

Identification of Key Candidate Biomarkers and Pathways in Atherosclerosis

Zhijun Meng

Thomas Jefferson University

Jia Gao

Shanxi Medical University

Hongping Liang

Shanxi Medical University

Caihong Liu

Shanxi Medical Univeristy

Jianli Zhao

Thomas Jefferson University

Zixuan Wang

Thomas Jefferson Unviersity

Xinliang Ma

Thomas Jefferson Unviersity

Xiangying Jiao

Shanxi Medical Univiversity

Jimin Cao

Shanxi Medical University

Yajing Wang (✉ yajing.wang@jefferson.edu)

Thomas Jefferson University <https://orcid.org/0000-0002-6358-6948>

Research

Keywords: Atherosclerosis, Bioinformatics analysis, Macrovascular disease, Gene expression profile, DEGs

Posted Date: September 18th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-72327/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Identification of key candidate biomarkers and pathways in atherosclerosis

Zhijun Meng^{1,2,3}; Jia Gao¹; Hongping Liang²; Caihong Liu¹; Jianli Zhao⁴; Zi-Xuan Wang⁴; Xinliang Ma³; Xiangying Jiao¹; Jimin Cao¹; Yajing Wang³

1. Department of Physiology, Key Laboratory of Cellular Physiology, Shanxi Medical University, Taiyuan, Shanxi, China
2. Clinical Laboratory, Shanxi Provincial People's Hospital of Shanxi Medical University, Taiyuan, Shanxi, China
3. Department of Emergency Medicine, Thomas Jefferson University, Philadelphia, PA
4. Department of Pathology, Anatomy & Cell biology, Thomas Jefferson University, Philadelphia, PA

Running title: Key candidate biomarkers in atherosclerosis

***Corresponding Author:**

Yajing Wang, MD, PhD
Department of Emergency Medicine
Thomas Jefferson University
Philadelphia, PA 19107
Tel: (215) 955-8895
Fax: (215) 503-4458
E-mail: yajing.wang@jefferson.edu

Jimin Cao, MD, PhD
Department of Physiology
Shanxi Medical University
Taiyuan, Shanxi 030001
Tel: +86 0351 4135423
E-mail: caojimin@126.com

Key words: Atherosclerosis; Biomarker; GSE profile; Genetic signals

Abstract

Background

Atherosclerosis is the leading cause of cardiovascular disease worldwide for which lacks effective prevention and therapeutic strategy. Therefore, clinical indicators for early diagnosis and screening are in great need. The present study aimed to elucidate the key genetic signatures and pathways identifying the key candidate biomarker in atherosclerosis by integrative bioinformatics analysis combining with experimental assay.

Methods

The gene expression profiles (GSE30169, GSE6584) were achieved from the Gene Expression Omnibus database. Functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed to examine the biological functions of identified differentially expressed genes (DEGs). A protein-protein interaction (PPI) network was mapped using Cytoscape software.

Results

91 DEGs were identified, including 68 up-regulated genes and 23 down-regulated genes. Functional enrichment analysis indicated that DEGs genes were significantly enriched in ferroptosis, TNF signaling pathway, IL-17 signaling pathway. 12 nodes with the highest degrees were selected as hub genes. CCL2, CXCL1, IL6, and DUSP1 can serve as a sensitive diagnostic indicator for the early stage of atherosclerosis; CEBPB and HMOX1 can serve as a diagnostic indicator for diabetic atherosclerosis; TRIB3 is a sensitive marker indicating atherosclerosis risks in diabetic women group.

Conclusions

In conclusion, we have identified key candidate genes that indicate the diagnosis of patients with atherosclerosis, and these genes may serve as potential therapeutic or drug development targets for atherosclerosis.

Key Words: Atherosclerosis; Bioinformatics analysis; Macrovascular disease; Gene expression profile; DEGs

Introduction

Cardiovascular diseases (CVD) are considered as the leading causes of morbidity and mortality in the world [1]. The exact cause of atherosclerosis isn't known. Certain traits, genetic and environmental factors raise the risks for developing atherosclerosis. Significant risk factors for atherosclerosis included diet, smoking, exercise, and infectious factors, and the candidate gene studies had shown that some of these factors interacted with genetic factors[2, 3].

As the primary pathological basis of CVD, atherosclerosis is a progressive inflammatory disease of the arteries caused by endothelial cell injury. Over the past three decades, it has become clear that oxidized low-density lipoproteins (oxLDLs) or oxidized lipids trapped in the vessel wall induce the overlying endothelial cells (ECs) to express adhesion molecules and cytokines that promote the recruitment of monocytes and lymphocytes to the vessel wall and brought inflammatory disorder [4, 5]. However, the mechanism by which oxidized lipids trigger a wide range of responses was still poorly understood. In addition, endothelial cell is the front line to defend all the stress in the vessel, and it is the basis for studying the progress of atherosclerosis. Therefore, in order to explore atherosclerosis, endothelial cell is crucial for seeking the early sensitive diagnostic marker and prevention strategies.

The development of gene microarray technology (identification of the changes in mRNA expression profiles), has been widely applied in the past decades [6]. It provided a unique opportunity to assess atherosclerosis's occurrence and development with lower expenses, more straightforward operation, high-throughput volume [7]. In recent years, numerous studies on the gene expression profile of atherosclerosis had revealed hundreds of differentially expressed genes (DEGs), which provided a large amount of practical information for gene regulatory network analysis [7, 8]. However, comparative DEGs analyses reported by independent research appear to have little substantial overlap, and the results are limited or inconsistent due to tissue or sample heterogeneity. Thus, there are no reliable biomarkers to distinguish atherosclerotic from non-atherosclerotic samples. The integrated bioinformatics approaches combining with expression profiling analysis will be innovative and may solve the disadvantages.

In this study, we analyzed in-depth on two microarray datasets, GSE30169 (Romanoski et al., 2011) and GSE6584 (Gong et al., 2007) from the Gene Expression Omnibus database (GEO), in which the

samples were collected from oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphatidylcholine (Ox-PAPC, a component in atherosclerotic lesion) treated human aortic endothelial cell (HAECs) and human microvascular endothelial cells (HMECs) [5]. Subsequently, extracted DEGs and integration of DEGs Protein-protein interaction (PPI) network and modular analysis were applied to identify hub genes in atherosclerosis. Identifying sensitive markers, DEGs and enriching their biological functions and key pathways will provide more accurate, practically reliable biomarkers for early diagnosis and individualized prevention and therapy as well as drug targets discovery.

Materials and methods

Data source and screening of DEGs

The GSE30169 and GSE6584 gene expression profiles and its relevant platform annotation files were obtained from GEO database. The GSE30169 dataset was submitted by Professor Romanoski CE and Lusis A on Jun 23th, 2011, last updated on Jan 17th, 2017 and stockpiled on the GPL3921 platform (HT_HG-U133A) Affymetrix HT Human Genome U133A Array (Affymetrix; Thermo Fisher Scientific, Inc., Waltham, MA, USA). This gene expression data consisted of 629 samples from the primary human aortic endothelial cell (HAECs) donated by 96 genetically identical patients, including 322 samples treated with 40ug/ml Ox-PAPC for 4 hours in media 199 containing 1% fetal bovine serum and 307 samples treated without Ox-PAPC in the same media. The GSE6584 dataset was submitted by Professor Gong K on Dec 20th, 2006, last updated on Mar 16th, 2012 and stockpiled on the GPL2700 platform Sentrix HumanRef-8 Expression BeadChip (Illumina Inc., San Diego, CA, USA). This gene expression data included human microvascular endothelial cells (HMECs) from 3 samples control and 9 samples treated by 40ug/ml oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphatidylcholine (Ox-PAPC) for 4 hours and 3 control samples. [9]

Data preprocessing and screening of DEGs

The DEGs were screened by using GEO2R to identify the different expression profiles between the HAECs and HMECs treated by 40ug/ml Ox-PAPC with control samples. In this study, DEGs were defined with cut-off criteria of $p < .05$ and $|\log_2 \text{fold-change} (\text{FC})| > .6$.

Functional annotation and pathway enrichment

Gene ontology analysis (GO) is a standard useful method for annotating genes and gene products to identify natural biological phenomena for high-throughput genomic or transcriptome data[10, 11]. To describe gene product attributes, GO provides three categories of defined terms, including molecular function (MF), cellular component (CC), and biological process (BP) [12]. Furthermore, the Kyoto Encyclopedia of Genes and Genomes (KEGG) is an integrated database resource with a powerful feature in the systematic analysis of gene functions, linking genomic information with higher-order functional information[13]. The above two analysis were obtainable in the Database for Annotation, Visualization and Integrated Discovery (DAVID; <https://david.ncifcrf.gov/>; date of access, 08/05/2020), which is also a bioinformatics data resource composed of an integrated biology knowledge base and analysis tools to extract biological meanings from large quantities of genes and protein collections through a novel agglomeration algorithm[14]. In the present study, GO term and KEGG pathway analysis was performed using the DAVID online tool and $P < .05$ was set as the cut-off criterion. Meanwhile, another available database REACTOME (available online: <http://www.reactome.org>) was carried out as a supplement of KEGG.

PPI network construction and analysis of modules

Search Tool for the Retrieval of Interacting Genes (STRING) database (<http://string-db.org/>) is an online software designed to evaluate protein-protein interaction (PPI) relationships between DEGs, including direct (physical) and indirect (functional) associations [15]. In the present study, STRING was used to map DEGs, and experimentally validated interactions with a combined score $> .4$ were selected as significant. Then, the PPI network was visually analyzed by Cytoscape 3.6.0[16]. To screen the hub genes, a node degree of ≥ 10 was selected as the threshold. Furthermore, the crucial modules' hub genes were further mapped to GO terms and KEGG pathways for functional analysis.

ROC curve analysis

GEO profiles were searched to analyze further the relationship between the hub genes and type 2 diabetes-related diseases. ROC (receiver operating characteristic) curves were constructed by the value of every sample of GDS to compare the diagnostic value of the hub genes in the related diseases. The diagnostic accuracy of the ROC curve was represented by the area under the curve (AUC). AUC values closer to 1 indicated that the diagnostic accuracy was reliable.

Type 2 diabetic atherosclerosis mice model and Real-time quantitative PCR

All experiments of this study were performed in adherence to the NIH Guidelines on the Use of Laboratory Animals and approved by the Thomas Jefferson University and Shanxi Medical University Committee on Animal Care. Apoe knockout mice (male Apoe^{-/-} mice, 8-10 weeks old) were utilized in this study. The mice were either fed the normal diet (ND), or high-fat diet (HFD) (60% kcal fat, 20% kcal protein, 20% kcal carbohydrate, Cat #D12492; Research Diets) for 20 weeks to induce atherosclerosis according to the previous study [17]. Total RNA was extracted from Apoe^{-/-} mice's artery using Trizol reagent (Invitrogen, Carlsbad, CA). Reverse transcription of 1µg total RNA samples was carried out by using RTScript cDNA synthesis kit (Takara) according to manufacturer's instructions. Real-time PCR reactions were prepared using SYBR-Green Master mix (Thermo Fisher Scientific) in a QuantStudio5 Real-Time PCR Detection System (Applied Biosystems). The relative amount of mRNA transcripts was quantified using the ΔCt method. The average Ct obtained in non-treated cells was used as a calibrator and 18s gene was used as the reference for normalization. Sequences of the forward (For) and reverse (Rev) primers were purchased from Integrated DNA Technologies (Table 5).

Statistical analysis

Quantitative results are expressed as mean±SEM. Comparisons between two groups were analyzed by t-test, P values less than 0.05 were considered statistically significant. All statistical analyses were performed via GraphPad Prism 8.

Results

Differentially expressed genes (DEGs) were identified in HAECs/HMECs treated with Ox-PAPC

Gene expression profiles of GSE30169 and GSE6584 were obtained from NCBI-GEO free database. The microarray profile of GSE30169 had 322 patients' tissues with Ox-PAPC treatment and 307 samples without Ox-PAPC treatment. The microarray profile of GSE6584 had 3 sample control and 9 patients issues treated with Ox-PAPC. With $p < 0.05$ and $[\log_2 FC] \geq 0.6$ as cut-off criteria, we extracted 166 and 2604 DEGs from the two expression datasets respectively (Figure 1A, 1B). Using integrated bioinformatical analysis, total 91 consistently expressed genes were identified from the two profile databases, including 68 up-regulated genes and 23 down-regulated genes in the human endothelial cells treated with Ox-PAPC compared to control (Figure 1C, 1D).

DEGs gene ontology enrichment analysis

Candidate DEGs functions and pathways enrichment analysis were assessed via online databases, including DAVID (<http://David.ncifcrf.gov>), Reactome (<http://www.reactome.org>), Panther (<http://www.pantherdb.org>), KEGG PATHWAY (<http://www.genome.jp/kegg>) and Gene Ontology website with $p < 0.05$ as the cut-off criterion. DEGs genes ontology (GO) terms were evaluated with DAVID, Panther and they were classified to three main categories, biological process (BP), cellular component (CC), and molecular function (MF) with showing top 10 significant GO items enriched based upon DEGs genes (Figure 2). As indicated in Figure 2, the up- and down-regulated DEGs were mainly involved in the positive regulation of transcription from RNA polymerase II promoter in the BP category; The altered DEGs were enriched in mitochondrial, and constituted nucleus and cytoplasm in the CC category, while the DEGs mainly enriched in binding, especially protein binding in the MF category (Figure 2). Gene ontology term enrichment analysis for the regulated DEGs was listed in Table 1. Therefore, the above results indicated that most of the DEGs were significantly enriched in cytoplasm mitochondrial, binding, and positive regulation of RNA polymerase II involved transcription.

Signaling pathway enrichment analysis

KEGG PATHWAY, Reactome, Panther, and Gene Ontology signaling pathway analysis tool were utilized to conduct DEGs functional and signaling pathway enrichment assay. As illustrated in Figure 3 and Table 2, the results indicated that the DEGs were significantly enriched in cytokine-cytokine receptor interaction, TNF signaling pathway, Influenza A, legionellosis, transcriptional dysregulation in cancer, protein processing in the endoplasmic reticulum, NOD-like receptor signaling pathway, Salmonella infection, mineral absorption signaling pathways. Furthermore, to clarify the relationship among signaling pathways, we analyzed the correlation among the pathways through deepen analyzing KEGG process in ClueGO online database (apps.cytoscape.org/apps/cluego). The results demonstrated that all DEGs were primarily associated with the ferroptosis, TNF signaling pathway, IL-17 signaling pathway, mineral absorption, prion diseases (Figure 3B, 3C).

Key candidate genes and pathways identified through the construction of DEGs PPI network and modular analysis

Using STRING online database (<http://string-db.org>) and Cytoscape online analyses tool, PPI network with 65 nodes and 168 edges was visually constructed. Total of 91 DEGs (68 up-regulated and 23

down-regulated genes) commonly altered DEGs was filtered into the DEGs PPI network complex with a PPI score of > 0.4 (Figure. 4). In the PPI network, the most significant 12 node degree genes with a degree of ≥ 10 were regarded as key genes (Figure. 4 and Table 3). They are interleukin 6 (IL6), prostaglandin-endoperoxide synthase 2 (PTGS2), DDIT8 (DNA damage-inducible transcript 3), C-X-C motif chemokine ligand 8 (CXCL8), heme oxygenase 1 (HMOX1), CCAAT/enhancer binding protein beta (CEBPB), HSPA1A (heat shock protein family A member 1A), NAD(P)H quinone dehydrogenase 1 (NQO1), dual specificity phosphatase 1 (DUSP1), C-X-C motif chemokine ligand 1 (CXCL1), C-C motif chemokine ligand 2 (CCL2), TRIB3 (tribbles pseudokinase 3).

Based upon the degree of importance, we chose one significant module from the PPI network complex for further analysis using Cytotype MCODE. Pathway enrichment analysis showed that Module 1 consisted of 12 nodes and 40 edges (Figure. 5A). GO term enrichment analysis further demonstrated that the 12 genes were significantly associated with inflammatory response, cellular response to interleukin-1, response to endoplasmic reticulum stress, PERK-mediated unfolded protein response, and cellular response to lipopolysaccharide (Table 4), in the BP category. In the CC category, the 12 genes were mainly enriched in CHOP-C/EBP complex, extracellular space, and caveola (Table 4). In the MF category, the analysis revealed that the genes were mainly related to chemokine activity, protein homodimerization activity, enzyme binding, receptor binding, and protein binding (Table 4). Pathway enrichment revealed that the 12 genes were mainly enriched in the TNF signaling pathway, Legionellosis, NOD-like receptor signaling pathway, and transcriptional misregulation pathway (Table 4).

Validation of the DEGs in atherosclerosis mice model

To confirm the reliability of the identified DEGs from the two databases, we verified the RNA expression level of hub genes in the aorta isolated from the normal diet (ND) or high-fat diet (HFD) feed Apoe^{-/-} mice (atherosclerosis). As shown in Figure 5B, the expression of IL6, CXCL1, CCL2, TRIB3 DUPS1, DDIT3, CEBPB and HMOX1 were ranked on the top up-regulated in Apoe^{-/-} HFD compared to Apoe^{-/-} ND. The total consistency of the up- and down-regulated genes was 90.7%, suggesting that our results are reliable.

The hub genes CCL2, CXCL1, IL6 and DUSP1 can serve as a diagnostic biomarker for obesity and type 2 diabetes mellitus

Since diabetic macroangiopathy, atherosclerosis secondary to diabetes[18], next, to explore the early diagnostic value of hub genes in the setting of diabetes or obesity, we further analyzed GEO profiles with the identified hub genes. Meanwhile, a receiver operating characteristic (ROC) curve was constructed to evaluate the diagnostic value of hub genes for obesity and type 2 diabetes. The results showed that CCL2, CXCL1, IL6 and DUSP1 in GDS3602 (adipocyte expression profile of obesity) were obviously up-regulated compared to control, suggesting that the expression of inflammation-related genes is up-regulated in obesity. The AUC (area under the curve) of CCL2, CXCL1, IL6 and DUSP1 was 0.93 (95% confidence interval 0.819-1.000, p=0.001, n=20), 0.90 (95% confidence interval 0.756-1.000, p=0.002, n=20), 0.84 (95% confidence interval 0.664-1.000, p=0.01, n=20) and 0.83 (95% confidence interval 0.625-1.000, p=0.013, n=20), respectively (Figure 5C-5F, 5J), confirming excellent specificity and sensitivity of CCL2, CXCL1, IL6 and DUSP1 as diagnostic biomarker for obesity. The AUC of CEBPB and HMOX1 in GDS3876 (liver from obese patients with and without type 2 diabetes) was 0.877 (95% confidence interval 0.715-1.000, p=0.016, n=18) and 0.846 (95% confidence interval 0.661-1.000, p=0.027, n=18), confirming excellent specificity and sensitivity of CEBPB and HMOX1 as diagnostic biomarker for the type 2 diabetes (Figure 5G-5H, 5J). TRIB3 was found in GDS3665 (Visceral adipose tissues of obese diabetic women) and the AUC was 0.880 (95% confidence interval 0.640-1.000, p=0.047, n=10) (Figure 5I, 5J). However, the AUC of ROC curve of DDIT3 in GDS3602 was 0.189 (95% confidence interval 0.000-0.425, p=0.022 n=19). Taken together, this data suggests CCL2, CXCL1, IL6 and DUSP1 can serve as a diagnostic indicator for obesity, CEBPB and HMOX1 can serve as a diagnostic indicator for type 2 diabetes, TRIB3 is a sensitive marker for indicating risks of diabetic women group.

Discussion

Numerous studies have been conducted to reveal the cause and underlying mechanisms of atherosclerosis formation and progression. However, the incidence and mortality of atherosclerosis remain high worldwide, because tissue differentiation and most studies focus on a single genetic event or the results are spawned from a single cohort study [19]. Our study integrated two expression profile datasets from different groups on vascular cells, the basis of diabetic macroangiopathy, utilized bioinformatics methods to analyze these datasets deeply, and identified 91 changed DEGs. Then DEGs PPI network complex was developed, and 65 nodes were identified with 168 edges. Among them, we further identified CCL2, CXCL1, IL6 and DUSP1 can serve as a diagnostic indicator for obesity,

CEBPP and HMOX1 can serve as a diagnostic indicator for type 2 diabetes, TRIB3 is a sensitive marker for indicating risks of the diabetic women group, which all the high-risk factors for atherosclerosis.

The changed DEGs were classified into three groups: molecular functions (MF), biological progress (BP) and cellular component (CC) groups, by GO term. Concerning BPs, the DEGs of atherosclerosis were mainly included in positive regulation of transcription from RNA polymerase II promoter, signal transduction, and immune response. It is well known that inflammation is an essential driver of atherosclerosis, and complex immune reactions drive the inflammation in the vascular wall in response to an atherosclerotic microenvironment[20]. There was compelling evidence emerging that the immune system and diabetes play a key role in human atherosclerotic CVD [21, 22] [23, 24] [25]. The present study, for the first time, revealed that the genes IL6 and CXCL1 involved in the crosstalk between endothelial cells and immune cells, such as driving the progression of atherosclerosis.

Next, KEGG pathway analysis was conducted by DAVID, and confirmed in ClueGO (plug-in of Cytoscape). The results showed that the 91 up- and down-regulated genes were enriched in the ferroptosis, TNF signaling pathway, IL-17 signaling pathway, mineral absorption, prion diseases. Ferroptosis is a recently discovered regulated form of necrosis pathway characterized by iron dependence and aggravation of lipid peroxidation [26-29]. Bruni reported that the utility of inhibitors of ferroptosis was capable of reducing intracellular lipid peroxidation [30]. This study found that GCLM, PRNP, SLC3A2, HMOX1, and FTH1 were up-regulated, indicating ferroptosis involvement. The TNF signaling pathway plays a crucial role in the inflammation. In our study, we found that the regulated cytokines, such as IL6, CCL2, CXCL1, and CXCL3 were involved in TNF signaling pathway. Of note, Interleukin-6 (IL-6) gene was first shown to regulate the acute phase response associated with inflammation [31] and considered as a mediator of inflammation, immune response, and hematopoiesis[32]. Various studies have demonstrated that interleukin-6 (Il-6) is an upstream inflammatory cytokine that plays a central role in the downstream inflammatory response responsible for atherosclerosis[33, 34]. In gene knockout models, only the IL-6 knockout animals showed impaired acute-phase response[35, 36]. Indeed, increased Il-6 concentrations found in downstream acute phase reactants were independent risk factors to predict overall mortality and cardiovascular mortality at 5 years in atherosclerosis[37]. Taking these results into account, IL-6 was an upstream

regulator that played a central role in propagating the downstream inflammatory response responsible for atherosclerosis.

Furthermore, by constructing the PPI network, a number of key genes were identified that might be useful in future therapeutic studies on atherosclerosis. Notably, key nodes in the PPI network and genes in the significant modules, including CXCL8, CXCL1, CCL2, may have specific contributions to the occurrence and development of atherosclerosis. CXCL8, also known as IL8, is an inflammatory

CXCL: C-X-C motif chemokine ligand 1

CCL2: C-C motif chemokine ligand 2

factor, known to play an important role in the development of atherosclerosis[38]. High levels of IL-8 are associated with an increased risk of coronary artery disease[39, 40]. CXCL1, a ligand of CXCR2, plays a vital role in atherogenic monocyte recruitment and other chemokines[41]. In the early stage of atherosclerosis, CXCL1 in the vascular wall promotes macrophages' aggregation and induces monocytes to arrest, thereby enhancing atherosclerosis[42]. The up-regulation of immobilized chemokine CCL2 released by endothelial or myeloid cells on arterial vessels can trigger cell arrest and promote leukocyte migration to atherosclerotic lesions[43]. Winter reported that Circulating myeloid cells deposit CCL2 rhythmically on the arterial endothelium and rhythmic arterial myeloid cell adhesion is triggered by the CCL2-CCR2 axis[44].

Next, to explore the difference between humans and animals, we analyze the RNA expression of hub genes from the artery of Apoe^{-/-} mice fed ND and HFD. The expression of IL6, CXCL1, CCL2, TRIB3, DUSP1, DDIT3, CEBPB and HMOX1 were up-regulated in HFD Apoe^{-/-} compared to ND mice. However, for CXCL15 (mouse homolog of human IL-8), no CT value was detected; this could be the difference between human and mouse. Finally, we searched the GEO profiles and found that some of the hub genes were related to obesity and type 2 diabetes. The area under the curve of CCL2, CEBPB and HMOX1 was 0.930, 0.877 and 0.846, respectively. This suggested that these genes could be the diagnostic indicator for diabetic atherosclerosis. However, further biological experimental evidence is required to determine the identified gene's causative function in atherosclerosis.

In conclusion, we conducted in-depth analysis of the DEGs identified in the human endothelial cells with Ox-PAPC compared with the matched without Ox-PAPC, which are involved in the

development of atherosclerosis. These DEGs and key nodes identified in the PPI network constructed by genes involved in important modules may play a vital role in atherosclerosis occurrence and development. Furthermore, these results may provide valuable clues for investigating the pathogenesis and drug targets of atherosclerosis.

Abbreviations

BP: Biological process

CC: Cellular component

CEPB: CCAAT/enhancer binding protein beta

CVD: Cardiovascular diseases

CXCL8: C-X-C motif chemokine ligand 8

DDIT8: DNA damage-inducible transcript 3

DEGs: Differentially expressed genes

DUSP1: Dual specificity phosphatase 1

ECs: Endothelial cells

HMECs: Human microvascular endothelial cells

HAECs: Human aortic endothelial cell

HSPA1A: Heat shock protein family A member 1A

HMOX1: Heme oxygenase 1

IL6: Interleukin 6

KEGG: Kyoto Encyclopedia of Genes and Genomes

MF: Molecular function

NQO1: NAD(P)H quinone dehydrogenase 1

Ox-PAPC: Oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphatidylcholine

oxLDLs: Oxidized low-density lipoproteins

PPI: Protein-protein interaction

PTGS2: Prostaglandin-endoperoxide synthase 2

ROC: Receiver operating characteristic

STRING: Search Tool for the Retrieval of Interacting Genes

TRIB3: Tribbles pseudokinase 3

Declaration

Ethics approval and consent to participate

This study were performed in adherence to the NIH Guidelines on the Use of Laboratory Animals and approved by the Thomas Jefferson University and Shanxi Medical University Committee on Animal Care.

Consent for publication

Not application

Availability of data and materials

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

Competing interests

The authors declared no conflict of interest.

Funding

This work was supported by the Scientific Research Project of the Shanxi Provincial Department of Health (Grant No. 2018023). Natural Science Foundation of China (81670278, 81670313, 81900892); Shanxi “1331 Project” Key Subjects Construction (1331KSC); Shanxi ‘Sanjin Scholars’ Program.

Authors' contributions

ZM, GJ and HL analyzed and interpreted the gene profiles data regarding the atherosclerosis disease and diabetes anima model. JZ, CL, and ZW performed the statistics analysis from the collected data. XM, XJ, JC and YW designed the experimental approaches. ZM wrote the manuscript. XM and YW edited the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

References

1. Joseph P, Leong D, McKee M, Anand SS, Schwalm JD, Teo K, Mente A, Yusuf S: **Reducing the Global Burden of Cardiovascular Disease, Part 1: The Epidemiology and Risk Factors.** *Circ Res* 2017, **121**:677-694.
2. Talmud PJ: **Gene-environment interaction and its impact on coronary heart disease risk.** *Nutr Metab Cardiovasc Dis* 2007, **17**:148-152.
3. Talmud PJ: **How to identify gene-environment interactions in a multifactorial disease: CHD as an example.** *Proc Nutr Soc* 2004, **63**:5-10.
4. Lusis AJ: **Atherosclerosis.** *Nature* 2000, **407**:233-241.
5. Berliner JA, Watson AD: **A role for oxidized phospholipids in atherosclerosis.** *N Engl J Med* 2005, **353**:9-11.
6. Guo Y, Sheng Q, Li J, Ye F, Samuels DC, Shyr Y: **Large scale comparison of gene expression levels by microarrays and RNAseq using TCGA data.** *PLoS One* 2013, **8**:e71462.
7. Zhang X, Sun R, Liu L: **Potentially critical roles of TNPO1, RAP1B, ZDHHC17, and PPM1B in the progression of coronary atherosclerosis through microarray data analysis.** *J Cell Biochem* 2019, **120**:4301-4311.
8. Tan X, Zhang X, Pan L, Tian X, Dong P: **Identification of Key Pathways and Genes in Advanced Coronary Atherosclerosis Using Bioinformatics Analysis.** *Biomed Res Int* 2017, **2017**:4323496.
9. Romanoski CE, Lee S, Kim MJ, Ingram-Drake L, Plaisier CL, Yordanova R, Tilford C, Guan B, He A, Gargalovic PS, et al: **Systems genetics analysis of gene-by-environment interactions in human cells.** *Am J Hum Genet* 2010, **86**:399-410.
10. Gene Ontology C: **The Gene Ontology (GO) project in 2006.** *Nucleic Acids Res* 2006, **34**:D322-326.
11. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al: **Gene ontology: tool for the unification of biology. The Gene Ontology Consortium.** *Nat Genet* 2000, **25**:25-29.
12. Harris MA, Clark J, Ireland A, Lomax J, Ashburner M, Foulger R, Eilbeck K, Lewis S, Marshall B, Mungall C, et al: **The Gene Ontology (GO) database and informatics resource.** *Nucleic Acids Res* 2004, **32**:D258-261.
13. Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, Katayama T, Kawashima S, Okuda S, Tokimatsu T, Yamanishi Y: **KEGG for linking genomes to life and the environment.** *Nucleic Acids Res* 2008, **36**:D480-484.
14. Huang da W, Sherman BT, Lempicki RA: **Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources.** *Nat Protoc* 2009, **4**:44-57.
15. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, et al: **STRING v10: protein-protein interaction networks, integrated over the tree of life.** *Nucleic Acids Res* 2015, **43**:D447-452.
16. Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T: **Cytoscape 2.8: new features for data integration and network visualization.** *Bioinformatics* 2011, **27**:431-432.
17. Liu J, Meng Z, Gan L, Guo R, Gao J, Liu C, Zhu D, Liu D, Zhang L, Zhang Z, et al: **C1q/TNF-related protein 5**

- contributes to diabetic vascular endothelium dysfunction through promoting Nox-1 signaling. *Redox Biol* 2020;101476.
- 18. Katakami N: Mechanism of Development of Atherosclerosis and Cardiovascular Disease in Diabetes Mellitus. *J Atheroscler Thromb* 2018, **25**:27-39.
 - 19. Libby P, Ridker PM, Hansson GK: Progress and challenges in translating the biology of atherosclerosis. *Nature* 2011, **473**:317-325.
 - 20. Ketelhuth DFJ, Lutgens E, Back M, Binder CJ, Van den Bossche J, Daniel C, Dumitriu IE, Hoefer I, Libby P, O'Neill L, et al: Immunometabolism and atherosclerosis: perspectives and clinical significance: a position paper from the Working Group on Atherosclerosis and Vascular Biology of the European Society of Cardiology. *Cardiovasc Res* 2019, **115**:1385-1392.
 - 21. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, et al: Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med* 2017, **377**:1119-1131.
 - 22. Koelwyn GJ, Corr EM, Erbay E, Moore KJ: Regulation of macrophage immunometabolism in atherosclerosis. *Nat Immunol* 2018, **19**:526-537.
 - 23. Hulkkonen J, Lehtimaki T, Mononen N, Juonala M, Hutili-Kahonen N, Taittonen L, Marniemi J, Nieminen T, Viikari J, Raitakari O, Kahonen M: Polymorphism in the IL6 promoter region is associated with the risk factors and markers of subclinical atherosclerosis in men: The Cardiovascular Risk in Young Finns Study. *Atherosclerosis* 2009, **203**:454-458.
 - 24. Maitra A, Shanker J, Dash D, John S, Sannappa PR, Rao VS, Ramanna JK, Kakkar VV: Polymorphisms in the IL6 gene in Asian Indian families with premature coronary artery disease--the Indian Atherosclerosis Research Study. *Thromb Haemost* 2008, **99**:944-950.
 - 25. Zhou Z, Subramanian P, Sevilmiss G, Globke B, Soehnlein O, Karshovska E, Megens R, Heyll K, Chun J, Saulnier-Blache JS, et al: Lipoprotein-derived lysophosphatidic acid promotes atherosclerosis by releasing CXCL1 from the endothelium. *Cell Metab* 2011, **13**:592-600.
 - 26. Cao JY, Dixon SJ: Mechanisms of ferroptosis. *Cell Mol Life Sci* 2016, **73**:2195-2209.
 - 27. Dixon SJ: Ferroptosis: bug or feature? *Immunol Rev* 2017, **277**:150-157.
 - 28. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, et al: Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 2012, **149**:1060-1072.
 - 29. Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, Fulda S, Gascon S, Hatzios SK, Kagan VE, et al: Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell* 2017, **171**:273-285.
 - 30. Bruni A, Bornstein S, Linkermann A, Shapiro AMJ: Regulated Cell Death Seen through the Lens of Islet Transplantation. *Cell Transplant* 2018, **27**:890-901.
 - 31. Rumin P, Gangneux C, Claeysens S, Scotte M, Daveau M, Salier JP: Gene transcription in hepatocytes during the acute phase of a systemic inflammation: from transcription factors to target genes. *Inflamm Res* 2001, **50**:383-390.
 - 32. Tanaka T, Narasaki M, Kishimoto T: IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol* 2014, **6**:a016295.
 - 33. Hartman J, Frishman WH: Inflammation and atherosclerosis: a review of the role of interleukin-6 in the development of atherosclerosis and the potential for targeted drug therapy. *Cardiol Rev* 2014, **22**:147-151.
 - 34. Akita K, Isoda K, Sato-Okabayashi Y, Kadoguchi T, Kitamura K, Ohtomo F, Shimada K, Daida H: An Interleukin-6 Receptor Antibody Suppresses Atherosclerosis in Atherogenic Mice. *Front Cardiovasc Med* 2017, **4**:84.
 - 35. Fantuzzi G, Zheng H, Faggioni R, Benigni F, Ghezzi P, Sipe JD, Shaw AR, Dinarello CA: Effect of endotoxin in IL-1

- beta-deficient mice.** *J Immunol* 1996, **157**:291-296.
36. Marino MW, Dunn A, Grail D, Inglese M, Noguchi Y, Richards E, Jungbluth A, Wada H, Moore M, Williamson B, et al: **Characterization of tumor necrosis factor-deficient mice.** *Proc Natl Acad Sci U S A* 1997, **94**:8093-8098.
37. Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger WH, Jr., Heimovitz H, Cohen HJ, Wallace R: **Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly.** *Am J Med* 1999, **106**:506-512.
38. Qin Y, Mao W, Pan L, Sun Y, Fan F, Zhao Y, Cui Y, Wei X, Kohama K, Li F, Gao Y: **Inhibitory effect of recombinant human CXCL8(3-72)K11R/G31P on atherosclerotic plaques in a mouse model of atherosclerosis.** *Immunopharmacol Immunotoxicol* 2019, **41**:446-454.
39. Boekholdt SM, Peters RJ, Hack CE, Day NE, Luben R, Bingham SA, Wareham NJ, Reitsma PH, Khaw KT: **IL-8 plasma concentrations and the risk of future coronary artery disease in apparently healthy men and women: the EPIC-Norfolk prospective population study.** *Arterioscler Thromb Vasc Biol* 2004, **24**:1503-1508.
40. Herder C, Baumert J, Thorand B, Martin S, Lowel H, Kolb H, Koenig W: **Chemokines and incident coronary heart disease: results from the MONICA/KORA Augsburg case-cohort study, 1984-2002.** *Arterioscler Thromb Vasc Biol* 2006, **26**:2147-2152.
41. Weber C, Zernecke A, Libby P: **The multifaceted contributions of leukocyte subsets to atherosclerosis: lessons from mouse models.** *Nat Rev Immunol* 2008, **8**:802-815.
42. Boisvert WA, Rose DM, Johnson KA, Fuentes ME, Lira SA, Curtiss LK, Terkeltaub RA: **Up-regulated expression of the CXCR2 ligand KC/GRO-alpha in atherosclerotic lesions plays a central role in macrophage accumulation and lesion progression.** *Am J Pathol* 2006, **168**:1385-1395.
43. Weber C, Noels H: **Atherosclerosis: current pathogenesis and therapeutic options.** *Nat Med* 2011, **17**:1410-1422.
44. Winter C, Silvestre-Roig C, Ortega-Gomez A, Lemnitzer P, Poelman H, Schumski A, Winter J, Drechsler M, de Jong R, Immmler R, et al: **Chrono-pharmacological Targeting of the CCL2-CCR2 Axis Ameliorates Atherosclerosis.** *Cell Metab* 2018, **28**:175-182 e175.

Legend

Figure 1. Identification of 91 commonly changes DEGs from the expression profile datasets (GSE30169 and GSE6584). A, Volcano plot analysis from GSE30169. B, Volcano plot analysis from GSE6584. Blue indicates down-regulated genes, red indicates up-regulated genes and gray indicates genes with unchanged expression. C, The commonly up-regulated DEGs from the two datasets. D, The commonly down-regulated DEGs from the two datasets. DEG, differentially expressed gene; FC, fold-change; down, down-regulated; non, no change; up, up-regulated.

Figure 2. Gene Ontology analysis and significant enriched GO term of DEGs based on their functions. DEG, differentially expressed gene; GO, Gene Ontology; MF, molecular function; CC, cellular component; BP, biological process.

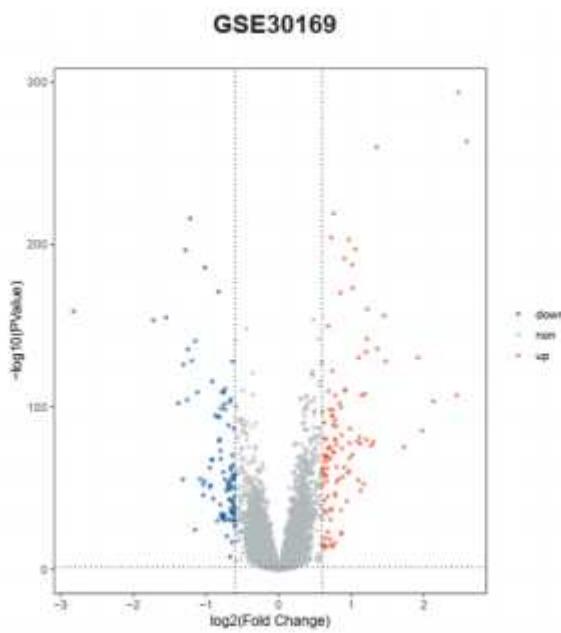
Figure 3. Significant enriched pathway terms of DEGs in two datasets. DEGs functional and signaling pathway enrichment were conducted via online KEGG, Kyoto Encyclopedia of Genes and Genomes. A, KEGG pathway enriched DEGs. B, ClueGo pathway enriched DEGs. C, Numbers of genes enriched in the identified ClueGo pathways. DEG, differentially expressed gene;

Figure 4. Protein-protein interaction network of DEGs. 68 up-regulated in Red nodes standing for upregulation and 23 down-regulated genes in Green standing for downregulation. Total of 91 DEGs were filtered into the DEGs PPI network complex. 65 nodes and 168 edges were constructed. DEG, differentially expressed gene;

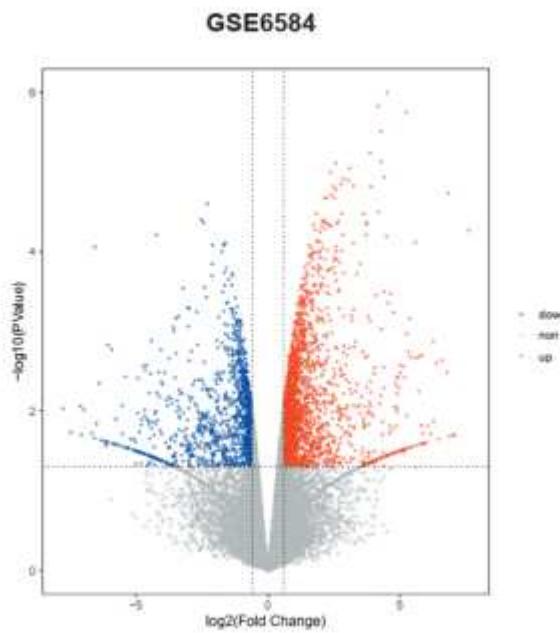
Figure 5. Identification of clinic indicators for atherosclerosis. A, 12 key nodes of significant module was selected from PPI network. B, Key nodes genes were analyzed by Real-time quantitative PCR. Results were expressed as mean \pm SD. n = 3. **p < 0.01 vs. Apoe^{-/-} ND. C-F, ROC curve analysis revealed that CCL2, CXCL1, IL6 and DUSP1 distinguished patients with and without obesity. G-H, ROC curve analysis revealed that HMOX1 and CEBPB distinguishes patients with and without diabetes. I, ROC curve analysis revealed that TRIB3 distinguishes women patients with and without obese diabetes. J, AUC of hub genes. PPI, protein-protein interaction network. Apoe^{-/-}, Apoe knockout; ND, normal diet; AUC, the area under of curve.

Figures

A

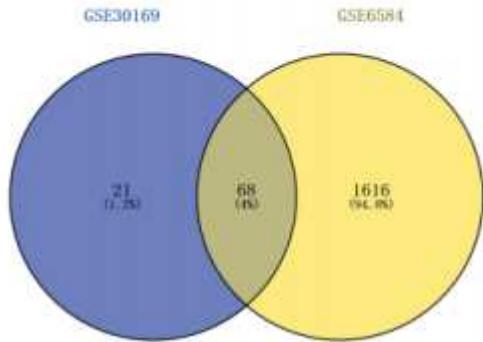


B



C

Up-regulated DEGs



D

Down-regulated DEGs

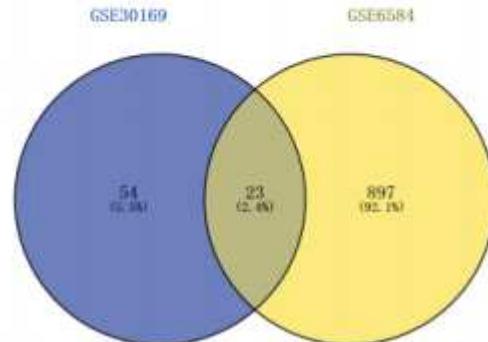


Figure 1

Identification of 91 commonly changes DEGs from the expression profile datasets (GSE30169 and GSE6584). A, Volcano plot analysis from GSE30169. B, Volcano plot analysis from GSE6584. Blue indicates down-regulated genes, red indicates up-regulated genes and gray indicates genes with unchanged expression. C, The commonly up-regulated DEGs from the two datasets. D, The commonly

down-regulated DEGs from the two datasets. DEG, differentially expressed gene; FC, fold-change; down, down-regulated; non, no change; up, up-regulated.

BP

CC

MF

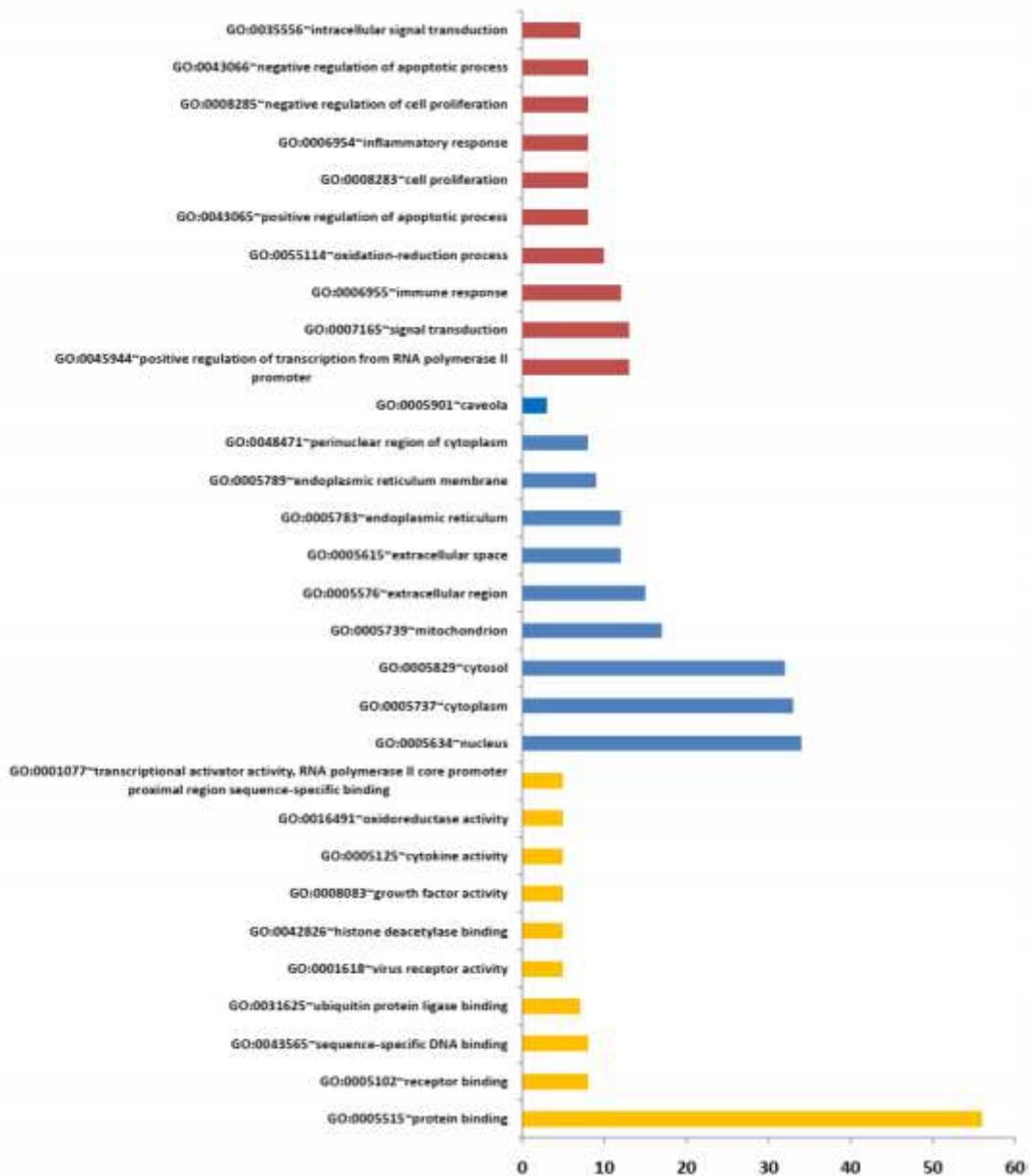
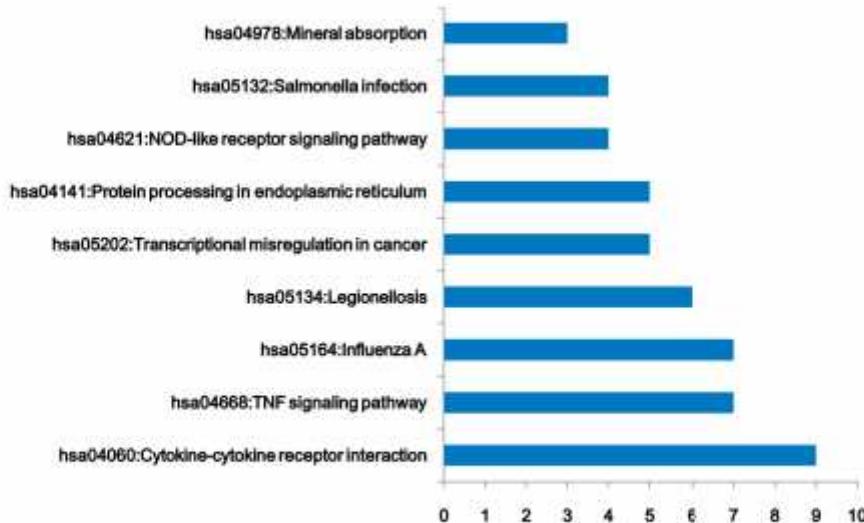


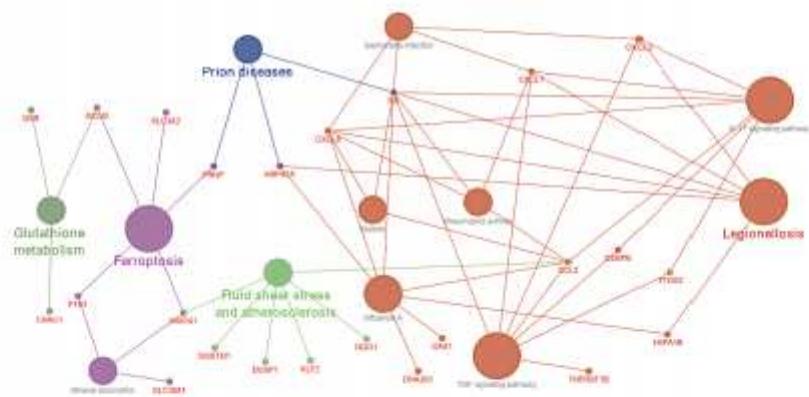
Figure 2

Gene Ontology analysis and significant enriched GO term of DEGs based on their functions. DEG, differentially expressed gene; GO, Gene Ontology; MF, molecular function; CC, cellular component; BP, biological process.

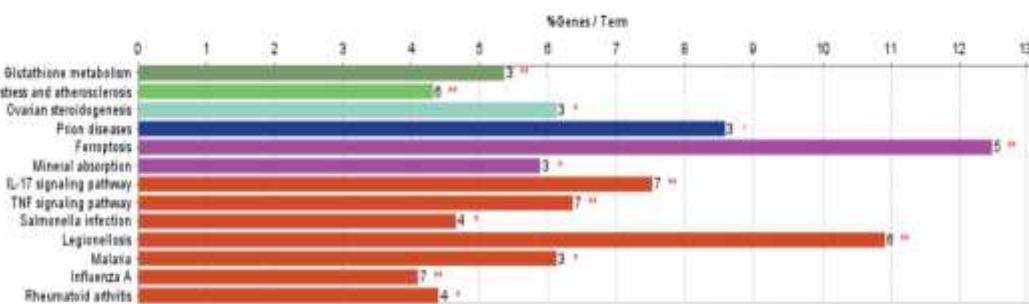
A



B



C

**Figure 3**

Significant enriched pathway terms of DEGs in two datasets. DEGs functional and signaling pathway enrichment were conducted via online KEGG, Kyoto Encyclopedia of Genes and Genomes. A, KEGG pathway enriched DEGs. B, ClueGo pathway enriched DEGs. C, Numbers of genes enriched in the identified ClueGo pathways. DEG, differentially expressed gene;

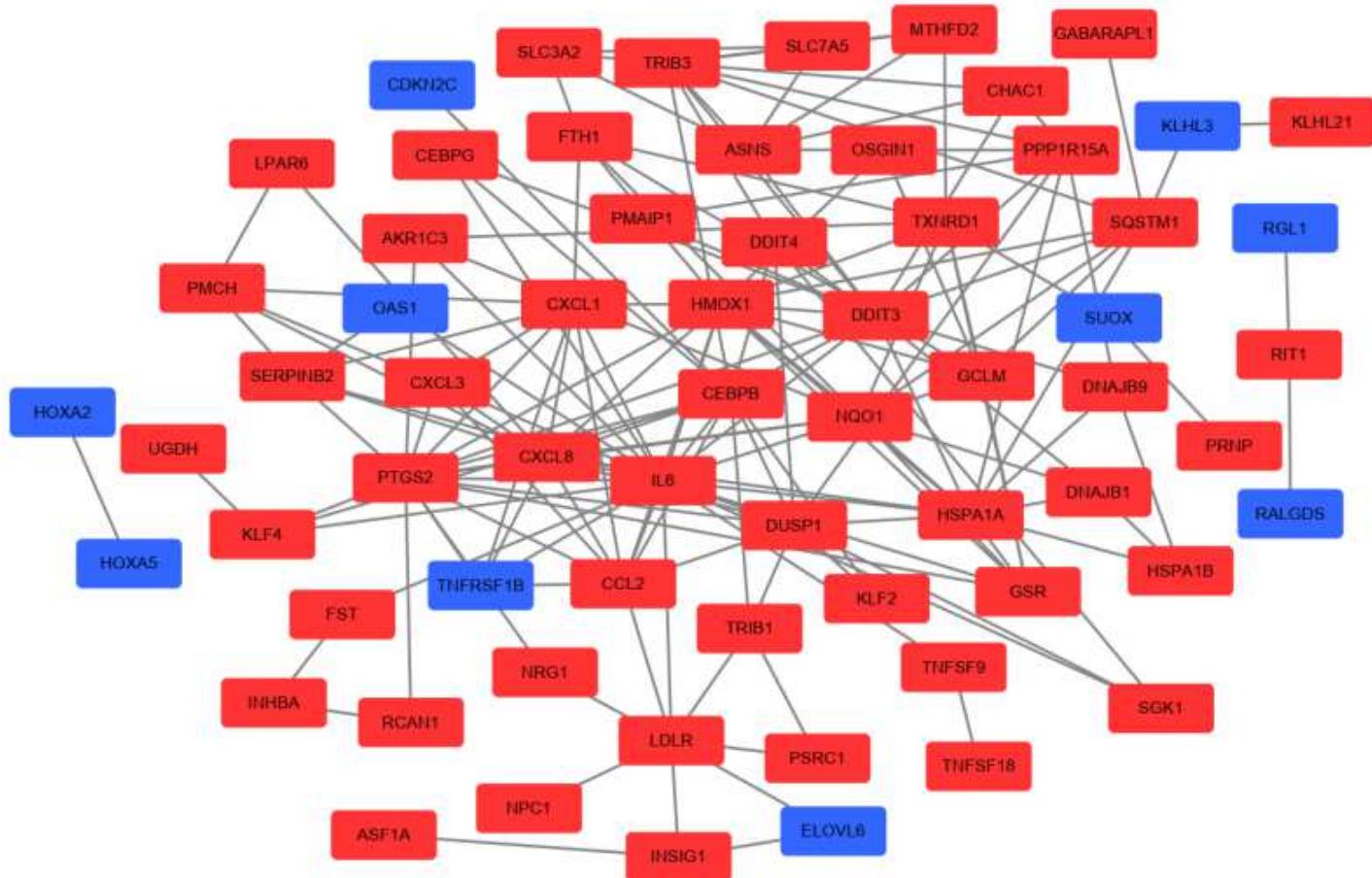


Figure 4

Protein-protein interaction network of DEGs. 68 up-regulated in Red nodes standing for upregulation and 23 down-regulated genes in Green standing for downregulation. Total of 91 DEGs were filtered into the DEGs PPI network complex. 65 nodes and 168 edges were constructed. DEG, differentially expressed gene;

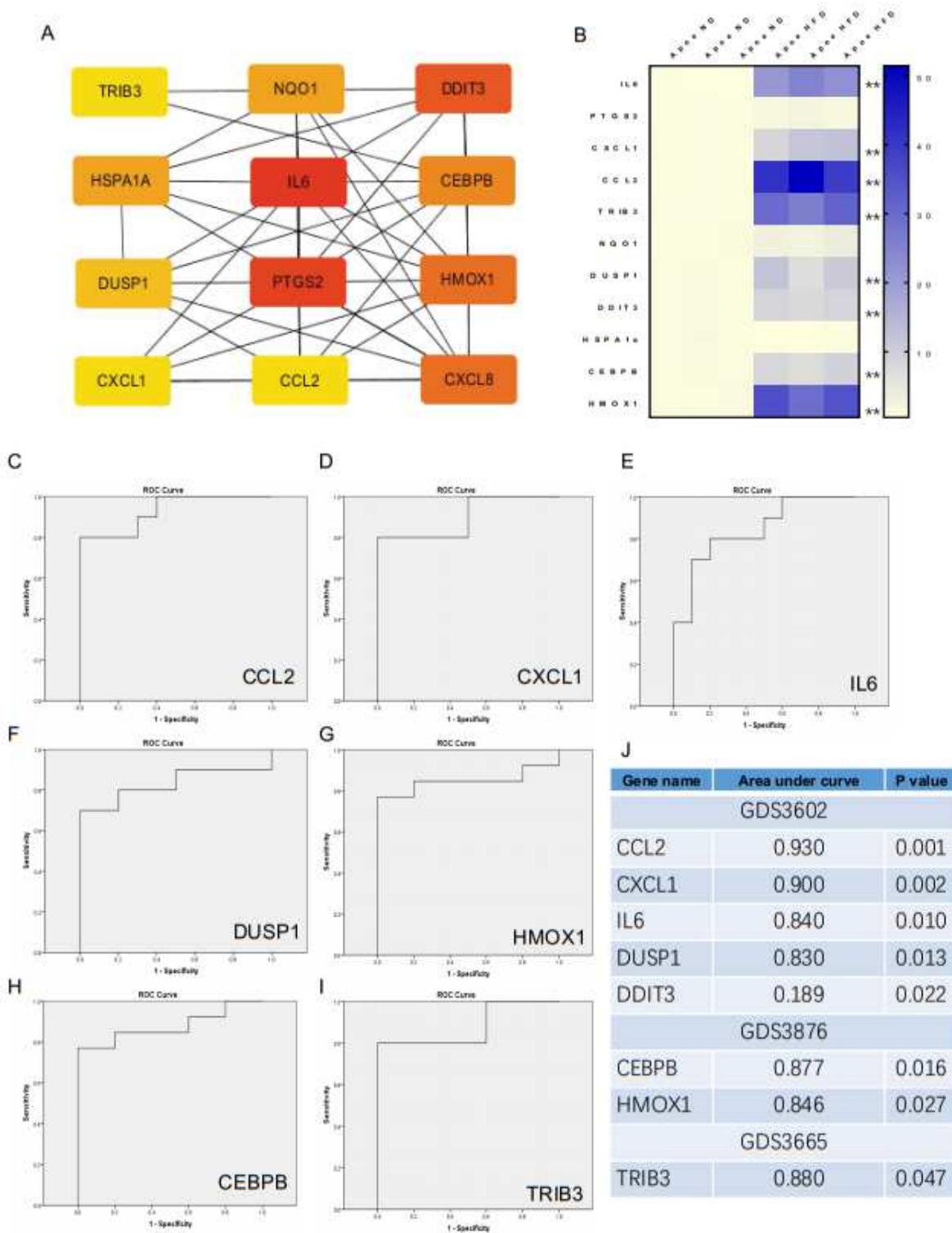


Figure 5

Identification of clinic indicators for atherosclerosis. A, 12 key nodes of significant module was selected from PPI network. B, Key nodes genes were analyzed by Real-time quantitative PCR. Results were expressed as mean \pm SD. n = 3. **p < 0.01 vs. Apoe-/ ND. C-F, ROC curve analysis revealed that CCL2, CXCL1, IL6 and DUSP1 distinguished patients with and without obesity. G-H, ROC curve analysis revealed that HMOX1 and CEBPB distinguishes patients with and without diabetes. I, ROC curve analysis revealed

that TRIB3 distinguishes women patients with and without obese diabetes. J, AUC of hub genes. PPI, protein-protein interaction network. Apoe-/-, Apoe knockout; ND, normal diet; AUC, the area under of curve.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Tables.pdf](#)