1,036 DEGs were identified in the CAD group compared with normal control samples, including 525 up regulated genes and 511 down regulated gene. Genes such as PTGDS (prostaglandin D2 synthase) [45] and PDE4D [46] were responsible for development of stroke. Oncostatin M receptor (OSMR) was liable for progression of atherosclerosis [47]. Genes such as SLC19A3 [48] and RCN2 [49] were liable for progression of diabetes, but these genes may be responsible for advancement of CAD. Genes such as KLKB1 [50], PRMT5 [51], F2R [52] and IL18RAP [53] were liable for progression of CAD. AKAP5 was associated with progression of hypertension [54], but this gene may be identified with progression of CAD.

Some of the up regulated genes enriched in pathways from different pathway databases. DIO2 was linked with development of hypertension [55], but this gene may be responsible for progression CAD. Enriched genes such as CCR2 [56], CCL19 [57], CX3CL1 [58], CXCL12 [59], IL20 [60], epidermal growth factor receptor (EGFR) [61], ERBB3 [62], adrenomedullin (ADM) [63], SCUBE1 [64], LMAN1L [65] and EGFL7 [66] were responsible for pathogenesis of CAD. Enriched genes such as CXCL6 [67], BMP7 [68], RXFP2 [69], BR50 [70], FFAR3 [71], neuropeptide B (NPB) [72], SPON2 [73], FCN3 [74], REG3A [75] and ornithine carbamoyltransferase (OTC) [76] were culpable for pathogenesis of diabetes, but these genes may be involved in development of CAD. Enriched genes such as COL18A1 [77], cortistatin (CORT) [78], guanine nucleotide binding protein (G protein) [79] and MUC2 [80] were involved in development of obesity, but these genes may be associated with pathogenesis of CAD. Enriched genes such as ADRA1A [81], corticotropin releasing hormone (CRH) [82], CRHR1 [83], GRIN1 [84], HSD3B1 [85] and nerve growth factor (beta polypeptide) (NGF) [86] were answerable for progression of hypertension, but these genes may be linked with development of CAD. ADAMTS2 was associated with progression of myocardial infarction [87], but this gene may be liable for progression of CAD. CFC1 was responsible for development of congenital cardiac disease [88], but this gene may be associated with progression of CAD. Our study found that KIT ligand (KITLG), IFNA4, IL13RA1, IL17B, thrombopoietin (THPO), nuclear factor I/B (NFIB), TFF1, OPN4, OR1J1, OR2J2, ADCY2, ADCYAP1R1, OR4C3, OR5C1, OR3A3, RASAL1, VIPR2, OR8U1, SPTBN4, OR10AD1, OR10W1, AVPR1B, RGS22, SSTR4, PTGER1, glial cell derived neurotrophic factor (GDNF), OR5H1, OR2AG1, GPR31, kalirin, RhoGEF kinase (KALRN), GPR32, OR2AG2, OR5A2, OR2T6, OR10H1, GNB4, OR52K2, GPHB5, CLEC1B, CLEC2L, SEMA5B, COL9A2, MXRA5, COL2BA1, SEMA4G, COL23A1, insulin-like growth factor binding protein, acid labile subunit (IGFALS), CLEC4G, MUC22, MUC3A, MUC4, MEGF11, IL36B, ADAM21, SMOC1, FREM1, TINAGL1, SRPX2, ADAMTS1L, transforming growth factor, alpha (TGFA), eyes shut homolog (Drosophila) (EYS), INSL6, CLEC3B, tenascin XB (TNXB) and guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type (GNAL) are up regulated in CAD and has potential as a novel diagnostic and prognostic biomarker, and therapeutic target. Similarly, some of the down regulated genes enriched in pathways from different
pathway data bases. Enriched genes such as HSPA8 [89], HIF1A [90], CCL4 [91], CCL20 [92], IL1B [93], NCAM1 [94], IL18R1 [95], CXCL1 [96], CXCL2 [97], oncostatin M (OSM) [98], CD80 [99], IL27 [100] and lamin A/C (LMNA) [101] were liable for progression of CAD. Enriched genes such as KIR2DL2 [102], KIR3DL1 [103], KLRC3 [104], KLRD1 [105], PIK3R1 [106] and PAK2 [107] were involved in the progression of diabetes, but these genes may be linked with progression of CAD. MAP2K4 was liable for progression of ischemic stroke [108]. Enriched genes such as S1PR1 [109] and CUL3 [110] were involved in progression of hypertension, but these genes may be associated with development of CAD. RAR-related orphan receptor A (RORA) was linked with development of obesity [111], but these genes may be involved in pathogenesis of CAD. Our study found that 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC), KIR2DS2, KIR2DL4, KIR2DS3, KIR2DS4, KIR3DL2, KLRC1, regulatory factor X-associated protein (RFXAP), PDIA3, KIR2DL5A, WIPF1, FYN oncogene related to SRC, FGR, YES (FYN), Cas-Br-M (murine) ecotropic retroviral transforming sequence b (CBLB), platelet-derived growth factor receptor, beta polypeptide (PDGFRB), CCL3L3, IL1A, PSMA1, SPTBN1, PSME4, BRWD1, IDS (iduronate 2-sulfatase), PARP4, PARP2, LMNB1, 6-pyruvoyltetrahydropterin synthase (PTS) and sarcosine dehydrogenase (SARDH) are down regulated in CAD and has potential as a novel diagnostic and prognostic biomarker, and therapeutic target.

Some of the up regulated genes enriched in GO terms. Enriched genes such as noggin (NOG) [112], very low density lipoprotein receptor (VLDLR) [113] and AQP10 [114] were responsible for progression of obesity, but these genes may be involved in development of CAD. Enriched genes such as TRPM5 [115], crystallin, alpha A (CRYAA) [116], PAX6 [117], SORBS1 [118], SLC38A1 [119], complement component 7 (C7) [120] and PAX8 [121] were linked with advancement of diabetes, but these genes may be associated with pathogenesis of CAD. Enriched genes such as KCNJ11 [122], PKD2L1 [123], CSMD1 [124], SLC6A2 [125] and ATP2B3 [126] were liable for advancement of hypertension, but these genes may be involved in progression CAD. ASGR1 was linked with advancement of CAD [127]. Our study found that SHROOM4, SHISA6, amphiphysin (AMPH), TMPRSS11E, CRYBB3, crystallin, gamma A (CRYGA), BARHL1, IGSF9B, LYNX1, LRRN4, SHANK1, GRIK5, LCN1, GRK1, GRIP2, LY6H, B3GNT3, KCNK9, PROM2, TRPV6, FXYD1, FXYD3, SYT3, AQP8, SLC4A1, ATP1A4, CALHM3, TSPAN9, PROM1, protein tyrosine phosphatase, receptor type, S (PTPRS), KCNJ16, PCDHB8, TM4SF5, SLC17A5, trehalase (brush-border membrane glycoprotein) (TREH), basal cell adhesion molecule (Lutheran blood group) (BCAM), SLC4A11, EPHA8, ANTXR1, PLA2R1 and IL17REL are up regulated in CAD and has potential as a novel diagnostic and prognostic biomarker, and therapeutic target. Similarly, some of the down regulated genes enriched in GO terms. Enriched genes such as CDKN1C [128], NR4A1 [129] and ZNF627 [130] were responsible for progression of myocardial infarction, but these genes may be associated with development of CAD. Enriched genes such as PPP1R15A [131], protein kinase C, theta (PRKACQ) [132], LPIN1 [133], NOTCH2 [134], Shwachman-Bodian-Diamond syndrome (SBDS) [135], SOX13 [136] and FOXP4 [137] were culpable for advancement of diabetes, but these genes may be linked with progression of CAD. Enriched genes such as ABCB1 [138], CAMK2N1 [139], HES1 [140], TNFAIP3 [141], proliferating cell nuclear antigen (PCNA) [142], IKZF2 [143], ZNF208 [144], NRF1 [145], EGR3 [146] and SMAD7 [147] were important for progression of CAD. Filamin A (FLNA) was involved in development of hypertension [148], but this gene may be responsible for
progression of CAD. Enriched genes such as PHLDA1 [149], PLK2 [150], IER3 [151] and thymopoietin (TMPO) [152] were identified with development of ischemic cardiomyopathy, but these genes may be involved in progression of CAD. TOR1AIP1 was associated with heart failure [153]. Enriched genes such as CERS6 [154], KLF3 [155] and NUCKS1 [156] were responsible for advancement of obesity, but these genes may be involved in progression of CAD. cAMP responsive element modulator (CREM) was linked with progression of cardiac arrhythmia [157], but this gene may be important for development CAD. Our study found that CHAF1B, CCND1, STAG1, RPS6, TFD3, NCAPH2, HIR histone cell cycle regulation defective homolog A (S. cerevisiae) (HIRA), BOD1, CEP78, HACE1, SERTAD1, farnesyltransferase, CAAX box, alpha (FNTA), WEE1, TRIAP1, PKP4, ZNF365, Wilms tumor 1 associated protein (WTAP), CCCTC-binding factor (zinc finger protein) (CTCF), CSNK1A1, PRIM2, PRKAA1, SENP6, CDK5RAP1, tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase (TNKS), RIOK2, MYBL1, TJPL, ZNF268, ZNF207, SMARCAD1, SMC5, junction mediating and regulatory protein, p53 cofactor (JMY), CEPI20, ANGEL2, CAB39L, NCAPD3, destrin (actin depolymerizing factor) (DSTN), CLASP1, CHMP1B, KIF3A, RNF2, KHLHC2, OSBP1L8, ZBTB1, FAM169A, suppressor of cancer cell invasion (SCAI), IP3R3, YBX1, SYNE1, ZNF671, recombination signal binding protein for immunoglobulin kappa J region-like (RBPJL), ZNF595, TXK tyrosine kinase (TXK), MLLT1, ZNF548, THAP6, ZNF567, ZNF680, ZFP37, zinc finger protein, X-linked (ZFX), ZNF430, ZNF26, ZFP1, ZNF83, ZBTB11, ligand dependent nuclear receptor corepressor (LCOR), BNC2, SP2, ZNF148, thymocyte selection-associated high mobility group box (TOX), HOP homeobox (HOPX), ZNF25, ZNF730, ZFP82, NR1D2, retinoic acid receptor, beta (RARB), ZNF354A, IKZF5, ZBED6, ZNF197 and RAR-related orphan receptor B (RORB) are down regulated in CAD and has potential as a novel diagnostic and prognostic biomarker, and therapeutic target.

In the PPI network, hub genes with a high node degree distribution, betweenness centrality, stress centrality, closeness centrality and low clustering coefficient were selected. GATA4 was important for progression of CAD [158]. Genes such as MAGEL2 [159], ADHFE1 [160] and neuromedin B (NMB) [161] were associated with development of obesity, but these genes may be liable for progression of CAD. SMURF1 was liable for advancement of hypertension [162], but this may be involved in pathogenesis of CAD. In addition, modules were extracted from PPI network, which involved 17 up regulated genes and 20 down regulated genes. TBX2 was involved in the progression of hypertension [163], but this gene may be associated with development of CAD. Genes such as podocan (PODN) [164] and PAS domain containing serine/threonine kinase (PASK) [165] were liable for progression of diabetes, but these genes may be linked with progression of CAD. In the target gene - miRNA regulatory network, 5 up regulated genes and 5 down regulated genes with a high node degree was chosen as target gene. TRIM72 was associates with development of cardiac fibrosis [166], but this gene may be liable for development of CAD. TET3 was responsible for progression of CAD [167]. PPP1R15B was important for progression of diabetes [168], but this gene may be involved in advancement of CAD. CAPN13, ACTBL2, ACTL8, ras homolog gene family, member V (RHOV), CHD5, THNSL2, SLC38A8, serine palmitoyltransferase, small subunit B (SPTSSB), SPATA21, DLG3, SLC25A36, ACTG2, ACTL6B and RAS, EF-hand domain containing (RASEF), LHX9, FOXJ1, TP73, CDK5R2, EIF1AX, HNRNPA0, RPS27, LGR6, granzyme B (GZMB), RPRD2 and SAR1A are the novel biomarkers for CAD.
In the target gene - TF regulatory network, 5 up regulated genes and 5 down regulated genes with a high node degree was chosen as target gene. GLI2 was linked with progression of obesity [169], but this gene may be responsible for advancement of CAD. Our study found that LHFPL3 is up regulated in CAD and has potential as a novel diagnostic and prognostic biomarker, similarly, our study found that EXOSC10, GDP-mannose 4,6-dehydratase (GMDS), C5orf58 and C10orf88 are down regulated in CAD and has potential as a novel diagnostic and prognostic biomarker, and therapeutic target.

We used immune histochemical (IHC) analysis, receiver operating characteristic (ROC) curve and RT-PCR to analyze the association of 5 up and 5 down regulated hub genes expression in CAD. The results showed that only 5 up (CAPN13, ACTBL2, ERBB3, GATA4 and GNB4) and 5 down (NOTCH2, EXOSC10, RNF2, PSMA1 and PRKAA1) regulated hub genes were related to the CAD. We then evaluated the prognostic value of these only 5 up (CAPN13, ACTBL2, ERBB3, GATA4 and GNB4) and 5 down (NOTCH2, EXOSC10, RNF2, PSMA1 and PRKAA1) regulated hub genes using the ROC curve, indicating that they have potential diagnostic value for CAD.

In conclusion, 1,036 DEGs (525 up rand 511 down regulated gene) were screened out from the GSE113079 dataset, which may contain hub genes contributing to the pathogenesis of CAD. Through our bioinformatics analysis, hub genes including CAPN13, ACTBL2, ERBB3, GATA4, GNB4, NOTCH2, EXOSC10, RNF2, PSMA1 and PRKAA1 might contribute to the progression of CAD, which could serve as novel diagnostic and prognostic biomarkers and therapeutic targets for CAD.

Declarations

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Conflict of interest

The authors declare that they have no conflict of interest.

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.

Availability of data and materials
The datasets supporting the conclusions of this article are available in the GEO (Gene Expression Omnibus) (https://www.ncbi.nlm.nih.gov/geo/) repository. [(GSE113079) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE113079)]

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Author Contributions

V. K. - Methodology and validation

B. V. - Writing original draft, and review and editing

C. V. - Software and investigation

I. K. - Supervision and resources

A. T. – Software, and review and editing

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Tables

Due to technical limitations, table docx is only available as a download in the Supplemental Files section.

Figures
Figure 1

Box plots of the gene expression data before (A) and after normalization (B). Vertical axis represents the sample symbol and the horizontal axis represents the gene expression values. The black line in the box plot represents the median value of gene expression. (A1-A48 = healthy controls; B1-B93 = CAD patients)
Figure 2

Volcano plot of differentially expressed genes. Genes with a significant change of more than two-fold were selected.

Figure 3

Heat map of up regulated genes. Legend on the top left indicate log fold change of genes. (A1-A48 = healthy controls; B1-B93 = CAD patients)
Figure 4

Heat map of down regulated genes. Legend on the top left indicate log fold change of genes. (A1-A48 = healthy controls; B1-B93 = CAD patients)

Figure 5
Protein–protein interaction network of up regulated genes. Green nodes denotes up regulated genes.

Figure 6

Scatter plot for up regulated genes. (A- Node degree; B- Betweenness centrality; C- Stress centrality ; D- Closeness centrality; E- Clustering coefficient)

Figure 7

Protein–protein interaction network of down regulated genes. Red nodes denotes down regulated genes.
Figure 8

Scatter plot for down regulated genes. (A- Node degree; B- Betweenness centrality; C- Stress centrality ; D- Closeness centrality; E- Clustering coefficient)

Figure 9

Modules in PPI network. The green nodes denote the up regulated genes
Figure 10

Modules in PPI network. The red nodes denote the down regulated genes.

Figure 11

The network of up regulated genes and their related miRNAs. The green circles nodes are the up regulated genes, and chocolate color diamond nodes are the miRNAs.
Figure 12

The network of down regulated genes and their related miRNAs. The red circles nodes are the down regulated genes, and blue diamond nodes are the miRNAs.

Figure 13

TF - gene network of predicted target up regulated genes. (Lavender triangle - TFs and green circles - target up regulated genes)
Figure 14

TF - gene network of predicted target down regulated genes. (Blue triangle - TFs and red circles- target up regulated genes)

Figure 15

Immune histochemical analyses of hub genes were produced using the human protein atlas (HPA) online platform.
Figure 16

ROC curve validated the sensitivity, specificity of hub genes as a predictive biomarker for CAD prognosis. A) CAPN13 B) ACTBL2 C) ERBB3 D) GATA4 E) GNB4 F) NOTCH2 G) EXOSC10 H) RNF2 I) PSMA1 J) PRKAA1

Supplementary Files

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