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

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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.

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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.

A, Upregulation

ID	LogFC	Gene symbol	Gene ontology: Biological function	Gene ontology: Cellular component	Gene ontology: Molecular function
11725632	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	CREBRF	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

Table I. Continued.

ID	LogFC	Gene symbol	Gene ontology: Biological function	Gene ontology: Cellular component	Gene ontology: 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 Receptor activity
11718939	2.723	TNFAIP3	B-1 B cell homeostasis	Nucleus	Protease binding
11736467	2.2	TAGAP	Signal transduction	Cytosol	Guanyl-nucleotide 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 diphosphorylase (Carboxylating) activity
11715673	2.002	JUNB	Negative regulation of transcription from RNA polymerase II pomoter	Chromatin	RNA polymerase II regulatory region Sequence-specific DNA binding
11717994	2.812	NR4A1	Positive regulation of endothelial cell proliferation	Nucleus	Transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding
11715691	2.008	ZFP36	Negative regulation of transcription from RNA polymerase II promoter	Nucleus	DNA binding
11733022	2.29	BTG1	Regulation of transcription, DNA-templated	Nucleus	Transcription cofactor activity
11724446	1.738	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 /// HCAR3	Neutrophil apoptotic process response to virus by host	Plasma membrane	Signal transducer activity

Table I. Continued.

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	Nuclear chromosome	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	Protein serine
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	C5AR1	Activation of MAPK activity	Cytosol	Complement component C5a binding
11759628	1.838	WIPF1	Actin cortical patch assembly	Ruffle	Actin binding
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

Table I. Continued.

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	Atpase activator activity
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 from RNA polymerase II promoter	Extracellular region	Receptor binding
11715817	1.18	ZFP36L2	Nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay	Nucleus	DNA binding
11743434	1.4	CHST11	Chondrocyte development	Golgi membrane	N-acetylgalactosamine 4-O-sulfotransferase activity
11724236	1.272	RIPK2	Activation of MAPK activity	Cytoplasm	Nucleotide binding
11720745	1.815	BCL6	Protein import into nucleus, translocation	Nucleus	RNA polymerase II regulatory region sequence-specific DNA 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 binding
11716048	2.357	TRIB1	Protein phosphorylation	Nucleus	Protein kinase activity
11756387	1.558	ARL4A	Intracellular protein transport	Intracellular	Nucleotide binding

Table I. Continued.

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	Posit		
tive regulation of transcription from		Nucleus	Transcription regulatory region RNA polymerase II promoter		sequence-specific DNA binding
11718061	1.88	PVALB	Cytosolic calcium ion homeostasis	Nucleus	Calcium ion binding
11721630	2.138	MAFB	Transcription, DNA-templated	Nucleus	RNA polymerase II core promoter proximal region sequence-specific DNA binding
11763755	1.068	GNLY	Cellular defense response	Extracellular region	
11757924	1.542	SIPA1L2	Positive regulation of gtpase activity		Gtpase activator activity
11732859	1.03	DNHD1	Microtubule-based movement	Dynein complex	Microtubule motor activity
11750016	1.498	MXD1	Negative regulation of transcription from RNA polymerase II promoter	Nucleus	RNA polymerase II core promoter 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		Catalytic activity
11756358	1.87	PLK3	G1	Chromatin	Nucleotide binding
11718397	3.16	JUN	Angiogenesis	Nuclear chromosome	RNA polymerase II core promoter proximal region sequence-specific DNA binding
11763954	1.04	SCARNA9L			
11730655	1.12	CNOT1	Negative regulation of transcription from RNA polymerase II promoter	Cytoplasmic mrna processing body	Poly(A)-specific ribonuclease activity

Table I. Continued.

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	NFIL3	Negative regulation of transcription from RNA polymerase II promoter	Nucleus	RNA polymerase II regulatory region sequence-specific DNA binding
11724036	4.27	PTGS2	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 /// FCGR2C	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
11716071	1.3	PIM3	Protein phosphorylation	Cytoplasm	Nucleotide binding
11720612	1.04	NAP1L5	Nucleosome assembly	Nucleus	
11755987	0.98	ANKRD44			Protein binding
11717895	1.285	PTP4A1	Protein dephosphorylation	Nucleus	Phosphoprotein phosphatase activity
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 /// FCGR2C	Immune system process	Cytoplasm	Transmembrane signaling receptor activity

Table I. Continued.

ID	LogFC	Gene symbol	Gene ontology: Biological function	Gene ontology: Cellular component	Gene ontology: Molecular function
11748516	0.932	NAP1L5	Nucleosome assembly	Nucleus	
11718927	0.975	ARID5B	Negative regulation of transcription from RNA polymerase II promoter	Nucleus	RNA polymerase II regulαtory region sequence-specific DNA binding
11716195	2.692	ID1	Negative regulation of transcription from RNA polymerase II promoter	Nucleus	Transcription factor activity, sequence-specific DNA binding
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	Nucleic acid binding
11741074	-1.407	METTL18	Methylation		Methyltransferase activity
11722367	-1.55	DLGAP5	Protein dephosphorylation	Nucleus	Phosphoprotein phosphatase activity
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 /// PRRT1	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, DNA damage recognition	Nuclear chromosome, telomeric region	Single-stranded DNA endodeoxyribonuclease activity
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	Guanyl-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		Protein binding
11727112	-1.397	SIT1	Adaptive immune response	Plasma membrane	Protein binding
11717301	-1.218	TACSTD2	Cell cycle	Extracellular space	Receptor activity
11734218	-1.38	ZNF681	Positive regulation of defense response to virus by host	Intracellular	Nucleic acid binding
11728339	-1.667	CENPBD1		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			Protein binding
11733069	-1.825	WDR5B			Protein binding
11730969	-1.765	THAP2		Nucleolus	Nucleic acid binding
11719780	-1.728	TNFAIP8L2	Immune system process	Cytoplasm	Protein binding
11758125	-1.32	DEPDC1B	Signal transduction	Intracellular	Gtpase activator activity
11733695	-1.055	UBE2C	Mitotic cell cycle	Nucleus	Nucleotide binding
11756100	-1.227	TMEM60		Membrane	
11732295	-1.455	ZNF566	Transcription, DNA-templated	Intracellular	Nucleic acid binding
11724984	-1.255	EXPH5	Keratinocyte development	Intracellular	Protein binding
11726757	-1.165	CDC25A	Regulation of cyclin-dependent protein serine	Intracellular	Phosphoprotein phosphatase activity
11727604	-1.278	EPB41L4A		Cytoplasm	Cytoskeletal protein binding
11716226	-1.147	LIMA1	Negative regulation of actin filament depolymerization	Stress fiber	Actin binding

Table I. Continued.

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 region	
11745077	-1.282	CRNKL1	Spliceosomal complex assembly	Prp19 complex	RNA binding
11730590	-1.198	KCTD21	Protein homooligomerization		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	Protein binding
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.

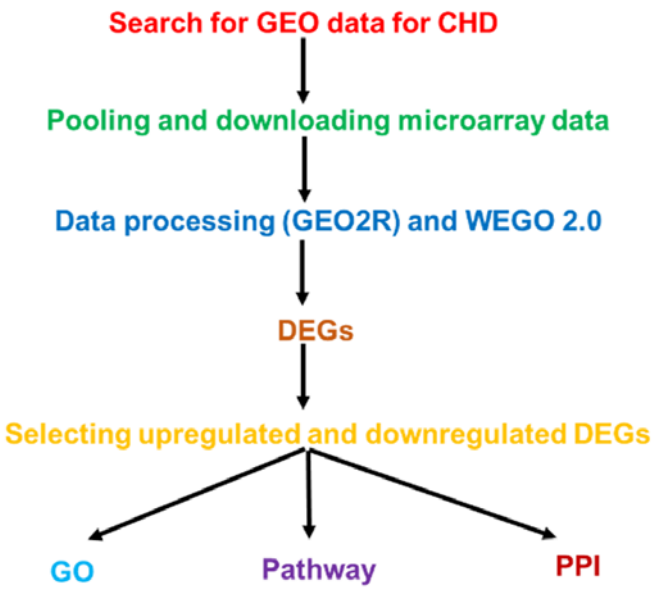

Figure 1. Designing of CHD data set for DEGs study.

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 adoptive immune system which may be a contributing factor

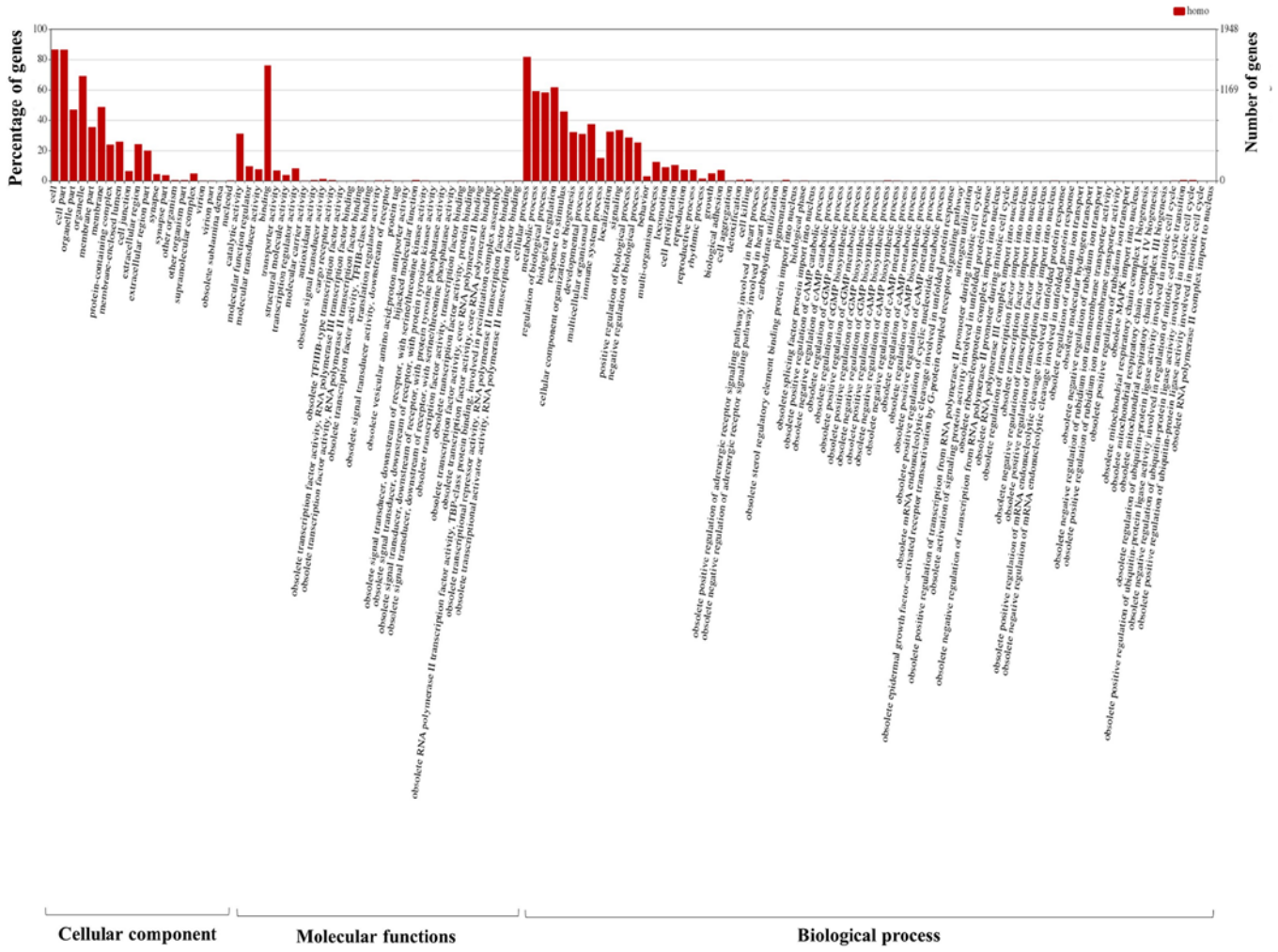

Figure 2. Up- and downregulated gene association comparing all the genetic data set.

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.

Analyzed 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

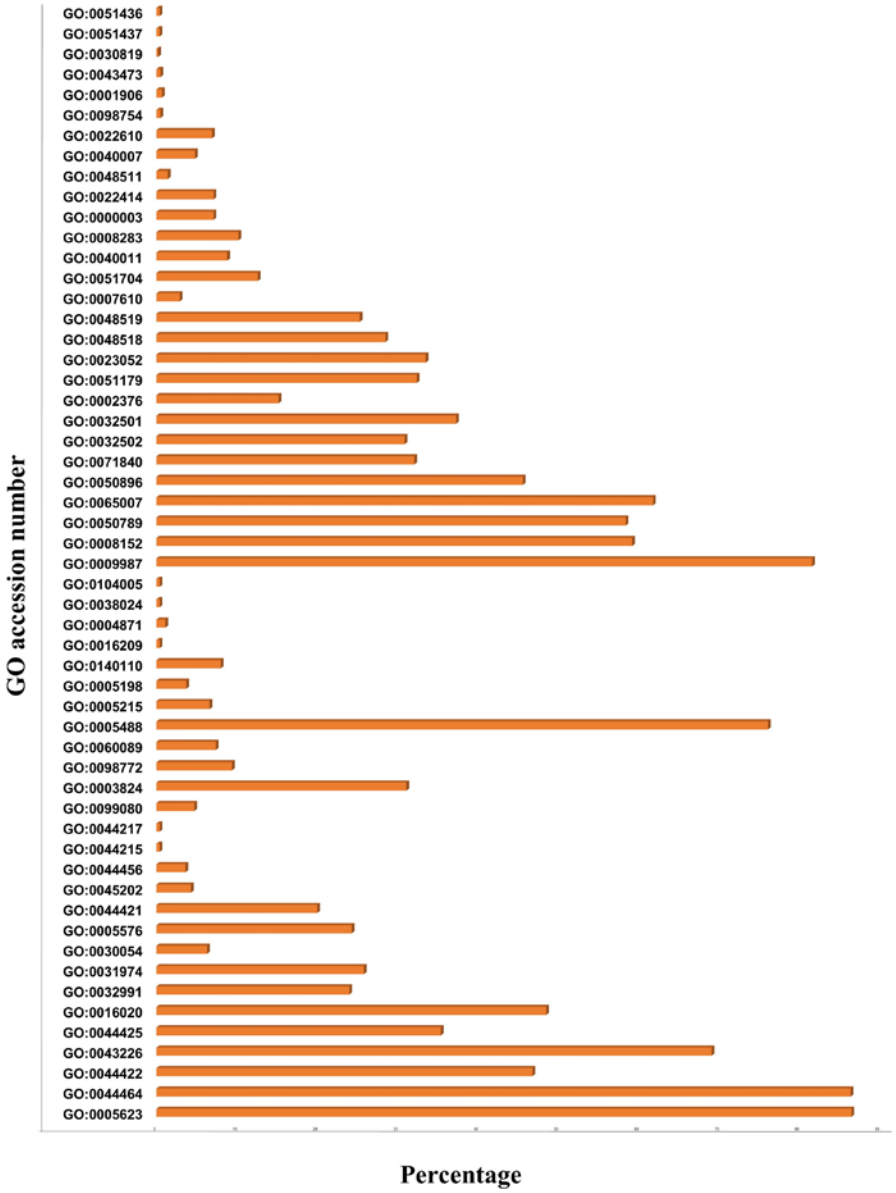

Figure 3. GO analysis of the microarray CHD data set. The x-axis shows selected GO terms, and y-axis is the percentage of the gene association from selected data set.

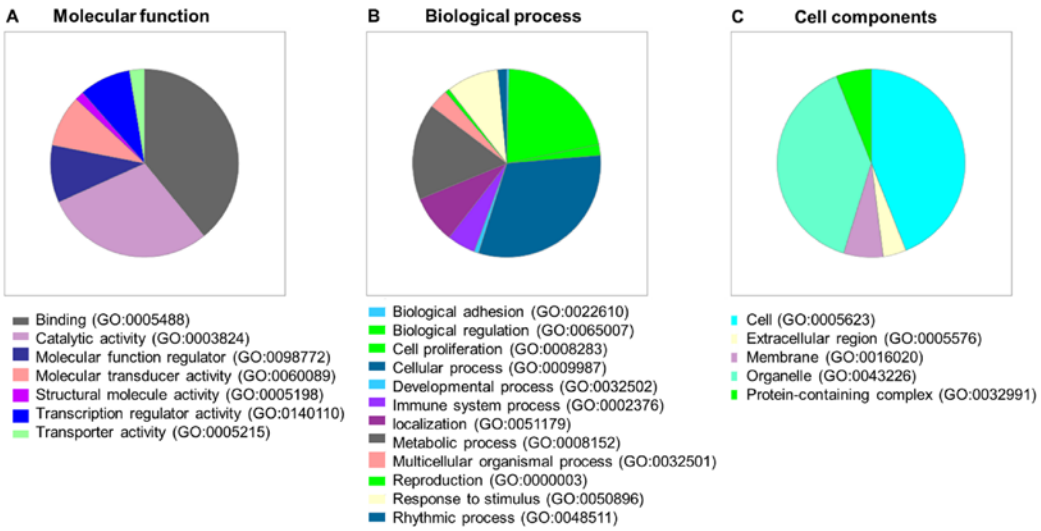

Figure 4. Analyzed Gene ontology (GO) of DEGs in CHD. Enriched GO terms in the (A) molecular function class, (B) biological process class, and (C) cellular component class of common DEGs.

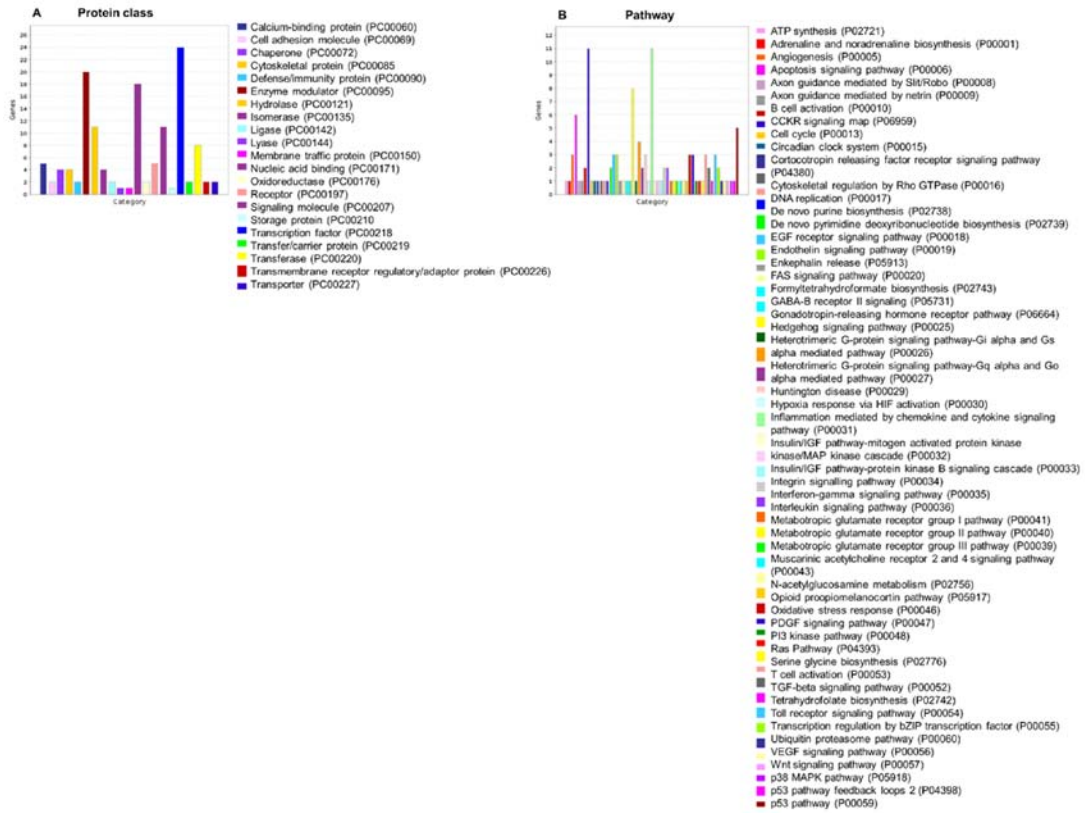

Figure 5. Analyzed protein class and pathways of DEGs in CHD. (A) The proteins of common DEGs were classified according to function. (B) Significantly enriched pathways of common DEGs.

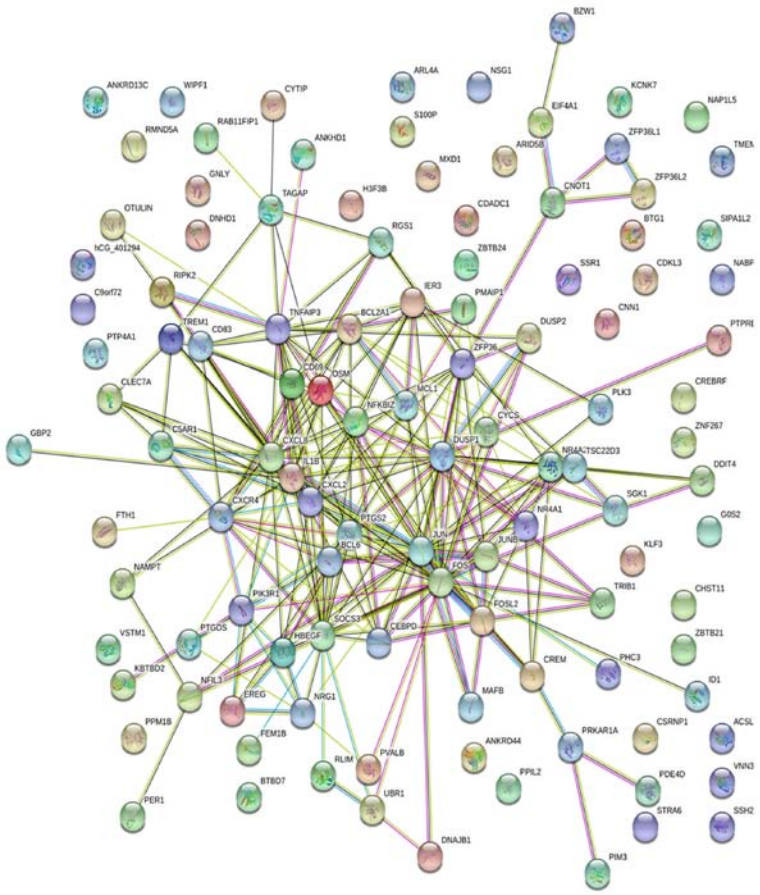

Figure 6. The CHD-associated data set PPI showing both gene interaction and binding properties.

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

Reactome pathways	Homo-sapiens REFLIST (20996)	Client text box Input (212)	Client text box input (expected)	Client text box input (over/under)	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	0.09	+	44.02	6.47E-06	3.54E-03
PI3K events in ERBB2 signaling (R-HSA-1963642)	13	4	0.13	+	30.47	2.09E-05	6.53E-03
ERBB2 activates PTK6 Signaling (R-HSA-8847993)	11	3	0.11	+	27.01	3.30E-04	4.53E-02
Chemokine receptors bind chemokines (R-HSA-380108)	48	6	0.48	+	12.38	1.61E-05	5.88E-03
Interleukin-10 signaling (R-HSA-6783783)	45	5	0.45	+	11	1.40E-04	2.79E-02
Interleukin-4 and Interleukin-13 signaling (R-HSA-6785807)	111	12	1.12	+	10.71	3.96E-09	8.68E-06
Peptide ligand-binding receptors (R-HSA-375276)	186	9	1.88	+	4.79	1.58E-04	2.66E-02
G alpha (i) signalling events (R-HSA-418594)	392	15	3.96	+	3.79	1.59E-05	6.98E-03
Signaling by Interleukins (R-HSA-449147)	449	17	4.53	+	3.75	4.90E-06	3.58E-03
Class A/1 (Rhodopsin-like receptors) (R-HSA-373076)	321	12	3.24	+	3.7	1.40E-04	2.56E-02
Cytokine signaling in Immune system (R-HSA-1280215)	669	23	6.76	+	3.4	4.96E-07	5.43E-04
Generic transcription Pathway (R-HSA-212436)	1,094	26	11.05	+	2.35	6.61E-05	1.45E-02
RNA polymerase II transcription (R-HSA-73857)	1,216	28	12.28	+	2.28	5.19E-05	1.26E-02
Gene expression (transcription) (R-HSA-74160)	1,351	29	13.64	+	2.13	1.95E-04	3.05E-02
Immune system (R-HSA-168256)	2,035	41	20.55	+	2	2.09E-05	5.73E-03
Signal transduction (R-HSA-162582)	2,667	46	26.93	+	1.71	2.66E-04	3.89E-02

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).

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:0000977	RNA polymerase II regulatory region sequence-specific DNA binding	16 of 647	0.00061
2	GO:0005515	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

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

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.

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Availability of data and materials

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

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.

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