In silico identification of common and specific signatures in coronary heart diseases

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Received January 3, 2020; Accepted April 15, 2020

DOI: 10.3892/etm.2020.9121

Abstract. Coronary heart disease (CHD) is on the increase in developing countries, where lifestyle choices such as smoking, bad diet, and no exercise contribute and increase the incidence of high blood pressure and high cholesterol levels to cause CHD. Through utilization of a biomarker-based approach for developing interventions, the aim of the study was to identify differentially expressed genes (DEGs) and their association and impact on various bio-targets. The microarray datasets of both healthy and CHD patients were analyzed to identify the DEGs and their interactions using Gene Ontology, PANTHER, Reactome, and STRING (for the possible associated genes with multiple targets). Our data mining approach suggests that the DEGs were associated with molecular functions, including protein binding (75%) and catalytic activity (56%); biological processes such as cellular process (83%), biological regulation (57%), and metabolic process (44%); and cellular components such as cell (65%) and organelle (58%); as well as other associations including apoptosis, inflammatory, cell development and metabolic pathways. The molecular functions were further analyzed, and protein binding in particular was analyzed using network analysis to determine whether there was a clear association with CHD and disease. The ingenuity pathway analysis revealed pathways related to cell cholesterol biosynthesis, the immune system including cytokinin signaling, in which, the understanding of DEGs is crucial to predict the advancement of preventive strategies. Results of the present study showed that, there is a need to validate the top DEGs to rule out their molecular mechanism in heart failure caused by CHD.

Introduction

Coronary heart disease (CHD), also known as coronary artery disease (CAD) is one of a group of diseases of the heart blood vessels affecting millions of individuals worldwide. According to the center for disease control (CDC) reports, each death out of four is related to heart diseases, leading to approximately 610,000 mortalities annually worldwide (1). Among the heart diseases, CAD is the most common, responsible for the death of 370,000 individuals annually worldwide (1). CAD occurs when the elasticity of arteries, as well as vein and vessel smoothing, become plaque in the inner wall, making them rigid and narrowed. This condition restricts the blood flow to the heart muscle, leading to oxygen starvation. The condition of plaque rupture leads to the heart failure or cardiac death (2).

Recently, there has been an increase in the incidence of CHD (also known as ischemic heart disease) in China (3). In addition, CHD has become the most common reason for death in middle and high-income countries (4). According to the data report by NHANES, CHD prevalence was higher in males than females across all ages (7.4 v/s 5.3%, respectively) (3). The American Heart Association explains ‘The important difference between sex and pathology’, clinical presentation and outcomes in CHD patients (5). Thus it is crucial to pay attention to sex disparities and subsequently to personalize treatment (6). Patients with CHD are also susceptible to more complicated clinical problems. Currently, the diagnosis and therapy of CHD is rare and costly as compared to coronary angiography, which is the most popular clinical management option (7). CHD is one of the leading causes of death, and markedly affects the immunity of the body, making it an economic burden worldwide (8,9). This is a complex disease involving multiple mechanisms and influenced by many risk factors, including physical activity, genetics, diet, and smoking (10,11). Recently, a genome-wide association study (GWAS) identified many candidate loci associated with CHD and myocardial infection (MI) (12-14). Although genetics play an important role, accounting for approximately 50% of CHD heritability, the exact mechanism and causative agent of CHD are not yet revealed clearly (15-17). In this regard, it is important to understand and address the candidate genome association in developing CHD.
Table I. Up - and down regulated genes with associated function in CHD.

| ID           | LogFC | Gene symbol | Gene ontology: Biological function                                                                 | Gene ontology: Cellular component | Gene ontology: Molecular function                                                                 |
|--------------|-------|-------------|----------------------------------------------------------------------------------------------------|----------------------------------|---------------------------------------------------------------------------------------------------|
| 11729172     | 3.963 | NR4A2       | Negative regulation of transcription from RNA polymerase II promoter                                 | Nucleus                          | Rna polymerase II regulatory region sequence-specific DNA binding                                  |
| 11716771     | 2.98  | LOC102724428| Negative regulation of transcription from RNA polymerase II promoter                                 | Intracellular                    | Nucleotide binding                                                                                |
| 11719898     | 3.593 | HBEGF       | MAPK CasCHDe receptor binding                                                                      | Extracellular region             | Epidermal growth factor                                                                          |
| 11718841     | 6.035 | CXCL8       | Angiogenesis                                                                                        | Extracellular region             | Cytokine activity                                                                                 |
| 11761272     | 2.91  | BCL2A1      | Apoptotic process                                                                                   | Cytoplasm                        | Protein binding                                                                                   |
| 11743972     | 2.917 | DDIT4       | Response to hypoxia                                                                                | Intracellular                    | 14-3-3 Protein binding                                                                            |
| 11732719     | 4.2   | EREG        | MAPK CasCHDe receptor binding                                                                      | Extracellular region             | Epidermal growth factor                                                                          |
| 11744219     | 6.925 | G0S2        | Apoptotic process                                                                                    | Mitochondrion                    | Protein binding                                                                                   |
| 11721695     | 3.385 | DUSP2       | Inactivation of MAPK activity                                                                       | Nucleus                          | Phosphoprotein phosphatase activity                                                                |
| 11742765     | 4.1   | RGS1        | Immune response                                                                                     | Cytoplasm                        | GTPase activator activity                                                                         |
| 11724037     | 5.573 | PTGS2       | Prostaglandin biosynthetic process                                                                 | Nucleus                          | Peroxidase activity                                                                               |
| 11746721     | 2.647 | TREM1       | Positive regulation of defense response to virus by host                                             | Extracellular region             | Receptor activity                                                                                  |
| 11759749     | 3.44  | KLF3        | Transcription, DNA-templated                                                                         | Nucleus                          | Nucleic acid binding                                                                              |
| 11744850     | 1.885 | SSH2        | Protein dephosphorylation                                                                           | Extracellular space              | DNA binding                                                                                        |
| 11743000     | 2.982 | CD83        | Regulation of cytokine production                                                                   | Plasma membrane                  | Protein binding                                                                                   |
| 11715931     | 3.478 | SGK1        | Regulation of cell growth                                                                           | Nucleus                          | Nucleotide binding                                                                                |
| 11724447     | 2.157 | PDE4D       | Regulation of heart rate                                                                            | Cytoplasm                        | Cyclic-nucleotide phosphodiesterase activity                                                       |
| 11737750     | 3.272 | SGK1        | Regulation of cell growth                                                                           | Nucleus                          | Nucleotide binding                                                                                |
| 11728190     | 2.058 | CXCR4       | Activation of MAPK activity                                                                          | Cytoplasm                        | Virus receptor activity                                                                            |
| 11743110     | 2.268 | NAMPT       | Vitamin metabolic process (carboxylating) activity                                                  | Extracellular region             | Nicotinate-nucleotide diphosphorylase                                                              |
| 11763170     | 2.103 | FOSL2       | Keratinocyte development sequence-specific DNA binding                                              | Nucleus                          | RNA polymerase II regulatory region                                                               |
| 11733698     | 3.143 | SGK1        | Regulation of cell growth                                                                           | Nucleus                          | Nucleotide binding                                                                                |
| 11744932     | 2.245 | CREB5F      | Negative regulation of transcription from RNA polymerase II promoter                                | Nucleus                          | Transcription factor activity, Sequence-specific DNA binding                                       |
| 11757721     | 2.265 | CSRNP1      | Transcription, DNA-templated                                                                        | Nucleus                          | Transcriptional activator activity, RNA polymerase II transcription regulatory region sequence-specific binding |
| ID       | LogFC | Gene symbol | Gene ontology: | Gene ontology: | Gene ontology: |
|----------|-------|-------------|----------------|----------------|----------------|
|          |       |             | Biological function | Cellular component | Molecular function |
| 11715766 | 2.552 | DUSP1       | Inactivation of MAPK activity | Nucleus | Phosphoprotein phosphatase activity |
| 11728191 | 2.465 | CXCR4       | Activation of MAPK activity | Cytoplasm | Virus receptor activity |
| 11719218 | 2.567 | SOCS3       | Response to hypoxia | Intracellular | Protein kinase inhibitor activity |
| 11739540 | 2.195 | PIK3R1      | Cellular glucose homeostasis | Nucleus | Transmembrane receptor protein tyrosine kinase adaptor activity |
| 11728189 | 2.54  | CXCR4       | Activation of MAPK activity | Cytoplasm | Virus receptor activity |
| 11743596 | 1.75  | PTPRE       | Protein dephosphorylation | Nucleus | Phosphoprotein phosphatase activity |
| 11717830 | 1.69  | TSC22D3     | Negative regulation of transcription from RNA polymerase II promoter | Nucleus | Transcription factor activity, sequence-specific DNA binding |
| 11752993 | 3.005 | DUSP1       | Inactivation of MAPK activity | Nucleus | Phosphoprotein phosphatase activity |
| 11717897 | 1.79  | PTP4A1      | Protein dephosphorylation | Nucleus | Phosphoprotein phosphatase activity |
| 11752039 | 1.468 | PHC3        | Multicellular organismal development | Nucleus | DNA Binding |
| 11756587 | 2.1   | PTGDS       | Prostaglandin biosynthetic process | Extracellular region | Prostaglandin-D synthase activity |
| 11723679 | 2.607 | CD69        | Signal transduction | Integral component of plasma membrane | Transmembrane signaling |
| 11718939 | 2.723 | TNFAIP3     | B-1 B cell homeostasis | Nucleus | Protease binding |
| 11736467 | 2.2   | TAGAP       | Signal transduction | Cytosol | Guanylnucleotide exchange factor activity |
| 11739094 | 2.712 | CXCR4       | Activation of MAPK activity | Cytoplasm | Virus receptor activity |
| 11763367 | 1.535 | NABP1       | Double-strand break repair via homologous recombination | Nucleus | DNA binding |
| 11743111 | 2.857 | NAMPT       | Vitamin metabolic process | Extracellular region | Nicotinate-nucleotide diphosphatase (Carboxylating) activity |
| 11715673 | 2.002 | JUNB        | Negative regulation of transcription from RNA polymerase II promoter | Chromatin | RNA polymerase II regulatory region |
| 11717994 | 2.812 | NR4A1       | Positive regulation of endothelial cell proliferation | Nucleus | DNA polymerase II core promoter proximal region sequence-specific binding |
| 11715691 | 2.379 | ZFP36       | Negative regulation of transcription from RNA polymerase II promoter | Nucleus | DNA binding |
| 11732045 | 2.29  | BTG1        | Regulation of transcription, DNA-templated | Nucleus | Transcription cofactor activity |
| 11724446 | 1.378 | PDE4D       | Regulation of heart rate | Cytoplasm | Cyclic-nucleotide phosphodiesterase activity |
| 11737176 | 2.052 | C9ORF72     | Endocytosis | Extracellular region | Protein binding |
| 11715487 | 1.405 | MCL1        | Cell fate determination | Intracellular | Protein binding |
| 11722615 | 2.275 | HCAR2 /// | Neutrophil apoptotic process | Plasma membrane | Signal transducer activity |
| ID       | LogFC | Gene symbol | Gene ontology: Biological function                                           | Gene ontology: Cellular component                  | Gene ontology: Molecular function                  |
|----------|-------|-------------|------------------------------------------------------------------------------|----------------------------------------------------|---------------------------------------------------|
| 11719862 | 2.093 | TREM1       | Positive regulation of defense response to virus by host                     | Extracellular region                                | Receptor activity                                  |
| 11734799 | 1.505 | RLIM        | Negative regulation of transcription from RNA polymerase II promoter          | Nucleus                                            | Transcription corepressor activity                 |
| 11763169 | 1.667 | FOSL2       | Keratinocyte development                                                     | Nucleus                                            | RNA polymerase II regulatory region sequence-specific DNA binding |
| 11718394 | 3.535 | JUN         | Angiogenesis                                                                  | Nucleus                                            | RNA polymerase II core promoter proximal region sequence-specific DNA binding |
| 11749652 | 1.69  | ZBTB21      | Transcription, DNA-templated                                                 | Nucleus                                            | RNA polymerase II regulatory region sequence-specific DNA binding |
| 11724038 | 4.715 | PTGS2       | Prostaglandin biosynthetic process                                            | Nucleus                                            | Peroxidase activity                                |
| 11753803 | 1.37  | CYCS        | Response to reactive oxygen species                                           | Protein phosphatase type 2A complex                |                                                   |
| 11725631 | 3.285 | NR4A2       | Negative regulation of transcription from RNA polymerase II promoter          | Nucleus                                            | RNA polymerase II regulatory region sequence-specific DNA binding |
| 11751242 | 1.28  | FCGR2A //FCGR2C | Immune system process                                                      | Cytoplasm                                          | Transmembrane signaling receptor activity          |
| 11721629 | 2.755 | MAFB        | Transcription, DNA-templated                                                 | Nucleus                                            | RNA polymerase II core promoter proximal region sequence-specific DNA binding |
| 11733355 | 1.655 | CSAR1       | Activation of MAPK activity                                                   | Cytosol                                            | Complement component C5a binding                  |
| 11759628 | 1.838 | WIPF1       | Actin cortical patch assembly                                                | Ruffle                                             |                                                   |
| 11726889 | 1.505 | ZFP36L1     | Nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay     | Nucleus                                            | DNA binding                                       |
| 11750700 | 1.375 | ACSL1       | Long-chain fatty acid metabolic process                                      | Mitochondrion                                      | Nucleotide binding                                |
| 11716602 | 1.895 | KBTBD2      | Protein ubiquitination                                                        | Cul3-RING ubiquitin ligase complex                 | Ubiquitin-protein transferase activity             |
| 11751415 | 1.688 | TSC22D3     | Negative regulation of transcription from RNA polymerase II promoter          | Nucleus                                            | Transcription factor activity, sequence-specific DNA binding |
| 11744775 | 1.497 | BZW1        | Transcription, DNA-templated                                                 | Cytoplasm                                          | Binding                                           |
| 11747736 | 1.2   | CNN1        | Regulation of smooth muscle contraction                                       | Cytoskeleton                                       | Actin binding                                     |
| 11727757 | 3.808 | OSM         | Positive regulation of acute inflammatory response                            | Extracellular region                                | Cytokine activity                                 |
| 11758730 | 1.39  | DUSP1       | Inactivation of MAPK activity                                                 | Nucleus                                            | Phosphoprotein phosphatase activity                |
| 11744810 | 1.312 | ZBTB24      | Hematopoietic progenitor cell differentiation                                 | Nucleus                                            | Nucleic acid binding                              |
| 11737147 | 1.357 | CLEC7A      | Response to yeast                                                            | Nucleoplasm                                        | Opsonin Binding                                   |
| ID       | LogFC | Gene symbol | Gene ontology: Biological function                      | Gene ontology: Cellular component | Gene ontology: Molecular function                        |
|----------|--------|-------------|----------------------------------------------------------|----------------------------------|----------------------------------------------------------|
| 11719713 | 1.662  | PPM1B       | Protein dephosphorylation                                | Cytoplasm                        | Magnesium ion binding                                     |
| 11715445 | 1.248  | DNAJB1      | Protein folding                                         | Nucleus                           | Actin binding                                             |
| 11720062 | 2.98   | IER3        | Response to protozoan                                    | Nucleus                           | Protein binding                                           |
| 11757513 | 2.262  | NFKBIZ      | Transcription, DNA-templated                            | Nucleus                           | Transcription cofactor activity                           |
| 11758522 | 1.92   | CREM        | Glucose metabolic process                                | Nucleus                           | Core promoter sequence-specific DNA binding               |
| 11724835 | 1.355  | HCAR2///HCAR3| Neutrophil apoptotic process                             | Plasma membrane                   | Signal transducer activity                                |
| 11762406 | 1.695  | GBP2        | Immune response                                         | Golgi membrane                    | Nucleotide binding                                       |
| 11752577 | 1.438  | FTH1        | Iron ion transport                                      | Cell                              | Ferroxidase activity                                     |
| 11744128 | 5.325  | CXCL2       | Response to molecule of bacterial origin                 | Extracellular region              | Cytokine activity                                        |
| 11760678 | 1.337  | PPIL2       | Protein polyubiquitination                               | Nucleus                           | Peptidyl-prolyl cis-trans isomerase activity             |
| 11727569 | 1.328  | OTULIN      | Angiogenesis                                             | Cytoplasm                         | Ubiquitin-specific protease activity                      |
| 11719916 | 4.9    | IL1B        | Negative regulation of transcription                     | Extracellular region              | Receptor binding                                         |
| 1175817  | 1.18   | ZFP36L2     | Nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay | Nucleus                           | DNA binding                                               |
| 11743434 | 1.4    | CHST11      | Chondrocyte development                                 | Golgi membrane                    | N-acetylglactosamine                                      |
| 11724236 | 1.272  | RIPK2       | Activation of MAPK activity                              | Cytoplasm                         | 4-O-sulfotransferase activity                             |
| 11720745 | 1.815  | BCL6        | Protein import into nucleus, translocation              | Nucleus                           | Nucleotide binding                                       |
| 11749291 | 1.75   | FOS         | Conditioned taste aversion                              | Nucleus                           | RNA polymerase II core promoter proximal region sequence-specific DNA binding |
| 11764029 | 1.58   | CEBPD       | Transcription from RNA polymerase II promoter            | Nucleus                           | RNA polymerase II core promoter proximal region sequence-specific DNA binding |
| 11718347 | 1.585  | S100P       | Response to organic substance                           | Nucleus                           | Magnesium ion binding                                     |
| 11724509 | 2.365  | PMAIP1      | Release of cytochrome c from mitochondria               | Nucleus                           | Protein binding                                           |
| 11734690 | 1.252  | CYTIP       | Regulation of cell adhesion                             | Nucleoplasm                       | Protein binding                                           |
| 11736782 | 1.395  | RAB11FIP1   | Transport                                               | Cytoplasm                         | Protein binding                                           |
| 11764030 | 1.27   | CEBPD       | Transcription from RNA polymerase II promoter            | Nucleus                           | RNA polymerase II core promoter proximal region sequence-specific DNA binding |
| 11743344 | 1.67   | RMND5A      | Protein phosphorylation                                 | Nucleus                           | Protein binding                                           |
| 11716048 | 2.357  | TRIB1       | Protein phosphorylation                                 | Nucleus                           | Protein kinase activity                                   |
| 11756387 | 1.558  | ARL4A       | Intracellular protein transport                          | Intracellular                     | Nucleotide binding                                       |
| ID         | LogFC | Gene symbol | Gene ontology: Biological function | Gene ontology: Cellular component | Gene ontology: Molecular function |
|------------|-------|-------------|------------------------------------|----------------------------------|----------------------------------|
| 11724510   | 2.298 | PMAIP1      | Release of cytochrome c from mitochondria | Nucleus                          | Protein binding                  |
| 11732665   | 1.782 | VSTM1       | Immune system process               | Extracellular region             | Cytokine activity                |
| 11759780   | 1.348 | ANKRD13C    | Protein retention in ER lumen       | Endoplasmic reticulum            | Receptor binding                 |
| 11737148   | 1.333 | CLEC7A      | Response to yeast                   | Nucleoplasm                      | Opsonin binding                  |
| 11758593   | 1.325 | H3F3B       | Chromatin silencing at rDNA         | Nuclear chromosome               | RNA polymerase II core promoter   |
|            |       |             |                                    |                                  | sequence-specific DNA binding    |
| 11763972   | 1.2   | SSR1        | Translation                         | Endoplasmic reticulum            | Protein binding                  |
| 11727523   | 1.36  | ZNF267      | Transcription, DNA-templated        | Intracellular                     | Nucleic acid binding             |
| 11718395   | 4.04  | JUN         | Angiogenesis                        | Nuclear chromosome               | RNA polymerase II core promoter   |
|            |       |             |                                    |                                  | proximal region sequence-specific DNA binding |
| 11727032   | -2.47 | NSG1        | Positive regulation of transcription from RNA polymerase II promoter | Nucleus                          | Calcium ion binding              |
| 11718061   | 1.88  | PVALB       | Cytosolic calcium ion homeostasis   | Nucleus                          | RNA polymerase II core promoter   |
| 11721630   | 2.138 | MAFB        | Transcription, DNA-templated        | Nucleus                          | proximal region sequence-specific DNA binding |
| 11763755   | 1.068 | GNLY        | Cellular defense response           | Extracellular region             | Gtpase activator activity        |
| 11757924   | 1.542 | SIPA1L2     | Positive regulation of gtpase activity | Dynein complex                   | Microtubule motor activity       |
| 11732859   | 1.03  | DNHD1       | Microtubule-based movement          | Nucleus                          | RNA polymerase II core promoter   |
| 11750016   | 1.498 | MXD1        | Negative regulation of transcription from RNA polymerase II promoter | Nucleus                          | proximal region sequence-specific DNA binding |
| 11744127   | 4.107 | CXCL2       | Response to molecule of bacterial origin | Extracellular region             | Cytokine activity                |
| 11763556   | 1.777 | EIF4A1      | Nuclear-transcribed mrna catabolic process, deadenylation-dependent decay | Nucleus                          | Nucleotide binding               |
| 11745466   | 1.252 | CDADC1      | Metabolic process                   | Chromatin                        | Catalytic activity               |
| 11756358   | 1.87  | PLK3        | G1                                  | Nucleotide binding               |                                 |
| 11718397   | 3.16  | JUN         | Angiogenesis                        | Nuclear chromosome               | RNA polymerase II core promoter   |
|            |       |             |                                    |                                  | proximal region sequence-specific DNA binding |
| 11763954   | 1.04  | SCARNA9L    | Negative regulation of transcription from RNA polymerase II promoter | Cytoplasmic mrna processing body | Poly(A)-specific ribonuclease activity |
| 11730655   | 1.12  | CNOT1       |                                     |                                  |                                 |
| ID       | LogFC | Gene symbol | Gene ontology: Biological function                                                                 | Gene ontology: Cellular component | Gene ontology: Molecular function                                                                 |
|----------|-------|-------------|------------------------------------------------------------------------------------------------------|-----------------------------------|-----------------------------------------------------------------------------------------------------|
| 11754074 | 4.195 | G0S2        | Apoptotic process                                                                                    | Mitochondrion                     | Protein binding                                                                                     |
| 11739230 | 1.132 | ARL4A       | Intracellular protein transport                                                                       | Intracellular                     | Nucleotide binding                                                                                    |
| 11743010 | 2.372 | NFL3        | Negative regulation of transcription from RNA polymerase II promoter                                   | Nucleus                           | RNA polymerase II regulatory region sequence-specific DNA binding                                    |
| 11724036 | 4.27  | PTG52       | Prostaglandin biosynthetic process                                                                   | Nucleus                           | Peroxidase activity                                                                                  |
| 11747474 | 1.64  | NR4A2       | Negative regulation of transcription from RNA polymerase II promoter                                   | Nucleus                           | RNA polymerase II regulatory region sequence-specific DNA binding                                    |
| 11740892 | 1     | KCNK7       | Transport                                                                                           | Plasma membrane                   | Voltage-gated ion channel activity                                                                  |
| 11733140 | 1.617 | ARL4A       | Intracellular protein transport                                                                       | Intracellular                     | Nucleotide binding                                                                                    |
| 11724769 | 0.978 | FCGR2A ///  | Immune system process                                                                                 | Cytoplasm                         | Transmembrane signaling receptor activity                                                           |
| 11760192 | 0.987 | TMEM68      | Metabolic process                                                                                    | Membrane                          | Transferase activity, transferring acyl groups                                                       |
| 11734988 | 1.285 | FEM1B       | Epithelial cell maturation                                                                           | Nucleus                           | Ubiquitin-protein transferase activity                                                              |
| 11757798 | 2.205 | MAFB        | Transcription, DNA-templated                                                                         | Nucleus                           | RNA polymerase II core promoter proximal region sequence-specific DNA binding                        |
| 11740347 | 1.2   | NRG1        | MAPK casCHDe                                                                                         | Extracellular region              | Transcription cofactor activity                                                                    |
| 11753791 | 2.535 | PRKAR1A     | Mesoderm formation                                                                                    | Immunological synapse             | Nucleotide binding                                                                                    |
| 11725114 | 1.45  | ANKHD1      | Regulation of translation                                                                             | Nucleoplasm                       | Nucleic acid binding                                                                                 |
| 11734659 | 1.54  | FOS         | Conditioned taste aversion                                                                           | Nucleus                           | RNA polymerase II core promoter proximal region sequence-specific DNA binding                        |
| 11740892 | 1.2   | PIM3        | Protein phosphorylation                                                                              | Cytoplasm                         | Nucleotide binding                                                                                    |
| 11720612 | 1.04  | NAP1L5      | Nucleosome assembly                                                                                   | Nucleus                           | Protein binding                                                                                      |
| 11755987 | 0.98  | ANKRD44     |                                                                                                      |                                   | Phosphoprotein phosphatase activity                                                                |
| 11717895 | 1.285 | PTP4A1      | Protein dephosphorylation                                                                            | Nucleus                           | Protein binding                                                                                      |
| 11725199 | 1.525 | BTBD7       | Multicellular organismal development                                                                  | Nucleus                           | Protein binding                                                                                      |
| 11746681 | 1.88  | VNN3        | Nitrogen compound metabolic process                                                                   | Extracellular space               | Hydrolase activity                                                                                   |
| 11720726 | 1.413 | UBR1        | Ubiquitin-dependent protein catabolic process                                                         | Ubiquitin ligase complex          | Ubiquitin-protein transferase activity                                                              |
| 11760818 | 1.05  | CDKL3       | Cellular protein modification process                                                                 | Cytoplasm                         | Nucleotide binding                                                                                    |
| 11733686 | 1.052 | STRA6       | Retinoid metabolic process                                                                            | Plasma membrane                   | Receptor activity                                                                                   |
| 11724768 | 0.958 | FCGR2A ///  | Immune system process                                                                                 | Cytoplasm                         | Transmembrane signaling receptor activity                                                           |
| ID        | LogFC | Gene symbol | Gene ontology: Biological function                          | Gene ontology: Cellular component | Gene ontology: Molecular function                                      |
|-----------|-------|-------------|-------------------------------------------------------------|-----------------------------------|------------------------------------------------------------------------|
| 11748516  | 0.932 | NAP1L5      | Nucleosome assembly                                         | Nucleus                           | RNA polymerase II regulatory region sequence-specific DNA binding       |
| 11718927  | 0.975 | ARID5B      | Negative regulation of transcription from RNA polymerase II promoter | Nucleus                           | Transcription factor activity, sequence-specific DNA binding            |
| 11716195  | 2.692 | ID1         | Negative regulation of transcription from RNA polymerase II promoter | Nucleus                           |                                                                        |
| B, Down regulation                              |                                               |                                                              |                                   |                                                                        |
| 11720657  | -3.328 | HLA-DRB5   | Immune system process                                       | Golgi membrane                    | Protein binding                                                        |
| 11724843  | -2.185 | CISH        | Regulation of cell growth                                    | Cytoplasm                         | protein kinase inhibitor activity                                       |
| 11762593  | -2.075 | NUMA1       | G2                                                           |                                   | Structural molecule activity                                            |
| 11742832  | -1.783 | ASPM        | Neuron migration                                             | Spindle pole                       | Binding                                                                |
| 11758261  | -2.223 | CEP55       | Mitotic cytokinesis                                         | Cytoplasm                         | Protein binding                                                        |
| 11758089  | -1.885 | HMMR        | Carbohydrate metabolic process                               | Cytoplasm                         | Protein binding                                                        |
| 11721145  | -1.542 | MKI67       | DNA metabolic process                                        | Chromosome, centromeric region     | Nucleotide binding                                                      |
| 11743423  | -2.188 | NSG1        | Positive regulation of receptor recycling                   | Golgi membrane                    | Receptor binding                                                       |
| 11736674  | -1.588 | KLHL35      |                                                              |                                   | Protein binding                                                        |
| 11759710  | -1.417 | TXNDC9      | Cell redox homeostasis                                       | Cell                              | Protein binding                                                        |
| 11735385  | -1.752 | DACT1       | Negative regulation of transcription from RNA polymerase II promoter | Nucleus                           | Protein kinase C binding                                                |
| 11748198  | -1.55  | NSG1        | Positive regulation of receptor recycling                   | Golgi membrane                    | Receptor binding                                                       |
| 11732363  | -1.775 | ZNF2        | Transcription, DNA-templated                                 | Intracellular                      | Methyltransferase activity                                              |
| 11741074  | -1.407 | METTL18     | Methylation                                                  |                                   | Phosphoprotein phosphatase activity                                    |
| 11722367  | -1.55  | DLGAP5      | Protein dephosphorylation                                    | Nucleus                           |                                                                        |
| 11751805  | -1.83  | TYMS        | G1                                                          | Nucleus                           | Nucleotide binding                                                      |
| 11764270  | -1.498 | PLGLB1 /// PLGLB2 |                                               | Extracellular region                |                                                                        |
| 11747230  | -1.518 | BUB1        | Mitotic cell cycle                                           | Chromosome, centromeric region     | Nucleotide binding                                                      |
| 11723209  | -1.732 | KBTBD6      | Protein ubiquitination                                       | Cul3-RING ubiquitin ligase complex | Ubiquitin–protein transferase activity                                  |
| 11732390  | -1.465 | CCR9        | Chemotaxis                                                  | Cytosol                           | Signal transducer activity                                              |
| 11716666  | -1.623 | ID3         | Negative regulation of transcription from RNA polymerase II promoter | Nucleus                           | Transcription factor activity, sequence-specific DNA binding            |
Table I. Continued.

| ID          | LogFC | Gene symbol | Gene ontology: Biological function | Gene ontology: Cellular component | Gene ontology: Molecular function |
|-------------|-------|-------------|------------------------------------|----------------------------------|----------------------------------|
| 11763252    | -1.705| PSPH        | Protein dephosphorylation          | Cytoplasm                        | Magnesium ion binding            |
| 11720240    | -1.307| TMSB15A     | Actin filament organization        | Cytoplasm                        | Actin binding                    |
| 11716793    | -1.518| CCNB2       | G2                                 | Nucleus                          | Protein binding                  |
| 11717163    | -1.375| CDC20       | Mitotic cell cycle                 | Spindle pole                     | Protein binding                  |
| 11716427    | -1.71  | POMC        | Generation of precursor metabolites and energy | Extracellular region | G-protein coupled receptor binding |
| 11755958    | -1.4   | ZNF691      | Regulation of transcription, DNA-templated | Nucleus                        | RNA polymerase II regulatory region sequence-specific DNA binding |
| 11724022    | -2.403| TRIM13      | Signal transduction               | Intracellular                     | Ubiquitin-protein transferase activity |
| 11730821    | -1.295| CDKN3       | Regulation of cyclin-dependent protein serine | Nucleus                        | Phosphoprotein phosphatase activity |
| 11726302    | -1.245| DTL         | Protein polyubiquitination         | Nucleus                          | Ubiquitin-protein transferase activity |
| 11744793    | -1.44  | DLGAP5      | Protein dephosphorylation          | Nucleus                          | Phosphoprotein phosphatase activity |
| 11718599    | -1.502| TM2D2       |                                      | Membrane                         |                                  |
| 11760734    | -1.272| GULP1       | Transport                          | Cytoplasm                        | Signal transducer activity        |
| 11718058    | -1.782| TYMS        | G1                                 | Nucleus                          | Nucleotide binding               |
| 11734748    | -1.245| LOC100507547| Response to biotic stimulus       | Plasma membrane                   |                                  |
| 11730796    | -1.165| PSPH        | Protein dephosphorylation          | Cytoplasm                        | Magnesium ion binding            |
| 11727968    | -1.148| ESCO2       | Mitotic cell cycle                 | Chromatin                        | Lysine N-acetyltransferase activity, acting on acetyl phosphate as donor |
| 11758219    | -1.48  | RRM2        | G1                                 | Nucleus                          | Ribonucleoside-diphosphate reductase activity thioredoxin disulfide as acceptor |
| 11755381    | -1.73  | PLGLA /// PLGLB1 /// PLGLB2 |                                      | Extracellular region |                                  |
| 11762018    | -1.635| DCLRE1C     | Nucleotide-excision repair,        | Nuclear chromosome, telomeric region DNA damage recognition | Single-stranded DNA |
| 11734263    | -1.97  | ZNF780A     | Transcription, DNA-templated       | Intracellular                     | Nucleic acid binding             |
| 11733149    | -1.62  | DDX58       | Positive regulation of defense response to virus by host | Cytoplasm                        | Nucleotide binding               |
| 11754360    | -1.762| RRM2        | G1                                 | Nucleus                          | Ribonucleoside-diphosphate reductase activity, thioredoxin disulfide as acceptor |
| 11758872    | -2.118| CDC37L1     | Protein folding                    | Cytoplasm                        | Protein binding                  |
| 11728830    | -1.47  | RAB3IP      | Protein targeting to membrane      | Nucleus                          | Guanylyl-nucleotide exchange factor activity |
Table I. Continued.

| ID            | LogFC | Gene symbol | Gene ontology: Biological function                                      | Gene ontology: Cellular component | Gene ontology: Molecular function                                      |
|---------------|-------|-------------|-------------------------------------------------------------------------|-----------------------------------|-------------------------------------------------------------------------|
| 11759287      | -1.37 | DNAJB4      | Protein folding                                                         | Nucleoplasm                       | Protein binding                                                          |
| 1172130009    | -1.157| ZNF174      | Negative regulation of transcription from RNA polymerase II promoter    | Nucleus                           | RNA polymerase II transcription factor activity, sequence-specific DNA binding |
| 11756778      | -1.195| NEFL        | MAPK casCHDe                                                            | Cytoplasm                         | Structural molecule activity                                             |
| 11740504      | -1.218| ZNF680      | Transcription, DNA-templated                                            | Intracellular                     | RNA polymerase II core promoter proximal region sequence-specific DNA binding |
| 11758615      | -1.177| FRMD4B      | Establishment of epithelial cell polarity                               | Ruffle                            | Protein binding                                                          |
| 11738883      | -1.055| TNFSF14     | Apoptotic process                                                       | Extracellular region               | Receptor binding                                                         |
| 11726443      | -1.183| KCTD6       | Protein homooligomerization                                             | Plasma membrane                   | Protein binding                                                          |
| 11727112      | -1.397| SIT1        | Adaptive immune response                                                | Extracellular membrane            | Receptor activity                                                        |
| 11717301      | -1.218| TACSTD2     | Cell cycle                                                              | Extracellular space               |                                                                           |
| 11734218      | -1.38  | ZNF681      | Positive regulation of defense response to virus by host                | Intracellular                     | Nucleic acid binding                                                     |
| 11728339      | -1.667| CENPBD1     | DNA damage checkpoint                                                   | Nucleus                           | DNA binding                                                              |
| 11764234      | -1.865| INTS7       | DNA damage checkpoint                                                   | Nucleus                           | Binding                                                                  |
| 11721932      | -1.09  | KIF23       | Mitotic spindle elongation                                              | Nucleus                           | Nucleotide binding                                                       |
| 11753763      | -1.18  | CDKN3       | Regulation of cyclin-dependent protein serine                           | Nucleus                           | Phosphoprotein phosphatase activity                                       |
| 11721190      | -1.312| TTC9C       | Protein peptidyl-prolyl isomerization                                   | Cytoplasm                         | Peptidyl-prolyl cis-trans isomerase activity                              |
| 11721418      | -1.2   | SH3RF3      |                                                                          |                                   |                                                                         |
| 11733069      | -1.825| WDR5B       |                                                                          |                                   |                                                                         |
| 11730969      | -1.765| THAP2       |                                                                          |                                   |                                                                         |
| 11719780      | -1.728| TNFAIP8L2   |                                                                          |                                   |                                                                         |
| 11758125      | -1.32  | DEPDC1B     |                                                                          |                                   |                                                                         |
| 11733695      | -1.055| UBE2C       |                                                                          |                                   |                                                                         |
| 11756100      | -1.227| TMEM60      |                                                                          |                                   |                                                                         |
| 11732295      | -1.455| ZNF566      |                                                                          |                                   |                                                                         |
| 11724984      | -1.255| EXPH5       |                                                                          |                                   |                                                                         |
| 11726757      | -1.165| CDC25A      |                                                                          |                                   |                                                                         |
| 11727604      | -1.278| EPB41L4A    |                                                                          |                                   |                                                                         |
| 11716226      | -1.147| LIMA1       |                                                                          |                                   |                                                                         |
| ID   | LogFC | Gene symbol | Gene ontology: Biological function | Gene ontology: Cellular component | Gene ontology: Molecular function |
|------|-------|-------------|-----------------------------------|----------------------------------|---------------------------------|
| 11760155 | -1.105 | FUBP1       | Transcription, DNA-templated      | Nucleus                          | Transcriptional activator activity, RNA polymerase II distal enhancer sequence-specific binding |
| 11725833 | -1.087 | ALKBH1      | In utero embryonic development    | Nucleus                          | Catalytic activity              |
| 11723939 | -1.115 | CCNB1       | G1                               | Spindle pole                     | Patched binding                 |
| 11722160 | -1.218 | GRB10       | Signal transduction              | Cytoplasm                        | Sh3                             |
| 11759488 | -1.133 | EYA3        | DNA repair                       | Nucleus                          | Chromatin binding               |
| 11739828 | -1.188 | CYS1        | Kidney development               | Cytoplasm                        | Protein binding                 |
| 11740637 | -1.258 | GPR19       | Signal transduction              | Plasma membrane                  | Signal transducer activity      |
| 11717629 | -1.82  | KIF1BP      | Mitochondrial transport          | Cytoplasm                        | Protein binding                 |
| 11753965 | -1.15  | MSL3P1      | Transcription, DNA-templated      | Nucleus                          |                                 |
| 11758149 | -0.975 | RACGAP1     | Mitotic cytokinesis              | Acrosomal vesicle                | Gtpase activator activity       |
| 11732175 | -1.44  | FANCF       | Ovarian follicle development      | Nucleus                          | Ubiquitin-protein transferase activity |
| 11750856 | -1.062 | CCR2        | Blood vessel remodeling          | Cytoplasm                        | Signal transducer activity      |
| 11757036 | -1.278 | SAC3D1      | Cell cycle                       | Nucleus                          | Protein binding                 |
| 11758529 | -1.192 | CENPA       | Establishment of mitotic spindle orientation | Chromosome, centromeric region | DNA binding                      |
| 11732544 | -1.292 | GPR18       | Signal transduction              | Plasma membrane                  | Signal transducer activity      |
| 11718213 | -1.118 | SLC27A2     | Very long-chain fatty acid metabolic process | Mitochondrion | Nucleotide binding               |
| 11725709 | -0.968 | WDHD1       | RNA processing                   | Chromosome, centromeric region   | DNA binding                      |
| 11739531 | -1.598 | PLGLB2      | Extracellular                     |                                 |                                 |
| 11745077 | -1.282 | CRNKLI      | Spliceosomal complex assembly    | Prp19 complex                    | RNA binding                      |
| 11730590 | -1.198 | KCTD21      | Protein homologimerization       |                                 | Protein binding                 |
| 11727196 | -1.37  | ZNF202      | Negative regulation of transcription from RNA polymerase II promoter | Intracellular | RNA polymerase II transcription factor activity, sequence-specific DNA binding |
| 11731676 | -1.567 | CCR2        | Blood vessel remodeling          | Cytoplasm                        | Signal transducer activity      |
| 11721473 | -1.388 | HCCS        | Metabolic process                | Mitochondrion                    | Holocytochrome-c synthase activity |
| 11737395 | -1.292 | SOWAHD      |                                 |                                 | Protein binding                 |
| 11737108 | -1.05  | ACKR4       | Receptor-mediated endocytosis    | Endosome                         | Signal transducer activity      |
| 11728404 | -1.113 | SHCBP1      | Fibroblast growth factor receptor signaling pathway |                                 | Protein binding                 |
| 11760278 | -0.968 | HCG8 /// ZNRD1-AS1 |                                 |                                 |                                 |
| 11728360 | -1.655 | BCDIN3D     | RNA methylation                  | Nucleus                          |                                 |
| 11743686 | -1.005 | ZNF436      | Transcription, DNA-templated     | Intracellular                    | Nucleic acid binding            |
| 11762571 | -1.425 | GNPDA2      | Carbohydrate metabolic process   | Nucleus                          | Glucosamine-6-phosphate deaminase activity |
MicroRNAs (miRNA) are small noncoding RNAs with a length of 22-25 nucleotide and which play a key role in the regulation of gene expression and have implications in many human disorders (18), including many biological processes such as cell differentiation, proliferation and apoptosis (19-21). To the best of the knowledge of the authors, the association pattern of miRNAs to CHD is lacking, leading to demand for specific CHD patients. Although relevant research has been undertaken to address DEGs associated with CHD, DEGs have only been used to check the expression pattern in case of CHD. In this study, we addressed the possible association of genes with CHD, which may be useful for the diagnosis and treatment of this disease in the near future. Additionally, analysis of gene expression data and network analysis were performed to gain a better understanding of CHD for the identification of differentially expressed genes (DEGs), biomarkers and therapeutic target options.

Materials and methods

Data availability. To identify key genes for the development of CHD biomarkers, we used gene expression datasets of 4 angiographically proven patients who were being treated for more than 3 months or from group-1 (n=100) compared to healthy control (n=50). This dataset was downloaded from the GEO module of National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE56885). Microarray gene expression profiles were downloaded and further analyzed for the identification of DEGs. In this dataset, GSM1370681 and GSM1370682 represent the replicate samples of healthy individuals and GSM1370683 to GSM1370686 of four patients as baseline associated with CAD.

Differential Expression Analysis (DEG). Using the default parameters, WEGO 2.0, and GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) were used to analyze the GEO series (22). The Benjamini and Hochberg false discovery rate method was utilized to adjust the P-values. NCBI-generated annotations were employed to display the DEG list by comparing the overall common gene expression pattern as compared to the control. On the basis of this analysis, possible associations related to CHD were reported. Although inappropriate to consider the data for analysis on inter-datasets, the average value of LogFC for all four datasets was assessed to represent the expression level.

Gene ontology (GO) analysis. The major bioinformatics tool GO was used as an initiative to understand the function of genes and gene products of Homo sapiens. The PANTHER (Protein ANalysis THrough Evolutionary Relationships) classification database (23) was used to perform the GO analysis, and the pathway analysis was performed using Reactome (24).

Protein-Protein Interaction (PPI) network construction analysis. An online freely available software package, STRING, was utilized to establish the PPI network (25), and all the cut-off points were combined to analyze the topology property of networks. Gene edges of >15 degrees were defined as hub genes.

Results and Discussion

Screening of differentially expressed common genes from microarray data sets. Atherosclerosis is one of the leading causes of cardiovascular diseases such as CHD (26). Understanding of the key players in expression, regulation and function, of GWAS CHD genes will provide the options to treat this disease, leading to further developments of novel therapeutic interventions (27). In this study, the first compressive investigation was conducted to identify the expression profile of collected microarray data sets of CHD. The dataset of two controls in replicate and four baseline test samples were used. We report an overall expression and function of genes associated with different biological processes, which may lead to CHD during pathological conditions. The overall study design is shown in Fig. 1, which presents CHD data of Homo sapiens from the GEO database, with four series of test samples and two control study sets. First, we used WEGO to visualize the GO annotations and the percentage of genetic association of different functions in cells to address the possible association with CHD.

A total of 52,998 genes sharing different functions such as cellular (18,476), biological (17,307) and molecular (17,215) functions were identified. Out of those, the highest gene association to cell, cell part, organelle, organelle part, membrane, binding, cellular process, biological regulation, and metabolic were topmost in the metadata of the CHD-associated data set (Fig. 2). The different GO representing the 0-90% range of gene expression as compared to control data set is shown in Fig. 3. The principal findings of this study confirm the association of immune system, inflammation, and apoptosis as mediators in the development of CHD. The impact of the immune system plays a key role in the development of heart failure. A transcriptomic study reported the sustained activation of the adaptive immune system which may be a contributing factor.
in the progression of CHD (28). Another report suggests that the imbalance in inflammatory and anti-inflammatory cytokines may lead to the onset of extensive fibrosis (29).

*From the GEO database, accession GSE56885 of CSD patients, who were being treated for more than 3 months was selected.* From the included patients, two controls in replicate and four overall test samples were used to consolidate data refining. The GEO2R was used to analyze the control and test data series by normalizing the microarray data for high quality. DEGs with different fold change confirm their crucial role in CHD (Table I).

In our study, many immune response processes were significantly changed and DEGs are associated with the metabolic process, which is associated with CHD. The CHDs of the innate immune system were largely mediated through neutrophils and monocyte, and macrophages (30), to contribute to the process of the chronic inflammation process.

*Functional enrichment and unified DEG analysis.* To precisely understand the gene changes during CHD, the DEGs GO was performed using the online PANTHER database for high-throughput analysis to classify the proteins and their genes into family and subfamily, molecular function, biological process, and pathway (31). In the dataset analyzed, the two significant changes in molecular function were protein binding (75%) and catalytic activity (56%), followed by molecular regulator, molecular transducer activity, structural activity, transcription regulator activity, and transporter activity (Fig. 4A). In terms of the biological process, the three most significant classes of CHD were cellular process (83%), biological regulation (57%), and metabolic process (44%) (Fig. 4B). Additionally, in terms of cellular components, another two more significant components are cell (65%) and organelle (58%), which were found to be associated with CHD (Fig. 4C). Many other target-associated DEGs were involved in the biological process, molecular function and cellular components.

*Analysed potential DEGs of the CHD data set shows protein classes distributed among transcription factor (24%), enzyme modulator (20%), nucleic acid binding (18%), and signaling molecules (18%) (Fig. 5A).* The DEGs mainly associated with CHD key pathways showed the significance are inflammation mediated by chemokine and cytokine signaling pathway (11%), CCKR signaling map (11%), gonadotropin-releasing hormone receptor pathway (8%), apoptosis signaling pathway (6%), and p53 pathway (5%) (Fig. 5B). This result was consistent with
Figure 3. GO analysis of the microarray CHD data set. The x-axis shows selected GO terms, and y-axis is the percentage of the gene association from selected data set.

Figure 4. Analyzed Gene ontology (GO) of DEGs in CHD. Enriched GO terms in the (A) molecular function class, (B) biological process class, and (C) cellular component class of common DEGs.
Figure 5. Analyzed protein class and pathways of DEGs in CHD. (A) The proteins of common DEGs were classified according to function. (B) Significantly enriched pathways of common DEGs.

Figure 6. The CHD-associated data set PPI showing both gene interaction and binding properties.
GO analysis, confirming the classes of proteins associated with CHD. Many genes associated with inflammatory roles, and a previous study showed a conserved signature of dilated cardiomyopathy (DCM) plays an important role in cell survival promotion during end-stage of heart failure (32). In the present study, we also revealed the expression pattern of apoptotic or inflammatory genes (Fig. 4) (33,34).

Pathway analysis. To address the overview of data insight into the pathways, which are associated and connected for CHD development (35), we analyzed 164 DEGs involved in different functional pathways compared to reference and expected genes for those pathways. A total of 13 pathways were found to be associated with signaling-, immune-, and transcription-related pathways (36). Genes were confirmed in the uploaded list over the expected one (number in the list divided by the expected number). If >1, it indicated that the category is over-represented in the experiment. Conversely, the category is under-represented if <1. In the future, overexpressed genes are likely to serve as the marker selected in the development of CHD interventions. The P-value indicates the Fisher’s exact test (37) or Binomial statistic in which the probability is the number of genes observed in this category occurred by chance (randomly), as determined by the reference list (Table II).

### Table II. Pathway enrichment and reactome selected for CHD associated pathways.

| Reactome pathways                                      | Homo-sapiens REF (20996) | Client text box Input (212) | Client text box input (fold enrichment) | Client text box input (raw P-value) | Client text box input (FDR) |
|--------------------------------------------------------|--------------------------|-----------------------------|----------------------------------------|-----------------------------------|---------------------------|
| PI3K events in ERBB4 signaling (R-HSA-1250342)         | 9                        | 4                           | +                                      | 44.02                             | 6.47E-06                  | 3.54E-03                 |
| PI3K events in ERBB2 signaling (R-HSA-1963642)         | 13                       | 4                           | +                                      | 30.47                             | 2.09E-05                  | 6.53E-03                 |
| ERBB2 activates PTK6                                    | 11                       | 3                           | +                                      | 27.01                             | 3.30E-04                  | 4.53E-02                 |
| Chemokine receptors bind chemokines (R-HSA-380108)     | 48                       | 6                           | +                                      | 12.38                             | 1.61E-05                  | 5.88E-03                 |
| Interleukin-10 signaling (R-HSA-6783783)               | 45                       | 5                           | +                                      | 11                                | 1.40E-04                  | 2.79E-02                 |
| Interleukin-4 and Interleukin-13 signaling (R-HSA-6785807) | 111                      | 12                          | +                                      | 10.71                             | 3.96E-09                  | 8.68E-06                 |
| Peptide ligand-binding receptors (R-HSA-375276)        | 186                      | 9                           | +                                      | 4.79                              | 1.58E-04                  | 2.66E-02                 |
| G alpha (i) signalling events (R-HSA-418594)           | 392                      | 15                          | +                                      | 3.79                              | 1.59E-05                  | 6.98E-03                 |
| Signaling by Interleukins (R-HSA-449147)               | 449                      | 17                          | +                                      | 3.75                              | 4.90E-06                  | 3.58E-03                 |
| Class A/1 (Rhodopsin-like receptors) (R-HSA-373076)    | 321                      | 12                          | +                                      | 3.7                               | 1.40E-04                  | 2.56E-02                 |
| Cytokine signaling in Immune system (R-HSA-1280215)    | 669                      | 23                          | +                                      | 3.4                               | 4.96E-07                  | 5.43E-04                 |
| Generic transcription Pathway (R-HSA-212436)           | 1,094                    | 26                          | +                                      | 2.35                              | 6.61E-05                  | 1.45E-02                 |
| RNA polymerase II transcription (R-HSA-73857)          | 1,216                    | 28                          | +                                      | 2.28                              | 5.19E-05                  | 1.26E-02                 |
| Gene expression (transcription) (R-HSA-74160)          | 1,351                    | 29                          | +                                      | 2.13                              | 1.95E-04                  | 3.05E-02                 |
| Immune system (R-HSA-168256)                           | 2,035                    | 41                          | +                                      | 2                                 | 2.09E-05                  | 5.73E-03                 |
| Signal transduction (R-HSA-162582)                     | 2,667                    | 46                          | +                                      | 1.71                              | 2.66E-04                  | 3.89E-02                 |

PPI analysis. To address the PPI of the CHD dataset in this study, STRING online suits was used to address the possible interaction of protein of CHD associated DEGs. A total of 112 nodes, 257 edges, 4.59 average node edge, 0.387 average clustering coefficient, 77 expected edge number, and <1.0e-16 PPI enrichment value were observed, and shown the network was significantly interacted than expected. Previous studies investigated the rare variants through targeted expression profiling across CHD relevant tissues from appropriate cases and controls (38,39). The PPI indicates the interaction of genes associated with multiple genes for outcome. In the present study, we identified 422 GO for biological process, 31 GO for molecular function, 12 GO for cellular component,
Table III. Protein-protein interaction network of CHD associated genes.

A, Biological process (GO).

| Sl. No | GO-term       | Description                              | Count in gene set | False discovery rate |
|--------|---------------|------------------------------------------|-------------------|----------------------|
| 1      | GO:0050789    | Regulation of biological process         | 100 of 11,116     | 2.85e-09             |
| 2      | GO:0065007    | Biological regulation                    | 101 of 11,740     | 2.13e-08             |
| 3      | GO:0050794    | Regulation of cellular process           | 95 of 10,484      | 2.13e-08             |
| 4      | GO:0048523    | Negative regulation of cellular process  | 59 of 4,454       | 2.13e-08             |
| 5      | GO:0048519    | Negative regulation of biological process| 62 of 4,953       | 2.13e-08             |

B, Molecular function (GO).

| Sl. No | GO-term       | Description                              | Count in gene set | False discovery rate |
|--------|---------------|------------------------------------------|-------------------|----------------------|
| 1      | GO:000977     | RNA polymerase II regulatory region       | 16 of 647         | 0.00061              |
|        |               | sequence-specific DNA binding            |                   |                      |
| 2      | GO:005515     | Protein binding                          | 62 of 6,605       | 0.00065              |
| 3      | GO:0043565    | Sequence-specific DNA binding            | 19 of 1,047       | 0.00083              |
| 4      | GO:0140110    | Transcription regulator activity          | 28 of 2,069       | 0.0011               |
| 5      | GO:0005488    | binding                                  | 89 of 11,878      | 0.0026               |

C, Cellular components (GO).

| Sl. No | GO-term       | Description                              | Count in gene set | False discovery rate |
|--------|---------------|------------------------------------------|-------------------|----------------------|
| 1      | GO:0005634    | Nucleus                                  | 67 of 6,892       | 8.05e-05             |
| 2      | GO:0035976    | Transcription factor AP-1 complex        | 3 of 5            | 0.0015               |
| 3      | GO:0005622    | Intracellular                            | 102 of 14,286     | 0.0015               |
| 4      | GO:0044424    | Intracellular part                       | 99 of 13,996      | 0.0064               |
| 5      | GO:0043227    | Membrane-bounded organelle               | 85 of 11,244      | 0.0067               |

D, KEGG pathways.

| Sl. No | GO-term       | Description                              | Count in gene set | False discovery rate |
|--------|---------------|------------------------------------------|-------------------|----------------------|
| 1      | hsa04668      | TNF signaling pathway                    | 9 of 108          | 4.47e-06             |
| 2      | hsa04380      | Osteoclast differentiation               | 8 of 124          | 8.58e-05             |
| 3      | hsa04657      | IL-17 signaling pathway                  | 7 of 92           | 9.94e-05             |
| 4      | hsa04621      | NOD-like receptor signaling pathway      | 8 of 166          | 0.00034              |
| 5      | hsa05210      | Colorectal cancer                        | 6 of 85           | 0.00051              |

E, Reactome pathways.

| Sl. No | GO-term       | Description                              | Count in gene set | False discovery rate |
|--------|---------------|------------------------------------------|-------------------|----------------------|
| 1      | HSA-6785807   | Interleukin-4 and Interleukin-13 signaling | 11 of 106         | 3.07e-08             |
| 2      | HSA-449147    | Signaling by Interleukins                | 14 of 439         | 6.23e-05             |
| 3      | HSA-1280215   | Cytokine Signaling in Immune system       | 17 of 654         | 6.23e-05             |
| 4      | HSA-1250342   | PI3K events in ERBB4 signaling           | 4 of 9            | 7.21e-05             |
| 5      | HSA-1963642   | PI3K events in ERBB2 signaling           | 4 of 13           | 0.00019              |
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33 pathways, 30 reactome pathways, 13 UniProt keywords, 11 PFAM protein domains, 29 INTERO protein domains, and 3 SMART protein domains in the analysis of CHD microarray data set. In those findings, associated edges shows physically binding protein and some of them were associated with but did not have physical binding. Of these, only the top-ranking ones have been presented (Fig. 6 and Table III).

Understanding and ruling the mechanism. There are several challenges to identifying the genetic basis of CHD that are also the determinants of this complex disease, including phenotypic and genetic heterogeneity, gene-environment, and etiological spectrum range and their effect. Considering research efforts involved in determining the genetic basis of this CHD, there is a need to understand the fine complexity of genetic association leading to mortality in developing countries. There is a need to focus on clinical manifestation rather than factors which influence or are heritable by genetic factors. There are many challenges in determining the genetic association of CHDs, such as phenotypic heterogeneity, genetic heterogeneity, small gene effects, gene-gene and gene-environment interactions and rare variants causing complex diseases. Some of the key points to be undertaken such as mortality, challenge in identifying the genetic determinants, studying linkage mapping through conventional approaches, and cataloguing of human diseases variation at single-nucleotide polymorphism (SNP), as well as genotyping will increase the likelihood of success.

In conclusion, we studied a comprehensive gene expression profile of microarray data of CHD. During the progression of CHD, there was a significant change in the expression of genes involved in the immune system, inflammation, and cell signaling through protein binding. This analysis provides valuable information for future research and in understanding the mechanism of CHD as well as identification of novel interventions for therapeutic application.

Acknowledgements

Not applicable

Funding

This research received no specific grants from any funding agencies.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Table III. Continued.

F,UniPort PFAM Protein domains

| Sl. No | Domain    | Description                        | Count in gene set | False discovery rate |
|-------|-----------|------------------------------------|-------------------|----------------------|
| 1     | PF07716   | Basic region leucine zipper        | 7 of 44           | 2.23e-06             |
| 2     | PF03131   | bZIP Maf transcription factor      | 5 of 33           | 0.00017              |
| 3     | PF00170   | bZIP transcription factor          | 5 of 36           | 0.00017              |
| 4     | PF04553   | Tis11B like protein, N terminus   | 2 of 2            | 0.0061               |
| 5     | PF00782   | Dual specificity phosphatase, catalytic domain | 4 of 45           | 0.0061               |

G, INTERPRO Protein Domains and Features

| Sl. No | Domain    | Description                                      | Count in gene set | False discovery rate |
|-------|-----------|--------------------------------------------------|-------------------|----------------------|
| 1     | IPR004827 | Basic-leucine zipper domain                      | 8 of 54           | 7.06e-07             |
| 2     | IPR029021 | Protein-tyrosine phosphatase-like                 | 5 of 101          | 0.0181               |
| 3     | IPR008917 | Transcription factor, Skn-1-like, DNA-binding domain superfamily | 3 of 16          | 0.0181               |
| 4     | IPR007635 | Tis11B-like protein, N-terminal                  | 2 of 2            | 0.0181               |
| 5     | IPR005643 | Jun-like transcription factor                    | 2 of 3            | 0.0181               |

SMART Protein Domains

| Sl. No | Domain    | Description                        | Count in gene set | False discovery rate |
|-------|-----------|------------------------------------|-------------------|----------------------|
| 1     | SM00338   | Basic region leucin zipper         | 8 of 53           | 1.47e-07             |
| 2     | SM00195   | Dual specificity phosphatase, catalytic domain | 3 of 28           | 0.0246               |
| 3     | SM00356   | Zinc finger                        | 3 of 42           | 0.0488               |
Authors' contributions

ZY conceived and designed the study, WL provided study materials. ZY, HM and WL were responsible for the collection and assembly of data, data analysis and interpretation. ZY was involved in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Patient consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

References

1. Underlying Cause of Death 1999-2017. Centers for Disease Control and Prevention 24/7.

2. Haukilahti MAE, Holmström L, Väätäjälä J, Kenttä T, Tikkanen J, Pakkanen L, Kortelainen ML, Perkkömäki J, Huikuri H, Myyryläinen RJ, et al: Sudden cardiac death in women. Circulation 139: 1012-1021, 2019.

3. Ding D, Wang M, Su D, Hong C, Li X, Yang G, Zhang Y, Hu G and Ling W: Body mass index, high-sensitivity C-reactive protein and mortality in Chinese with coronary artery disease. PLoS One 10: e0135713, 2015.

4. Yusuf S, Rangarajan S, Teo K, Islam S, Li W, Liu L, Bo J, Lou Q, Lu F, Liu Y, et al: Cardiovascular risk and events in 17 low-, middle-, and high-income countries. N Engl J Med 371: 818-827, 2014.

5. Benjamin EJ, Blaha MJ, Cushman M, Daniels SR, Finucane MM, Franklin SS, Gardenswartz LM, Giles WH, Goldstein JA, Greenlund KJ, et al: Heart disease and stroke statistics-2017 Update: A report from the American Heart Association. Circulation 135: e146-e603, 2017.

6. Mehta LS, Beckie TM, DevOn HA, Grines CL, Krumholz HM, Johnson MN, Lindley KJ, Vaccarino V, Wang TY, Watson KE, et al: American Heart Association Cardiovascular Disease in Women and Special Populations Committee of the Council on Clinical Cardiology, Council on Epidemiology and Prevention, Council on Cardiovascular and Stroke Nursing, and Council on Quality of Care and Outcomes Research: Acute myocardial infarction in women: A scientific statement from the American Heart Association. Circulation 133: 916-947, 2016.

7. Fyraz S, Rauf A, Hudson J and Olahintan O: 107 Nice 2016 stable chest pain guidelines: Improved yield of severe coronary artery disease at invasive coronary angiography. Heart 105: 88, 2019.

8. Timmis A, Townsend N, Gale C, Grobbee DE, Maniadakis N, Flather M, Wilkins E, Wright L, Vos R, Bax J, et al: ESC Scientific document group: European society of cardiology: Cardiovascular Disease 2017. Eur Heart J 39: 508-579, 2018.

9. Erbel R, Aboyans V, Boileau C, Bosone E, Bartolomeo RD, Eggebrecht H, Evangelista A, Falk V, Frank H, Gaemperli O, et al: ESC Committee for Practice Guidelines: 2014 ESC Guidelines on the diagnosis and treatment of aortic diseases: Document covering aortic and chronic aortic diseases of the thoracic and abdominal aorta of the adult. Eur Heart J 33: 787-929, 2014.

10. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, Cooney MT, Corrà U, Cosyns B, Deaton C, et al: ESC Scientific Document Group: 2016 European guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur Heart J 37: 2315-2381, 2016.

11. Kwak BR, Bäck M, Bochaton-Piallat ML, Caligiuri G, Daemen MJAP, Davies PF, Hoefere IJ, Holvoet P, Jo H and Krans R: Biomechanical factors in atherosclerosis: Mechanisms and clinical implications. Eur Heart J 37: 2315-2381, 2016.

12. CARDIoGRAMplusC4D Consortium, Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, Ingelsson E, Saleheen D, Erdmann J: Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet 45: 25-33, 2013.

13. Nikpay M, Goel A, Won H-H, Hall LM, Willenborg C, Kanoni S, Saleheen D, Kyriakou T, Nelson CP, Hopewell JC, et al: A comprehensive 1,000 genomes-based genome-wide association meta-analysis of coronary artery disease. Nat Genet 47: 1121-1130, 2015.

14. Kessels T, Vilne B and Schunkert H: The impact of genome-wide association studies on the pathophysiology and therapy of cardiovascular disease. EMBO Mol Med 8: 688-701, 2016.

15. Roberts R: Genetics of coronary artery disease. Circ Res 114: 1890-1903, 2014.

16. Zhao Y, Chen J, Freudenberg JM, Meng Q, Rajpal DK and Yang X: Network-based identification and prioritization of key regulators of coronary artery disease loci. Arterioscler Thromb Vasc Biol 36: 928-941, 2016.

17. Miller CL, Fearnhead P and Quertermous T: From locus association to mechanism of gene causality: The devil is in the details. Arterioscler Thromb Vasc Biol 35: 2079-2080, 2015.

18. Cheng Y and Zhang C: MicroRNA-21 in cardiovascular disease. J Cardiovasc Transl Res 3: 251-255, 2010.

19. Chen CZ, Li L, Lodish H and Bartel DP: MicroRNAs modulate cardiomyopathic lineage differentiation. Science 303: 83-86, 2003.

20. Johnson CD, Esquesa-Kerscher A, Stefani G, Byrom M, Kelnar K, Ovarenkenko D, Wilson M, Wang X, Shelton J, Shingara J, et al: The let-7 microRNA represses cell proliferation pathways in human cells. Cancer Res 67: 7713-7725, 2007.

21. Cheng AM, Byrom MW, Shelton J and Ford LP: Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. Nucleic Acid Res 33: 1290-1297, 2005.

22. Smyth GK, Michaud J and Scott HS: Use of within-array replicate spots for assessing differential expression in microarray experiments. Bioinformatics 21: 2067-2075, 2005.

23. Mi H, Muruganjanu A, Casagrande JT and Thomas PD: Large-scale gene function analysis with the PANTHER classification system. Nat Protoc 8: 1551-1566, 2013.

24. Jupe S, Fabregat A and Hermjakob H: Expression data analysis with reactome. Curr Protoc Bioinformatics 49: 8.20.1-8.20.9, 2015. https://doi.org/10.1002/0471290953.bio0820s49.

25. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, et al: The STRING database in 2017: Quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 45: D362-D368, 2017.

26. Rana JS, Nieuworp D, Jukema JW and Kastelein JJ: Cardiovascular metabolic syndrome - an interplay of, obesity, inflammation, diabetes and coronary heart disease. Diabetes Obes Metab 9: 218-232, 2007.

27. Kessels T, Vilne B and Schunkert H: The impact of genome-wide association studies on the pathophysiology and therapy of cardiovascular disease. EMBO Mol Med 8: 688-701, 2016.

28. Torre-Amione G: Immune activation in chronic heart failure. Am J Cardiol 95 (11A): 2C-8C, discussion 38C-40C, 2005.

29. Breses-Castro D, Castillo EC, Vázquez-Garza E, Torre-Amione G and García-Rivas G: Temporal frame of immune cell infiltration during heart failure establishment: Lessons from animal models. Int J Mol Sci 19, 2018.

30. Flores-Arredondo JH, García-Rivas G and Torre-Amione G: Immune modulation in heart failure: Past challenges and future hopes. Curr Heart Fail Rep 8: 27-38, 2011.

31. Gu X, Li B, Jiang M, Fang M, Ji J, Wang A, Wang M, Jiang X and Gu X: RNA sequencing reveals differentially expressed genes in the parathyroid gland of diabetic rats. J Am Coll Cardiol 61: 1890-1903, 2013.

32. Hoque MM, Fazal A, Ah탄n E, Celik M, Yildirim D and Yildirim M: The let-7 microRNA represses cell proliferation pathways in human cells. Cancer Res 67: 7713-7725, 2007.

33. Cheng AM, Byrom MW, Shelton J and Ford LP: Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. Nucleic Acid Res 33: 1290-1297, 2005.
34. Sinnaeve PR, Donahue MP, Grass P, Seo D, Vonderscher J, Chibout SD, Kraus WE, Sketch M Jr, Nelson C, Ginsburg GS, et al: Gene expression patterns in peripheral blood correlate with the extent of coronary artery disease. PLoS One 4: e7037, 2009.

35. Gotoh T, Endo M and Oike Y: Endoplasmic reticulum stress-related inflammation and cardiovascular diseases. Int J Inflamm 2011: 1-9, 2011.

36. Haas MJ and Mooradian AD: Regulation of high-density lipoprotein by inflammatory cytokines: Establishing links between immune dysfunction and cardiovascular disease. Diabetes Metab Res Rev 26: 90-99, 2010.

37. Costa THR, de Figueiredo Neto JA, de Oliveira AEF, Lopes e Maia MF and de Almeida AL: Association between chronic apical periodontitis and coronary artery disease. J Endod 40: 164-167, 2014.

38. Jensen MK, Pers TH, Dworzynski P, Girman CJ, Brunak S and Rimm EB: Protein interaction-based genome-wide analysis of incident coronary heart disease. Circ Cardiovasc Genet 4: 549-556, 2011.

39. Duan S, Luo X and Dong C: Identification of susceptibility modules for coronary artery disease using a genome wide integrated network analysis. Gene: 531: 347-357, 2013

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