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A Network Pharmacology-Based Study on Active Ingredients of Corydalis Rhizoma on Coronary Heart Disease

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Keywords: Chinese Traditional Medicine, Corydalis Rhizoma, Network pharmacology, coronary heart disease

Posted Date: August 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-41449/v1

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Abstract

Background. Corydalis Rhizoma(CR) showed a high efficacy for coronary heart disease (CHD). However, the interaction between the active ingredients of CR and the targets of CHD has not been unequivocally explained in previous researches. To study the active components and potential targets of Corydalis Rhizoma and to determine the mechanism underlying the exact effect of Corydalis Rhizoma on coronary heart disease, a method of network pharmacology was used.

Materials and Methods. The active components of CR and targets corresponding to each component were scanned out from Traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP), and target genes of CHD were searched on GeneCards database and Online Mendelian Inheritance in Man(OMIM) database. The active components and common targets of CR and CHD were used to build the "CR-CHD" network through Cytoscape (version 3.2.1) software as well as protein-protein interaction(PPI) network on String database. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes(KEGG) pathway enrichment analysis was executed by clusterProfiler(version 3.8) and DOSE(version 3.6) package on R platform.

Results. 49 active ingredients and 394 relevant targets of CR and the 7173 CHD-related genes were retrieved. 40 common genes were selected for subsequent analysis. Crucial biological processes and pathways were obtained and analyzed, including DNA-binding transcription activator activity, RNA polymerase II-specific, RNA polymerase II transcription factor binding, kinase regulator activity, ubiquitin-like protein ligase binding, fluid shear stress and atherosclerosis, TNF signaling pathway, apoptosis, MAPK signaling pathway and PI3K-Akt signaling pathway.

Conclusions. Overall, CR could alleviate CHD through the mechanisms predicted by network pharmacology, laying the foundation for future development of new drugs from traditional Chinese medicine on CHD.

1. Background

Coronary heart disease (CHD) accounts for the greatest proportion of cardiovascular diseases(CVD) which cause over 1.7 million deaths (nearly one-third of all deaths) worldwide, and this figure was expected to grow to 23.6 million by 2030[1]. Despite the positive trend that during the past 25 years, both incidence and mortality (age standardized) have decreased globally, the burden remains disproportionately high in Eastern Europe and Central Asia and age-standardized mortality also remains high in South Asia, North Africa, and the Middle East[2]. Some countries reported increases mortality (particularly those in Eastern Europe and Asia) [3]. Moreover, despite advances in current secondary prevention therapies of CHD, including cholesterol-lowering medications, beta-blockers, calcium antagonists, antiplatelet agents, anticoagulant drugs, these drugs can also triggered some serious side effects like bleeding, muscle pain or weakness, new-onset diabetes

mellitus[4, 5, 6]. Therefore, there is an urgent need to find more effective and sustainable prevention and treatment strategies for CHD.

Traditional Chinese Medicine(TCM), compared with modern medicine, provides a better long-term curative effect on chronic conditions, as the Danshen dripping pill provides a better long-term curative effect than isosorbide dinitrate does for chronic angina pectoris[7]. Thus, it is necessary to follow up the study and further develop traditional Chinese herbal medicine.

Corydalis Rhizom(CR) , recommended in Pharmacopoeia of the People's Republic of China for treatment of chest pain[8], is one of the most commonly prescribed Chinese herbal medicine, according to reviews and data mining of TCM masters(nationally certified in China)'s practice experience, for the treatment of Coronary heart disease[9, 10, 11, 12]. However, the mechanism of the effectiveness of CR on treating Coronary heart disease, since ingredients of CR are complex and their action targets numerous, has not yet been well defined. Therefore, we intend to study the pharmacological mechanism of CR when treating CHD, with the approach of network pharmacology which can provide both complementary support for the development of drug design and better understanding of the mechanisms underlying the actions of traditional Chinese herbal medicine[13, 14, 15]. In present study, we aimed to elucidate the mechanism of CR on treatment of CHD with a comprehensive network pharmacology-based approach. A flowchart of the network pharmacology approach is shown in Figure 1.

2. Materials And Methods

In this study, we constructed an active components of CR-CHD disease targets network which can directly observed the distribution of active components acting on CHD related targets. Moreover, construction of PPI network enabled us to reveal interactions of relative target proteins, by which we got the core genes with calculation. At last, Gene Ontology(GO) function and Kyoto Encyclopedia of Genes and Genomes(KEGG) pathway enrichment analysis were performed for functional annotation of the relative target genes in CR-CHD network.

2.1.Establishment of active components of CR-relative targets database. Traditional Chinese medicine systems pharmacology database and analysis platform(TCMSP, http://tcmspw.com/index.php, last updated on May 31, 2014) was built for herbal medicines, based on systems pharmacology. It contains all the 499 Chinese herbs registered in the Chinese pharmacopoeia with 29,384 ingredients, 3,311 targets and 837 associated diseases[16]. We performed a research on TCMSP with the keyword of "Corydalis Rhizoma" and the screening conditions were drug similarity (DL) greater than 0.18 and oral bioavailability (OB) greater than 30%[17, 18]. The active components of CR-relative targets database was established according to the search result which contained active components of CR and targets corresponding to each component, of which target names were converted into Universal Protein Resource (Uniprot, http://www.uniprot.org/, last updated on November 29, 2019) symbols for subsequent analysis.

2.2. Establishment of disease targets database of CHD. To get a comprehensive collection, the search of disease targets was performed , with the key word of "coronary heart disease", on GeneCards database(https://www.genecards.org/, Version 4.13) which enables researchers to effectively navigate and

inter-relate a large number of human genes, diseases, variants, proteins, cells, and biological pathways[19] as well as Online Mendelian Inheritance in Man(OMIM, https://omim.org/, last updated on February 5, 2020) which is the primary repository of comprehensive, curated information on genes and genetic phenotypes and the relationships between them[20]. Database of relative gene targets was built with the obtained targets, converted into Uniprot symbols, from both GeneCards and OMIM database.

2.3. Construction of active components of CR-disease targets of CHD network. Intersection between two gene sets of active components of CR-relative targets database and disease targets database of CHD was employed to draw a Venn diagram[21] showing the distribution of these genes. Cytoscape (version 3.2.1) [22] software was used to construct the active components of CR-disease targets of CHD network, with components and targets are represented as “nodes” while associations between them are encoded by “edges”.

2.4. Construction of protein-protein interaction(PPI) network. The String database aims to collect and integrate this information, by consolidating known and predicted protein-protein association data for a large number of organisms[23]. Using the “CR-CHD” common target genes, PPI network[24] was constructed by String database (https://string-db.org/, Version 11.0), with all the targets were only limited the species as “Homo sapiens”, setting combined score≥0.4, from which disconnected nodes were hidden. We calculated the number of adjacent nodes of each node in the PPI network, and drew a histogram.

2.5. GO Enrichment and KEGG Pathway Analysis. In order to understand the biological function of “CR-CHD” common genes, we performed the Gene Ontology (GO, http://geneontology.org/, last updated on January 1, 2020)[25] enrichment and Kyoto Encyclopedia of Genes and Genomes(KEGG, https://www.kegg.jp/, last updated on February 18, 2020)[26] pathway analysis using clusterProfiler(version 3.8) and DOSE(version 3.6) software package on R platform. As increasing quantitative data generated from transcriptomics and proteomics requiring integrative strategies for analysis, clusterProfiler automates the process of biological-term classification and the enrichment analysis of gene clusters[27] while DOSE provides semantic similarity computations among disease ontology(DO) terms, which is important annotation in translating molecular findings from high-throughput data to clinical relevance, and genes which allows biologists to explore the similarities of diseases and of gene functions in disease perspective[28]. Threshold values were set as *P* value=0.05 and Q value=0.05. A target gene-molecular function and pathway network was built by using Cytoscape (version 3.2.1) software for a more intuitive visualization, with key modules screened from the network by MCODE plugin of Cytoscape (version 3.2.1) [29, 30], setting degree cutoff=2≥node score cutoff=0.2≥K-score=2≥max depth=100.

3. Results

3.1. Active components of CR-relative targets database. A total of 77 reported ingredients of CR were retrieved by searching the TCMSP database, and 49 active ingredients were screened out using OB and DL from ADME parameters. And 394 related targets of the active components were searched from TCMSP. Details of these components are provided in Table 1.

TABLE 1: Active ingredients of CR(OB≥30%, DL≥0.18)

No	Molecule name	OB(%)	DL	No	Molecule name	OB(%)	DL
1	bicuculline	69.67	0.88	26	CORYDALINE	65.84	0.68
2	coptisine	30.67	0.86	27	dehydrocorydaline	41.98	0.68
3	Dihydrosanguinarine	59.31	0.86	28	isocorybulbine	40.18	0.66
4	sanguinarine	37.81	0.86	29	palmatine	64.6	0.65
5	pseudocoptisine	38.97	0.86	30	Hyndarin	73.94	0.64
6	Tetrahydrocorysamine	34.17	0.86	31	Dehydrocorybulbine	46.97	0.63
7	18797-79-0	46.06	0.85	32	13-methyldehydrocorydalmine	35.94	0.63
8	stylopine	48.25	0.85	33	13-methylpalmatrubine	40.97	0.63
9	24240-05-9	53.75	0.83	34	Corynoloxine	38.12	0.6
10	Fumarine	59.26	0.83	35	Corydalmine	52.5	0.59
11	Dihydrochelerythrine	32.73	0.81	36	Dehydrocorydalmine	43.9	0.59
12	Cavidine	35.64	0.81	37	Isocorypalmine	35.77	0.59
13	dehydrocavidine	38.99	0.81	38	N-methyllaurotetanine	41.62	0.56
14	(-)-alpha-N-methylcanadine	45.06	0.8	39	norglaucing	30.35	0.56
15	saulatine	42.74	0.79	40	ST057701	31.87	0.56
16	berberine	36.86	0.78	41	Corydine	37.16	0.55
17	(R)-Canadine	55.37	0.77	42	(S)-Scoulerine	32.28	0.54
18	Stigmasterol	43.83	0.76	43	Clarkeanidine	86.65	0.54
19	sitosterol	36.91	0.75	44	demethylcorydalmatine	38.99	0.54
20	2,3,9,10-tetramethoxy-13-methyl-5,6-dihydroisoquinolino[2,1-b]isoquinolin-8-one	76.77	0.73	45	Izoteolin	39.53	0.51
21	Cryptopin	78.74	0.72	46	methyl-[2-(3,4,6,7-tetramethoxy-1-phenanthryl)ethyl]amine	61.15	0.44
22	(1S,8'R)-6,7-dimethoxy-2-methylspiro[3,4-dihydroisoquinoline-1,7'-6,8-dihydrocyclopenta[g][1,3]benzodioxole]-8'-ol	43.95	0.72	47	tetrahydroprotopapaverine	57.28	0.33
23	pontevedrine	30.28	0.71	48	quercetin	46.43	0.28
24	Capaurine	62.91	0.69	49	leonticine	45.79	0.26

25	C09367		47.54	0.69

3.2. Disease targets database of CHD. Removing the same genes, a total of 7173 target genes of CHD were screened from GeneCards database and OMIM database. All of these genes were used for construction of disease targets database of CHD.

3.3. Active components of CR-disease targets of CHD network. As shown in Figure 2, we found out that there are 40 "CR-CHD" common target genes, namely target genes with possible roles in treatment of CHD. The common genes correspond to 48 active components of CR. The common genes and the components corresponded to them were converted into a visualized active components of CR-disease targets of CHD network, shown in Figure 3. The distribution of correspondence between ingredients and target genes can be observed intuitively through the network. According to the analysis of the network, distribution of node degree, namely the number of connections or edges the node has to other nodes, of the network is shown in Figure 4. There are 3 components with node degree equal or more than 5, including quercetin, C09367 and stigmasterol, while 3 genes with node degree equal or more than 3, which are *PTGS1, CHRM3, AR*, of which details are listed in Table 2 and Table 3.

FIGURE 2: Intersection of gene targets of CR and CHD

FIGURE 3: Network of CR active ingredients and CHD disease targets

FIGURE 4: Distribution of node degree of CR active ingredients-CHD disease targets network

TABLE 2: Ingredients with node degree ≥10 in CR active ingredients-CHD disease targets network

TABLE 2: Ingredients with node degree ≥10 in CR active ingredients-CHD disease targets network

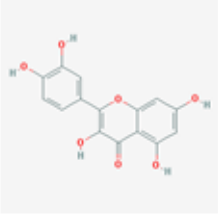
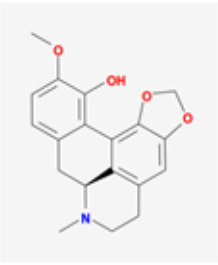
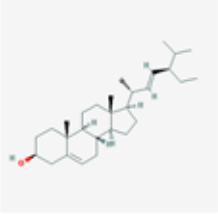
Mol ID	Molecule name	Structure	Node degree
MOL000098	Quercetin		38
MOL000793	C09367		6
MOL000449	Stigmasterol		5

TABLE 3: Gene with node degree ≥10 in CR active ingredients-CHD disease targets network

Gene symbol	Node degree
PTGS1	42
CHRM3	30
AR	23

3.4. Protein-protein interaction network. As can be seen from Figure 5, the protein-protein interaction of "CR-CHD" common target genes are displayed in the network clearly. Each linkage of target genes represents a meaning, such as protein homology, gene co-expression, inter-genetic adjacency. The more adjacent nodes one node has, the more prominent it is. We can view the key genes with equal or more than 8 adjacent nodes in Figure 6.

FIGURE 5: PPI network of CR-CHD shared targets

FIGURE 6: Histogram of genes with the number of adjacent genes (≥8) in the PPI network

3.5. GO enrichment and KEGG pathway analysis of "CR-CHD" common target genes. A total of 70 biological processes and 67 signaling pathways were obtained through GO enrichment analysis and KEGG pathway analysis with the *P* value 0.05. The first 20 biological processes were visualized in Figure 7, ranking based on the *P* values in the order from small to large. Top 5 biological processes which are DNA-binding transcription activator activity, RNA polymerase II-specific with the highest enrichment score, and the next most critical processes, which were RNA polymerase II transcription factor binding, kinase regulator activity, ubiquitin-like protein ligase binding can be used for the subsequent analysis. A total of 12 related signaling pathways, after removing of the extensive pathway of KEGG pathway enrichment by literature search on PubMed and Embase, are shown in Table 4. Fluid shear stress and atherosclerosis signaling pathway with the most abundant gene enrichment can be the significant pathway for further analysis, secondly TNF signaling pathway, apoptosis, MAPK signaling pathway and PI3K-Akt signaling pathway, of which details are visualized in Figure 8. The target gene-molecular function and pathway network was constructed by Cytoscape 3.6.0 software. The network can be seen from Figure 9 that biological processes are represented by small red dots arrayed at the upper part within the circle, while pathways arrayed at the lower part, and dots in the circle indicate genes. Dot size represents a node degree. The bigger the dot is, the more significant the gene is. And the genes with node degree equal or more than 10 in target gene-molecular function and pathway network were listed in Table 5. In addition, the network was analyzed by MCODE plugin, and 3 significant modules were selected as shown in Figure 10.

FIGURE 7: Dotplot of the first 20 biological processes with CR-CHD common genes gathering

TABLE 4: Pathways related with CHD

ID	Term	Count	Value
hsa05418	Fluid shear stress and atherosclerosis	10	1.35E-07
hsa04668	TNF signaling pathway	8	2.07E-06
hsa04210	Apoptosis	7	8.67E-05
hsa04010	MAPK signaling pathway	7	2.48E-03
hsa04151	PI3K-Akt signaling pathway	7	6.30E-03
hsa04115	p53 signaling pathway	5	2.71E-04
hsa04657	IL-17 signaling pathway	5	6.31E-04
hsa04064	NF-kappa B signaling pathway	5	8.05E-04
hsa04218	Cellular senescence	5	4.58E-03
hsa04066	HIF-1 signaling pathway	4	7.66E-03
hsa04370	VEGF signaling pathway	3	1.10E-02
hsa04012	ErbB signaling pathway	3	2.56E-02

FIGURE 8: Fluid shear stress and atherosclerosis pathway

FIGURE 9: Network of target-molecular function-pathway

FIGURE 10: Modules of target- molecular function-pathway based on MCODE

TABLE 5: Genes with node degree ≥10 in target gene-molecular function and pathway network

Uniprot symbol	Node degree	Uniprot symbol	Node degree
CASP3	17	ERBB3	11
PPARG	15	CAP1A1	11
NOS3	14	AR	10
CASP8	13	AHR	10
FOS	12	HIF1A	10
GSTP1	11		

3.6. Active ingredients of CR relative to the hub genes. Based on the above analysis, we listed the hub genes, including *AR*, *FOS*, *CASP3*, *IL6*, *MYC*, *PPAG*, *NOS3*, *CASP8*, *GSTP1*, *CYP1A1*, *AHR*, *HIF1A*, *ERBB2*, *IGF2*, *HSPB1*, *CRP*, *CHRM3*, *DUOX2*, *PTGS1*, which played important roles in each analysis, and active ingredients corresponding to these genes in Table 6. These hub genes and ingredients are the main focus in this study.

TABLE 6: Active ingredients of CR relative to the hub genes

Gene symbol	Ingredient	Analysis
AR	Berberine, coptisine, Dihydrochelerythrine, Dihydrosanguinarine, Corydine, Corynoloxine, Dehydrocorybulbine, dehydrocorydaline, Dehydrocorydalmine, 13-methyldehydrocorydalmine, Izoteolin, 13-methylpalmatrubine, N-methyllaurotetanine, norglaucing, pseudocoptisine, ST057701, 2,3,9,10-tetramethoxy-13-methyl-5,6-dihydroisoquinolino[2,1-b]isoquinolin-8-one, palmatine, bicuculline, C09367	PPI network, GO and KEGG enrichment
FOS	Bicuculline, quercetin	PPI network, GO and KEGG enrichment
CASP3	Quercetin	PPI network, GO and KEGG enrichment
IL6	Quercetin	PPI network
MYC	Quercetin	PPI network
PPAG	Dihydrochelerythrine, Dihydrosanguinarine, Corydalmine, quercetin	GO and KEGG enrichment
NOS3	Quercetin	GO and KEGG enrichment
CASP8	Quercetin	GO and KEGG enrichment
GSTP1	Quercetin	GO and KEGG enrichment
CYP1A1	Quercetin	GO and KEGG enrichment
AHR	Quercetin	GO and KEGG enrichment
HIF1A	Quercetin	GO and KEGG enrichment
ERBB2	Quercetin	PPI network, MCODE analysis(Cluster 1)
IGF2	Quercetin	MCODE analysis(Cluster 1)
HSPB1	Quercetin	MCODE analysis(Cluster 1)
CRP	Quercetin	MCODE analysis(Cluster 2)
CHRM3	Cryptopin, (S)-Scoulerine, Cavidine, (R)-Canadine, Hyndarin, (-)-alpha-N-	MCODE

	methylcanadine, Capaurine, Clarkeanidine, CORYDALINE, Corydalmine, Corydine, 18797-79-0, dehydrocavidine, demethylcorydalmatine, Izoteolin, isocorybulbine, leonticine, N-methyllaurotetanine, norglaucing, 24240-05-9, saulatine, stylopine, Tetrahydrocorysamine, tetrahydroprotopapaverine, ST057701, Stigmasterol, Fumarine, Isocorypalmine, C09367	analysis(Cluster 2)
DUOX2	Quercetin	MCODE analysis(Cluster 3)
PTGS1	Berberine, coptisine, Cryptopin, Dihydrochelerythrine, Dihydrosanguinarine, sanguinarine, (S)-Scoulerine, Cavidine, (R)-Canadine, Hyndarin, Capaurine, Clarkeanidine, Corydalmine, Corydine, 18797-79-0, Corynoloxine, methyl-[2-(3,4,6,7-tetramethoxy-1-phenanthryl)ethyl]amine, dehydrocavidine, Dehydrocorybulbine, dehydrocorydaline, Dehydrocorydalmine, demethylcorydalmatine, 13-methyldehydrocorydalmine, Izoteolin, isocorybulbine, leonticine, 13-methylpalmatrubine, N-methyllaurotetanine, norglaucing, pontevedrine, pseudocoptisine, 24240-05-9, stylopine, Tetrahydrocorysamine, ST057701, 2,3,9,10-tetramethoxy-13-methyl-5,6-dihydroisoquinolino[2,1-b]isoquinolin-8-one, Stigmasterol, palmatine, Fumarine, Isocorypalmine, C09367	MCODE analysis(Cluster 3)

4. Discussion

In this study, we attempted to explore the mechanism of action by which CR treating CHD, with the method of network pharmacology.

In the active components of CR-disease targets of CHD network, quercetin, C09367 and stigmasterol are key ingredients as they are closely related to the most target genes compared to other ingredients of CR. Also, quercetin relates to the majority of hub genes as we concluded in Table 6, while C09367 relates to the rest of hub genes. Quercetin presents significant functions as inhibition of LDL oxidation, reduction of inflammatory markers, endothelium-independent vasodilator effects, protection on endothelial function and nitric oxide, and antiplatelet effect, proved by vitro and some animal models, showing the potential to treat cardiovascular diseases[31, 32, 33]. Also, quercetin showed the analgesic property in various models of inflammation[34]. It is worth mentioning that stigmasterol is related to almost the same genes as C09367, excepting the AR gene. Mouse experiments demonstrated that stigmasterol prevented the HFWD-induced elevation of some di- and triacylglycerol species, decreased serum levels of ceramides, inhibited intestinal absorption of cholesterol and plant sterol, and suppressed hepatic cholesterol[35, 36]. Therefore, these key ingredients can achieve a good effect on CHD by lowering cholesterol, inhibiting platelet aggregation, suppressing inflammatory response and relieving the pain.

After analysis of the active components of CR-disease targets of CHD network, we found out that, *PTGS1*, *CHRM3* and *AR* are the top three most significant genes, for a total of 40 “CR-CHD” common target genes, with the most relative ingredients of CR. Prostaglandin G/H synthase 1 protein corresponding to *PTGS1* gene, is involved in the generation of thromboxane A2 (TXA2), which promotes platelet activation and aggregation, vasoconstriction and proliferation of vascular smooth muscle cells. Therefore, we expect a modulating effect of CR on the expression of *PTGS1* encoding the Prostaglandin G/H synthase 1, proving in future experiments. *CHRM3* encodes Muscarinic acetylcholine receptor M3 protein proved to cause an endothelium-independent vasodilatation in a mouse experiment[37]. The muscarinic acetylcholine receptor mediates various cellular

responses, including inhibition of adenylate cyclase, breakdown of phosphoinositide and modulation of potassium channels through the action of G proteins[38]. As to *AR* gene, which encodes androgen receptor protein, is verified in some investigations directly that it has atheroprotective effects through both *AR*-dependent and *AR*-independent mechanisms, causing different incidence of cardiovascular disease between men and women[39, 40]. When analyzing the PPI network, genes with the node degree equal or more than 8 were selected as key genes, including *CASP3*, *IL6*, *MYC*, *ERBB2*, *AR*, *FOS*. A member of caspase family, caspase-3, encoded by *CASP3* gene, in neuronal cells, has been identified as a key mediator of apoptosis[41]. Encoded by *IL6*, interleukin-6 is increased in a number of cardiovascular diseases, and also associates with a higher incidence of future cardiovascular events, which effects on activity and expression of endothelial nitric oxide synthase and increases vascular superoxide, thus inactivating NO thereby and limiting NO bioavailability[42]. A Swedish cohort indicated that interleukin-6 trans-signaling driven by the *IL6* and soluble *IL6* receptor binary complex, could be a promising marker of cardiovascular events risk and possibly be used for anti-inflammatory therapy[43]. Expression of *ERBB2* relates to mitochondrial function in cardiomyocytes, according to a mouse experiment[44], encoding receptor tyrosine-protein kinase which has a protective effect on cardiomyocytes[45] as well as regulates outgrowth and stabilization of peripheral microtubules. *FOS* gene could be one of the indexes reflecting myocardial ischemia[46]. By analyzing the target gene-molecular function and pathway network, there were 11 genes, including *CASP3*, *PPARG*, *NOS3*, *CASP8*, *FOS*, *GSTP1*, *ERBB3*, *CYP1A1*, *AR*, *AHR*, *HIF1A*, found out to act important roles in the network, as each of them associating with equal or more than 10 molecular functions and pathways. *PPARG* might increase the risk of CHD in Asian population, as suggested in a meta-analysis[47], as well as *PPARGC161T CT/TT* was associated with lower levels of blood TC and LDL-C in Han population[48]. *NOS3* is significantly altered in patients with CHD[49], of which expression reduces in patients with atherosclerosis[50]. Caspase-8, encoded by *CASP8* gene controls apoptosis, necroptosis and pyroptosis as a switch[51]. *CYP1A1* showed an increased expression in females compared to males under the situation of ischemic heart disease[52]. *AHR* regulates the expression of members in *CYP1* family, including *CYP1A1* and *CYP1A2*. It was demonstrated that the *AHR* system could induce the reporter gene expression by acute hypoxia, of which induction was transient, in an ischemic hind limb model[53]. Hypoxia inducible factors, including *HIF1A*, are key oxygen sensors that mediate the ability of the cell to deal with hypoxia[54]. Moreover, 3 modules derived from MCODE analysis, which are module 1 consisting of *ERBB2*, *IGF2*, *HSPB1*, module 2 consisting of *CHRM3*, *CRP*, and module 3 consisting of *DUOX2*, *PTGS1*. *IGF2* may be relevant to the regeneration of the mammalian heart after injury[55], of which expression is induced to increase by hypoxia in rat hearts[56]. An experiment suggested that *HSPB1* acts a role that reduced inflammation and healed wound after myocardial infarction(MI), expected to be a target for myocardial repair in MI patients[57]. *CRP*, which is one of the inflammatory biomarkers, is considered to be an indicator for evaluating severity and prognosis of CHD, as lipoproteins inflammation is considered significant in the pathogenesis of CHD[58]. The key genes mentioned above reveal mechanisms underlying the therapeutic effect of CR in the treatment of CHD, participating, to varying degrees, in the process of platelet aggregation, vasoconstriction and vasodilatation, proliferation and repair of vascular endothelial cells and cardiomyocytes, lipid metabolism and in inflammation in the heart. CR may play a full part in the treatment by acting on these key genes. Furthermore, taking these results together, it was concluded that *AR*, *FOS*, *CASP3* were the 3 most critical genes that played roles in the underlying mechanisms for CR treatment on CHD.

We scanned out the biological process of DNA-binding transcription activator activity, RNA polymerase II-specific with the highest enrichment score, and the next most critical processes, which were RNA polymerase II transcription factor binding, kinase regulator activity, ubiquitin-like protein ligase binding after GO enrichment analysis. GO enrichment analysis showed that CR may achieve effects on CHD through the bi-directional regulation of DNA-binding transcription activator activity, RNA polymerase II-specific, RNA polymerase II transcription factor binding, kinase regulator activity, ubiquitin-like protein ligase binding, though regulation mechanisms still waiting for strong scientific evidence. After KEGG enrichment analysis and removal of wide range of metabolic pathways, we concluded 5 top pathways with the highest enrichment scores, including fluid shear stress and atherosclerosis, TNF signaling pathway, apoptosis, MAPK signaling pathway, PI3K-Akt signaling pathway. Sensing of fluid shear stress and atherosclerosis is considered important in processes of vascular development and remodeling[59, 60]. Studies demonstrated that TNF antagonists has a potent effect of anti-inflammatory and antioxidant[61, 62]. MAPK signaling pathway is activated to regulate apoptosis of cardiac myocytes and angiogenic response of microvascular endothelial cells by related factors, for example, nicotine and protein phosphatase 2A, demonstrating that activation of the MAPK signaling pathway appeared to be a key process in microvascular endothelial cells and cardiac myocytes life-death decisions[63]. The PI3K/Akt signal pathway regulates survival, apoptosis, cell morphology, protein synthesis, and integration of metabolism in cardiomyocytes[64], involved in regulating inflammatory responses, playing a critical role in cardioprotection of preconditioning against ischemia injury[65]. These researches indicate that CR acts on CHD at multiple levels through multiple biological processes and mainstream signaling pathways, mainly regulating vascular development and remodeling, inflammatory process, oxidation, endothelial cell and cardiacmyocyte apoptosis and protein synthesis. Furthermore, we can understand the advantages of CR in the treatment of CHD and its potential in new drug development by this study.

5. Conclusions

Traditional Chinese medicine itself with diversified structures have multi-targets and multi-pathways effect, thus increasing the difficulty to identify mechanisms of active ingredients in CR acting on CHD. Network pharmacology offers a promising new way to solve this problem, basing on the network of “drug-target-disease-pathway” interactions and bioinformatics analysis. Combined with results of above analyses, it suggests that quercetin plays a dominant role in therapy of CR on CHD, as it relates to the most target genes of CHD. And also, some of these genes corresponding to quercetin are demonstrated to take a critical part in the subsequent PPI network, GO and KEGG enrichment analyses. Among them are *FOS* and *CASP3*, which involve in fluid shear stress and atherosclerosis, TNF signaling pathway. The next significant ingredients are C09367 and stigmasterol, for their common target genes involve in the control of the same biological processes, for example, *DUOX2* and *PTGS1* participant in heme binding and tetrapyrrole binding, *CHRM3* and *CRP* involve in neurotransmitter binding and ammonium ion binding. Moreover, the *AR* gene, related to C09367, is the key player in PPI network, GO and KEGG enrichment. In this study, we analyzed the active components of CR as well as the possible mechanisms of these components on CHD, forming the basis of a future research projects.

Abbreviations

CR
Corydalis Rhizoma
CHD
Coronary heart disease
CVD
Cardiovascular diseases
MI
Myocardial infarction
TCM
Traditional Chinese medicine
OMIM
Online Mendelian inheritance in man
Uniprot
Universal Protein Resource
KEGG
Kyoto Encyclopedia of Genes and Genomes
PPI
Protein-protein interaction
GO
Gene Ontology
DL
Drug similarity
OB
Oral bioavailability
DO
Disease ontology

Declarations

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Ying Li and Zhenkun Zhuang searched articles in electronic databases and wrote the manuscript. Mingtai Chen, Haidan Lin and Changjian Yuan analyzed the data. Meihuan Li and Yanhui Wu performed the data extraction. Zhong Zhang designed the study and amended the paper.

Acknowledgments

This study was supported by the SanMing Project of Shenzhen City of China (grant number: SZSM201612033).

Supplementary Materials

Supplementary Table 1: Ingredients and related genes of CR. Supplementary Table 2: Genes of CHD. Supplementary Table 3: Common genes of CR and CHD. Supplementary Table 4: Biological processes and pathways from KEGG and GO. *(Supplementary Materials)*

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Figures

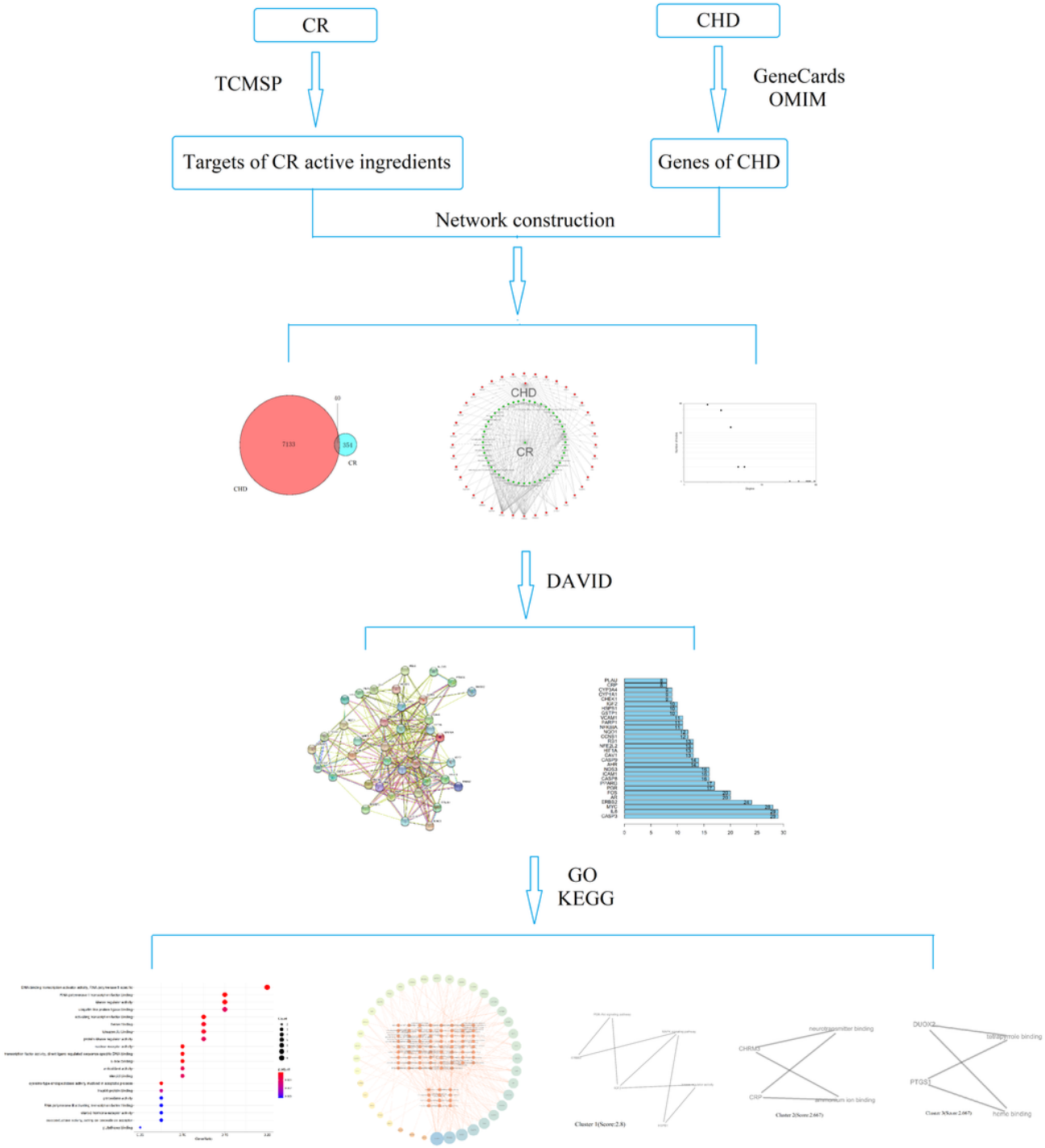

Figure 1

Flow chart of the research

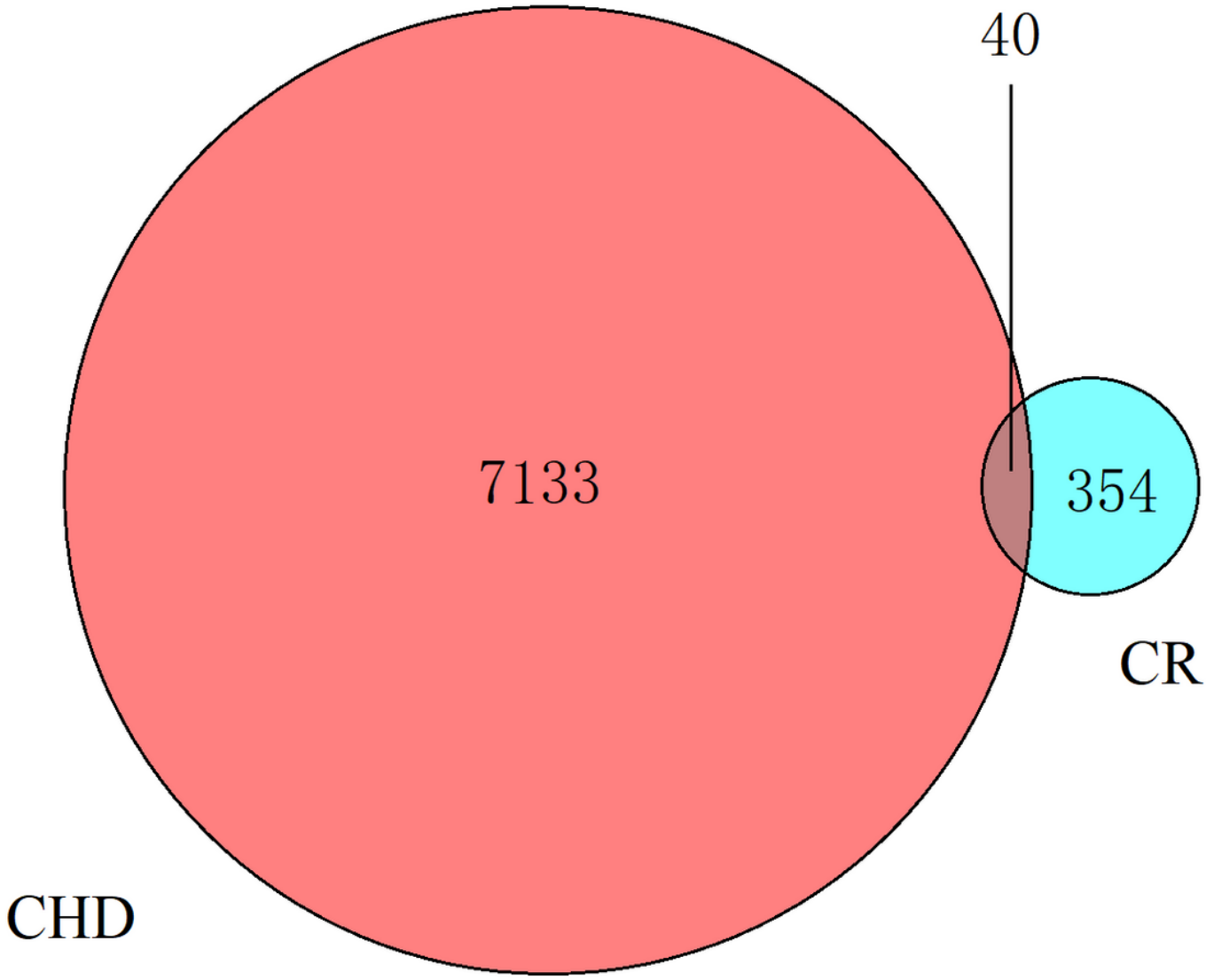

Figure 2

Intersection of gene targets of CR and CHD

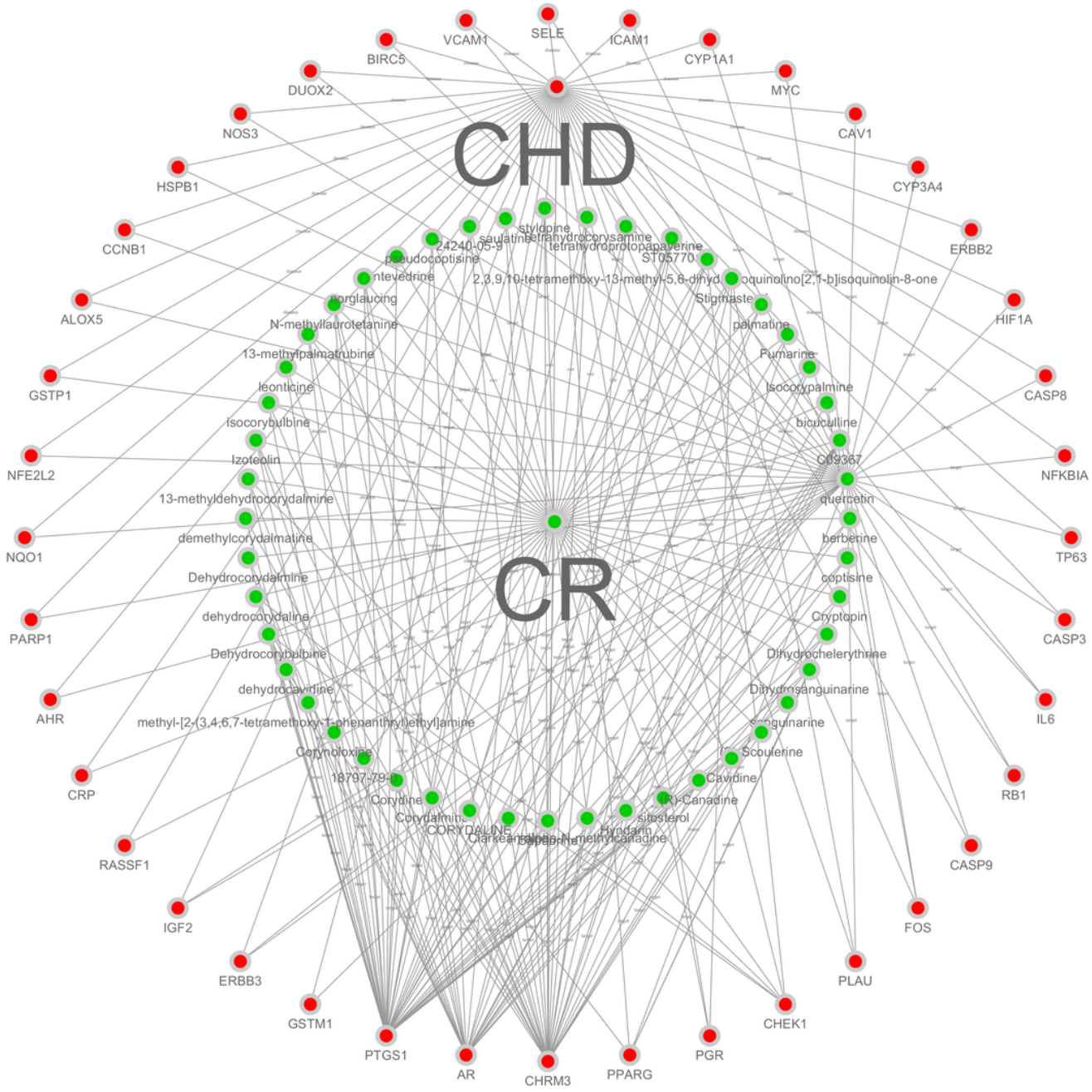

Figure 3

Network of CR active ingredients and CHD disease targets

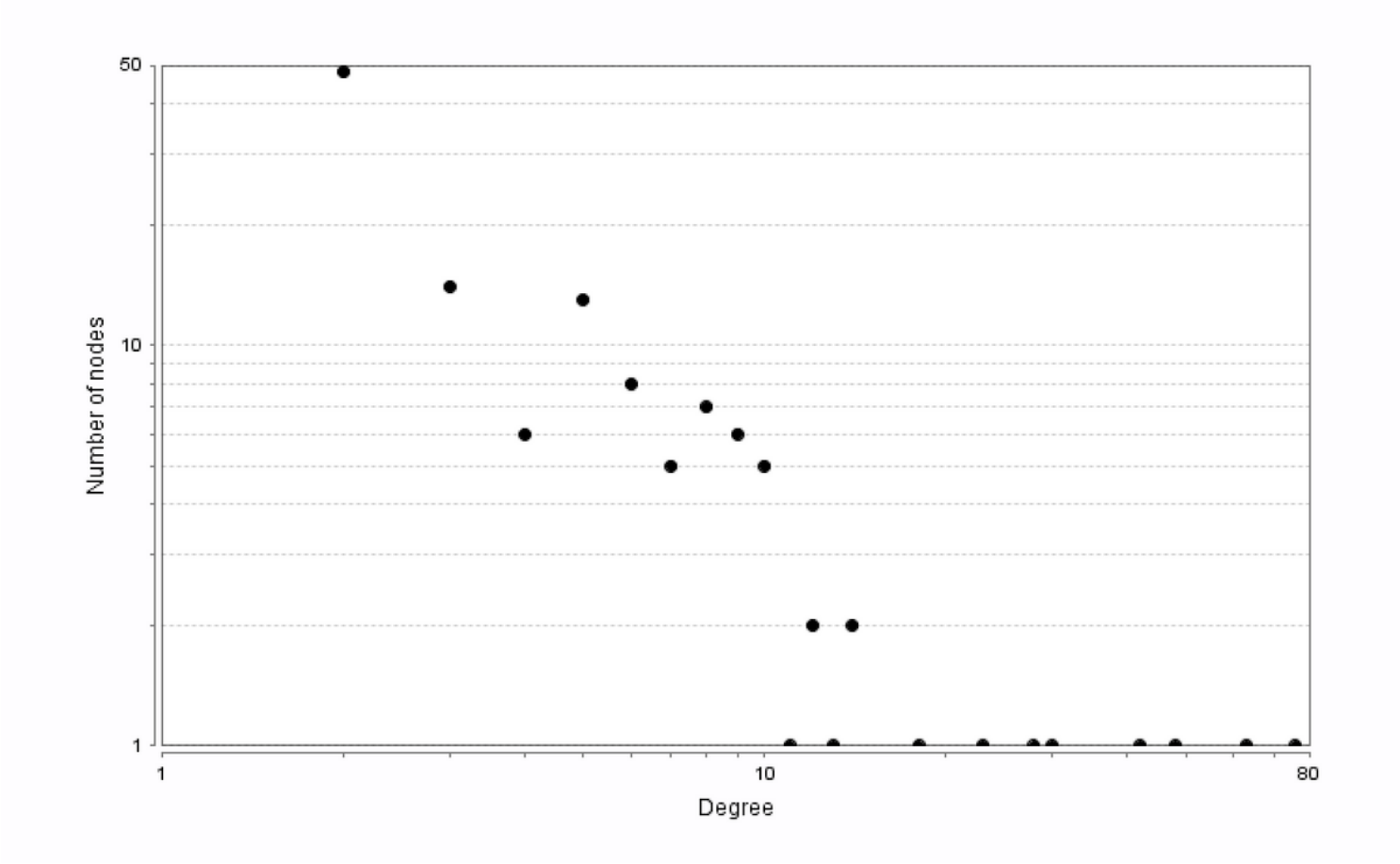

Figure 4

Distribution of node degree of CR active ingredients-CHD disease targets network

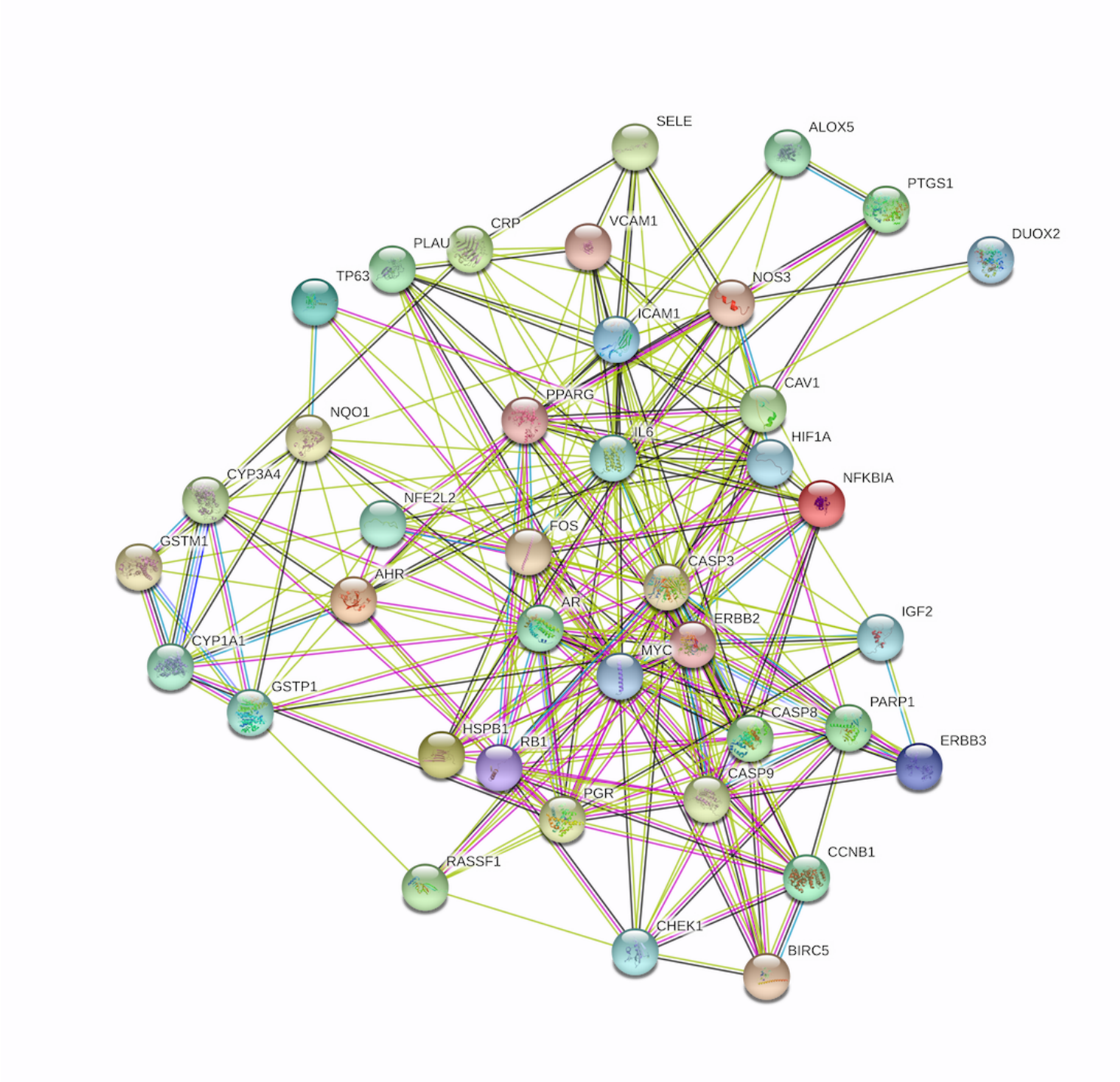

Figure 5

PPI network of CR-CHD shared targets

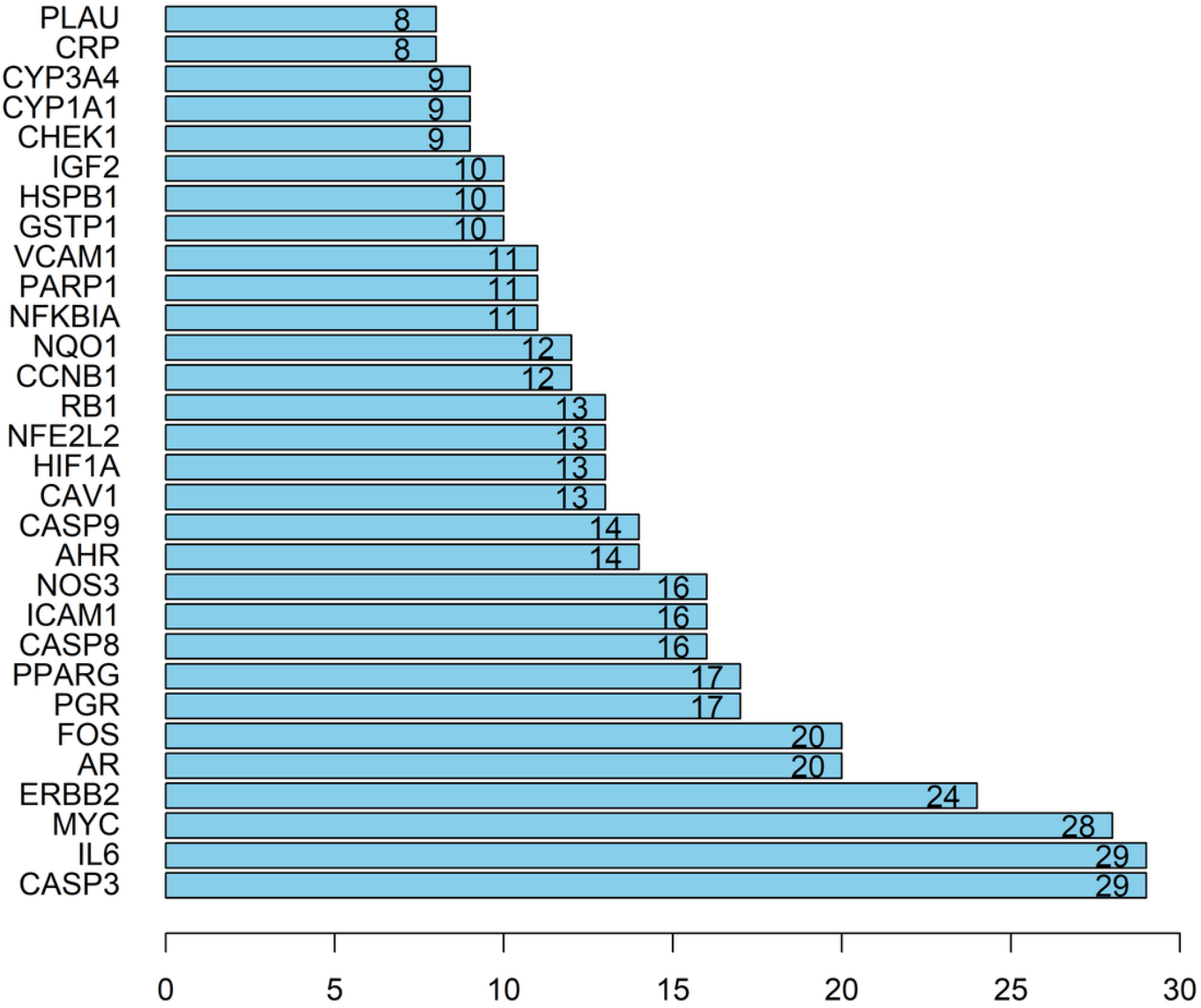

Figure 6

Histogram of genes with the number of adjacent genes (≥8) in the PPI network

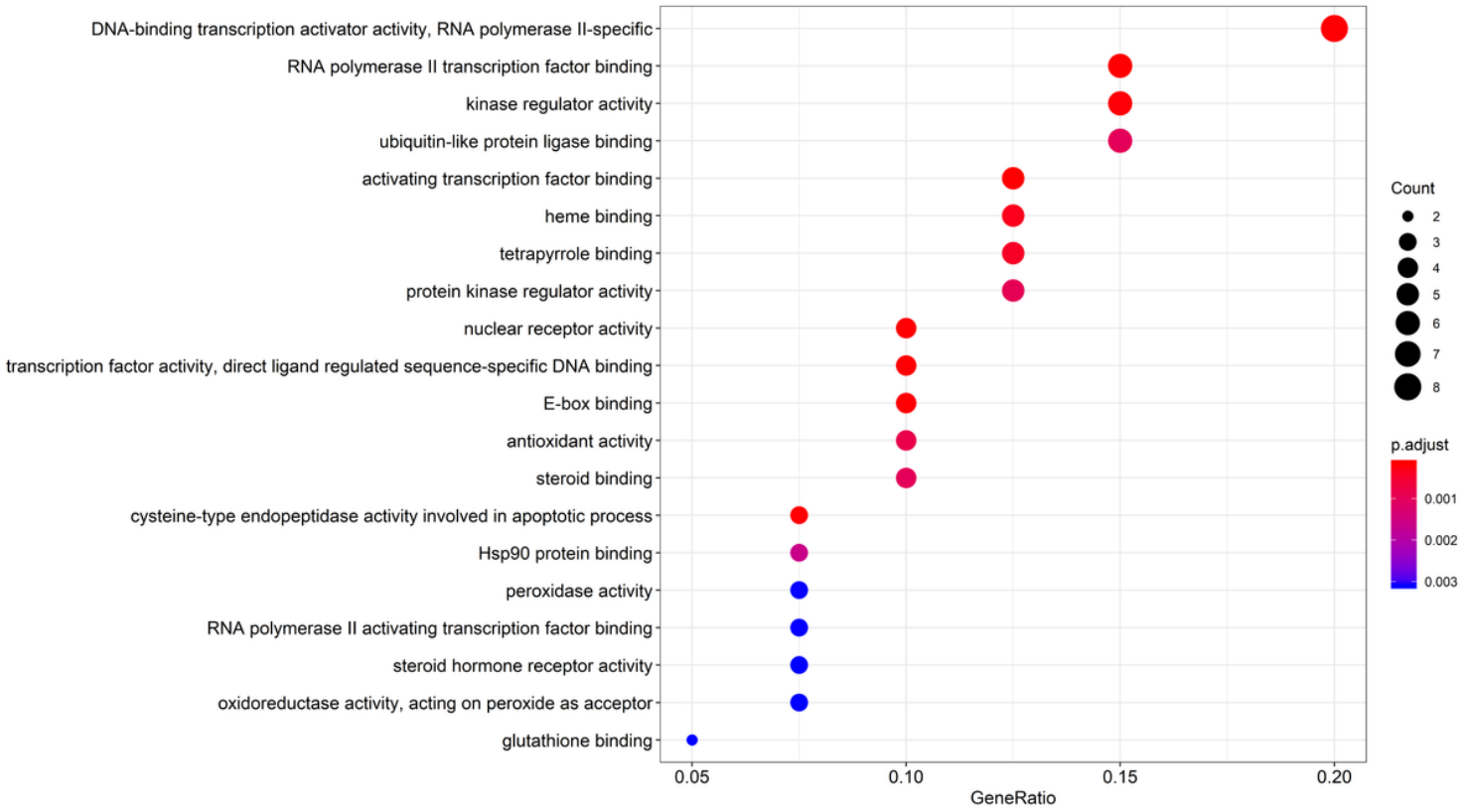

Figure 7

Dotplot of the first 20 biological processes with CR-CHD common genes gathering

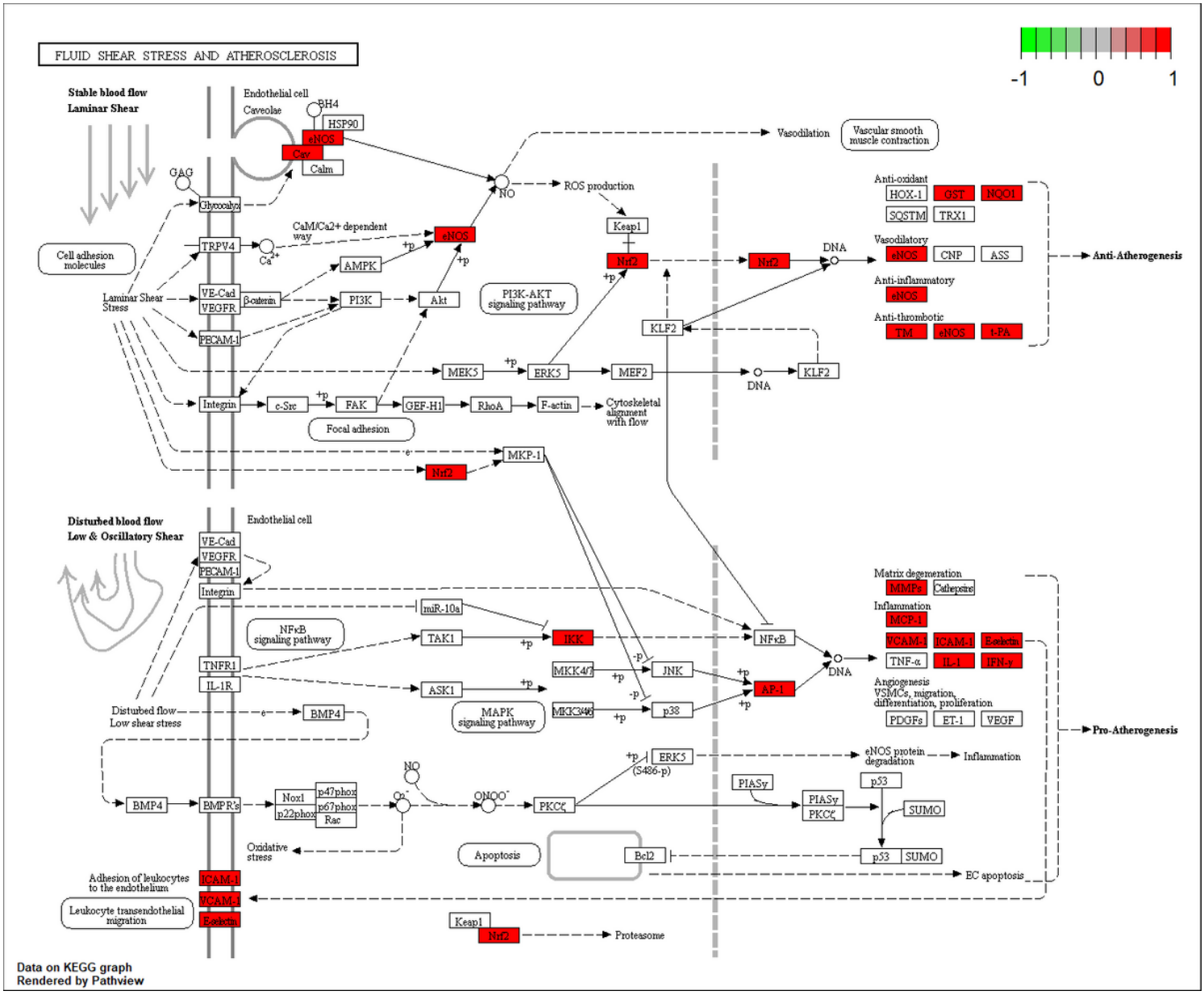

Figure 8

Fluid shear stress and atherosclerosis pathway

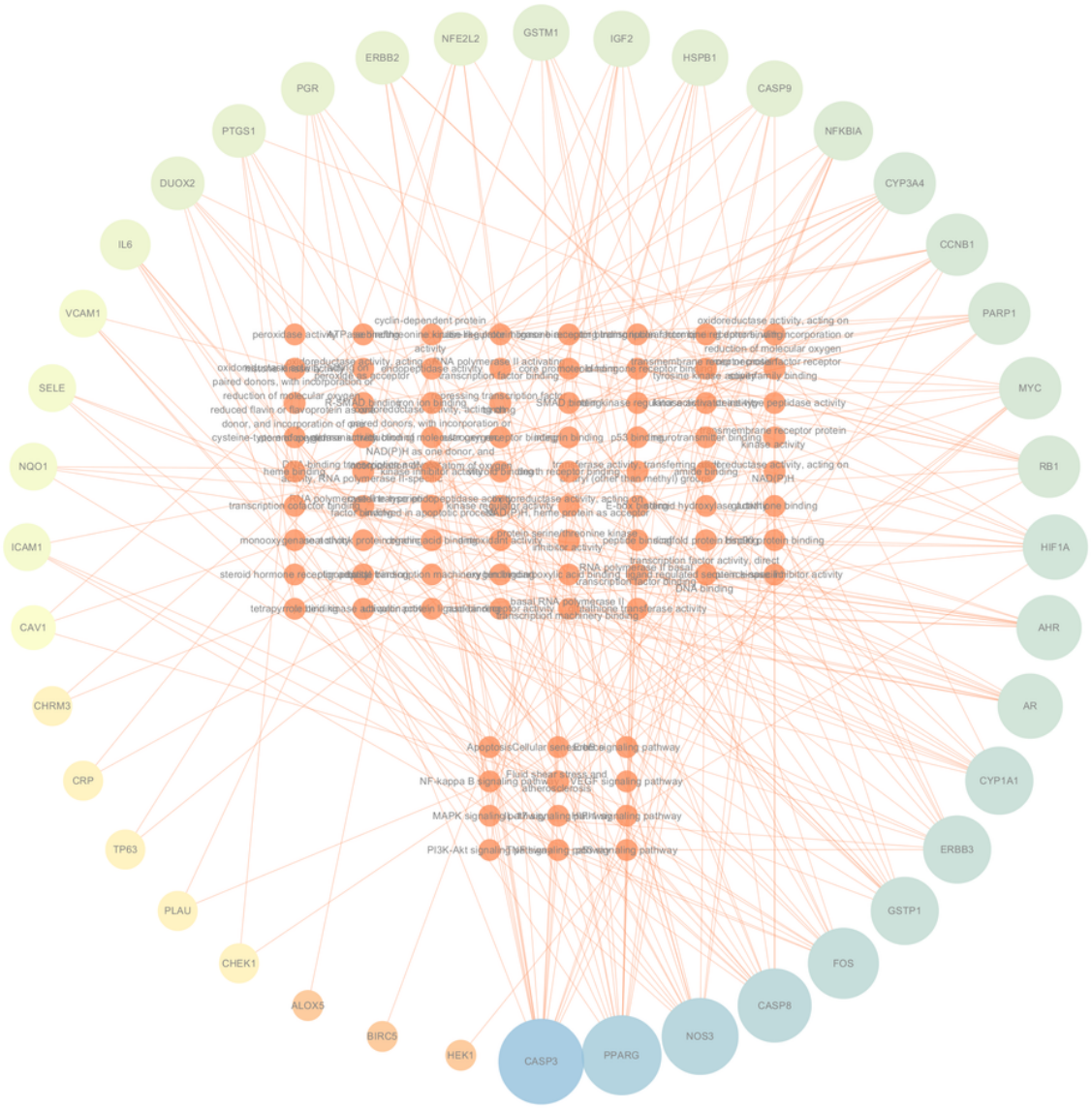

Figure 9

Network of target-molecular function-pathway

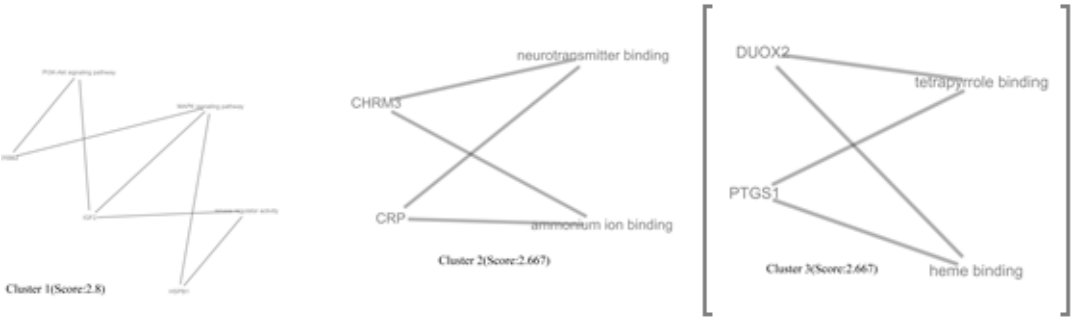

Figure 10

Modules of target- molecular function-pathway based on MCODE

Supplementary Files

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- Supplementary2.xlsx
- Supplementary1.xlsx