Exploring the mechanism of Danggui Buxue Decoction in regulating atherosclerotic disease network based on integrated pharmacological methods

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Running Title: Mechanism of DGBXD on Atherosclerosis

Abstract:

Objective: To explore the mechanism of Danggui Buxue Decoction (DGBXD) in regulating Atherosclerosis (AS) network based on integrated pharmacological methods.

Methods: The active ingredients and targets of DGBXD are obtained from TCMSP database and ETCM. AS-related targets were collected from the Genecards and OMIM databases. The drug-disease protein interaction (PPI) networks were constructed by Cytoscape. Meanwhile, it was used to screen out densely interacting regions, namely clusters. Finally, Gene Ontology (GO) annotations are performed on the targets and genes in the cluster to obtain biological processes, and Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations are performed on the targets of the PPI network to obtain signaling pathways.

Results: A total of 212 known targets, 265 potential targets and 229 AS genes were obtained. The “DGBXD known-AS PPI network” and “DGBXD-AS PPI Network” were constructed and analyzed. DGBXD can regulate inflammation, platelet activation, endothelial cell apoptosis, oxidative stress, lipid metabolism, vascular smooth muscle proliferation,
angiogenesis, TNF, HIF-1, FoxO signaling pathway, etc. The experimental data showed that compared with the model group, the expressions of ICAM-1, VCAM-1 and IL-1β protein and mRNA in the DGBXD group decreased (P<0.05). However, plasma IL-1β, TNF-α and MCP-1 in the DGBXD group were not significantly different from the model group (P>0.05).

**Conclusion:** The mechanism of DGBXD in the treatment of AS may be related to the improvement of extracellular matrix deposition in the blood vessel wall and the anti-vascular local inflammatory response, which may provide a reference for the study of the mechanism of DGBXD.

**Key Words:** Danggui Buxue Decoction; Atherosclerosis; Integrated pharmacological; Bioinformatics; Chinese Medicine; Herb Medicine

1 Introduction

Atherosclerosis (AS) is a common disease that seriously harms human health, and is the most common and important type of arteriosclerosis [1-2]. There are lipid deposits in the arterial intima, accompanied by the proliferation of smooth muscle cells (SMC) and connective tissue, which results in the formation of fibrous plaques on the intima, causing the vessel wall to thicken, harden, and narrow the lumen. Then, the connective tissue that deposits a large amount of lipids in the plaque undergoes necrosis to form atheroma [2-3]. AS mainly involves large and medium-sized arteries, namely the aorta and its main branches (brain, kidney, arteries of the limbs, and coronary arteries, etc.) [3]. The etiology and pathogenesis of AS have not yet been fully understood, but it is unanimously recognized that hyperlipidemia, smoking, and hypertension are the main risk factors for the disease [4-5].

The current treatment measures are mainly lipid-lowering, anticoagulant and thrombolytic drugs, but the treatment effect is not ideal due to poor compliance and drug side effects [6-7]. Danggui Buxue Decoction (DGBXD) is composed of *Angelicae Sinensis Radix* (Danggui) and *Hedysarum Multijugum Maxim.* (Huangqi) [8]. Modern pharmacological research shows that DGBXD has pharmacological effects such as improving blood rheology and hemodynamics, regulating blood lipids, improving vascular endothelial function, and regulating inflammation; It has a significant effect on the treatment of hyperlipidemia,
In recent years, clinical and experimental studies have shown that DGBXD can improve AS by inhibiting inflammation, protecting endothelial cell function, and improving hemodynamics [9-12]. However, the therapeutic effect and mechanism of DGBXD on AS are still unclear. In view of the multi-component, multi-effect, multi-target and overall regulatory effects of traditional Chinese medicine (TCM), this study uses TCM to integrate pharmacological strategies to explore the key targets and signal pathways of DGBXD intervention in AS, to explore its molecular mechanism, in order to provide a basis for the development and development of DGBXD drugs [13].

Integrated pharmacology is a new model of modern TCM research [14]. The law of interaction between the substance entity of TCM prescriptions and the body is one of the key scientific issues in the study of integrated pharmacology. It is an interdisciplinary integration of TCM chemistry, pharmacokinetics, pharmacology, systems biology, and computational science [15]. Our previous research has applied integrated pharmacology to the herbal formulae to intervene in cardiovascular, tumor and endocrine diseases by developing new methodology [16-18]. Therefore, this study will explore the mechanism of DGBXD on AS through integrated pharmacological strategies. The idea and process of this research was shown in Figure 1.
Figure 1 The idea and process of this research
2 Materials and Methods

2.1 Known and Potential Targets Collection

The components and known targets of DGBXD were collected from TCMSP database (http://tcmspw.com/index.php) [19] with their oral bioavailability (OB) ≥ 30%, Caco-2 permeability > -0.4 and drug-likeness (DL) ≥ 0.18 (Table 1). The potential targets were collected from ETCM (http://www.tcmip.cn/ETCM/index.php) [20], which is a database including comprehensive and standardized information for the commonly used herbs and formulas of TCM, as well as their ingredients. (Table S1 and S2).

Table 1 The Potential components

| Molecule Name | MW   | OB (%) | Ca | DL  |
|---------------|------|--------|----|-----|
| (3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol (64474-51-7) | 302.35 | 67.67 | 0.9 | 0.26 |
| (3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol (64997-52-0) | 428.82 | 36.23 | 1.4 | 0.78 |
| (6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofuro[3,2-c]chroman-3-ol (73340-41-7) | 300.33 | 64.26 | 0.9 | 0.42 |
| 1,7-Dihydroxy-3,9-dimethoxy pterocarpene | 314.31 | 39.05 | 0.8 | 0.48 |
| 3,9-di-O-methylnissolin | 314.36 | 53.74 | 1.1 | 0.48 |
| 7-O-methylisomucronulatol | 316.38 | 74.69 | 1.0 | 0.3 |
| beta-sitosterol | 414.79 | 36.91 | 1.3 | 0.75 |
| Bifendate | 418.38 | 31.1 | 0.1 | 0.67 |
| Compound       | MW     | Molecular Weight | I  | Neutral Loss |
|----------------|--------|------------------|----|--------------|
| Calycosin      | 284.28 | 47.75            | 0.5| 0.24         |
| formononetin   | 268.28 | 69.67            | 0.7| 0.21         |
| hederagenin    | 414.79 | 36.91            | 1.3| 0.75         |
| isoflavanone   | 316.33 | 109.99           | 0.5| 0.3          |
| isorhamnetin   | 316.28 | 49.6             | 0.3| 0.31         |
| Jaranol        | 314.31 | 50.83            | 0.6| 0.29         |
| kaempferol     | 286.25 | 41.88            | 0.2| 0.24         |
| Mairin         | 456.78 | 55.38            | 0.7| 0.78         |
| quercetin      | 302.25 | 46.43            | 0.0| 0.28         |
| Stigmasterol   | 412.77 | 43.83            | 1.4| 0.76         |
| Ferulic acid   | 194.2  | 39.56            | 0.4| 0.06         |
| Butylenephthalide | 188.24 | 42.44          | 1.3| 0.07         |
| Senkyunolide I | 204.24 | 46.8             | 0.8| 0.08         |

2.2 AS Gene Collection

The AS-related gene were collected from Genecards (http://www.genecards.org) [21] and
Online Mendelian Inheritance in Man (OMIM) (http://omim.org/) databases [22] with keywords “Atherosclerosis”. The genes with relevance score ≥ 6.0 were selected for sequence research. (Table S3)

2.3 Network Construction and Analysis Methods

The protein-protein interaction (PPI) data of DGBXD targets and AS genes were collected from String 11.0 (https://string-db.org) [23]. According to DGBXD target and AS gene information, Cytoscape 3.7.0 software is used to construct a drug target-disease gene network (ie DGBXD Known Target-AS PPI network and DGBXD-AS PPI network) [24]. Then, the DGBXD-AS PPI network were analyzed by the “Network Analyzer” and “MCODE” to collect the degree and betweenness of nodes and the cluster of this PPI network [24]. The DAVID ver 6.8 (https://david.ncifcrf.gov/) was utilized to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and Gene Ontology (GO) enrichment analysis [25].

2.4 Molecular docking analysis

The molecular structure of DGBXD components were collected from TCMSP. The PDB database (https://www.rcsb.org/) was used to retrieve the 3D structure of PIK3R1 (PDB ID: 1H9O) and AKT1 (PDB ID: 1H10), and download the file in the "pdb" format [26]. Discovery Studio Client Ver. 4.5 was used to hydrogenate, remove water, and remove ligand molecules from receptor molecules. Auto Dock ver. 4.2 software was used for molecular docking, supplemented by SwissDock [27]. If the binding energy of the receptor and the ligand is ≤ -5.0 kCal/mol, it is considered that the ligand can bind to the receptor stably [28-29].

2.5 Experimental Materials

2.5.1 Experimental Animals

Forty (40) specific pathogen free (SPF) Sprague-Dawley (SD) male rats, weighing 220~250g, were purchased from Hunan Slack Jingda Experimental Animal Co., Ltd. [Quality Certificate Number: SCXK (Xiang) 2013-0004]. The rats were bred adaptively for 1 week before the experiment with a humidity of 45%-65% and a room temperature of 25°C. All animal experiments took place at the Experimental Animal Center of Hunan University of
Chinese Medicine, License number: SCXK (Xiang) 2013-0005. Animal experiments were approved by the Animal Ethics Committee of Hunan University of Chinese Medicine (Ethical approval number: HUCM-15021) and were in accordance with the National Institute of Health’s Guide for the Care and Use of Laboratory Animals.

2.5.2 Experimental Drugs

Angelicae Sinensis Radix is produced in Gansu Province (batch number: 20170501); Hedysarum Multijugum Maxim. is produced in Neimenggu Province (batch number: 20171607). Herbs was purchased by the Department of Pharmacy of the First Affiliated Hospital of Hunan University of Chinese Medicine and appraised by Professor Zuo Yajie of the Department of Pharmacy of the First Affiliated Hospital of Hunan University of Chinese Medicine. Atorvastatin calcium is produced by Zhejiang Xindonggang Pharmaceutical Co., Ltd. (batch number: 20160803, specification: 10mg/tablet).

2.5.3 Reagents and Instruments

Mouse anti-rat intercellular cell adhesion molecule-1 (ICAM-1) monoclonal antibody, rabbit anti-rat vascular cell adhesion molecule-1 (VCAM-1) monoclonal antibody, rabbit anti-rat interleukin-1β (IL-1β) polyclonal antibodies were purchased from Abcam company. DAB color reagent kit (batch number: SP-900D) and immunohistochemical staining kit (batch number: SP-9001) were purchased from Beijing Zhongshan Jinqiao Biological Co., Ltd. Rat tumor necrosis factor-α (TNF-α) ELISA assay kit (lot number: E-EL-R0019c), rat monocyte chemotactic protein-1 (MCP-1) ELISA assay kit (lot number: E-ELR0633c), rat IL-1β ELISA assay kit (batch number: E-EL-R0012c) were purchased from Elite Biotech Co., Ltd. Ferulic acid reference substance (batch number 0773-9910, for content determination) was purchased from China Institute for the Control of Pharmaceutical and Biological Products.

The 2.0mm×15mm Runjin medical balloon catheter, Runthrough guide wire were purchased from Japan Terumo Co., Ltd., and the medical balloon dilatation pressure pump was purchased from the Department of Apparatus, Jiangxi Provincial Hospital of Traditional Chinese Medicine. 1260 type High Performance Liquid Chromatograph (HPLC) (Aglient Company), Waters 2996 Diode Array Detector (PDA); Model 98-1-B Heating Mantle (Tianjin
Test Instrument Co., Ltd.); Model N-1000 Rotary Evaporator (RIKAKIKAI Company)

2.6 Experimental Methods

2.6.1 Preparation of Drugs

The herbs were accurately weighed and extracted 3 times by hydrothermal reflux method (the first time was extracted with 8 times the amount of water for 1 hour; the second and third times were extracted with 6 times the amount of water for 1 hour). The extracts of three times were combined and filtered. Then, the extracts was evaporated and concentrated under vacuum at 60°C to make the DGBXD extracts, and the DGBXD extracts were taken out to a constant volume with distilled water 0.39g of crude drug/mL. 0.1% sodium benzoate is added to the medicinal solution and stored in a refrigerator at -4°C for later use.

2.6.2 Animal Modeling, Grouping and Intervention

The current balloon injury model is a hyperplasia/neointima model, and it is also a mainstream model in the early stage of AS [30]: After the rats were anesthetized with 4% sodium pentobarbital (50 mg/kg), the left common carotid artery was surgically exposed. The distal end of the left common carotid artery was ligated, the proximal end was clamped by an arterial clip, and then a "V"-shaped incision was made at the distal end. The balloon tube was then inserted through the incision, crossing the aortic arch to a depth of about 6-7 cm. The balloon was pressurized with a medical balloon expansion pressure pump to maintain the pressure at 8 bar, and the balloon catheter is repeatedly pulled back and forth to the aortic arch 4 times. Then rotate the balloon catheter 180° and perform the same operation 4 times. After the completion of the balloon expansion pressure pump back to the negative pressure state, the catheter was withdrawn.

The rats were randomly divided into 4 groups: sham operation group, model group, DGBXD group and atorvastatin group. According to the pre-experimental modeling situation, each group contains 8-10 rats. Sham operation group: only the left common carotid artery was separated and exposed and then sutured without balloon injury; the same amount of distilled water was given after the operation. Model group: Balloon injury of the thoracic and abdominal aorta was performed without drug intervention after operation; the same amount of distilled water was given after operation. Atorvastatin group: After thoracic and abdominal
aortic balloon injury, atorvastatin was administered. DGBXD group: After thoracic and abdominal aortic balloon injury, the DGBXD 3.9 of crude drug g/kg was administered.

2.6.3 Specimen collection

After 14 days of intragastric administration (the day after the last administration), blood was collected from the abdominal aorta under anesthesia with 4% sodium pentobarbital (50 mg/kg), and then the thoracic and abdominal aortic vessels were intercepted. After that, the rats were sacrificed by cervical dislocation. After the blood is centrifuged at low temperature (4°C, 2000rpm/min for 15 min), the plasma is collected and stored in a refrigerator at -80°C for ELISA testing. The blood vessels used for immunohistochemical detection were fixed with 4% paraformaldehyde and stored in a refrigerator at 4°C.

2.6.4 Vascular intimal hyperplasia index Measurement

After the thoracic-abdominal aorta was taken out, it was rinsed with normal saline to remove the connective tissue outside the blood vessel, and fixed in 4% paraformaldehyde for 24 hours. Then, the ethanol gradient dehydration was carried out, and the paraffin was embedded vertically, and 8 slices of each segment of blood vessel were cut uniformly for Masson staining. After staining, observe under a light microscope, and the MIAS medical image analysis system was used to take pictures. Image-pro plus6.0 image analysis software is used to measure the media area (MA), the perimeter of the midline of the media, the intimal area (IA), and the perimeter of the midline of the intima. Media thickness (MT) = Media area/Median midline circumference. Intimal thickness (IT)=intimal area/intimal midline circumference. Hyperplasia ratio of intimal area (HRIA) and hyperplasia ratio of intimal thickness (HRIT) were also calculated.

2.6.5 Detection of ICAM-1, VCAM-1, IL-1β mRNA expression in the intima of hyperplastic vessels

The total RNA of the aortic tissues of each group was extracted by Trizol method. Then reverse transcription of RNA into cDNA according to the operating instructions of the kit. The RT-PCR reaction was performed using the two-step chimeric fluorescence quantitative RT-PCR kit (TaKaRa Company, SYBR Green chimeric fluorescence method). RT-PCR conditions: perform reverse transcription reaction in a constant temperature water bath at 37°C.
for 15 min; then place it in 85 °C water for 5 s to inactivate the reverse transcriptase; Rotor-Gene 3000 Real-Time PCR amplification analysis system is used for RT-PCR. Reaction conditions (40 cycles in total): pre-denaturation 95 °C 30s, denaturation 95 °C 10 s, annealing 60 °C 30 s, extension 72 °C 30 s, extension 72 °C 5 min. The primers were synthesized by Shanghai Bioengineering Company and passed quality inspection (Table 2). The relative expression of target gene mRNA was calculated by 2-△△Ct.

| Gene   | Sequence                      | Length/bp |
|--------|-------------------------------|-----------|
| IL-1β  | F:5′-CCTGTGGCCTTGGGCCTCAA -3′ | 204       |
|        | R:5′-GGTGCTGATGTACCAGTTGGG-3′ |           |
| ICAM-1 | F:5′-GCCGGCTTGGAGGTGGAT-3′    | 485       |
|        | R:5′-GGAGGCCGGGGCTTGTACC-3′   |           |
| VCAM-1 | F:5′-CCTGTCCCAGAGGAGGGC-3′   | 500       |
|        | R:5 -CAACTGCGAGCGACTTCG -3′  |           |
| β -actin| F:5 -AGCTGAGAGGGAATCGTGCG -3 | 204       |
|        | R:5 -GTGCCACCAGACAGCAGCTTG -3 |          |

2.6.6 Detection of ICAM-1, VCAM-1, IL-1β protein expression in the intima of hyperplastic vessels

The expression of ICAM-1, VCAM-1, IL-1β in the blood vessels with intimal hyperplasia was determined by immunohistochemistry (primary antibody dilution ratio: ICAM-1 1:100, VCAM-1 1:500, IL-1β 1:100, FN 1:100, Col-I 1:100); The operation is carried out according to the instructions of the kit. Under the light microscope, it can be seen that brown-yellow spot or fibrous staining is concentrated in the cell membrane, cytoplasm or between cells, which is positive expression, and negative is no brown-yellow staining. The slices were photographed with MIAS medical image analysis system, and then analyzed with Image-pro plus 6.0: Under a 400-fold light microscope, three different fields of view were selected for each slice, and the integrated optical density (IOD) of positive staining per unit area was measured, and then the average was taken for statistical analysis.
2.6.7 Detection of plasma inflammatory response related factors

The plasma levels of IL-1β, TNF-α, and MCP-1 were measured by ELISA to reflect the state of systemic inflammatory response. The specific operation is carried out according to the instructions of the kit.

2.7 HPLC Methods

2.7.1 Sample Preparation

DGBXD sample: 1 g of the extract was dissolved in 30 ml of distilled water, ethanol was added to 80%, precipitated, filtered, the filtrate was evaporated to dryness and the volume was adjusted to 100 ml with 70% methanol. Finally, the extract was filtered through a 0.45 μm membrane.

Ferulic acid reference sample: 1.05 mg of ferulic acid was adjusted to 5 ml with 70% methanol, and then 3 ml was accurately measured, and the volume was adjusted to 50 ml with 70% methanol, and the concentration was 0.0126 mg/ml. Finally, it was filtered with a 0.45 μm filter membrane.

2.7.2 HPLC Condition

Column: Agilent ZORBAX Eclipse XDB-C18 column (250mm × 4.6mm, 5μm); mobile phase A: 0.2% formic acid solution, mobile phase B: 0.2% formic acid acetonitrile solution, flow rate: 10 ml/min; Column temperature: 30 ℃; Injection volume: 10 μl. The chromatogram is shown in Figure 2. After determination, the content of ferulic acid in DGBXD is 0.590 0 mg/g.

Figure 2 The results of HPLC (A: Ferulic acid reference sample; B: DGBXD sample)
2.8 Statistical Analysis

SPSS 17.0 statistical software was used for analysis, and the experimental data were expressed as mean ± SD. One-way ANOVA was used to compare the means among multiple groups. For pairwise comparisons between two group, if the variances are uniform, the LSD test is used, and if the variances are not uniform, Dunnett’s T3 test is used. P<0.05 was considered statistically significant.

3 Results and Discussion

3.1 The Known Targets and Potential Targets of DGBXD and AS Genes

A total of 212 known targets, 265 potential targets and 229 AS genes were obtained. There is overlap between the target of each target set (Figure 3a). The compounds and known targets were input into Cytoscape to construct compound-known target network, which consists of 18 compound nodes, 212 known target nodes and 537 edges (Figure 3b). In this network, some targets can be regulated by a lot of compounds (for example, PTGS1 can be regulated by Quercetin, Kaempferol, 7-O-methylisomucronulatol, Formononetin, Isorhamnetin, Stigmasterol, 3,9-di-O-methylnissolin, 73340-41-7, Butylidenephthalide, Calycosin, Hederagenin, Ferulic acid, Jaranol, Senkyunolide I, Bifendate), while other targets were regulated by only one compound (for example, CASP9 is regulated by Quercetin.).

Figure 3 The Known Targets and Potential Targets of DGBXD and AS Genes (a: Venn diagram of Known Targets and Potential Targets of DGBXD and AS Genes; b: Compound-Known target network. Pink
hexagon stand for known targets; Red and orange circle stands for *Hedysarum Multijugum Maxim.* and *Angelicae Sinensis Radix* components, respectively.)

### 3.2 DGBXD Known Target-AS PPI Network Analysis

#### 3.2.1 DGBXD Known Target-AS PPI Network Construction
The DGBXD Known Target-AS PPI network was composed of 165 DGBXD known target nodes, 162 AS gene nodes, 46 DGBXD known-AS targets and 9094 edges (Figure 4a). The targets are arranged in descending order according to their degree, the top 20 can be divided into 3 category: (1) DGBXD known target: CASP3 (147 edges), EGF (145 edges), CXCL8 (144 edges), EGFR (143 edges), MAPK8 (135 edges), JUN (133 edges); (2) AS genes: INS (234 edges), ALB (217 edges), MMP9 (147 edges), APOE (134 edges), APP (133 edges), TLR4 (129 edges); (3) DGBXD known-AS target: IL6 (207 edges), AKT1 (195 edges), TNF (178 edges), VEGFA (175 edges), TP53 (161 edges), CCL2 (137 edges), MAPK1 (136 edges), IL1B (131 edges). The topological property of this network was assessed by network analyzer tool, and the result demonstrates that DGBXD Known Target-AS PPI network meets the power-law distribution ($R^2=0.377, y = 13.382x^{-0.459}$) (Figure 4b).

3.2.2 Biological Processes of DGBXD Known Target-AS PPI Network
were obtained (Figure 5 and Table 3). The targets and genes of each cluster were input into DAVID to perform GO enrichment analysis so as to obtain the biological processes of each cluster.

**Table 3 Clusters of DGBXD Known Target-AS PPI Network**

| Cluster | Score | Nodes | Edges | Targets and Genes |
|---------|-------|-------|-------|-------------------|
| 1       | 54.344| 65    | 1739  | PTEN, IL1B, AGT, MPO, CXCL10, HMOX1, SPP1, PTGS2, SMAD3, MMP1, MAPK14, TIMP1, TGFB1, APOE, JUN, IL4, PPARG, CCL2, CRP, SELE, PLG, ESR1, TP53, NOS3, AKT1, IL6, INS, CASP3, TNF, MAPK8, ADIPOQ, ACE, KDR, VCAM1, VEGFA, SERPINE1, MMP9, ICAM1, NOTCH1, TLR4, EDN1, IFNG, CDKN2A, ALB, EGFR, CCND1, MAPK1, BCL2L1, FOS, LEP, EGF, CXCL12, ELN, VWF, CASP8, PECAM1, LOX, MMP2, MYC, CXCL8, MMP3, CCL5, CAT, IL10, IL2 |
| 2       | 13.707| 42    | 281   | HMGCR, APOC2, ABCG2, APOC3, PON1, CHEK2, CST3, E2F1, SREBF2, NAMPT, APOA5, CTSD, NR1H4, SLC2A4, PPBP, ITIH4, ITGB3, NR1H3, PLTP, TOP2A, CDKN2B, ABCG1, NPC1L1, COG2, TOP1, LDLR, SERPIND1, APOA1, CETP, LPL, FGA, ABCG8, LIPC, SERPINC1, ABCG5, OLR1, F13A1, BIRC5, PCSK9, LCAT, ANGPTL3, LPA |
| 3       | 10.111| 37    | 182   | AGTR1, RELA, IL1A, HSP90AA1, PGR, STAT1, AR, RUNX2, CAV1, IGFBP3, GPT, IRF1, NOS2, NFE2L2, SELP, NFKB1, CCNA2, CDKN1A, MET, CASP9, NFKB1A, CD40LG, APOB, F2, PLAU, KNG1, HIF1A, ENG, SIRT1, ERBB2, APP, GJA1, RETN, HSPB1, REN, F3, CCNB1 |
| 4       | 9     | 9     | 36    | HTR2A, ADRA1A, ADRA1B, ADRA1D, UTS2, CHRM3, CHRM1, SAA1, CHRM5 |
| 5       | 6.692 | 53    | 174   | NQO1, PARP1, OPRM1, CXCL11, CXCL2, CHRM4, OPRD1, CHRM2, IGF2, APOA4, ERBB3, DPP4, CYBA, GSK3B, CDK2, CHEK1, NPY, ADRA2A, HP, ADRA2B, APOH, TGFB2, CDH5 |
Cluster 1 is related to inflammation, platelet activation, endothelial cell apoptosis, oxidative stress, lipid metabolism, vascular smooth muscle proliferation, angiogenesis, NFkB signaling pathway, leukocyte migration and rolling. Cluster 2 is related to lipid metabolism such as cholesterol and triglycerides, foam cell differentiation, and platelet degranulation. Cluster 3 is related to angiogenesis, endothelial cell proliferation, active oxygen metabolism, foam cell differentiation, oxidative stress, hypoxia, and cholesterol metabolism. Cluster 4 is related to vascular smooth muscle contraction. Cluster 5 is related to inflammatory chemotaxis, blood coagulation, oxidative stress, and cholesterol metabolism. Cluster 7 is related to steroid metabolism and redox. Cluster 10 is related to endoplasmic reticulum stress, cholesterol efflux, coagulation, and hypoxia. Cluster 6, 8 and 9 failed to return any AS-related biological processes (Table S4). The P-value, fold enrichment and count of biological processes in cluster 1 were shown in figure 6b as an example.
3.2.3 Pathway of DGBXD Known Target-AS PPI Network

The pathway enrichment analysis showed that DGBXD can regulate a lot of AS-related signaling pathways, such as TNF signaling pathway, HIF-1 signaling pathway, FoxO signaling pathway, Toll-like receptor signaling pathway, PI3K-Akt signaling pathway, PPAR signaling pathway, NF-kappa B signaling pathway, Complement and coagulation cascades, Adipocytokine signaling pathway, MAPK signaling pathway (Figure 6a and Table S5). The P-value, fold enrichment and count of each signaling pathways were shown in figure 6c. The PI3K-Akt signaling pathway was shown in figure 6d. The DGBXD potential targets were marked in red; the AS genes were marked in blue; the DGBXD-AS targets were marked in purple.

3.3 DGBXD-AS PPI Network Analysis

3.3.1 DGBXD-AS PPI Network Construction
The DGBXD-AS PPI network was composed of 225 DGBXD target nodes, 188 AS gene nodes, 20 DGBXD-AS targets and 7081 edges (Figure 7a). The targets are arranged in descending order according to their degree, the top 21 can be divided into 3 category: (1) DGBXD target: CASP3 (102 edges); (2) AS genes: IL6 (173 edges), AKT1 (153 edges), VEGFA (134 edges), APOE (130 edges), APP (118 edges), MAPK1 (115 edges), CCL2 (115 edges), TP53 (114 edges), IL1B (112 edges), APOB (112 edges), MMP9 (109 edges), IL10 (105 edges), CRP (103 edges), NOS3 (102 edges), LEP (100 edges), SERPINE1 (100 edges); (3) DGBXD-AS target: INS (196 edges), ALB (189 edges), TNF (142 edges), TLR4 (106 edges).

The topological property of this network was assessed by network analyzer tool, and the result demonstrates that DGBXD-AS PPI network meets the power-law distribution ($R^2=0.611, y = 38.943x^{-0.694}$) (Figure 7b)

### 3.3.2 Biological Processes of DGBXD-AS PPI Network

The DGBXD-AS PPI network was analyzed by MCODE and 18 clusters were obtained (Figure 8 and Table 4). The targets and genes of top 10 clusters were input into DAVID to perform GO enrichment analysis so as to obtain the biological processes of each cluster.

| Cluster Score | Nodes | Edges | Targets and Genes |
|---------------|-------|-------|-------------------|
|               |       |       |                   |

- **Table 4 Clusters of DGBXD-AS PPI Network**

![Figure 8 Clusters of DGBXD-AS PPI Network (Pink circles stand for DGBXD target, Blue circles stand for AS genes, purple circles stand for DGBXD-AS target.)](image)
|   | Score  | Rank | Gene(s)                                                                 |
|---|--------|------|-------------------------------------------------------------------------|
| 1 | 45.373 | 52   | TP53, AKT1, CCL2, CRP, SELE, KDR, NOS3, NOTCH1, IL6, ADIPOQ, ACE, IFNG, VEGFA, SERPINE1, MMP9, ICAM1, MAPK1, EDN1, CXCL12, SELP, LEP, ELN, VWF, CASP3, PPARG, PECAM1, MMP2, CCL5, RETN, MMP3, CAT, IL10, F3, TNF, IL1B, AGT, MPO, HMOX1, SPP1, PTGS2, MMP1, TIMP1, TGFB1, INS, ESRI, TLR4, KNG1, ALB, APOE, PLG, REN |
| 2 | 12.231 | 27   | CETP, ABCG8, LIPC, PTX3, CYP7A1, PCSK9, LCAT, APOA2, SOD1, APOC2, APOC3, LPL, SREBF1, MMP8, NFE2L2, APOA5, SCARB1, PLTP, NR1H4, ABCG1, NR3C1, HPGDS, NFKB1, GGT1, NOX1, APOB, APOA1 |
| 3 | 9.657  | 36   | SOAT2, GABRQ, FGB, ATIC, F5, CST3, GABRB1, CYP27A1, GABRA1, GABRB2, GABRA2, GABRB3, GABRA3, MTHFR, GABRA4, GABRA5, HNF4A, GABRD, GABRE, GABRA6, SDHC, TGFB1, ITGB3, SERPIND1, TGFB2, GLRA3, GABRG1, NQO1, HSPD1, GABRG2, F13A1, COL3A1, GABRG3, F7, VDR, GABRP |
| 4 | 8.242  | 34   | SCN1A, FOLR3, SCN1B, MTHFD1, MTRR, HBB, MTHFD2, HBA1, OGDH, ACO2, SCN2B, SCN3A, TCN1, SCN3B, SCN4A, SCN10A, MTFMT, SCN4B, SUCLG1, ALDH1L1, SUCLG2, SCN5A, SDHA, SDHB, AMT, SDHD, LTF, TYMS, MTR, SUCLA2, SHMT1, SCN8A, SHMT2, SCN9A |
| 5 | 6.833  | 25   | ABCA1, ANGPTL3, CD163, OLR1, CX3CR1, LPA, HMGCR, HCAR2, PON1, P2RY12, HCAR3, CDKN2A, SUCNR1, CASR, PPBP, PPARA, NR1H3, FBN1, AR, FGA, PF4, SERPINC1, PGR, LDLR, Cnr2 |
| 6 | 5.926  | 28   | ABCG5, AGTR1, MSR1, SAA1, MTTP, SREBF2, GPT, CAV1, SMAD3, NOS2, APOA4, THBD, PLAT, HP, AGER, NPC1L1, PLA2G7, CD40LG, APOH, DCN, APP, CDH5, SEL1, F2, LOX |
Cluster 1 is related to apoptosis, inflammatory chemokines and their mediated severe inflammatory response, smooth muscle proliferation, hypoxia, endothelial cell proliferation and apoptosis, vasodilation, and oxidative stress. Cluster 2 is related to cholesterol and other lipid anabolism and inflammation. Cluster 3 is related to coagulation reaction and hypoxia reaction. Cluster 5 is related to cholesterol metabolism, macrophage foam cell transformation, inflammatory chemotaxis, and blood coagulation. Cluster 6 is related to hypoxia, cholesterol metabolism and blood coagulation. Cluster 7 is related to calcium ion transport across membranes. Cluster 9 is related to calcium ions. Cluster 4, 8 and 10 failed to return any AS-related biological processes (Table S6). The P-value, fold enrichment and count of biological processes in cluster 1 were shown in figure 9b as an example.
Figure 9 Enrichment Analysis results (a: Pathways of DGBXD-AS PPI Network; Red diamonds stand for signaling pathways. Pink circles stand for DGBXD target, Blue circles stand for AS genes, purple circles stand for DGBXD-AS target. b: Bubble chart of biological processes in cluster 1; c: Bubble chart of signaling pathway. X-axis stand for fold enrichment)

3.3.3 Pathway of DGBXD-AS PPI Network

The pathway enrichment analysis showed that DGBXD can regulate a lot of AS-related signaling pathways, such as PPAR signaling pathway, One carbon pool by folate, Fat digestion and absorption, Complement and coagulation cascades, Metabolic pathways, cAMP signaling pathway, TNF signaling pathway, Adipocytokine signaling pathway, HIF-1 signaling pathway, NF-kappa B signaling pathway (Figure 9a and Table S7). The P-value, fold enrichment and count of each signaling pathways were shown in figure 9c.

The results of network pharmacology suggest that DGBXD may have anti-AS effects. Astragaloside IV is the main medicinal substance of *Hedysarum Multijugum Maxim.*, which has cardiovascular protective effects such as strengthening the heart, protecting myocardial cells, protecting vascular endothelial cells, inhibiting the proliferation of vascular smooth muscle cells, and regulating blood pressure; its mechanism is related to anti-oxidation, scavenging free radicals, anti-inflammatory, anti-apoptosis, etc. [31-32]. Current studies also show that stigmasterol can regulate fatty acid synthesis and cholesterol metabolism, lipid metabolism, inhibit inflammation, and thus play a cardiovascular protective effect [33]. Current research shows that Butylidenephthalide has its anti-platelet activity [34], as well as anti-tumor [35] and anti-inflammatory properties [36]. 3,9-di-O-methylnissolin belongs to isoflavones. Current research shows that isoflavones have good antioxidant and anti-inflammatory functions [37]. Studies have also shown that Calycosin can inhibit inflammation through NF-KB signaling pathway and MAPK signaling pathway [38-39]. Ferulic acid has many physiological functions (anti-inflammatory, anti-oxidant, anti-diabetic effect and free radical scavenger, scavenging lipids) [40-42]. The current research progress shows its anti-fatty liver effect. Formononetin has anti-inflammatory properties [43]. In the process of protecting endothelial damage, Formononetin improves the endothelial dysfunction induced by high glucose by inhibiting the JAK/STAT signaling pathway [44]. In terms of
oxidative stress, Hederagenin prevents atherosclerosis by inhibiting the Nrf2-ARE antioxidant pathway [45-46]. Current research shows that Isorhamnetin has a wide range of pharmacological activities, such as protecting cardiovascular and cerebrovascular, anti-tumor, anti-inflammatory, antioxidant, organ protection, and preventing obesity. The mechanism involves PI3K/AKT/PKB pathway, NF-κB pathway, MAPK pathway and other signal pathways, as well as the expression of related cytokines and kinases [47-49]. A large number of pre-clinical studies have shown that Kaempferol has anti-oxidant, anti-inflammatory, anti-microbial, anti-tumor, cardioprotective, neuroprotective, anti-diabetic, anti-osteoporotic, and estrogen/anti-estrogen effects [50-51]. As a flavonoid, quercetin has been shown to have significant heart-related benefits, such as inhibiting LDL oxidation, non-endothelial-dependent vasodilation, reducing adhesion molecules and other inflammatory markers, and protecting endothelial function [52-53]. Senkyunolide I is one of the biologically active ingredients. Current studies have shown that it has anti-inflammatory, anti-oxidative damage [54], anti-platelet, anti-coagulation [55] and alleviates cerebral ischemia reperfusion injury [56]. Therefore, it is speculated that the above active ingredients may be the key ingredients of DGBXD anti-AS.

The results of GO and pathway enrichment analysis showed that DGBXD can mainly regulate inflammatory chemokines and their mediated signal transduction, blood coagulation, smooth muscle proliferation, endothelial cell proliferation, angiogenesis, leukocyte adhesion, migration and activation, oxidative stress and other biological modules. The signaling pathways that DGBXD can regulate mainly include PI3K/Akt signal pathway, TNF signal pathway, NF-κB signal pathway, HIF-1 signal pathway, FoxO signal pathway / PPAR signal pathway, etc. Current research shows that AS is a chronic inflammatory disease of the blood vessel wall, and the inflammatory response plays an important role in different stages of AS [57-58]. Cytokines, which are important mediators of inflammation, can be secreted by a variety of activated cells in AS. At the same time, these cytokines can activate different types of cells and play a key role in atherosclerosis [59]. Cytokines realize biological activity through their related signal pathways. These pathways include PI3K/AKT signaling pathway, nuclear factor-κB pathway, TGF-beta/Smad signaling pathway, JAK/STAT signaling
pathway, HIF signaling pathway and Toll signaling pathway, MAPK signaling pathway, etc. [60-63]. The above results suggest that DGBXD can act on multiple signal pathways to play an anti-AS effect through a variety of biological processes. There is a complex interaction relationship between these pathways, which reflects the characteristics of multi-component, multi-target, and multi-path cooperative treatment of diseases in traditional Chinese medicine. Next, we would further verify the prediction results of network pharmacology through animal experiments.

3.4 Effect of DGBXD on the pathological morphology of rat vascular intima

In the sham operation group, the elastic membrane in the vascular intima was intact, in a single layer, and there was no hyperplasia. In the model group, the angiogenesis intima showed uniform or uneven thickening, a large number of proliferated vascular smooth muscle cells (VSMC) existed, the arrangement was disordered, the lumen showed centripetal or eccentric stenosis, and the intimal hyperplasia was obvious. In the DGBXD group and the atorvastatin group, the vascular intima showed proliferative changes, but the degree of proliferation was less than that of the model group.(Figure 10a).
Figure 10 Effect of DGBXD on the pathological morphology of rat vascular intima (a: pathological morphology; Masson staining, ×100. The black arrow points to the hyperplasia. b: Comparison of vascular intimal hyperplasia; compared with sham operation group, ** P<0.01; compared with model group,▲▲P<0.01)

The vascular morphometric analysis showed that compared with the sham operation group, the vascular IA, IT, HRIA and HRIT of the model group increased significantly (P<0.01), indicating that the model was successful. The rat thoracoabdominal aorta showed obvious intimal hyperplasia after balloon injury. Compared with the model group, the vascular tissue IA, IT, HRIA and HRIT of the DGBXD group and the atorvastatin group were significantly reduced (P<0.01) (Figure 10b).

3.5 Expression of ICAM-1, VCAM-1 and IL-1β protein in blood vessels

Compared with the sham operation group, the expression of ICAM-1, VCAM-1 and IL-1β in the local blood vessels of the model group was significantly increased (P<0.01).
Compared with the model group, the vascular ICAM-1, VCAM-1 and IL-1β expression intensity in the atorvastatin group and DGBXD group were significantly lower than those in the model group (P<0.01, P<0.05). There was no statistical difference of ICAM-1, VCAM-1 and IL-1β between the DGBXD group and the atorvastatin group (P>0.05). (Figure 11).

Figure 11 Expression of ICAM-1, VCAM-1 and IL-1β in blood vessels (A: sham operation group; B: model group; C: atorvastatin group; D: DGBXD group. Immunohistochemistry, ×400)
Figure 12 Inflammatory factor expression (a: Expression of ICAM-1, VCAM-1 and IL-1β mRNA; b: plasma inflammatory factors IL-1β, TNF-α, MCP-1 content. compared with sham operation group, ** P<0.01; compared with model group, ▲ P<0.05, ▲▲ P<0.01)

3.6 Expression of ICAM-1, VCAM-1 and IL-1β mRNA in blood vessels

Compared with the sham operation group, the expression of ICAM-1, VCAM-1 and IL-1β mRNA in the blood vessels of the model group was significantly increased (P<0.01). Compared with the model group, the vascular ICAM-1, VCAM-1 and IL-1β mRNA expression intensity in the atorvastatin group and DGBXD group were significantly lower than those in the model group (P<0.01, P<0.05). There was no statistical difference of ICAM-1, VCAM-1 and IL-1β mRNA between the DGBXD group and the atorvastatin group (P>0.05). (Figure 12a).

3.7 Detection of plasma inflammatory factors IL-1β, TNF-α, MCP-1 content

Compared with the sham operation group, the plasma levels of IL1β, TNF-α and MCP-1 in the model group were significantly increased (P<0.01). Compared with the model group, the plasma levels of IL-1β, TNF-α, and MCP-1 in the atorvastatin group were significantly reduced (P<0.01). Compared with the atorvastatin group, the plasma levels of IL-1β, TNF-α,
and MCP-1 in the DGBXD group were significantly increased (P<0.01) (Figure 12b).

3.8 Molecular docking results of DGBXD components and PIK3R1 and AKT1

The top 10 DGBXD components in compound-known target network were selected for molecular docking. The results show that the top 10 components may be stably combined with PIK3R1 and AKT1 (Figure 13). This suggests that DGBXD may act on PIK3R1 and Akt through these active components, thereby regulating the PI3K-Akt signaling pathway.

Current research shows that the main biological process of AS is the inflammatory response of the vascular intima. After vascular intima injury, the inflammatory cells are activated and the blood vessels are mechanically expanded, which increases the release of inflammatory factors and chemotactic factors, and exposes the subintimal tissues, induces platelet adhesion and aggregation at the damaged intima, forming a platelet covering layer [64-66]. The activated platelets adhere to circulating white blood cells through platelet receptors, and mediate white blood cells to roll along the damaged endothelial surface. Damaged endothelial cells, VSMCs, and activated inflammatory cells secrete inflammatory chemokines and inflammatory mediators through the adherent platelet-fibrin layer and
exudate to the intima, causing an inflammatory reaction in the blood vessel wall [67-68]. Cytokines and inflammatory response mediators can also induce monocytes, lymphocytes and other inflammatory cells to chemotaxis to the injury site, resulting in the adsorption of a large number of monocytes and leukocytes on the surface of blood vessels, which induces an early inflammatory response [69-70]. In the following days to weeks, macrophages infiltrate the vascular intima and cluster around the scaffold. Platelets, macrophages, and histiocytes gathered at the injured site secrete a large number of chemokines and growth factors to induce VSMCs to migrate from the media into the inner membrane, and the VSMC changes from the contractile type to the synthetic type. VSMC proliferates in large quantities, synthesizes a large amount of extracellular matrix (ECM) components and deposits on the vascular wall, forming a neointima [71-72]. VSMC can maintain its activation state in the inflammatory environment of the intima, continuously synthesize cytokines, growth factors and ECM [73-74]. In addition, under the influence of various factors such as leukocyte, platelet aggregation and vascular intima injury, it can cause the increase of the expression of various inflammatory response factor ligands such as macrophage surface antigen-1 and L-selectin receptor on the surface of leukocytes [72-73]. Mediated by pro-inflammatory response mediators such as interleukin-6 (IL-6), IL-1, MCP-1, TNF-α, ICAM-1 and VCAM-1, local inflammatory reactions can develop. It is a systemic inflammatory reaction [74-75]. Therefore, when the vascular intima is injured, the inflammatory reaction can also aggravate the vascular intimal hyperplasia.

In the occurrence of AS, local inflammatory reaction is the cause, and vascular intimal hyperplasia is the result, which constitutes a causal relationship. Current studies have found that some natural active ingredients can inhibit the cascade of inflammatory reactions, inhibit inflammatory stress and counter-inflammatory adverse events. For example, ligustrazine can inhibit endothelial cell inflammation and leukocyte adhesion response induced by oxidized low density lipoprotein (ox-LDL), and inhibit the activation of mitogen-activated protein kinase (MAPK) and nuclear factor κB (NF-κB) signaling pathways, which can inhibit the inflammatory response in the initial stage of inflammatory response [76-78]. Quercetin [79], emodin [80], triptolide [81], icariin [82], and total ginsenosides [83] also have similar effects.
Lin [84] et al. showed that DGBXD may improve inflammation in AS mice by affecting the activity of NF-kB. Ma et al. [85] and Huang et al. [86] used immunoblotting to detect the effect of DGBXD on the expression of p38 MAPK in RAW264.7 cells activated by ox-LDL. They speculated that the mechanism of DGBXD’s prevention and treatment of AS and related diseases may be to down-regulate the activity of p38 MAPK, thereby blocking the cascade of this pathway to reduce the AS inflammatory response. This prevents ox-LDL from inducing monocytes to accumulate to the vascular endothelium, which not only blocks the early stage of development of AS lesions, but also alleviates the progression of the disease.

This study found that after balloon catheter injury, the expression of inflammatory response factors ICAM-1, VCAM-1, and IL-1β in the locally hyperplastic intima was significantly increased. It shows that the vascular intima is injured and produces a local inflammatory response of the blood vessel, which can promote the proliferation and migration of VSMC and cause intimal hyperplasia. Atorvastatin and DGBXD can inhibit the expression of ICAM-1, VCAM-1 and IL-1β in the vascular intima, indicating that atorvastatin and DGBXD can reduce the local inflammatory response of blood vessels by inhibiting the expression of local inflammatory response factors in blood vessels. Studies have also found that the local inflammatory response after vascular injury can induce systemic chronic inflammatory response, which in turn promotes the migration and proliferation of VSMCs and causes intimal hyperplasia [87-88]. In addition, atorvastatin can inhibit the increase of plasma IL-1β, TNF-α, and MCP-1 levels, indicating that atorvastatin can inhibit the systemic chronic inflammatory response after vascular intimal injury, while DGBXD does not seem to inhibit systemic inflammation.

4 Conclusion

The mechanism of DGBXD in the treatment of AS may be related to the improvement of extracellular matrix deposition in the blood vessel wall and the anti-vascular local inflammatory response. This may provide a reference for the study of the mechanism of DGBXD.

Data Availability Statements

All datasets for this study are included in the manuscript and the supplementary files.
Declare

The work described has not been submitted elsewhere for publication, in whole or in part, and all the authors listed have approved the manuscript that is enclosed.

Competing Interests

All authors have no financial or scientific conflict of interest with regard to the research described in this manuscript.

Author contributions

Hao Xu, Tianqing Zhang, Ling He, Mengxia Yuan, You Yuan, Shanshan Wang participant in the concept and design. Hao Xu, Tianqing Zhang, Ling He, Mengxia Yuan, You Yuan, Shanshan Wang are responsible for data analysis and interpretation in the chemical informatics section; Hao Xu, You Yuan, Shanshan Wang are responsible for data analysis and interpretation in experiments; Hao Xu, Tianqing Zhang, Ling He, Mengxia Yuan drafted the paper; Shanshan Wang supervised the study; all authors participated in the analysis and interpretation of data and approved the final paper. Hao Xu, Tianqing Zhang, Ling He, Mengxia Yuan, You Yuan should be considered joint first author.

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Danggui Buxue Decoction

DGBXD Targets Collection

DGBXD targets and AS genes

DGBXD known-AS PPI Network Analysis

Enrichment Analysis

DGBXD-AS PPI Network

Enrichment Analysis

In vivo experimental and molecular docking verification
| Compounds               | Targets               |
|-------------------------|-----------------------|
| 3,9-di-O-methylnissol   | NOS2                  |
|                         | PTGS1                 |
|                         | CHRM3                 |
|                         | F2                    |
|                         | CHRM1                 |
|                         | ESR1                  |
|                         | ADRB1                 |
|                         | SCN5A                 |
|                         | PTGS2                 |
|                         | NOS3                  |
|                         | HTR3A                 |
|                         | ADRA2C                |
|                         | RXRA                  |
|                         | ACHE                  |
|                         | PDE3A                 |
|                         | ADRA1B                |
|                         | ADRB2                 |
|                         | ADRA1D                |
|                         | OPRM1                 |
|                         | GABRA1                |
|                         | PRSS1                 |
|                         | NCOA2                 |
|                         | CALM1/CALM2/CALM3     |
| 64997-52-0              | PGR                   |
| 7-O-methylisomucron     | NOS2                  |
|                         | PTGS1                 |
|                         | DRD1                  |
|                         | CHRM3                 |
|                         | F2                    |
|                         | KCNH2                 |
|                         | CHRM1                 |
|                         | ESR1                  |
|                         | AR                    |
|                         | ADRB1                 |
|                         | SCN5A                 |
|                         | PPARG                 |
|                         | F10                   |
|                         | CHRM5                 |
|                         | PTGS2                 |
|                         | NOS3                  |
|                         | ADRA2C                |
|                         | CHRM4                 |
|                         | RXRA                  |
|                         | OPRD1                 |
|                         | PDE3A                 |
|                         | HTR2A                 |
|                         | ADRA1A                |
|                         | CHRM2                 |
| Gene Abbreviation | Gene Symbol |
|------------------|-------------|
| ADRA1B           | SLC6A3      |
| ADRA1D           | SLC6A4      |
| ADRA1D           | ESR2        |
| ADRA2B           | DPPIV       |
| MAPK14           | CHEK1       |
| RXRB             | PRSS1       |
| PIM1             | CCNA2       |
| CDK2             | NCOA2       |
| GSK3B            | TXK         |
| CHEK1            | CHEK1       |
| RXRB             | RXRB        |
| PIM1             | PIM1        |
| PRSS1            | PRSS1       |
| CCNA2            | CCNA2       |
| NCOA2            | NCOA2       |
| TXK              | TXK         |
| CHEK1            | CHEK1       |
| RXRB             | RXRB        |
| PIM1             | PIM1        |
| PRSS1            | PRSS1       |
| CCNA2            | CCNA2       |
| NCOA2            | NCOA2       |
| TXK              | TXK         |

**Chemicals**

- **Beta-sitosterol**
- **Bifendate**
- **Butylenephthalide**

**Chemical IDs**

- 73340-41-7 NOS2
- PTGS1
- CHRM3
- PTGS2
- PTGS1
- CALM2/CALM3

**Additional Chemicals**

- PGR
- NCOA2
- NF2
- PTGS1
- TOP2A
- NCOA1
- PTGS1
CHRM3
CHRM1
ADRB1
SCN5A
PTGS2
ADRA2A
ADRA2C
PDE3A
HTR2A
SLC6A2
ADRA1A
CHRM2
ADRA2B
ADRA1B
SLC6A3
ADRB2
SLC6A4
GABRA1
MAOB
MAOA
RXRA
PKIA
Calycosin
NOS2
PTGS1
ESR1
AR
PPARG
PTGS2
RXRA
PDE3A
ESR2
DPP4
MAPK14
GSK3B
CDK2
CHEK1
PRSS1
PIM1
CCNA2
NCOA2
CALM1/CALM2/CALM3
ADRB2
Ferulic acid
PTGS1
PTGS2
NOS3
ADRA2A
SLC6A2
ADRA1A
SLC6A3
ADRB2
LTA4H
MAOB
MAOA
CTRB1
ADRA2B
PLAU
Formononetin
NOS2
PTGS1
CHRM1
ESR1
AR
PPARG
PTGS2
RXRA
PDE3A
ADRA1A
SLC6A3
ADRB2
SLC6A4
ESR2
DPP4
MAPK14
GSK3B
HSP90AB1
CDK2
MAOB
CHEK1
PRSS1
PIM1
CCNA2
CALM1/CALM2/CALM3
CALM1/CALM2/CALM3
CALM1/CALM2/CALM3
PKIA
F2
NOS3
ACHE
JUN
PPARG
IL4
SIRT1
ATP5B
MT-ND6
HSD3B2
HSD3B1
Hederagenin
PGR
NCOA2
CHRM3
CHRM1
GABRA2
| Gene       | Isorhamnetin | Jaranol |
|------------|--------------|---------|
| GABRA3     |              |         |
| CHRM2      |              |         |
| ADRA1B     |              |         |
| GABRA1     |              |         |
| GRIA2      |              |         |
| GABRA6     |              |         |
| GABRA5     |              |         |
| IGHG1      |              |         |
| ADH1B      |              |         |
| ADH1C      |              |         |
| PTGS1      |              |         |
| SCN5A      |              |         |
| PTGS2      |              |         |
| RXRA       |              |         |
| PDE3A      |              |         |
| SLC6A2     |              |         |
| NOS2       |              |         |
| PTGS1      |              |         |
| ESR1       |              |         |
| AR         |              |         |
| PPAR1      |              |         |
| PTGS2      |              |         |
| ESR2       |              |         |
| DPP4       |              |         |
| MAPK14     |              |         |
| GSK3B      |              |         |
| CDK2       |              |         |
| PIK3CG     |              |         |
| PRSS1      |              |         |
| PIM1       |              |         |
| CCNA2      |              |         |
| NCOA2      |              |         |
| CALM1/CALM2/CALM3 |  |         |
| PYGM       |              |         |
| PPAR1      |              |         |
| CHEK1      |              |         |
| AKR1B1     |              |         |
| NCOA1      |              |         |
| F7         |              |         |
| F2         |              |         |
| NOS3       |              |         |
| ACHE       |              |         |
| GABRA1     |              |         |
| MAOB       |              |         |
| GRIA2      |              |         |
| RELA       |              |         |
| XDH        |              |         |
| NCF1       |              |         |
| OLR1       |              |         |
| NOS2       |              |         |
PTGS1
AR
SCN5A
PTGS2
ESR2
DPP4
CDK2
CHEK1
PRSS1
NCOA2
CALM1/CALM2/CALM3

Kaempferol
NOS2
PTGS1
AR
PPARG
PTGS2
HSP90AA1
PIK3CG
NCOA2
DPP4
PRSS1
PGR
F2
CHRM1
NOS3
GABRA2
ACHE
SLC6A2
CHRM2
ADRA1B
GABRA1
TOP2A
TOP2A
F7
CALM1/CALM2/CALM3
CALM1/CALM2/CALM3
CALM1/CALM2/CALM3
RELA
IKBKB
AKT1
BCL2
BAX
TNF
JUN
AHSA1
CASP3
MAPK8
XDH
MMP1
STAT1
CDK1
PPARG
HMOX1
CYP3A4
CYP1A2
CYP1A1
ICAM1
SELE
VCAM1
NR1I2
CYP1B1
ALOX5
HAS2
GSTP1
AHR
PSMD3
SLC2A4
NR1I3
INSR
DIO1
PPP3CA
GSTM1
GSTM2
AKR1C3
SLPI
PGR
PTGS1
AR
PPARG
PTGS2
HSP90AA1
PIK3CG
NCOA2
DPP4
AKR1B1
PRSS1
TOP2A
F2
KCNH2
SCN5A
F10
ADRB2
MMP3
F7
NOS3
RXRA
ACHE
GABRA1
MAOB
RELA

Mairin
Quercetin
EGFR
AKT1
VEGFA
CCND1
BCL2
BCL2L1
FOS
CDKN1A
EIF6
BAX
CASP9
PLAU
MMP2
MMP2
MAPK1
IL10
EGF
RB1
TNF
JUN
IL6
AHSA1
CASP3
TP53
ELK1
NFKBIA
POR
ODC1
XDH
CASP8
TOP1
RAF1
SOD1
PRKCA
MMP1
HIF1A
STAT1
RUNX1T1
CDK1
HSPA5
ERBB2
PPARG
ACACA
HMOX1
CYP3A4
CYP1A2
CAV1
MYC
F3
GJA1
CYP1A1
ICAM1
IL1B
CCL2
SELE
VCAM1
PTGER3
CXCL8
PRKCB
BIRC5
DUOX2
NOS3
HSPB1
TGFB1
SULT1E1
MGAM
IL2
NR1I2
CYP1B1
CCNB1
PLAT
THBD
SERPINE1
COL1A1
IFNG
ALOX5
PTEN
IL1A
MPO
TOP2A
NCF1
ABCG2
HAS2
GSTP1
NFE2L2
NQO1
PARP1
AHR
PSMD3
SLC2A4
COL3A1
CXCL11
CXCL2
DCAF5
NR1I3
CHEK2
INSR
CLDN4
PPARA
PPARD
LTA4H
MAOB
MAOA
CTRB1
CHRM3
CHRM1
ADRB1
SCN5A
HTR2A
ADRA1A
GABRA3
CHRM2
ADRA1B
GABRA1
CHRNA7
Table S2 Potential Targets from ETCM

| Gene         |
|--------------|
| HSD17B1      |
| PLA2G2D      |
| CALM1        |
| SCN2A        |
| PLA2G2E      |
| SCN2B        |
| BCHE         |
| ASPH         |
| MIF          |
| P4HA1        |
| HSD17B6      |
| CYTH2        |
| P4HA2        |
| PLD1         |
| PLD2         |
| SCN4A        |
| MMACHC       |
| CTRB1        |
| SCN4B        |
| PLOD1        |
| SUCNR1       |
| GABRD        |
| TMLHE        |
| GABRE        |
| ECI2         |
| PPARA        |
| SLC13A1      |
| SDHA         |
| PLOD3        |
| SDHB         |
| SLC13A2      |
| RDH5         |
| SDHC         |
| TYR          |
| SLC13A3      |
| PPARD        |
| SDHD         |
| MMAA         |
| AKR1D1       |
| RDH8         |
| SULT2B1      |
| ATP2A1       |
| MMAB         |
| GLRA3        |
| CLEC4E       |
| CASP3        |
| PPARG        |
| SRD5A2       |
| NQO1         |
ESR1
SLCO1B3
CYP2C8
ESR2
PPT1
HAO1
ARF6
ALDH5A1
PAEP
TYMS
PPP1CC
PNP
ANXA1
UL30
NR3C1
GABRG1
NR3C2
GABRG2
ABAT
PLA2G1B
GABRG3
CNR1
SOAT1
MUT
VDR
ACADS
CNR2
SOAT2
SCN1A
HA
SCN1B
MTHFD1
QPT
MTHFD2
FAAH
ACSL3
GUCA1A
ATIC
ALDH1A1
ACSL4
ALDH1A2
TRAPPC3
ALDH1A3
E
SCN3A
HDAC2
ALAD
SCN3B
LPL
HCAR2
| Gene          | Gene          | Gene          | Gene          | Gene          |
|--------------|--------------|--------------|--------------|--------------|
| SREBF1       | P2RY12       | AMN          | HCAR3        | TRPV1        |
|              |              |              |              | SEC14L2      |
|              |              |              |              | MTHFR        |
|              |              |              |              | S100B        |
|              |              |              |              | HNF4A        |
|              |              |              |              | DHRS4L1      |
|              |              |              |              | TRPV3        |
|              |              |              |              | LTB4R        |
|              |              |              |              | SCN5A        |
|              |              |              |              | HDAC9        |
|              |              |              |              | AKR1C1       |
|              |              |              |              | PKIA         |
|              |              |              |              | RCVRN        |
|              |              |              |              | SULT2A1      |
|              |              |              |              | AKR1C2       |
|              |              |              |              | AMT          |
|              |              |              |              | LRAT         |
|              |              |              |              | PHOSPHO1     |
|              |              |              |              | PMP2         |
|              |              |              |              | LTF          |
|              |              |              |              | HNF4G        |
|              |              |              |              | PPP3R1       |
|              |              |              |              | DPYD         |
|              |              |              |              | SCN7A        |
|              |              |              |              | PVR          |
|              |              |              |              | P3H1         |
|              |              |              |              | P3H2         |
|              |              |              |              | NR1H4        |
|              |              |              |              | MPN348       |
|              |              |              |              | P3H3         |
|              |              |              |              | HSD11B1      |
|              |              |              |              | RHO          |
|              |              |              |              | SUCLA2       |
|              |              |              |              | HSD11B2      |
|              |              |              |              | PPA_RS05235  |
|              |              |              |              | RETSAT       |
|              |              |              |              | NFKB1        |
|              |              |              |              | LY96         |
|              |              |              |              | DHRS3        |
|              |              |              |              | NFKB2        |
|              |              |              |              | SCN9A        |
|              |              |              |              | DHRS4        |
|              |              |              |              | FTCD         |
|              |              |              |              | NNMT         |
|              |              |              |              | PGR          |
|              |              |              |              | FADS1        |
PRKACA
FADS2
TM1468
SIGMAR1
MTRR
HSD3B1
PCYT1A
BLLF1
RDH11
PCYT1B
RDH12
GLTP
RDH13
ACO2
CUBN
RDH14
APOD
MTTP
TCN1
GABRB1
GRIN2A
GABRB2
SCN11A
GRIN2B
GABRB3
MTFMT
GRIN2C
XDH
PPP3CA
SUCLG1
GRIN2D
THYA
SUCLG2
RBP1
SHBG
RLBP1
C8G
RBP3
RXRA
RXRB
INS
RORA
TTHA0718
YWHAE
TK
IGHG1
ATP1A1
ATP1A2
FFAR1
ATP1A3
RXRG  
TLR4  
FKBP1A  
MTR  
AR  
CPA1  
ALB  
ACOX1  
SLC25A10  
GM2A  
VLDLR  
SHMT1  
SHMT2  
PLA2G2A  
LTB4R2  
ABL1
| Table S3 AS Genes |
|------------------|
| APOE      |
| APOB      |
| LDLR      |
| APOA1     |
| ABCA1     |
| CETP      |
| LPL       |
| PPARG     |
| LMNA      |
| ABCG8     |
| LIPC      |
| ABCG5     |
| OLR1      |
| CCL2      |
| CYP7A1    |
| CRP       |
| SELE      |
| PCSK9     |
| LCAT      |
| ESR1      |
| APOA2     |
| LPA       |
| HMGCR     |
| NOS3      |
| APOC2     |
| APOC3     |
| CYP27A1   |
| PON1      |
| ATHS      |
| LDLRAP1   |
| IL6       |
| INS       |
| TNF       |
| ADIPOQ    |
| ACE       |
| WRN       |
| VCAM1     |
| VEGFA     |
| SERPINE1  |
| MMP9      |
| ICAM1     |
| APOA5     |
| PPARA     |
| THBD      |
| TLR4      |
| PLAT      |
| SCARB1    |
| EDN1      |
| LIPA      |
CST3
HMOX1
SPP1
PTGS2
SOAT2
KCNJ5
CAV1
MIR145
NAMPT
NR1H4
MIR21
SMAD3
APOA4
TNXB
CYBA
MMP1
PPBP
NPPB
NFE2L2
MIR155
ITIH4
TIMP1
H19
NPC1
TGFBI
CBS
LTA
NPY
PPARD
NR1H3
CEP19
TGFBR1
TUG1
TP53COR1
HP
GHR
NPC1L1
TGFBR2
CD40LG
APOH
TGFBI2
ZNF687
CD14
KNG1
CES1
CDH5
FGA
HULC
MGP
HSPD1
MAPK1
GCLC
PON3
ADRB2
ACAT1
SLC10A2
CXCL12
CDKN2B
BGN
UTS2
PSEN1
PSMA6
LGALS2
MIAT
SERPIND1
ANTXR1
AGXT
TGFBR3
HGD
ANGPTL6
DCN
APP
LRP8
MIR210
DYNC2LI1
SELL
GP1BA
NPPA
CCL5
SHBG
| Cluster Term                                      | Biological Processes          | Count | %     | Pvalue       |
|--------------------------------------------------|-------------------------------|-------|-------|--------------|
| GO:0045429 positive regulation of nitric oxide biosynthesis | 12 | 23.07692 | 3.40E-19 |
| GO:0006954 inflammatory response                   | 16 | 30.76923  | 2.32E-13 |
| GO:0008217 regulation of blood pressure            | 9  | 17.30769  | 1.80E-11 |
| GO:0002576 platelet degranulation                   | 10 | 19.23077  | 2.12E-11 |
| GO:0001666 response to hypoxia                     | 11 | 21.15385  | 8.69E-11 |
| GO:0048661 positive regulation of smooth muscle c  | 8  | 15.38462  | 5.31E-10 |
| GO:0032496 response to lipopolysaccharide          | 10 | 19.23077  | 1.39E-09 |
| GO:0022617 extracellular matrix disassembly         | 8  | 15.38462  | 2.90E-09 |
| GO:0051092 positive regulation of NF-kappaB trans  | 10 | 19.23077  | 5.99E-09 |
| GO:0043066 negative regulation of apoptotic process| 13 | 25       | 8.26E-09 |
| GO:0001525 angiogenesis                            | 10 | 19.23077  | 2.05E-08 |
| GO:0014068 positive regulation of phosphatidylinos | 7  | 13.46154  | 4.17E-08 |
| GO:0032496 response to lipopolysaccharide          | 8  | 15.38462  | 4.70E-08 |
| GO:0050901 leukocyte tethering or rolling          | 5  | 9.615385  | 5.29E-08 |
| GO:0030198 extracellular matrix organization       | 9  | 17.30769  | 1.24E-07 |
| GO:0071456 cellular response to hypoxia             | 7  | 13.46154  | 4.36E-07 |
| GO:0045427 positive regulation of JAK-STAT cascade | 5  | 9.615385  | 5.30E-07 |
| GO:0035924 cellular response to vascular endothelium| 5  | 9.615385  | 6.40E-07 |
| GO:0007159 leukocyte cell-cell adhesion             | 5  | 9.615385  | 9.11E-07 |
| GO:0045766 positive regulation of angiogenesis     | 7  | 13.46154  | 1.27E-06 |
| GO:0010888 negative regulation of lipid storage    | 4  | 7.692308  | 1.46E-06 |
| GO:0001938 positive regulation of endothelial cell f | 6  | 11.53846  | 2.05E-06 |
| GO:0045080 positive regulation of chemokine biosynthesis | 4  | 7.692308  | 3.12E-06 |
| GO:0030195 negative regulation of blood coagulatic | 4  | 7.692308  | 5.70E-06 |
| GO:0001938 positive regulation of endothelial cell f | 6  | 11.53846  | 2.05E-06 |
| GO:0045080 positive regulation of chemokine biosynthesis | 4  | 7.692308  | 3.12E-06 |
| GO:0030195 negative regulation of blood coagulatic | 4  | 7.692308  | 5.70E-06 |
| GO:0001938 positive regulation of endothelial cell f | 6  | 11.53846  | 2.05E-06 |
| GO:0045080 positive regulation of chemokine biosynthesis | 4  | 7.692308  | 3.12E-06 |
| GO:0030195 negative regulation of blood coagulatic | 4  | 7.692308  | 5.70E-06 |
| GO:0001938 positive regulation of endothelial cell f | 6  | 11.53846  | 2.05E-06 |
| GO:0045080 positive regulation of chemokine biosynthesis | 4  | 7.692308  | 3.12E-06 |
| GO:0030195 negative regulation of blood coagulatic | 4  | 7.692308  | 5.70E-06 |
| GO:0001938 positive regulation of endothelial cell f | 6  | 11.53846  | 2.05E-06 |
| GO:0045080 positive regulation of chemokine biosynthesis | 4  | 7.692308  | 3.12E-06 |
| GO:0030195 negative regulation of blood coagulatic | 4  | 7.692308  | 5.70E-06 |
| GO:0001938 positive regulation of endothelial cell f | 6  | 11.53846  | 2.05E-06 |
| GO:0045080 positive regulation of chemokine biosynthesis | 4  | 7.692308  | 3.12E-06 |
| GO:0030195 negative regulation of blood coagulatic | 4  | 7.692308  | 5.70E-06 |
| GO:0001938 positive regulation of endothelial cell f | 6  | 11.53846  | 2.05E-06 |
| GO:0045080 positive regulation of chemokine biosynthesis | 4  | 7.692308  | 3.12E-06 |
| GO:0030195 negative regulation of blood coagulatic | 4  | 7.692308  | 5.70E-06 |
| GO:0001938 positive regulation of endothelial cell f | 6  | 11.53846  | 2.05E-06 |
| GO:0045080 positive regulation of chemokine biosynthesis | 4  | 7.692308  | 3.12E-06 |
GO:0032700 negative regulation of interleukin-17 3 5.769231 4.89E-04
GO:0002003 angiotensin maturation 3 5.769231 4.89E-04
GO:0050927 positive regulation of positive chemotaxis 3 5.769231 4.89E-04
GO:0010745 negative regulation of macrophage differentiation 3 5.769231 6.90E-04
GO:0051926 negative regulation of calcium ion transport 3 5.769231 6.90E-04
GO:0043627 response to estrogen 4 7.692308 0.00101
GO:0050995 negative regulation of lipid catabolic process 3 5.769231 0.001056
GO:0032722 positive regulation of chemokine production 3 5.769231 0.001195
GO:0050927 positive regulation of positive chemotaxis 3 5.769231 0.002392
GO:0010575 positive regulation of vascular endothelial growth factor 3 5.769231 0.003024
GO:0032715 negative regulation of interleukin-6 production 3 5.769231 0.00325
GO:0030168 platelet activation 4 7.692308 0.005131
GO:0032943 mononuclear cell proliferation 2 3.846154 0.006065
GO:0002548 monocyte chemotaxis 3 5.769231 0.007206
GO:0001781 neutrophil apoptotic process 2 3.846154 0.009084
GO:0010572 positive regulation of platelet activation 2 3.846154 0.009084

GO:0042632 cholesterol homeostasis 15 35.71429 9.62E-25
GO:0042157 lipoprotein metabolic process 11 26.19048 1.03E-18
GO:0070328 triglyceride homeostasis 9 21.42857 9.20E-16
GO:0043691 reverse cholesterol transport 8 19.04762 9.38E-15
GO:0008203 cholesterol metabolic process 10 23.80952 5.34E-14
GO:0033475 high-density lipoprotein particle remodeling 7 16.66667 7.12E-13
GO:0033444 cholesterol efflux 7 16.66667 2.47E-11
GO:0033472 very-low-density lipoprotein particle remodeling 5 11.90476 1.06E-09
GO:0006869 lipid transport 7 16.66667 2.79E-08
GO:0033700 phospholipid efflux 5 11.90476 3.01E-08
GO:0030301 cholesterol transport 5 11.90476 5.45E-08
GO:0006629 lipid metabolic process 8 19.04762 9.40E-08
GO:0019433 triglyceride catabolic process 5 11.90476 3.73E-07
GO:0055091 phospholipid homeostasis 4 9.52381 1.12E-06
GO:0030299 intestinal cholesterol absorption 4 9.52381 1.12E-06
GO:0001937 negative regulation of endothelial cell proliferation 4 10.81081 3.18E-05
GO:2000379 positive regulation of reactive oxygen species 4 10.81081 3.53E-05
GO:0042127 regulation of cell proliferation 6 16.21622 4.39E-05
GO:0071456 cellular response to hypoxia 5 13.51351 5.14E-05
GO:0006954 inflammatory response 7 18.91892 1.40E-04
| GO          | Description                                                        | Value | p-value       |
|-------------|--------------------------------------------------------------------|-------|---------------|
| GO:0006915  | apoptotic process                                                  | 8     | 21.62162      |
| GO:0051092  | positive regulation of NF-kappaB trans                             | 5     | 13.51351      |
| GO:0043066  | negative regulation of apoptotic process                          | 7     | 18.91892      |
| GO:1902176  | negative regulation of oxidative stress                           | 3     | 8.108108      |
| GO:0071260  | cellular response to mechanical stimulus                           | 4     | 10.81081      |
| GO:0001666  | response to hypoxia                                                 | 5     | 13.51351      |
| GO:0035994  | response to muscle stretch                                         | 3     | 8.108108      |
|             | positive regulation of macrophage derivative                       |       | 5.26E-04      |
| GO:0071356  | cellular response to tumor necrosis factor                         | 4     | 10.81081      |
| GO:0050900  | leukocyte migration                                                | 4     | 10.81081      |
| GO:0032481  | positive regulation of type I interferon                           | 3     | 8.108108      |
| GO:0042632  | cholesterol homeostasis                                            | 3     | 8.108108      |
| GO:0030512  | negative regulation of transforming growth                         | 3     | 8.108108      |
| GO:0014068  | positive regulation of macrophage derivative                       | 3     | 8.108108      |
| GO:0006642  | triglyceride mobilization                                          | 2     | 5.405405      |
| GO:2001028  | positive regulation of endothelial cell c                         | 2     | 5.405405      |
| GO:0045084  | positive regulation of interleukin-12 binding                      | 2     | 5.405405      |
| GO:0002576  | platelet degranulation                                             | 3     | 8.108108      |
| GO:0001649  | osteoblast differentiation                                          | 3     | 8.108108      |
| GO:0010884  | positive regulation of lipid storage                               | 2     | 5.405405      |
| GO:0010875  | positive regulation of cholesterol efflux                         | 2     | 5.405405      |
| GO:0030194  | positive regulation of blood coagulation                          | 2     | 5.405405      |
| GO:0071354  | cellular response to interleukin-6                                 | 2     | 5.405405      |
| GO:0045987  | positive regulation of smooth muscle c                            | 4     | 44.44444      |
| GO:0007204  | positive regulation of cytosolic calcium                           | 5     | 55.55556      |
| GO:0045907  | positive regulation of vasoconstriction                           | 4     | 44.44444      |
| GO:0007197  | adenylate cyclase-inhibiting G-protein                             | 3     | 33.33333      |
| GO:0007207  | phospholipase C-activating G-protein c                            | 3     | 33.33333      |
| GO:0008283  | cell proliferation                                                  | 5     | 55.55556      |
| GO:003056   | regulation of vascular smooth muscle c                            | 2     | 22.22222      |
| GO:0042554  | superoxide anion generation                                        | 4     | 7.54717       |
| GO:0032496  | response to lipopolysaccharide                                     | 7     | 13.20755      |
| GO:0006940  | regulation of smooth muscle contract                               | 4     | 7.54717       |
| GO:0006954  | inflammatory response                                              | 9     | 16.98113      |
| GO:0030168  | platelet activation                                                | 6     | 11.32075      |
| GO:0006801  | superoxide metabolic process                                       | 4     | 7.54717       |
| GO:0042127  | regulation of cell proliferation                                   | 6     | 11.32075      |
| GO:0002576  | platelet degranulation                                             | 5     | 9.433962      |
| GO:0019430  | removal of superoxide radicals                                     | 3     | 5.660377      |
| GO:0045730  | respiratory burst                                                  | 3     | 5.660377      |
| GO:0042632  | cholesterol homeostasis                                            | 4     | 7.54717       |
| GO:0055114  | oxidation-reduction process                                        | 7     | 13.20755      |
| GO:0042542  | response to hydrogen peroxide                                      | 3     | 5.660377      |
| GO:1902177  | positive regulation of oxidative stress-i                         | 2     | 3.773585      |
| GO:0034197  | triglyceride transport                                             | 2     | 3.773585      |
| GO:0016525  | negative regulation of angiogenesis                                | 3     | 5.660377      |
| GO:0070098  | chemokine-mediated signaling pathway                               | 3     | 5.660377      |
| GO:0010886  | positive regulation of cholesterol storage                         | 2     | 3.773585      |
| GO:0032930  | positive regulation of superoxide anion | 2 | 3.773585 | 0.027534 |
|-------------|------------------------------------------|---|-----------|-----------|
| GO:0006707  | cholesterol catabolic process             | 2 | 3.773585 | 0.033551 |
|-------------|------------------------------------------|---|-----------|-----------|
| GO:0008202  | steroid metabolic process                 | 4 | 3.33333  | 2.54E-06  |
| GO:0051583  | dopamine uptake involved in synaptic      | 2 | 16.666667| 0.003271  |
| GO:0042420  | dopamine catabolic process                | 2 | 16.666667| 0.003271  |
|-------------|------------------------------------------|---|-----------|-----------|
| GO:0055114  | oxidation-reduction process               | 4 | 3.33333  | 0.00582   |
| GO:0008210  | estrogen metabolic process                | 2 | 16.666667| 0.007184  |
| GO:0042136  | neurotransmitter biosynthetic process     | 2 | 16.666667| 0.007835  |
|-------------|------------------------------------------|---|-----------|-----------|
| GO:0032469  | endoplasmic reticulum calcium ion homeo  | 3 | 17.64706 | 8.87E-05  |
| GO:2001234  | negative regulation of apoptotic signali | 3 | 17.64706 | 1.01E-04  |
| GO:0033344  | cholesterol efflux                       | 3 | 17.64706 | 2.52E-04  |
| GO:0043200  | response to amino acid                    | 3 | 17.64706 | 3.89E-04  |
| GO:0031100  | organ regeneration                        | 3 | 17.64706 | 8.97E-04  |
| GO:0042632  | cholesterol homeostasis                   | 3 | 17.64706 | 0.001658  |
| GO:0051384  | response to glucocorticoid                | 3 | 17.64706 | 0.00171   |
| GO:0043627  | response to estrogen                      | 3 | 17.64706 | 0.00171   |
|-------------|------------------------------------------|---|-----------|-----------|
| GO:0008203  | cholesterol metabolic process             | 3 | 17.64706 | 0.001869  |
| GO:0006808  | regulation of nitrogen utilization        | 2 | 11.76471 | 0.001905  |
| GO:0043085  | positive regulation of catalytic activity | 3 | 17.64706 | 0.002639  |
| GO:0007598  | blood coagulation, extrinsic pathway      | 2 | 11.76471 | 0.004756  |
|-------------|------------------------------------------|---|-----------|-----------|
| GO:0009651  | response to salt stress                   | 2 | 11.76471 | 0.008545  |
|-------------|------------------------------------------|---|-----------|-----------|
| GO:0006888  | ER to Golgi vesicle-mediated transport    | 3 | 17.64706 | 0.009918  |
| GO:0017187  | peptidyl-glutamic acid carboxylation      | 2 | 11.76471 | 0.010434  |
| GO:0001666  | response to hypoxia                      | 3 | 17.64706 | 0.011391  |
| GO:0033700  | phospholipid efflux                      | 2 | 11.76471 | 0.013262  |
| GO:0043691  | reverse cholesterol transport             | 2 | 11.76471 | 0.017021  |
| GO:0001782  | B cell homeostasis                       | 2 | 11.76471 | 0.017021  |
| GO:0008637  | apoptotic mitochondrial changes           | 2 | 11.76471 | 0.017959  |
| GO:0001836  | release of cytochrome c from mitochon     | 2 | 11.76471 | 0.021701  |
| GO:0070059  | intrinsic apoptotic signaling pathway ii  | 2 | 11.76471 | 0.030998  |
| GO:2001244  | positive regulation of intrinsic apoptoti | 2 | 11.76471 | 0.030998  |
| GO:0002931  | response to ischemia                      | 2 | 11.76471 | 0.030998  |
| GO:008015   | blood circulation                         | 2 | 11.76471 | 0.042045  |
| GO:0008630  | intrinsic apoptotic signaling pathway ii  | 2 | 11.76471 | 0.043874  |
| GO:0042542  | response to hydrogen peroxide             | 2 | 11.76471 | 0.047524  |
| Genes                                      | Fold Enrichment | Bonferroni |
|-------------------------------------------|-----------------|------------|
| AKT1, ICAM1, IL6, TNF, PTGS2, INS, AGT, EDN1, IFNG, E | 90.11806798     | 5.20E-16   |
| KNG1, SELP, IL6, TNF, CCL2, PTGS2, CRP, TLR4, CCL5, C | 13.63263649     | 3.56E-10   |
| LEP, ACE, PTGS2, REN, AGT, HMOX1, EDN1, PPARG, NO | 44.71242604     | 2.76E-08   |
| KNG1, SELP, VWF, ALB, PECAM1, VEGFA, SERPINE1, T | 31.35175504     | 3.24E-08   |
| LEP, VCAM1, CASP3, CCL2, HMOX1, VEGFA, CAT, CXC1 | 20.62501572     | 1.33E-07   |
| AKT1, RETN, IL6, TNF, PTGS2, HMOX1, EDN1, CCL5  | 43.05641026     | 8.13E-07   |
| VCM1, SELP, NOTCH1, CASP3, PTGS2, REN, EDN1, MP6 | 19.69043152     | 2.13E-06   |
| MMP9, ELN, MMP3, MMP2, PLG, MMP1, SPP1, TIMP1 | 33.99109283     | 4.44E-06   |
| ICAM1, IL6, TNF, INS, AGT, IL1B, TLR4, CAT, TGFB1 | 21.85193754     | 9.18E-06   |
| LEP, AKT1, CASP3, IL6, ALB, MMP9, VEGFA, TP53, MPO | 9.22637364     | 1.26E-05   |
| LEP, CCL2, PTGS2, HMOX1, PECAM1, VEGFA, SERPINE1 | 14.48085547     | 3.14E-05   |
| LEP, SELP, INS, AGT, CAT, CCL5, KDR | 34.77631366 | 6.40E-05   |
| ICAM1, IL6, TNF, CCL2, SERPINE1, IFNG, TLR4, IL10 | 22.86180768 | 7.20E-05   |
| AKT1, TNF, CCL2, IL1B, CCL5, TGFB1 | 58.71328671 | 7.21E-05   |
| ICAM1, IL6, TNF, CCL2, VEGFA, TLR4, CCL5, TGFB1, KD | 16.60747253 | 7.89E-05   |
| LEP, VCAM1, SELP, TNF, SELE | 124.21018343 | 8.10E-05   |
| VCM1, ICAM1, VWF, TNF, PECAM1, ELN, SERPINE1, SI | 14.82810047 | 1.90E-04   |
| AKT1, ICAM1, PTGS2, HMOX1, EDN1, VEGFA, TP53 | 23.54674034 | 6.68E-04   |
| LEP, NOTCH1, IL6, CCL5, IL10 | 73.39160839 | 8.12E-04   |
| VCM1, AKT1, NOTCH1, VEGFA, KDR | 70.20066891 | 9.81E-04   |
| VCMC1, ICAM1, SELP, CCL5, SELE | 64.58415385 | 0.001394213 |
| F3, HMOX1, VEGFA, SERPINE1, IL1B, NOS3, KDR | 19.65861279 | 0.001940028 |
| LEP, IL6, TNF, CRP | 161.4631583 | 0.002237727 |
| AKT1, CCL2, F3, VEGFA, CXCL12, KDR | 28.08026756 | 0.003137587 |
| TNF, HMOX1, IFNG, IL1B | 129.1692308 | 0.004768545 |
| KNG1, APOE, EDN1, SERPINE1 | 107.6410256 | 0.008687782 |
| LEP, CASP3, PTGS2, ESR1, CAT, TGFB1 | 21.29163145 | 0.012290391 |
| AGT, MMP9, MMP2, IL10 | 86.11282051 | 0.017770944 |
| ICAM1, SELP, MMP9, PECAM1, SELE, MMP1 | 15.88146282 | 0.050061282 |
| TNF, PTGS2, IL1B, TLR4 | 61.50915751 | 0.050427816 |
| AKT1, APOE, INS, ESR1 | 58.71328671 | 0.058036324 |
| CCL2, EDN1, IFNG, IL1B, CCL5 | 24.46369646 | 0.069945392 |
| LEP, RETN, IL6, CAT, IL10 | 24.09873708 | 0.074076161 |
| LEP, TNF, IFNG, IL1B, CCL5 | 24.09873708 | 0.074076161 |
| LEP, AKT1, TNF, INS, ADIPOQ | 24.09873708 | 0.074076161 |
| CASP3, PTGS2, ADIPOQ, SELE | 51.66769231 | 0.084974274 |
| ICAM1, IL6, CCL2, EDN1, CCL5 | 22.74106176 | 0.092156864 |
| LEP, AKT1, IL1B, NOS3 | 49.68047337 | 0.095232211 |
| VCM1, ICAM1, SELP, VWF, CCL2, PECAM1, CXCL12, SI | 6.331825038 | 0.096875958 |
| CCL2, AGT, SERPINE1, TLR4, CCL5 | 22.11801897 | 0.102213324 |
| ICAM1, SERPINE1, IL10, KDR | 46.13186813 | 0.117963151 |
| MAPK1, TNF, CCL2, INS, IL1B, CCL5, TGFB1 | 8.627715979 | 0.188357184 |
| TNF, VEGFA, CCL5, CXCL12 | 30.03959999 | 0.367516979 |
| AGT, CRP, TGFB1 | 107.6410256 | 0.388673926 |
| IL6, TNF, IL1B, TLR4 | 28.7042735 | 0.408149055 |
| AKT1, PTGS2, APOE, HMOX1, MPO | 14.76832168 | 0.409405386 |
| AGT, EDN1, VEGFA, KDR | 28.03826756 | 0.428734833 |
| LEP, CCL2, IFNG, TLR4 | 27.48281506 | 0.449433235 |
| Gene Symbols | p-Value | Log2 Fold Change |
|--------------|---------|-----------------|
| IFNG, TLR4, TGFB1 | 8.806993007 | 0.527161643 |
| ACE, REN, AGT | 8.806993007 | 0.527161643 |
| F3, VEGFA, KDR | 8.806993007 | 0.527161643 |
| CRP, PPARg, ADIPOQ | 7.452071006 | 0.652922048 |
| ICAM1, PTGS2, NOS3 | 7.452071006 | 0.652922048 |
| MAPK1, HMOX1, PPARg, ESR1 | 1.987218935 | 0.787228306 |
| TNF, INS, IL1B | 6.054807692 | 0.801872394 |
| IL6, TNF, TLR4 | 5.968642534 | 0.839787975 |
| AKT1, AGT, MMP9 | 4.36538462 | 0.974483599 |
| PTGS2, IL1B, TGFB1 | 3.88034188 | 0.990339486 |
| TNF, TLR4, IL10 | 3.5989011 | 0.993177316 |
| AKT1, MAPK1, VWF, IL6 | 1.123210702 | 0.999622113 |
| ACE, TGFB1 | 3.229230769 | 0.999910413 |
| IL6, CCL2, CCL5 | 2.06534047 | 0.999984564 |
| IL6, IFNG | 2.215820513 | 0.999999152 |
| SELP, TLR4 | 2.215820513 | 0.999999152 |

| Gene Symbols | p-Value | Log2 Fold Change |
|--------------|---------|-----------------|
| LPL, LDLR, APOC2, ABCG1, ABCG8, APOA1, ABCG5, LC | 9.70353714 | 4.77E-22 |
| LPL, APOA1, LPA, LCAT, APOC3, APOA5, APOC2, NPC1L1 | 1.15734358 | 5.09E-16 |
| LPL, APOA1, APOC3, APOA5, APOC2, CETP, ANGPTL3, L | 1.389566044 | 4.41E-13 |
| APOA1, LCAT, APOC3, APOA5, APOC2, CETP, LIPC, ABC | 1.776931217 | 4.63E-12 |
| APOA1, LCAT, LCAT, PON1, PCSK9, CETP, ANGPTL3, LII | 5.79551821 | 2.65E-11 |
| APOA1, LCAT, APOC3, CETP, LIPC, PLTP, ABCG1 | 1.86577777 | 3.53E-10 |
| ABCG8, APOA1, ABCG5, APOC3, APOA5, APOC2, ABCG1 | 1.119466667 | 1.23E-08 |
| LPL, LCAT, APOC2, CETP, LIPC | 2.285782313 | 5.28E-07 |
| APOA1, LPA, APOC3, APOA5, APOC2, CETP, PLTP | 3.68245614 | 1.38E-05 |
| APOA1, APOC3, APOA5, APOC2, ABCG1 | 1.427891156 | 1.49E-05 |
| APOA1, LDLR, LCAT, NPC1L1, CETP | 1.249404762 | 2.70E-05 |
| LPL, LPA, LDLR, LCAT, APOC2, LIPC, PLTP, SREBF2 | 2.037245981 | 4.66E-05 |
| LPL, APOA1, APOC3, APOA5, LIPC | 7.96190476 | 1.85E-04 |
| APOA1, CETP, ANGPTL3, ABCG1 | 1.776931217 | 5.57E-04 |
| ABCG8, ABCG5, LDLR, NPC1L1 | 1.776931217 | 5.57E-04 |
| LDLR, NR1H4, NR1H3, SREBF2 | 1.776931217 | 5.57E-04 |
| LPL, APOC3, APOA5, PCSK9, CETP | 5.711564626 | 7.52E-04 |
| APOA1, APOA5, APOC2, NR1H3 | 1.599238095 | 7.94E-04 |
| LCAT, APOA5, PON1, CETP | 1.599238095 | 7.94E-04 |
| APOA1, APOA5, APOC2, NR1H3 | 1.599238095 | 7.94E-04 |
| CETP, ITGB3, ABCG1, NR1H3 | 1.23018315 | 0.00188253 |
| PON1, PLTP, ABCG1, NR1H3 | 1.142312925 | 0.002391274 |
| APOA1, FGA, PPBP, F13A1, ITIH4, ITGB3 | 2.328978517 | 0.002452888 |
| ABCG8, TOP1, LPL, APOA1, ABCG5, CST3, NPC1L1, ABC | 1.052130362 | 0.003904569 |
| LPL, APOA1, LCAT, PCSK9, ANGPTL3 | 3.701490035 | 0.004382303 |
| ABCG8, APOA1, ABCG5, HMGCR, SERPINC1 | 2.701415701 | 0.015307783 |

| Gene Symbols | p-Value | Log2 Fold Change |
|--------------|---------|-----------------|
| HIF1A, F3, HSPB1, NFE2L2, ENG, SIRT1, IL1A | 2.762491187 | 1.37E-04 |
| CAV1, GJA1, STAT1, ENG | 6.259832246 | 0.028791354 |
| AGTR1, CDKN1A, F2, NFE2L2 | 6.051171171 | 0.031892573 |
| AGTR1, NFKBIA, NOS2, ENG, SIRT1, PLAU | 1.471906501 | 0.039501011 |
| CCNB1, HIF1A, NFE2L2, CCNA2, SIRT1 | 2.367387391 | 0.046049807 |
| KNG1, SELP, CD40LG, RELA, NFKB1, NFE2L2, IL1A | 8.382229195 | 0.120235506 |
| Gene 1          | Gene 2          | p-value 1    | p-value 2    |
|----------------|----------------|-------------|-------------|
| CASP9, IRF1, NFKBIA, GJA1, NFKB1, STAT1, IGFBP3, IL6 | 6.403355737 | 0.145773429 |
| AR, CD40LG, RELA, NFKBIA, NFKB1 | 17.06157285 | 0.153895353 |
| CDKN1A, CD40LG, RELA, NFKBIA, HSPB1, NFKB1, SIRT1 | 6.982120582 | 0.290629749 |
| HSPB1, NFE2L2, SIRT1 | 97.25096525 | 0.349270728 |
| IRF1, GJA1, NFKB1, ENG | 25.56832889 | 0.39270728 |
| RELA, HIF1A, NOS2, ENG, PLA2 | 13.1929604 | 0.359273718 |
| RELA, NFKBIA, NFKB1 | 85.09459459 | 0.383190626 |
| AGTR1, APOB, NFKB1 | 85.09459459 | 0.383190626 |
| APOB, RELA, NFE2L2, SIRT1 | 16.5031941 | 0.784121252 |
| SELP, CAV1, APOB, F2 | 14.87992911 | 0.872698492 |
| RELA, IRF1, NFKB1 | 26.6963434 | 0.992642363 |
| CAV1, APOB, SIRT1 | 21.27364865 | 0.999519187 |
| CAV1, ENG, SIRT1 | 21.27364865 | 0.999519187 |
| SELP, F2, SIRT1 | 20.94636175 | 0.999611915 |
| APOB, SIRT1 | 226.9189189 | 0.999623235 |
| MET, HSPB1 | 181.5351351 | 0.99947365 |
| RELA, IRF1 | 151.2792793 | 0.999992665 |
| KNG1, SELP, APP | 13.2185778 | 0.999999994 |
| GJA1, RUNX2, IGFBP3 | 13.09147609 | 0.999999996 |
| APOB, NFKB1 | 90.76756757 | 0.999999997 |
| NFKBIA, SIRT1 | 64.8397683 | 1.0 |
| F2, NFE2L2 | 64.8397683 | 1.0 |
| RELA, NFKB1 | 64.8397683 | 1.0 |
| CHRM3, ADRA1B, ADRA1A, ADRA1D | 355.3862434 | 1.18E-05 |
| UTS2, SAA1, ADRA1B, ADRA1A, ADRA1D | 69.6185738 | 3.31E-05 |
| ADRA1B, ADRA1A, ADRA1D, HTR2A | 233.2222222 | 4.37E-05 |
| CHRM5, CHRM3, CHRM1 | 799.6190476 | 5.21E-04 |
| CHRM5, CHRM3, CHRM1 | 699.6666667 | 6.94E-04 |
| CHRM5, CHRM3, CHRM1, ADRA1B, ADRA1D | 25.48876746 | 0.001811122 |
| CHRM3, CHRM1 | 932.8888889 | 0.21203273 |
| CYBA, NCF1, NOX1, SOD1 | 0.004310286 | 0.015440401 |
| OPRM1, TNFRSF11B, THBD, CXCL2, PF4, DCN, CXCL11 | 0.00321163 | 0.017247493 |
| CHRM2, ADRA2A, ADRA2C, ADRA2B | 0.00302799 | 0.023649793 |
| CYBA, TNFRSF11B, PTGER3, CXCL2, NOX1, PF4, CXCL1 | 0.003424417 | 0.030651592 |
| ADRA2A, RAF1, PF4, ADRA2C, ADRA2B, COL1A1 | 0.004006697 | 0.043046129 |
| CYBA, NCF1, NOX1, SOD1 | 0.003807616 | 0.047719294 |
| TNFRSF11B, ERBB3, CXCL2, CHEK1, PF4, CXCL11 | 0.020517582 | 0.406767558 |
| APOH, IGF2, PF4, SOD1, TGFB2 | 0.020638955 | 0.446301428 |
| APOA4, SOD1, NQO1 | 0.037093879 | 0.94061921 |
| CYBA, NCF1, NOX1 | 0.040700327 | 1.107853271 |
| APOA4, CYP7A1, SCARB1, MTTP | 0.053907207 | 1.572965689 |
| CYBA, NCF1, CYP7A1, NOX1, ALOX5, SOD1, NQO1 | 0.276002231 | 13.93688959 |
| HP, COL1A1, SOD1 | 0.296612643 | 15.61660755 |
| NOX1, SOD1 | 0.319292498 | 17.50985147 |
| APOH, MTTP | 0.361567755 | 21.8909506 |
| APOH, PF4, DCN | 0.35974724 | 28.60125842 |
| CXCL2, PF4, CXCL11 | 0.375468255 | 27.36611544 |
| MSR1, SCARB1 | 0.383247209 | 28.60125842 |
| Gene Combination | p-value | Log10(p-value) |
|------------------|---------|---------------|
| CYBA, SOD1       | 0.416407276 | 35.15497902   |
| CYP7A1, SCARB1   | 0.447024993 | 41.10780763   |
| AKR1C3, CYP3A4, CYP1B1, SULT1E1 | 130.1705426 | 5.39E-04 |
| SLC6A2, SLC6A3   | 0.500770107 | 559.7333333   |
| SLC6A3, MAOB     | 0.500770107 | 559.7333333   |
| AKR1C3, CYP3A4, CYP1B1, MAOB | 0.709904457 | 9.454954955   |
| CYP1B1, SULT1E1  | 0.783158398 | 254.4242424   |
| ACHE, SLC6A3     | 0.811300673 | 233.2222222   |
| PSEN1, BAX, BCL2 | 0.042888897 | 197.5529412   |
| PSEN1, BAX, BCL2 | 0.048837666 | 185.2058824   |
| SOAT2, APOA2, ABCA1 | 0.117115719 | 118.5317647   |
| MTHFR, GSTP1, CHUK | 0.175056468 | 95.59013283   |
| APOA2, F7, GSTP1 | 0.3582304   | 63.04881101   |
| SOAT2, APOA2, ABCA1 | 0.559431606 | 46.30147059   |
| APOA2, BCL2, HSPD1 | 0.57056016  | 45.58914027   |
| APOA2, F7, HSPD1 | 0.57056016  | 45.58914027   |
| SOAT2, APOA2, ABCA1 | 0.603175056 | 43.57785467   |
| BAX, BCL2        | 0.610104885 | 987.7647059   |
| APOA2, PSEN1, BCL2 | 0.728997745 | 36.5838788    |
| F10, F7          | 0.905097408 | 395.1058824   |
| HSP90AB1, BAX    | 0.98582795  | 219.503268    |
| F10, F5, F7      | 0.992731132 | 18.52058824   |
| F10, F7          | 0.994381642 | 179.5935829   |
| MTHFR, F7, HSPD1 | 0.996515669 | 17.22845417   |
| APOA2, ABCA1     | 0.998633498 | 141.1092437   |
| APOA2, ABCA1     | 0.999792617 | 109.751634    |
| BAX, BCL2        | 0.999792617 | 109.751634    |
| BAX, HSPD1       | 0.99987057  | 103.9752322   |
| BAX, BCL2        | 0.99990368  | 85.89258312   |
| BAX, BCL2        | 0.999999824 | 59.86452763   |
| BAX, BCL2        | 0.999999824 | 59.86452763   |
| BCL2, HSPD1      | 0.999999999 | 43.90065359   |
| MTHFR, F5        | 1          | 42.03254068   |
| BAX, BCL2        | 1          | 38.73587082   |
| BCL2, HSPD1      | 1          | 38.73587082   |
| Term       | Pathway                                   | Count | %    | Pvalue   |
|------------|-------------------------------------------|-------|------|----------|
| hsa04668   | TNF signaling pathway                     | 29    | 0.052112 | 1.30E-14 |
| hsa04066   | HIF-1 signaling pathway                   | 27    | 0.048518 | 4.89E-14 |
| hsa04068   | FoxO signaling pathway                     | 28    | 0.050315 | 3.25E-11 |
| hsa04620   | Toll-like receptor signaling pathway      | 24    | 0.043127 | 2.08E-10 |
| hsa04151   | PI3K-Akt signaling pathway                | 43    | 0.07727 | 2.61E-09 |
| hsa03320   | PPAR signaling pathway                    | 18    | 0.032346 | 4.04E-09 |
| hsa04641   | NF-kappa B signaling pathway              | 19    | 0.034143 | 4.51E-08 |
| hsa04610   | Complement and coagulation cascades       | 17    | 0.030549 | 4.70E-08 |
| hsa04920   | Adipocytokine signaling pathway          | 16    | 0.028752 | 3.78E-07 |
| hsa04010   | MAPK signaling pathway                    | 31    | 0.055706 | 1.05E-06 |
| hsa04370   | VEGF signaling pathway                    | 14    | 0.025158 | 2.45E-06 |
| hsa04060   | Cytokine-cytokine receptor interaction    | 29    | 0.052112 | 4.31E-06 |
| hsa04975   | Fat digestion and absorption              | 11    | 0.019767 | 6.27E-06 |
| hsa04062   | Chemokine signaling pathway               | 22    | 0.039534 | 9.45E-05 |
| hsa04923   | Regulation of lipolysis in adipocytes     | 11    | 0.019767 | 1.77E-04 |
| hsa04270   | Vascular smooth muscle contraction        | 15    | 0.026955 | 7.79E-04 |
| hsa04152   | AMPK signaling pathway                    | 15    | 0.026955 | 0.001279 |
| hsa04350   | TGF-beta signaling pathway                | 12    | 0.021564 | 0.001342 |
| hsa04670   | Leukocyte transendothelial migration      | 14    | 0.025158 | 0.002011 |
| hsa04611   | Platelet activation                       | 15    | 0.026955 | 0.002178 |
| hsa04150   | mTOR signaling pathway                    | 9     | 0.016173 | 0.004331 |
| hsa04630   | Jak-STAT signaling pathway                | 14    | 0.025158 | 0.014145 |
| hsa00480   | Glutathione metabolism                    | 7     | 0.012579 | 0.026821 |
| Genes                          | Fold Enrichment | Bonferroni |
|-------------------------------|-----------------|------------|
| TNF, CCL2, PTGS2, MMP9, EDN1, CXCL2, NFKBIA, NFKE | 5.95655493 | 3.14E-12 |
| ERBB2, EDN1, HK2, NFKB1, TLR4, TIMP1, AKT1, INS, HM | 6.181210064 | 1.18E-11 |
| PTEN, IL10, TGFB1, TGFB2, AKT1, CDKN2B, SLC2A4, IN | 4.592341805 | 7.86E-09 |
| PIK3CG, IL6, TNF, RELA, CXCL8, NFKBIA, NFKB1, TLR4 | 4.976068479 | 5.03E-08 |
| HSP90AB1, COL3A1, NFKB1, TLR4, BCL2L1, ITGB3, PTE | 2.739241561 | 6.32E-07 |
| PPARA, LPL, PPARD, OLR1, RXRB, RXRA, PPARG, ADIP | 5.904439464 | 9.78E-07 |
| ICAM1, TNF, PTGS2, RELA, CXCL8, NFKBIA, NFKB1, TL | 4.799713562 | 1.09E-05 |
| KNG1, PLAT, F10, F13A1, F7, PLG, VWF, THBD, F5, FGA | 5.14779831 | 1.14E-05 |
| PPARA, TNF, RXRB, RXRA, RELA, NFKBIA, NFKB1, ADI | 5.023459607 | 9.16E-05 |
| TNF, ELK1, NFKB1, TGFB1, TGFB2, AKT1, FOS, CASP3, PIK3CG, PRKCA, PTGS2, RA | 2.692911894 | 2.53E-04 |
| NFKB1, TL | 5.044047557 | 5.94E-04 |
| KL, CCL2, TNF, CXCL2, BMPR2, CXCL8, PF4, CXCL11, CCL5, APOA4, ABCG8, APOB, APOAI, CD36, ABCG5, NPC1L1, S | 2.622845423 | 0.001041535 |
| PIK3CG, CCL2, NCF1, RELA, CXCL2, CXCL8, RAF1, NF | 6.198820349 | 0.00151573 |
| KI PIK3CG, AKT1, ADRB2, PTGER3, ADRB1, NPY, PTGS2, IN | 2.599505308 | 0.022614095 |
| KCNMA1, PRKCA, ACTA2, RAF1, PRKG1, PRKCB, MAPK | 4.3170356 | 0.041858195 |
| PIK3CG, HMGCR, PPARG, ACACA, SIRT1, ADIPOQ, LEP, MAPK1, TNF, CDKN2B, TGFB1, TGFB2, IFNG, BMPR2, PIK3 | 2.817645613 | 0.171828968 |
| PIK3CG, PRKCA, ICAM1, CLDN4, NCF1, MMP9, CXCL12 | 2.680199486 | 0.266361594 |
| PIK3CG, PRKCA, IPI, PTGS1, ITGB3, PRKG1, AKT1, MAPK1 | 1.39662255 | 0.277479665 |
| PIK3CG, PRKCA, AKT1, MAPK1, TNF, INS, IKBKB, PTEN IL4, PIK3CG, IL6, PIM1, BCL2L1, STAT1, IL10, AKT1, LEP | 2.675538269 | 0.385686932 |
| PIK3CG, COL3A1, PTGS1, ITGB3, PRKG1, AKT1, MAPK1, PRKCA, PIK3CG, AKT1, MAPK1, TNF, INS, IKBKB, PTEN | 2.535881052 | 0.410050764 |
| IL4, PIK3CG, IL6, PIM1, BCL2L1, STAT1, IL10, AKT1, LEP | 3.410322794 | 0.650157826 |
| PIK3CG, COL3A1, PTGS1, ITGB3, PRKG1, AKT1, MAPK1, PRKCA, PIK3CG, AKT1, MAPK1, TNF, INS, IKBKB, PTEN | 2.121978627 | 0.968175299 |
| GSTM1, GSTM2, ODC1, GCLC, GGT1, GCLM, GSTP1 | 3.016538245 | 0.998611344 |
| Cluster        | Term                                                                 | Biological Processes               | Count | %       | Pvalue   |
|---------------|----------------------------------------------------------------------|-------------------------------------|-------|---------|----------|
| GO:0045429    | positive regulation of nitric oxide biosynthesis                      | 14                                 | 21.53846 | 3.25E-22 |
| GO:0043066    | negative regulation of apoptotic process                             | 21                                 | 32.30769 | 1.86E-16 |
| GO:0001525    | angiogenesis                                                         | 15                                 | 23.07692 | 9.39E-14 |
| GO:0006954    | inflammatory response                                                | 17                                 | 26.15385 | 6.03E-13 |
| GO:0008284    | positive regulation of cell proliferation                            | 18                                 | 27.69231 | 1.07E-12 |
| GO:0007568    | aging                                                                | 13                                 | 20      | 1.14E-12 |
| GO:0048661    | positive regulation of smooth muscle cell                            | 10                                 | 15.38462 | 4.39E-11 |
| GO:0001666    | response to hypoxia                                                  | 12                                 | 18.46154 | 5.29E-11 |
| GO:0070374    | positive regulation of ERK1 and ERK2                                 | 12                                 | 18.46154 | 4.78E-10 |
| GO:0006955    | immune response                                                      | 15                                 | 20.15385 | 2.76E-12 |
| GO:0008217    | regulation of blood pressure                                         | 8                                  | 12.30769 | 4.88E-09 |
| GO:0002576    | platelet degranulation                                               | 9                                  | 13.84615 | 5.07E-09 |
| GO:0022617    | extracellular matrix disassembly                                     | 8                                  | 12.30769 | 1.48E-08 |
| GO:0042060    | wound healing                                                        | 8                                  | 12.30769 | 2.13E-08 |
| GO:0035924    | cellular response to vascular endothelia                             | 6                                  | 13.84615 | 3.82E-08 |
| GO:0051092    | positive regulation of NF-kappaB trans                               | 10                                 | 15.38462 | 5.30E-08 |
| GO:0030198    | extracellular matrix organization                                    | 10                                 | 15.38462 | 7.59E-08 |
| GO:0071456    | cellular response to hypoxia                                          | 8                                  | 12.30769 | 7.62E-08 |
| GO:0071347    | cellular response to interleukin-1                                   | 7                                  | 10.76923 | 2.85E-07 |
| GO:0050729    | positive regulation of inflammatory response                         | 7                                  | 10.76923 | 3.36E-07 |
| GO:0090026    | positive regulation of monocyte chemotaxis                           | 5                                  | 7.692308  | 3.37E-07 |
| GO:0006935    | chemotaxis                                                           | 8                                  | 12.30769 | 3.96E-07 |
| GO:0046427    | positive regulation of JAK-STAT cascade                              | 5                                  | 7.692308  | 1.33E-06 |
| GO:0048146    | positive regulation of fibroblast proliferation                      | 6                                  | 9.230769  | 1.88E-06 |
| GO:0034612    | response to tumor necrosis factor                                    | 5                                  | 7.692308  | 2.29E-06 |
| GO:0032757    | positive regulation of interleukin-8 protein                          | 5                                  | 7.692308  | 2.69E-06 |
| GO:2000352    | negative regulation of endothelial cell ;                            | 5                                  | 7.692308  | 3.67E-06 |
| GO:0045909    | positive regulation of vasodilation                                  | 5                                  | 7.692308  | 4.24E-06 |
| GO:0045766    | positive regulation of angiogenesis                                  | 7                                  | 10.76923 | 4.91E-06 |
| GO:0030593    | neutrophil chemotaxis                                                | 6                                  | 9.230769  | 5.13E-06 |
| GO:0001938    | positive regulation of endothelial cell ;                            | 6                                  | 9.230769  | 6.39E-06 |
| GO:0050901    | leukocyte tethering or rolling                                       | 4                                  | 6.153846  | 1.47E-05 |
| GO:0043536    | positive regulation of blood vessel end                               | 4                                  | 6.153846  | 4.90E-05 |
| GO:0006979    | response to oxidative stress                                         | 6                                  | 9.230769  | 6.17E-05 |
| GO:0042346    | positive regulation of NF-kappaB imp                                 | 4                                  | 6.153846  | 6.69E-05 |
| GO:0007159    | leukocyte cell-cell adhesion                                         | 4                                  | 6.153846  | 1.14E-04 |
| GO:0042632    | cholesterol homeostasis                                              | 15                                 | 55.55556  | 2.76E-28 |
| GO:0042157    | lipoprotein metabolic process                                         | 12                                 | 44.44444  | 1.21E-23 |
| GO:0043691    | reverse cholesterol transport                                         | 10                                 | 37.03704  | 5.16E-22 |
| GO:0034375    | high-density lipoprotein particle remod                              | 9                                  | 33.33333  | 6.37E-20 |
| GO:0033344    | cholesterol efflux                                                   | 9                                  | 33.33333  | 1.06E-17 |
| GO:0070328    | triglyceride homeostasis                                              | 8                                  | 29.62963  | 5.68E-15 |
| GO:0008203    | cholesterol metabolic process                                         | 9                                  | 33.33333  | 6.97E-14 |
| GO:0033700    | phospholipid efflux                                                  | 6                                  | 22.22222  | 1.17E-11 |
| GO:0034372    | very-low-density lipoprotein particle r                               | 5                                  | 18.51852  | 1.58E-10 |
| GO:0019433    | triglyceride catabolic process                                        | 6                                  | 22.22222  | 3.08E-10 |
| GO ID                  | Description                                                                 | Count | Log2 Fold Change | p Value          |
|-----------------------|-----------------------------------------------------------------------------|-------|------------------|------------------|
| GO:0034374            | low-density lipoprotein particle remodeling                                | 5     | 18.51852         | 1.48E-09         |
| GO:0006869            | lipid transport                                                             | 7     | 25.92593         | 1.51E-09         |
| GO:0006641            | triglyceride metabolic process                                              | 6     | 22.22222         | 1.86E-09         |
| GO:0001523            | retinoid metabolic process                                                  | 6     | 22.22222         | 3.32E-08         |
| GO:0034384            | high-density lipoprotein particle clearance                                 | 4     | 14.81481         | 6.57E-08         |
| GO:0042158            | lipoprotein biosynthetic process                                            | 4     | 14.81481         | 2.75E-07         |
| GO:0030301            | cholesterol transport                                                       | 4     | 14.81481         | 1.82E-06         |
| GO:0006629            | lipid metabolic process                                                     | 6     | 22.22222         | 3.76E-06         |
| GO:0034370            | triglyceride-rich lipoprotein particle remodeling                           | 3     | 11.11111         | 6.91E-06         |
| GO:0030300            | regulation of intestinal cholesterol absorption                              | 3     | 11.11111         | 2.30E-05         |
| GO:0034382            | chylomicron remnant clearance                                               | 3     | 11.11111         | 3.44E-05         |
| GO:0010886            | positive regulation of cholesterol storage                                  | 3     | 11.11111         | 4.82E-05         |
| GO:0034380            | high-density lipoprotein particle assembly                                  | 3     | 11.11111         | 6.42E-05         |
| GO:0048261            | negative regulation of receptor-mediated                                   | 3     | 11.11111         | 6.42E-05         |
| GO:0010898            | positive regulation of triglyceride catalysis                               | 3     | 11.11111         | 6.42E-05         |
| GO:0006644            | phospholipid metabolic process                                              | 4     | 14.81481         | 7.76E-05         |
| GO:0055091            | phospholipid homeostasis                                                    | 3     | 11.11111         | 8.24E-05         |
| GO:0010873            | positive regulation of cholesterol ester                                    | 3     | 11.11111         | 8.24E-05         |
| GO:0042493            | response to drug                                                             | 6     | 22.22222         | 9.06E-05         |
| GO:0046470            | phosphatidylcholine metabolic process                                       | 3     | 11.11111         | 1.03E-04         |
| GO:0051006            | positive regulation of lipoprotein lipase                                    | 3     | 11.11111         | 1.03E-04         |
| GO:0045723            | positive regulation of fatty acid biosynthesis                              | 3     | 11.11111         | 1.03E-04         |
| GO:0010744            | positive regulation of macrophage derivation                                | 3     | 11.11111         | 2.73E-04         |
| GO:0006656            | phosphatidylcholine biosynthetic process                                     | 3     | 11.11111         | 6.77E-04         |
| GO:0015914            | phospholipid transport                                                       | 3     | 11.11111         | 6.77E-04         |
| GO:0006954            | inflammatory response                                                       | 5     | 18.51852         | 0.002577         |
| GO:0071420            | cellular response to histamine                                              | 5     | 13.88889         | 1.10E-09         |
| GO:0007596            | blood coagulation                                                           | 7     | 19.44444         | 1.99E-06         |
| GO:0002576            | platelet degranulation                                                       | 5     | 13.88889         | 6.04E-05         |
| GO:0051932            | synaptic transmission, GABAergic                                            | 3     | 8.333333         | 8.81E-05         |
| GO:0001666            | response to hypoxia                                                          | 5     | 13.88889         | 4.34E-04         |
| GO:0042060            | wound healing                                                                | 4     | 11.11111         | 6.11E-04         |
| GO:0060384            | innervation                                                                  | 3     | 8.333333         | 6.32E-04         |
| GO:0030168            | platelet activation                                                          | 4     | 11.11111         | 0.001746         |
| GO:0007179            | transforming growth factor beta receptor                                     | 3     | 8.333333         | 0.015711         |
| GO:0007186            | G-protein coupled receptor signaling p                                       | 9     | 36              | 2.23E-05         |
| GO:0010887            | negative regulation of cholesterol stora                                    | 3     | 12              | 2.93E-05         |
| GO:0042632            | cholesterol homeostasis                                                      | 4     | 16              | 1.01E-04         |
| GO:0008203            | cholesterol metabolic process                                               | 4     | 16              | 1.21E-04         |
| GO:0010745            | negative regulation of macrophage der                                        | 3     | 12              | 1.51E-04         |
| GO:0010875            | positive regulation of cholesterol efflux                                    | 3     | 12              | 1.76E-04         |
| GO:0006367            | transcription initiation from RNA poly                                      | 4     | 16              | 0.00128          |
| GO:0042157            | lipoprotein metabolic process                                               | 3     | 12              | 0.001334         |
| GO:0030522            | intracellular receptor signaling pathway                                      | 3     | 12              | 0.001334         |
| GO:0043401            | steroid hormone mediated signaling pathwa                                    | 3     | 12              | 0.002978         |
| GO:0070098            | chemokine-mediated signaling pathwa                                          | 3     | 12              | 0.004581         |
| GO ID | Description                                                                 | Count | P Value            |
|-------|------------------------------------------------------------------------------|-------|--------------------|
| GO:0007584 | response to nutrient                                                        | 3     | 0.004966           |
| GO:0050728 | negative regulation of inflammatory response                                | 3     | 0.00564            |
| GO:0002576 | platelet degranulation                                                       | 3     | 0.009418           |
| GO:0071222 | cellular response to lipopolysaccharide                                      | 3     | 0.011247           |
| GO:0045944 | positive regulation of transcription from 6 to 24                           | 6     | 0.011296           |
| GO:0055091 | phospholipid homeostasis                                                     | 2     | 0.012793           |
| GO:2000188 | regulation of cholesterol homeostasis                                       | 2     | 0.012793           |
| GO:0010867 | positive regulation of triglyceride biosynthesis                            | 2     | 0.015615           |
| GO:0006954 | inflammatory response                                                       | 4     | 0.016245           |
| GO:0030595 | leukocyte chemotaxis                                                         | 2     | 0.017022           |
| GO:0006629 | lipid metabolic process                                                      | 3     | 0.020952           |
| GO:0032496 | response to lipopolysaccharide                                               | 3     | 0.02273            |
| GO:0002690 | positive regulation of leukocyte chemotaxis                                  | 2     | 0.025429           |
| GO:007596  | blood coagulation                                                           | 3     | 0.028141           |
| GO:0001666 | response to hypoxia                                                          | 6     | 2.142857 7.15E-06 |
| GO:0051918 | negative regulation of fibrinolysis                                          | 3     | 10.71429 1.11E-04  |
| GO:0042632 | cholesterol homeostasis                                                      | 4     | 14.28571 1.45E-04  |
| GO:0008203 | cholesterol metabolic process                                                | 4     | 14.28571 1.73E-04  |
| GO:0042060 | wound healing                                                                | 4     | 14.28571 2.80E-04  |
| GO:0030301 | cholesterol transport                                                        | 3     | 10.71429 2.95E-04  |
| GO:0002227 | innate immune response in mucosa                                             | 3     | 10.71429 7.30E-04  |
| GO:0044267 | cellular protein metabolic process                                           | 4     | 14.28571 8.75E-04  |
| GO:0050900 | leukocyte migration                                                          | 4     | 14.28571 9.64E-04  |
| GO:0001937 | negative regulation of endothelial cell                                     | 3     | 10.71429 9.84E-04  |
| GO:0006641 | triglyceride metabolic process                                               | 3     | 10.71429 0.001434  |
| GO:0006953 | acute-phase response                                                        | 3     | 10.71429 0.001778  |
| GO:0044240 | multicellular organism lipid catabolic pathway                               | 2     | 7.142857 0.003213  |
| GO:0006898 | receptor-mediated endocytosis                                                | 4     | 14.28571 0.003216  |
| GO:0030512 | negative regulation of transforming growth factor                           | 3     | 10.71429 0.00472  |
| GO:0015918 | sterol transport                                                             | 2     | 7.142857 0.008015  |
| GO:0034197 | triglyceride transport                                                       | 2     | 7.142857 0.008015  |
| GO:0007179 | transforming growth factor beta receptor                                     | 3     | 10.71429 0.009534  |
| GO:0010886 | positive regulation of cholesterol storage                                  | 2     | 7.142857 0.011203  |
| GO:0051001 | negative regulation of nitric-oxide synth                                    | 2     | 7.142857 0.012794  |
| GO:0010544 | negative regulation of platelet activator                                   | 2     | 7.142857 0.012794  |
| GO:0035235 | ionotropic glutamate receptor signaling                                      | 4     | 80 1.03E-08        |
| GO:0070588 | calcium ion transmembrane transport                                         | 2     | 40 0.028049        |
| GO:0007268 | chemical synaptic transmission                                               | 2     | 40 0.055961        |
| GO:0000165 | MAPK cascade                                                                 | 2     | 40 0.06097         |
| GO:0070588 | calcium ion transmembrane transport                                         | 3     | 60 2.96E-04        |
| GO:0043065 | positive regulation of apoptotic process                                     | 3     | 60 0.001864        |
| Genes                                | Fold Enrichment | Bonferroni |
|--------------------------------------|-----------------|------------|
| AKT1, EGFR, ICAM1, IL6, TNF, PTGS2, INS, AGT, EDN1, IL1 | 84.11091678     | 5.98E-19   |
| IL4, EGFR, IL6, MMP9, TP53, SMAD3, BCL2L1, PDE4D, IL1 | 11.92331361     | 4.09E-13   |
| CCL2, PTGS2, CXL8, PDE4D, MMP2, KDR, LEP, HMOX1, IL1 | 17.37702656     | 1.73E-10   |
| IL6, TNF, CCL2, PTGS2, CRP, CXL8, TLR4, CCL5, CXCL5 | 11.58774102     | 1.11E-09   |
| EGFR, IL6, EDN1, BCL2L1, PDE4D, KDR, CXL8, TLR4, IL1 | 9.978738858     | 1.97E-09   |
| AKT1, EGFR, IL6, TNF, PTGS2, JUN, HMOX1, EDN1, CCL5 | 20.35393939     | 2.10E-09   |
| LEF, VEGFA, SMAD3, CCL2, BCL2L1, PTEN, IL1 | 43.05641026     | 2.21E-09   |
| IL6, TNF, CCL2, PTGS2, CRP, CXL8, TLR4, CCL5, CXCL5 | 17.71463736     | 9.74E-08   |
| EGFR, ICAM1, IL6, TNF, CCL2, JUN, VEGFA, TLR4, CCL5 | 9.20445825      | 8.80E-07   |
| CCL2, PTGS2, CXCL8, SMAD3, TLR4, CCL5, CXCL5 | 31.79550296     | 9.89E-06   |
| IL4, IL6, TNF, CCL2, EDN1, CXCL8, TLR4, CCL5, CXCL5 | 22.57326363     | 9.33E-06   |
| ICAM1, IL6, TNF, INS, AGT, IL1B, TLR4, CAT, TGFBI | 58.71328671     | 0.002451057|
| VCAM1, ICAM1, VWF, TNF, PECAM1, ELN, SERPINE1, IL1 | 27.19352227     | 2.73E-05   |
| EGFR, CCND1, HMOX1, MYC, TGFBI, IL10 | 53.44936387     | 1.40E-04   |
| AKT1, ICAM1, PTGS2, HMOX1, EDN1, VEGFA, TP53, PTE | 21.52803513     | 1.40E-04   |
| ICAM1, IL6, CCL2, EDN1, CXCL8, CCL5, MYC | 25.46998917     | 5.24E-04   |
| EGFR, CCL2, AGT, SERPINE1, TLR4, CCL5, IL2 | 13.18053575     | 9.75E-05   |
| CCL2, SERPINE1, CCL5, CXCL12, CXCL10, IL2 | 24.77218124     | 6.19E-04   |
| IL4, MAPK1, CCL2, MAPK14, CXCL8, CCL5, CXCL12, C5X | 80.73076923     | 6.21E-04   |
| LEP, NOTCH1, IL6, CCL5, IL10 | 58.71328671     | 0.002451057|
| EGFR, AGT, JUN, ESR1, MYC, TGFBI | 28.70427350     | 0.003452291|
| CASP3, PTGS2, CASP8, ADIPOQ, SELE | 51.66769231     | 0.004198768|
| TNF, SERPINE1, IL1B, TLR4, ADIPOQ | 49.68047337     | 0.004961818|
| IL4, ICAM1, SERPINE1, IL10, KDR | 46.13186813     | 0.006794686|
| EGFR, INS, AGT, HMOX1, NOS3 | 44.51114063     | 0.007779792|
| HMOX1, VEGFA, SERPINE1, CXL8, IL1B, NOS3, KDR | 15.72494983     | 0.00899528 |
| CCL2, EDN1, IFNG, CXL8, IL1B, CCL5 | 23.48531469     | 0.009390795|
| AKT1, CCL2, JUN, VEGFA, CXCL12, KDR | 22.4621405     | 0.01169268 |
| LEP, VCAM1, TNF, SELE | 79.8857745     | 0.026693173|
| AKT1, MAPK14, VEGFA, TGFBI | 54.38704435     | 0.086236853|
| AKT1, EGFR, PTGS2, APOE, VEGFA, HMOX1, MPO | 14.09118881    | 0.107410642|
| TNF, PTGS2, IL1B, TLR4 | 49.20732601     | 0.115834231|
| VCAM1, ICAM1, CCL5, SELE | 41.33415385     | 0.189900508|

LPL, APOC2, ABCG1, ABCG8, APOB, APOA2, APOA1, LC | 145.7638889     | 1.20E-25   |
LPL, APOA2, APOB, APOA1, LCAT, APOC3, APOA5, APO | 196.3976608     | 5.27E-21   |
APO2, APOA1, LCAT, APOC3, APOC5, SCARB1 | 345.514033      | 2.24E-19   |
APO2, APOA1, LCAT, APOC3, SCARB1, CETP, LIPC, PL | 373.1555555     | 2.77E-17   |
ABCG8, APO2, APOB, APOA1, APOC3, APOA5, APOC2, LPL, APOA1, APOC3, APOA5, APOC2, SCARB1 | 223.8933333     | 4.60E-15   |
LPL, APOA1, APOC3, APOC5, SCARB1, CETP, LIPC, PL | 373.1555555     | 2.77E-17   |
SREBF1, APO2, APOB, APOA1, LCAT, PCSK9, CETP, LIPC, PL | 82.31372549     | 3.03E-11   |
APO2, APOA1, APOC3, APOC5, APOC2, ABCG1 | 266.536825      | 5.09E-09   |
LPL, LCAT, APOC2, CETP, LIPC | 444.2320842     | 6.84E-08   |
LPL, APOB, APOA1, APOC3, APOC5, LIPC | 149.2622222     | 1.34E-07   |
| Protein Combinations | p-value | Adjusted p-value |
|----------------------|---------|------------------|
| APOA2, APOB, CETP, LIPC, ABCG1 | 282.6936027 | 6.42E-07 |
| APOA2, APOA1, APOC3, APOA5, APOC2, CETP, PLTP | 57.28265107 | 6.54E-07 |
| LPL, APOA2, APOC3, APOA5, PCSK9, CETP | 106.615873 | 8.08E-07 |
| LPL, APOA2, APOB, APOA1, APOC3, APOC2 | 61.17304189 | 1.44E-05 |
| APOA2, APOA1, APOC2, SCARB1 | 414.617284 | 2.85E-05 |
| APOA2, APOB, APOA1, LCAT | 276.4115226 | 1.19E-04 |
| APOB, APOA1, LCAT, CETP | 155.4814815 | 7.90E-04 |
| SREBF1, LPL, LCAT, APOC2, LIPC, PLTP | 466.4444444 | 0.005973788 |
| APOA2, APOA5, APOC2 | 621.9259259 | 0.002994207 |
| APOA2, APOA1, APOA5 | 373.1555556 | 0.0099271 |
| APOC3, APOC2, LIPC | 310.962963 | 0.014839672 |
| LPL, APOB, SCARB1 | 266.5396825 | 0.020694309 |
| APOA2, APOA1, APOA5 | 233.2222222 | 0.02747119 |
| APOA2, APOA1, APOA5 | 207.308642 | 0.035147972 |
| GABRG2, GABRA1, GABRB2 | 291.5277778 | 4.74E-07 |
| F5, HNF4A, FGB, F13A1, F7, SERPIND1, ITGB3 | 17.74516908 | 8.57E-04 |
| F5, FGB, F13A1, ITGB3, TGFB2 | 22.6429342 | 0.025694099 |
| GABRG2, GABRA1, GABRB2 | 199.9047619 | 0.03724106 |
| MTHFR, CST3, F7, HSPD1, TGFB2 | 13.59943152 | 0.170769717 |
| TGFB1, COL3A1, ITGB3, TGFB2 | 23.32222222 | 0.23443018 |
| GABRB3, GABRB2, GABRA5 | 77.74074074 | 0.238624443 |
| F5, FGB, COL3A1, ITGB3 | 16.22415459 | 0.529074577 |
| TGFB1, COL3A1, TGFB2 | 15.21014493 | 0.9989138 |

P-values and adjusted p-values for the association of different protein combinations with a specific condition or phenotype are provided. The table lists the combinations along with their respective p-values and adjusted p-values. The adjusted p-values are used to correct for multiple comparisons, reducing the likelihood of false positives.
| Genes                  | Score 1 | Score 2 |
|-----------------------|---------|---------|
| HMGCR, SERPINC1, ABCA1| 27.230  | 0.839923513  |
| PPARA, CNR2, NR1H3    | 25.506  | 0.875256643  |
| FGA, PPBP, PF4        | 19.563  | 0.969262223  |
| CX3CR1, ABCA1, NR1H3  | 17.832  | 0.984431171  |
| PGR, PPARA, AR, CDKN2A, PF4, NR1H3 | 4.108134557 | 0.984710568 |
| ABCA1, ANGPTL3        | 149.262 | 0.991245596  |
| LDLR, NR1H3           | 149.262 | 0.991245596  |
| LDLR, NR1H3           | 122.123 | 0.99694653   |
| OLR1, PPBP, CNR2, PF4 | 7.089   | 0.97587499   |
| CNR2, PF4             | 111.946 | 0.998196747  |
| PPARA, LPA, LDLR      | 12.834  | 0.999587097  |
| PPBP, CNR2, PF4       | 12.286  | 0.999788506  |
| PPBP, PF4             | 74.631  | 0.999923555  |
| P2RY12, FGA, SERPINC1 | 10.951  | 0.99972586   |

| Genes                  | Score 1 | Score 2 |
|-----------------------|---------|---------|
| PLAT, CAV1, SMAD3, NOS2, AGER, ENG | 20.92026578 | 0.003741898  |
| THBD, F2, APOH         | 179.914 | 0.056583474 |
| APOA4, CAV1, ABCG5, MTTP | 37.48214286 | 0.073015356 |
| APOA4, APP, VLDLR, SREBF2 | 35.27731092 | 0.08689049 |
| SMAD3, LOX, DCN, ENG   | 29.98571429 | 0.136689763 |
| CAV1, MSR1, NPC1L1     | 112.446286 | 0.14308667 |
| APOA4, PLA2G1B, NOS2   | 71.96751429 | 0.31792036 |
| APOA4, APP, SAA1, F2   | 20.32929782 | 0.367839106 |
| CAV1, THBD, SELL, F2   | 19.66276347 | 0.396575386 |
| CAV1, APOH, ENG        | 62.03940887 | 0.40358343 |
| CAV1, APOH, MTTP       | 51.40408163 | 0.52843815 |
| SAA1, F2, HP           | 46.13186813 | 0.606523969 |
| APOA4, PLA2G1B         | 599.7142857 | 0.814832523 |
| MSR1, SAA1, HP, VLDLR  | 12.89708141 | 0.815112675 |
| CAV1, SMAD3, ENG       | 28.11160714 | 0.916199011 |
| ABCG5, NPC1L1          | 239.8857143 | 0.985251494 |
| APOH, MTTP             | 239.8857143 | 0.985251494 |
| SMAD3, ENG, CDH5       | 19.55590062 | 0.993394306 |
| MSR1, SREBF2           | 171.3469388 | 0.997270429 |
| CAV1, ENG              | 149.9285714 | 0.99882582 |
| THBD, F2               | 149.9285714 | 0.99882582 |

| Genes                  | Score 1 | Score 2 |
|-----------------------|---------|---------|
| GRIN2C, GRIN2D, GRIN3B, GRIN3A | 559.7333333 | 3.48549E-07 |
| GRIN3B, GRIN3A         | 56.44369748 | 0.619895962 |
| GRIN2C, GRIN2D         | 27.98666667 | 0.858857251 |
| GRIN2C, GRIN2D         | 25.63664122 | 0.882213757 |

| Genes                  | Score 1 | Score 2 |
|-----------------------|---------|---------|
| PSEN1, GRIN1, GRIN2A  | 84.66554622 | 0.042307922 |
| PSEN1, GRIN1, GRIN2A  | 33.584  | 0.238442902 |
| Term   | Pathway                                      | Count | %     | Pvalue   |
|--------|----------------------------------------------|-------|-------|----------|
| hsa03320 | PPAR signaling pathway                       | 23    | 0.035099 | 6.82E-13 |
| hsa00670 | One carbon pool by folate                    | 12    | 0.018313 | 5.99E-10 |
| hsa04975 | Fat digestion and absorption                 | 15    | 0.022891 | 3.07E-09 |
| hsa04610 | Complement and coagulation cascades          | 16    | 0.024417 | 1.45E-06 |
| hsa01100 | Metabolic pathways                           | 95    | 0.144974 | 1.08E-05 |
| hsa04024 | cAMP signaling pathway                       | 26    | 0.039677 | 2.29E-05 |
| hsa04668 | TNF signaling pathway                        | 17    | 0.025943 | 9.54E-05 |
| hsa04920 | Adipocytokine signaling pathway              | 13    | 0.019839 | 1.93E-04 |
| hsa04066 | HIF-1 signaling pathway                      | 15    | 0.022891 | 3.39E-04 |
| hsa04064 | NF-kappa B signaling pathway                 | 13    | 0.019839 | 0.001468 |
| hsa04611 | Platelet activation                          | 16    | 0.024417 | 0.002507 |
| hsa04146 | Peroxisome                                   | 12    | 0.018313 | 0.003157 |
| hsa04350 | TGF-beta signaling pathway                   | 11    | 0.016786 | 0.010197 |
| hsa04060 | Cytokine-cytokine receptor interaction       | 22    | 0.033573 | 0.012527 |
| hsa04270 | Vascular smooth muscle contraction           | 13    | 0.019839 | 0.016115 |
| hsa04923 | Regulation of lipolysis in adipocytes        | 8     | 0.012208 | 0.023139 |
| hsa04370 | VEGF signaling pathway                       | 8     | 0.012208 | 0.03509  |
| hsa04620 | Toll-like receptor signaling pathway         | 11    | 0.016786 | 0.04404  |
| hsa04931 | Insulin resistance                           | 11    | 0.016786 | 0.048998 |
| hsa04068 | FoxO signaling pathway                       | 12    | 0.018313 | 0.081132 |
| Genes                                      | Fold Enrichment | Bonferroni |
|--------------------------------------------|-----------------|------------|
| LPL, ACOX1, PPARA, PPARD, ORL1, RXRB, RXRA, PPAR | 6.689653714     | 1.71E-10   |
| MTHFD1, MTHFD2, TYMS, SHMT1, SHMT2, MTHFR, ALI | 11.69235127     | 1.50E-07   |
| ABCA1, MTTP, ABCG8, APOA4, APOB, APOA1, ABCG5, C | 7.495096971     | 7.70E-07   |
| KNG1, PLAT, F13A1, F7, PLG, C8G, VWF, THBD, F5, FGA, NAMPT, ACOX1, ALAD, PTGS2, HMGCR, AMT, PTGS1, L PPARA, ACOX1, GRIN3B, NFKB1, GRIN3A, AKT1, GRIN2 ICAM1, IL6, TNF, CCL2, PTGS2, MMP9, EDN1, NFKB1, MI PPARA, TNF, RXRB, RXRA, RXRG, NFKB1, ADIPOQ, AK’ IL6, EDN1, NFKB1, TLR4, TIMP1, AKT1, MAPK1, INS, HM VCAM1, ICAM1, TNF, PTGS2, CD40LG, LY96, IL1B, NFKI COL3A1, PTGS1, ITGB3, PPP1CC, PRKG1, P2RY12, AKT1, HAO1, XDH, EC12, ACOX1, DHRS4, EPHX2, CAT, NOS2, MAPK1, TNF, CDKN2B, TGFBR1, TGFBR2, IFNG, BMPR2, IL6, TNF, CCL2, TNFSF4, TGFBR1, TGFBR2, BMPR2, PF4, AGTR1, MAPK1, PTGIR, ACTA2, PLA2G2A, PLA2G1B, PR AKT1, ADRB2, NPY, PTGS2, INS, PTGS1, PRKACA, PRKG AKT1, MAPK1, PTGS2, VEGFA, PPP3R1, NOS3, PPP3CA, AKT1, MAPK1, IL6, TNF, LY96, IL1B, NFKB1, TLR4, CCL1, AKT1, SREBF1, PPARA, IL6, TNF, CD36, INS, NFKB1, NO’ AKT1, MAPK1, IL6, CDKN2B, INS, TGFBR1, TGFBR2, SM | 4.518783101     | 3.64E-04 |
| 1.518694792 | 0.002703389 |
| 2.558932097 | 0.005741483 |
| 3.096105478 | 0.023666114 |
| 3.619061109 | 0.047241037 |
| 3.044831444 | 0.08156863 |
| 2.911888249 | 0.308468462 |
| 3.298431031 | 0.467453977 |
| 2.817434042 | 0.547859408 |
| 2.551902064 | 0.923661933 |
| 1.76427797  | 0.957747264 |
| 2.165250236 | 0.983057549 |
| 2.783893161 | 0.997194458 |
| 2.555705197 | 0.999872294 |
| 2.022262013 | 0.999987688 |
| 1.984812716 | 0.999996662 |
| 1.745127056 | 0.999999999 |