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DATABASE ANALYSIS

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Crucial Gene Identification in Carotid Atherosclerosis Based on Peripheral Blood Mononuclear Cell (PBMC) Data by Weighted (Gene) Correlation Network Analysis (WGCNA)

Authors' Contribution:
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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: Many patients are not responsive or tolerant to medical therapies for carotid atherosclerosis. Thus, elucidating the molecular mechanism for the pathogenesis and progression of carotid atherosclerosis and identifying new potential molecular targets for medical therapies that can slow progression of carotid atherosclerosis and prevent ischemic events are quite important.

Material/Methods: We downloaded the expression profiling data of PBMC in Biobank of Karolinska Endarterectomy (BiKE, GSE21545) for GEO. The WGCNA and DEG screening were conducted. The co-expression pattern between patients with ischemic events (the events group) and patients without ischemic events (the no-events group) were compared. Then, we identified hub genes of each module. Finally, the DEG co-expression network was constructed and MCODE was used to identify crucial genes based on this co-expression network.

Results: In the study, 183 DEGs were screened and 8 and 6 modules were assessed in the events group and no-events group, respectively. Compared to the no-events group, genes associated with inflammation and immune response were clustered in the green-yellow module of the events group. The hub gene of the green-yellow module of the events group was *KIR2DL5A*. We obtained 1 DEG co-expression network, which has 16 nodes and 24 edges, and we detected 5 crucial genes: *SIRT1*, *THRAP3*, *RBM43*, *PEX1*, and *KLHDC2*. The upregulated genes (*THRAP3* and *RBM43*) showed potential diagnostic and prognostic value for the occurrence of ischemic events.

Conclusions: We detected 8 modules for the events group and 6 modules for the no-events group. The hub genes for modules and crucial genes of the DEG co-expression network were also identified. These genes might serve as potential targets for medical therapies and biomarkers for diagnosis and prognosis. Further experimental and biological studies are needed to elucidate the role of these crucial genes in the progression of carotid atherosclerosis.

MeSH Keywords: Carotid Artery Diseases • Gene Expression Profiling • Gene Regulatory Networks • Microarray Analysis

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Background

Atherosclerosis is an inflammatory disease that involves the accumulation of fibrous and/or fatty components in the intima of medium and large arteries such as the coronary artery, carotid artery, and peripheral artery, and the clinical manifestations vary with the arteries affected [1,2]. Ischemic strokes and transient ischemic attacks may occur if the carotid artery is involved, and carotid atherosclerotic disease accounts for approximately 18–25% of all ischemic strokes [3]. Prevention of stroke in patients with carotid atherosclerosis depends on the degree of carotid stenosis. These preventive methods mainly include carotid endarterectomy, carotid stenting, and medical management such as with statins and antiplatelet agents [4,5]. Although the medical management is effective and may even serve as an alternative to carotid endarterectomy in patients with asymptomatic carotid atherosclerosis, patients who are nonresponsive to medical therapies or not tolerant of the adverse effects may not benefit from present medical therapies [6–8]. Therefore, elucidating the molecular mechanism of the pathogenesis and progression of carotid atherosclerosis and identifying new potential molecular targets for medical therapies that can slow progression of carotid atherosclerosis and prevent ischemic events are quite important. The molecular mechanism mainly includes abnormal accumulation of lipids, immune response, and inflammation, and monocytes play an important role [1,9]. Induced by chemokines, circulating monocytes can bind to adhesion molecules expressed by endothelial cells, migrating into the arterial wall and differentiating into macrophages. Previous studies focused on the role of circulating monocytes in the pathogenesis and progression of carotid atherosclerosis; however, few researchers have used weighted (gene) correlation network analysis (WGCNA) to construct gene co-expression networks for carotid atherosclerosis based on high-throughput data of peripheral blood mononuclear cells (PBMCs) in patients.

Zhang and Horvath first developed the WGCNA algorithm in 2005, which can be used for gene co-expression network construction, gene module detection, and hub gene identification, based on gene expression data [10–12]. Furthermore, gene modules and hub genes can be correlated with clinical traits if these data are available. The WGCNA R package was developed on the official R website (*https://cran.r-project.org/*), making it more convenient for researchers to conduct WGCNA. Although WGCNA was first developed for analyzing gene expression data, it can also be used for miRNA, lncRNA, and even metabolome [13–15].

Previous studies screened differentially expressed genes (DEGs) using microarray data of carotid atherosclerotic plaques. For instance, Razuvaev et al. identified 11 downregulated genes and 19 upregulated genes by comparing the gene expression profile between symptomatic and asymptomatic patients [16]. However, DEG screening cannot reveal the interaction among genes or identify genes with crucial biological functions.

In the present study, we focused on the possible underlying molecular mechanism of the occurrence of ischemic events. The mRNA microarray data of the Biobank of Karolinska Endarterectomies (BiKE) were included. The expression data of peripheral blood mononuclear cells for patients with ischemic events (the events group) and patients without ischemic events (the no-events group) during follow-up [17] were used in our analysis. The genes in the gene modules were subjected to functional enrichment analysis. Then, we mapped DEGs into the co-expression network of events group and obtained 1 DEG co-expression network. Furthermore, we identified crucial genes based on the DEG co-expression network. The potential diagnostic and prognostics values of the upregulated crucial genes were identified.

Material and Methods

Datasets

The dataset GSE21545, from the Biobank of Karolinska Endarterectomy (BiKE), was selected from the Gene Expression Omnibus (GEO) (*http://www.ncbi.nlm.nih.gov/geo/*). Series matrix file and platform data tables (GPL570) were downloaded.

DEG analysis

The series matrix file was annotated with GPL570 platform data tables, and the probe names in the matrix file were replaced by the gene symbols. Then, the 97 peripheral blood mononuclear cell (PBMC) samples were included in our analysis, in which 21 were samples of the events group and 76 were samples of the no-events group. Differentially expressed genes (DEGs) were screened using the "limma" R package. $|\log_2(\text{fold-change})|>2$ and adjusted $p<0.01$ were set as the threshold of DEG screening.

Construction of co-expression network by WGCNA

Co-expression networks for both PBMC and plaque samples were constructed using the "WGCNA" R package. The algorithm filtered genes with the top 25% variance for further analysis, and WGCNA analysis was conducted for the events group (21 samples) and the no-events group (76 samples). The soft-power threshold β was chosen to ensure a scale-free topology. A topological overlap measure (TOM) matrix was created from the adjacency matrix to estimate the network's connectivity property. A clustering dendrogram was constructed using average linkage hierarchical clustering based on the

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TOM matrix. The threshold for modules size was set as 50 for both groups to generate modules with proper size, and similar modules were merged.

GO and KEGG pathway enrichment of gene modules

Gene ontology (GO) and Kyoto Encyclopedia of Genes Genomes (KEGG) pathway analyses were conducted for genes in modules we detected using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 (*https://david.ncifcrf.gov/*) to determine the biological function and signaling pathway involved in these modules. Count number >2 and $p<0.05$ were set as thresholds for the analysis. The differences between co-expression networks for the events group and no-events group were compared based on the results of functional enrichment analysis.

Identification of hub genes and crucial genes

Hub genes were considered to be the gene which had the largest intramodular connectivity in each module. Then, we mapped the DEGs into a co-expression network in the events group using Cytoscape v3.7.0, and we obtained 1 DEG co-expression network. Isolated nodes and isolated nodes pairs were removed from the network. The Molecular Complex Detection (MCODE), a plugin in Cytoscape to detect core subnetworks, was used to identify crucial gene clusters based on the DEG co-expression network. Receiver operating characteristic (ROC) analysis and survival analysis were also conducted using the combination of the upregulated genes in the crucial gene cluster by SPSS 25.0 to show the potential diagnostic and prognostic value of upregulated crucial genes.

Results

Flowchart

The flowchart of our study is shown in Figure 1. We constructed the co-expression networks for the events group and no-events group and detected gene modules. Then, DEG screening was conducted, and 183 DEGs were screened. The DEG co-expression network were constructed by mapping DEGs into the whole co-expression network of the events group. Based on the DEG co-expression network, crucial genes were identified, and their clinical significance was evaluated by ROC and survival analysis.

Screening of DEGs

With the threshold of $|\log_2(\text{fold-change})|>2$ and $p<0.01$, 183 DEGs were screened with 122 upregulated and 61 downregulated genes. The heatmap and the volcano plot showed the

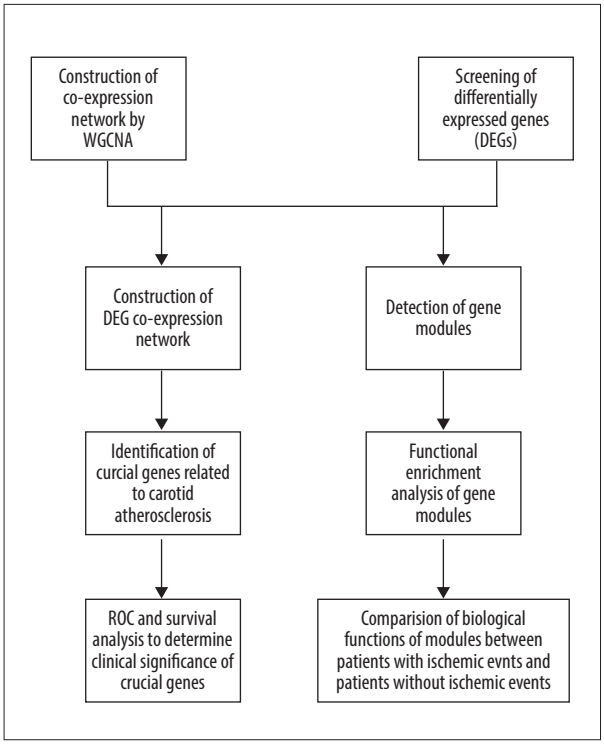

Figure 1. Flowchart for the study.

expression pattern of DEGs (Figure 2). Upregulated DEGs and downregulated DEGs with the top 10-fold-change are shown in Supplementary Table 1.

Construction of the co-expression network for the events group and no-events group

One outlier (GSM892524) in the events group was removed, while all samples in the no-events group were included for further analysis, as shown in the sample clustering dendrogram (Figure 3A and Supplementary Figure 1A). The power of $\beta=10$ and 16 were chosen as the soft-threshold for the network of the events group and no-events group, respectively (Figure 3B and Supplementary Figure 1B). And the both the co-expression networks we constructed met the requirements of scale-free topology (Figure 3C–3E and Supplementary Figure 1C–1E). We detected 8 gene modules for the events group and 6 gene modules for the no-events group (Figure 3F and Supplementary Figure 1F).

Comparison of co-expression patterns

KEGG pathway and GO-BP analysis were used to assess the biological function of genes for modules. Results of GO-BP and KEGG analyses are shown in Supplementary Tables 2 and 3. The green-yellow module may be related to the occurrence of ischemic events. The green-yellow module is mainly associated

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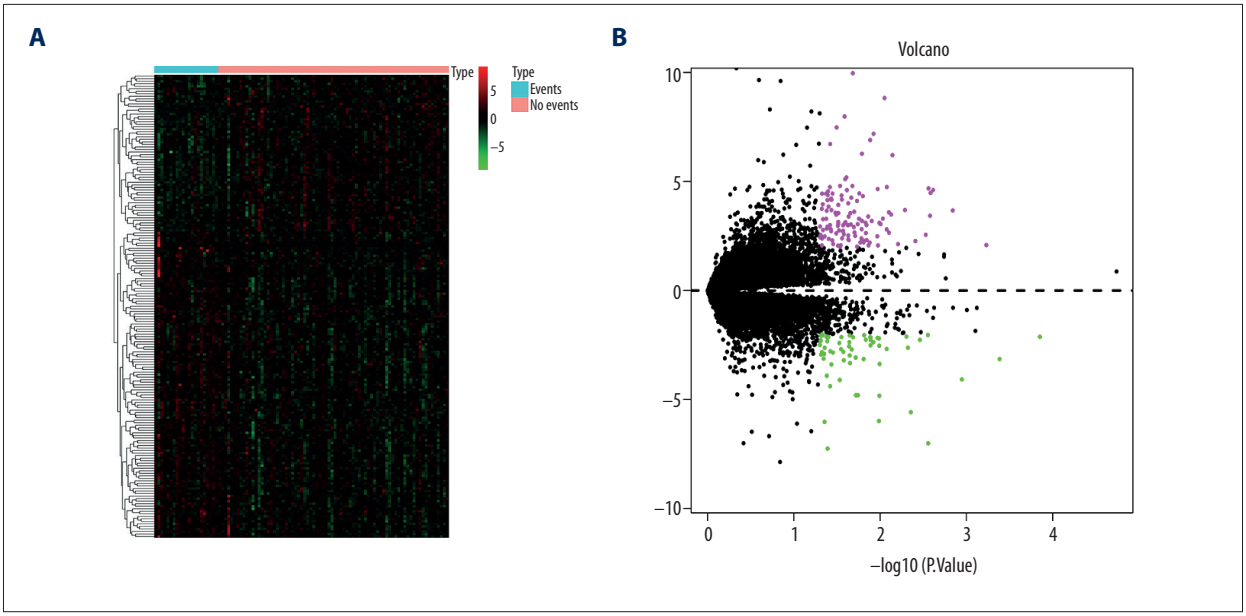

Figure 2. DEG screening. (**A**) Heatmap for the DEGs we screened. (**B**) Volcano plots for the DEGs. The X-axis represents –log(P.val) and Y-axis represents logFC.

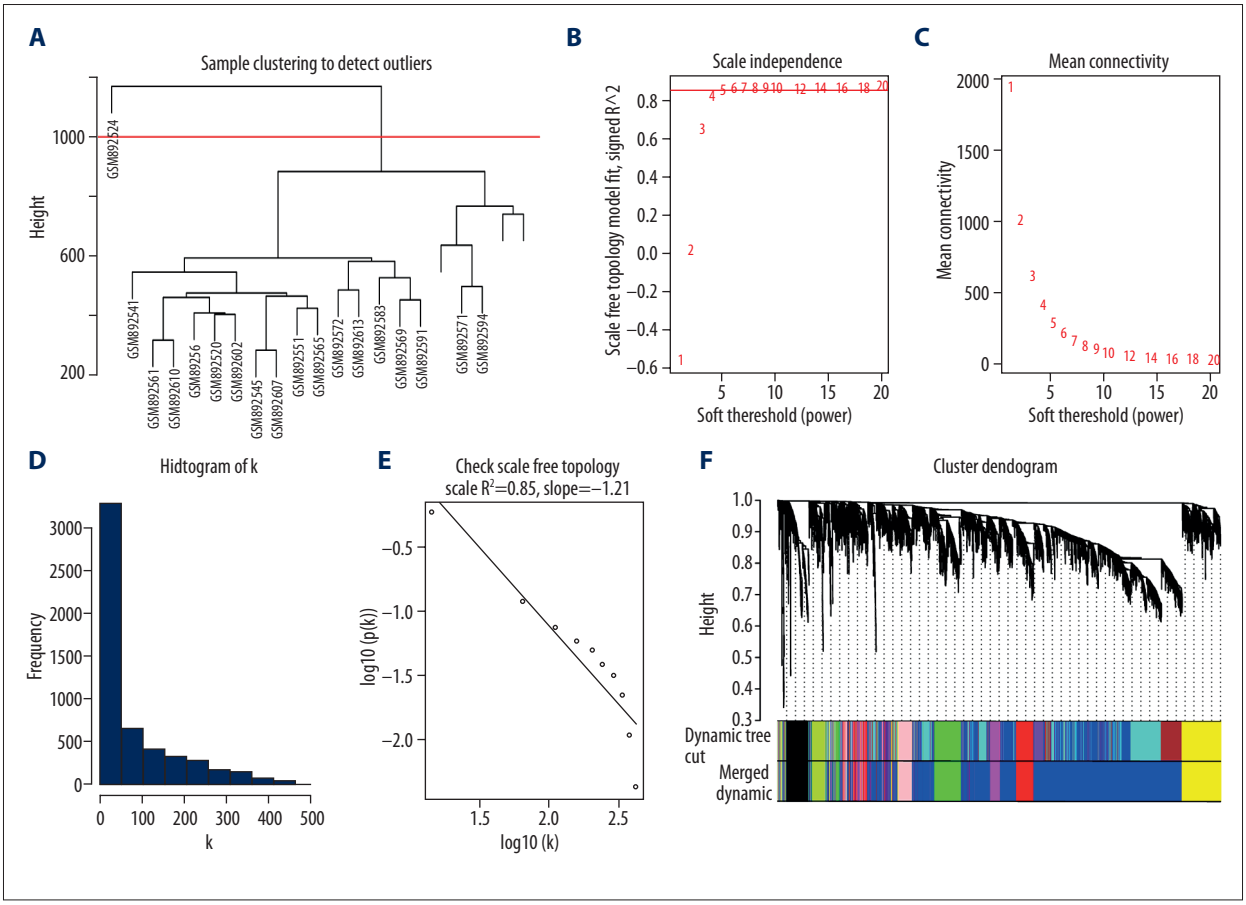

Figure 3. WGCNA of event group. (**A**) One outlier (GSE89254) was delected by sample clustering. (**B, C**) Selection of soft-threshold β. (**D, E**) Fitness for scale free topology when β 10. (**F**) Cluster dendrogram. Each module was represented by WGCNA.

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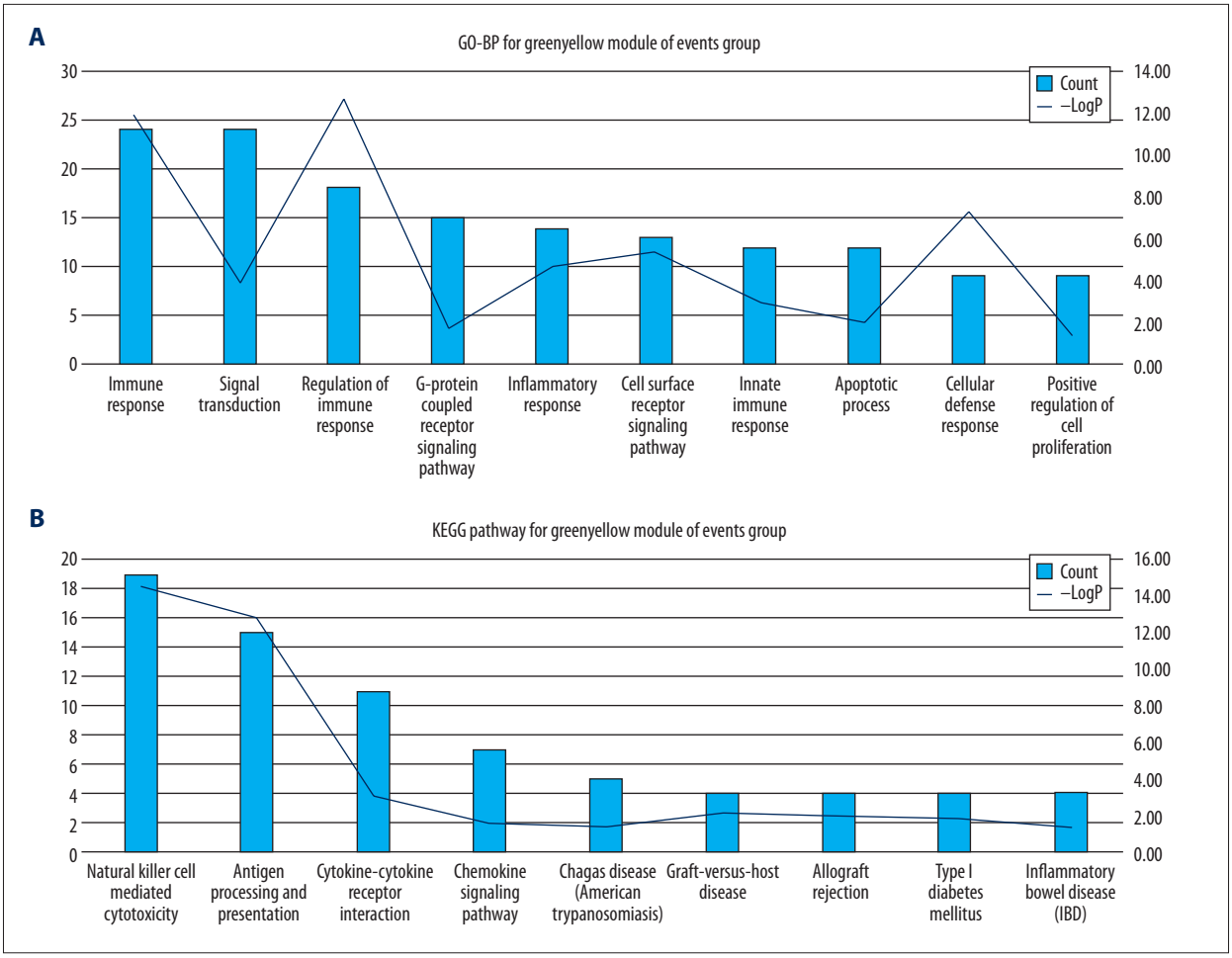

Figure 4. Enriched GO-BP terms and KEGG pathways with top10 count number for greenyellow module of events group. (**A**) GO-BP terms; (**B**) KEGG terms.

with inflammation and immune response. Nonetheless, pathways associated with inflammation and immune response were scattered in modules of the no-events group. The KEGG pathway GO-BP terms with the top 10 count numbers for greenyellow modules of the events group are shown in Figure 4 and Table 1. These results indicate that PBMC might play a role in the occurrence of ischemic events through regulating inflammation and immune response.

Hub genes in modules of the events group and no-events group

Hub genes for modules of the events group and no-events group are shown in Table 2. The hub genes of the green-yellow modules of the events group were killer cell immunoglobulin-like receptor, 2 Ig domains, and long cytoplasmic tail 5A (*KIR2DL5A*), which are killer cell immunoglobulin-like receptors (KIRs) and are mainly expressed by natural killer cells and subsets of T cells.

Identification of crucial genes mediating ischemic events

The DEG co-expression network was obtained by mapping DEGs into the whole co-expression network of the events group. The threshold for weighted edge was set as 0.1. After removing isolated nodes and isolated nodes pairs, a network with 16 nodes and 24 edges was generated (Figure 5A). MCODE detected 1 significant cluster consisting of 5 genes for the DEG co-expression network (Figure 5B, Table 3). Among these 5 genes, 2 genes were upregulated (*THRAP3* and *RBM43*) and 3 genes were downregulated (*SIRT1*, *PEX1*, and *KLHDC2*). Sirtuin 1 (*SIRT1*), a member of the sirtuin family, had the highest connectivity among the 5 crucial genes.

Combination of the 2 upregulated genes showed potential diagnostic and prognostic value (Figure 6).

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Table 1. GO-BP KEGG pathways terms with top 10 count number of black module for events group.

ID	Terms	Count	-LogP
GO-BP			
GO: 0006955	Immune response	24	11.80
GO: 0007165	Signal transduction	24	3.82
GO: 0050776	Regulation of immune response	18	12.65
GO: 0007186	G-protein coupled receptor signaling pathway	15	1.63
GO: 0006954	Inflammatory response	14	4.60
GO: 0007166	Cell surface receptor signaling pathway	13	5.34
GO: 0045087	Innate immune response	12	2.89
GO: 0006915	Apoptotic process	12	1.99
GO: 0006968	Cellular defense response	9	7.24
GO: 0008284	Positive regulation of cell proliferation	9	1.31
KEGG			
hsa04650	Natural killer cell mediated cytotoxicity	19	14.53
hsa04612	Antigen processing and presentation	15	12.70
hsa04060	Cytokine-cytokine receptor interaction	11	3.16
hsa04062	Chemokine signaling pathway	7	1.59
hsa05142	Chagas disease (American trypanosomiasis)	5	1.42
hsa05332	Graft-versus-host disease	4	2.13
hsa05330	Allograft rejection	4	1.99
hsa04940	Type I diabetes mellitus	4	1.84
hsa05321	Inflammatory bowel disease (IBD)	4	1.37

Table 2. Hub genes of each module for events group and no-events group.

Module	Gene symbol	Official full gene name
Events group		
Black	ACRBP	Acrosin binding protein
Blue	AP2M1	Adaptor related protein complex 2 subunit mu 1
Green	DOCK10	Dedicator of cytokinesis 10
Greenyellow	KIR2DL5A	Killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 5A
Magenta	GNS	Glucosamine (N-acetyl)-6-sulfatase
Pink	FHOD1	Formin homology 2 domain containing 1
Red	ITGA5	Integrin subunit alpha 5
Yellow	MPEG1	Macrophage expressed 1
No-events group		
Black	CTTN	Cortactin
Green	PRKCSH	Protein kinase C substrate 80K-H
Magenta	MAPRE1	Microtubule associated protein RP/EB family member 1
Red	FAM103A1	RNA Guanine-7 Methyltransferase Activating Subunit
Tan	ZHX1	Zinc fingers and homeoboxes 1
Yellow	ZBTB20	Zinc finger and BTB domain containing 20

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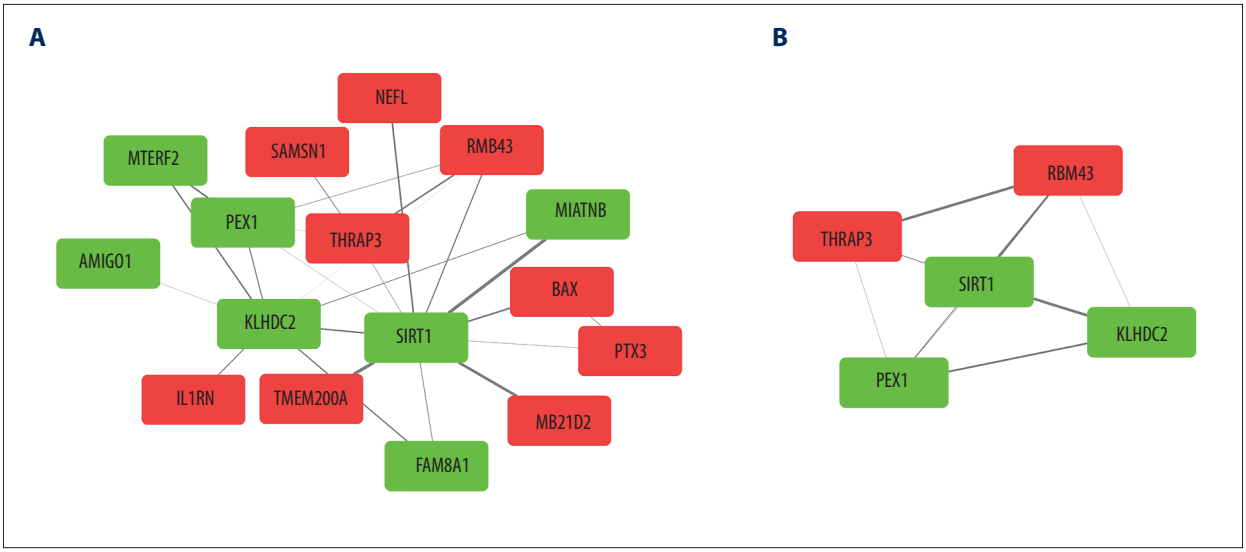

Figure 5. DEG co-expression network and crucial genes. (**A**) Red boxes represent up-regulated genes. Green boxes represent down-regulated genes. (**B**) Crucial genes generated by MCODE.

Table 3. Crucial genes detected by MCODE.

Entrez ID	Gene symbol	Official full gene name
23411	SIRT1	Sirtuin 1
9967	THRAP3	Thyroid Hormone Receptor Associated Protein 3
375287	RBM43	RNA Binding Motif Protein 43
23588	KLHDC2	Kelch Domain Containing 2
5189	PEX1	Peroxisomal Biogenesis Factor 1

Discussion

We screened 183 DEGs, among which 122 were upregulated and 61 were downregulated (Figure 2 and Supplementary Table 1). Weighted co-expression networks were constructed using the WGCNA algorithm. We detected 8 modules for the events group and 6 modules for the no-events group.

We also conducted KEGG pathway and GO-BP analysis (Supplementary Tables 2 and 3) and found that pathways related to inflammation and immune response were mainly enriched in the green-yellow module of the events group. However, these pathways were dispersed in modules of the no-events group. Hub genes were considered to be genes which had the highest connectivity in each module (Table 2). Then, the DEG co-expression network was obtained by mapping DEGs into the whole co-expression network of the events group, and crucial genes were identified by MCODE based on the DEG co-expression network. These crucial genes were *THRAP3, RBM43, SIRT1, PEX1,* and *KLHDC2. SIRT1* had the highest connectivity among the 5 crucial genes, and the combination of 2 upregulated genes (*THRAP3* and *RBM43*) showed potential prognostic and diagnostic value.

Perisic et al. used the same dataset and analyzed the expression signature of PBMCs, and the DEGs they screened were different from the DEGs in our study. They grouped patients into a symptomatic group and an asymptomatic group. In the symptomatic group, patients already had plaque instability, which was defined as transient ischemic attack (TIA), minor stroke (MF), and amaurosis fugax (AF) [18]. However, unlike the previous study, we classified patients into an events group and a no-events group, depending on the occurrence of ischemic events during follow-up [17]. The difference in grouping patients may account for the difference in DEG screening results.

Several previous studies conducted WGCNA on expression data of atherosclerosis. Using aortic samples from Apob^{tm2Sgy}Ldlr^{tm1Her} knockout mice, Deshpande et al. discovered that inflammation and immune response might play a role in the pathogenesis and progression of atherosclerosis, and identified several related genes *(TM9SF1, LEPR, WIF1, and SP1*). In contrast to the sample Desphande et al. used, some researchers used human atherosclerotic samples from the GEO website and also found that inflammation and immune response might have important roles. Zhang et al. discovered crucial genes such as *TNPO1* and *ZDHHC17,* while Wang et al. found that a lncRNA module was associated with inflammation and immune response. However, they did not elucidate the molecular mechanism based on the expression profiling of PBMC samples, and the grouping was also different [19–22].

The gene module detection and functional enrichment analysis indicated that the co-expression patterns in the events

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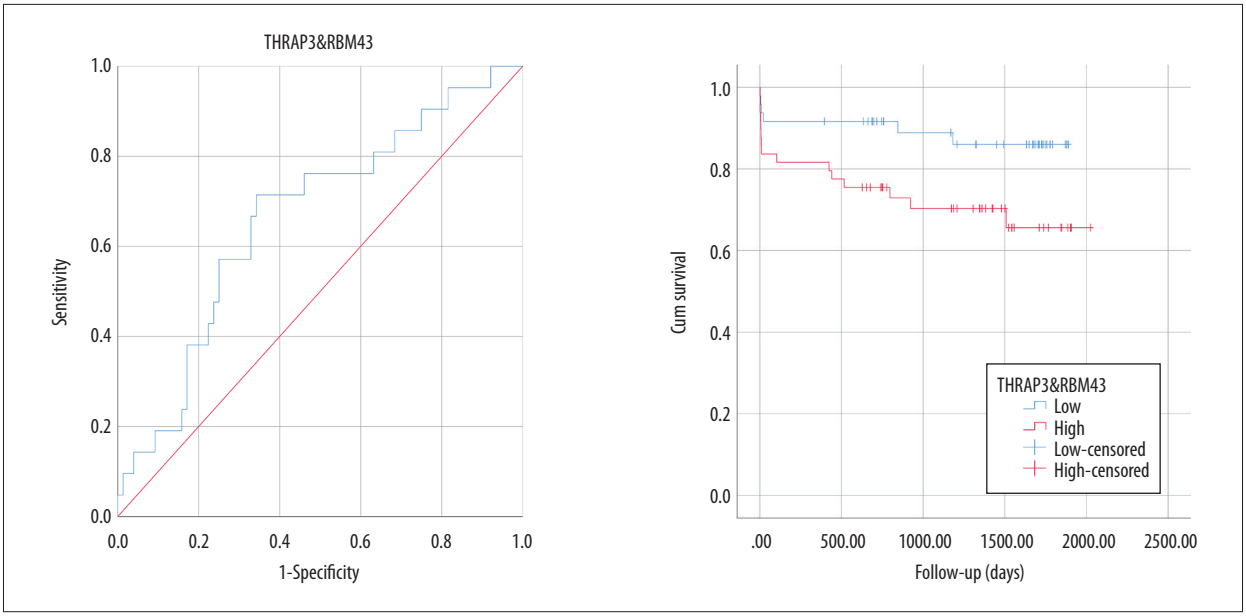

Figure 6. ROC and survival analysis of up-regulated crucial genes.

group and no-events group were different. We found that inflammation and immune response were clustered in the green-yellow module of the events group. A previous study showed that $CD14^{+}CD16^{-}$ monocyte has a proinflammatory phenotype, and increased circulating proinflammatory monocytes were observed in the atherosclerotic models of $ApoE^{-/-}$ mice [23,24]. Belge et al. also discovered that proinflammatory cytokines such as TNF-α can be produced by activated $CD14^{hi}CD16^{+}$ monocytes, which might participate in atherosclerosis progression [25]. In addition, monocytes are involved in regulation of immune response in atherosclerosis. Some tissue macrophages and dendritic cells in the lesion originated from monocytes [26,27]. Evans et al. found that T cell response can be regulated by monocytes [28]. Furthermore, the *TLR-4* expression in $CD14^{hi}CD16^{+}$ monocytes were correlated with occurrence of plaque progression and ischemic events in coronary artery disease [29]. In a recent experimental study, Bruen et al. showed that conjugated linoleic acid (CLA), which is an anti-inflammatory lipid, can induce regression of atherosclerosis in $ApoE^{-/-}$ mice. In mice fed CLA, more monocytes differentiated into anti-inflammatory M2 macrophages [30]. Sun et al. fed $ApoE^{-/-}$ model mice phenytoin, a non-selective voltage-gated sodium channels antagonist, and the mice subsequently exhibited increased levels of anti-inflammatory monocytes and decreased levels of proinflammatory monocytes [31]. Statins were also found to affect monocytes in atherosclerosis. Using samples from patients, Gasbarrino et al. discovered that intensive statins therapy can downregulate the expression of the anti-inflammatory adiponectin-AdipoR pathway in monocytes and macrophages, instead of positively regulating this pathway, which may explain part of the residual cardiovascular risk in patients using statins [32]. These studies, together with our findings, suggest that monocytes participate in the pathogenesis and progression of atherosclerosis via mediating inflammation and immune response, both directly and indirectly.

The hub gene of the green-yellow module was *KIR2DL5A*, belonging to the KIR family, and it is mainly expressed by natural killer cells and T cells. *KIR2DL5A* is an inhibitory receptor of immune response [33] and it is involved in immune response to viral infection and prognosis of certain malignant diseases. Shan et al. reported that patients with $KIR2DL5A^{-}/2DL5B^{+}$ genotype had increased HCV clearance [34]. In colorectal cancer, the presence of *KIR2DL5A* is related to increased complete response rate in patients treated with FOLFIRI chemotherapy [35], and *KIR2DL5A* is also a protective factor against breast cancer [36], while in pediatric leukemia patients after hematopoietic stem cell transplantation, the presence of *KIR2DL5A* is associated with higher relapse rate [37]. However, few studies had reported the role of *KIR2DL5A* in monocytes or its role in atherosclerosis, and it might be a promising target to elucidate the molecular mechanism for the progression of carotid atherosclerosis.

SIRT1 was the gene having the highest degree among the 5 crucial genes, and it was downregulated in the events group. *SIRT1* is a type of NAD-dependent histone deacetylase [38] and participates in regulating inflammation, apoptosis, and cell senescence [39,40]. It also plays roles in stress response, aging, and longevity [41,42]. *SIRT1* can also slow the progression of atherosclerosis by lipid modification, oxidative stress reduction, anti-inflammatory actions, foam cells, and autophagy regulation, and downregulation of *SIRT1* was observed in a atherosclerotic mouse model [43], which is consistent with

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our findings. Recently, Lee et al. discovered that *SIRT1* inhibits the adhesion of monocytes to vascular endothelia cells by suppressing *MAC-1* expression in monocytes [44]. In addition, Nguyen et al. discovered that a dipeptidyl peptidase 4 inhibitor, evogliptin, can inhibit monocytes adhesion to vascular endothelial cells in an ApoE$^{-/-}$ mouse model, and this effect is associated with regulation of *NF-κB* by *SIRT1* [45]. Therefore, *SIRT1* might also slow the progression of atherosclerosis by preventing monocytes adhesion, which is the one of the initiation steps in the pathogenesis of atherosclerosis.

The upregulated genes, *THRAP3* and *RBM43*, showed potential diagnostic and prognostic value. *THRAP3*, thyroid hormone receptor-associated protein 3, is an RNA-processing factors and can also participate in the DNA damage response (DDR) pathway and transcription regulation [46–49]. Mutations in *THRAP3* may cause DNA damage repair defects, and Vohhodina reported that loss of *THRAP3* made 293T and U2OS cells more susceptible to DNA-damaging factors [49]. Ino et al. used LNCaP and LNCaP-AI prostate cancer cell lines to demonstrate that *THRAP3* phosphorylation can contribute to the acquisition of androgen independence in prostate cancer via transcriptional regulation [48]. Another study, using high-fat-fed mice, found that *THRAP3* can act as a transcriptional regulator in diabetes and can control diabetic gene programming [47]. *RBM43* is an RNA binding motif protein 43 and its detailed biological function is not known. At present, it is unclear whether *THRAP3* and *RBM43* participates the pathogenesis of atherosclerosis, although they were found to have potential clinical significance for the occurrence of ischemic events in carotid atherosclerosis patients.

In the present study, for the first time, we constructed a co-expression network, detected genes modules, and identified hub genes and crucial genes in carotid atherosclerosis using PBMC expression data. However, datasets in GEO lack clinical information; therefore, it is difficult to correlate traits with clinical importance with gene modules in WGCNA analysis.

The events group and no-events group had different co-expression patterns, and these differences suggest that monocytes are of vital importance in the pathogenesis and progression of carotid atherosclerosis via mediating inflammation and immune response. Then, we identified hub genes and crucial genes, which might have crucial biological functions in the pathogenesis of carotid atherosclerosis or potential diagnostic and prognostic value for ischemic events.

Conclusions

We detected 8 modules for the events group and 6 modules for the no-events group. The hub genes for each module and crucial genes of the DEG co-expression network were also identified. These genes might serve as potential targets for medical therapies and as biomarkers for diagnosis and prognosis. Further mechanism studies are needed to explore the biological function of these genes in the pathogenesis and progression of carotid atherosclerosis.

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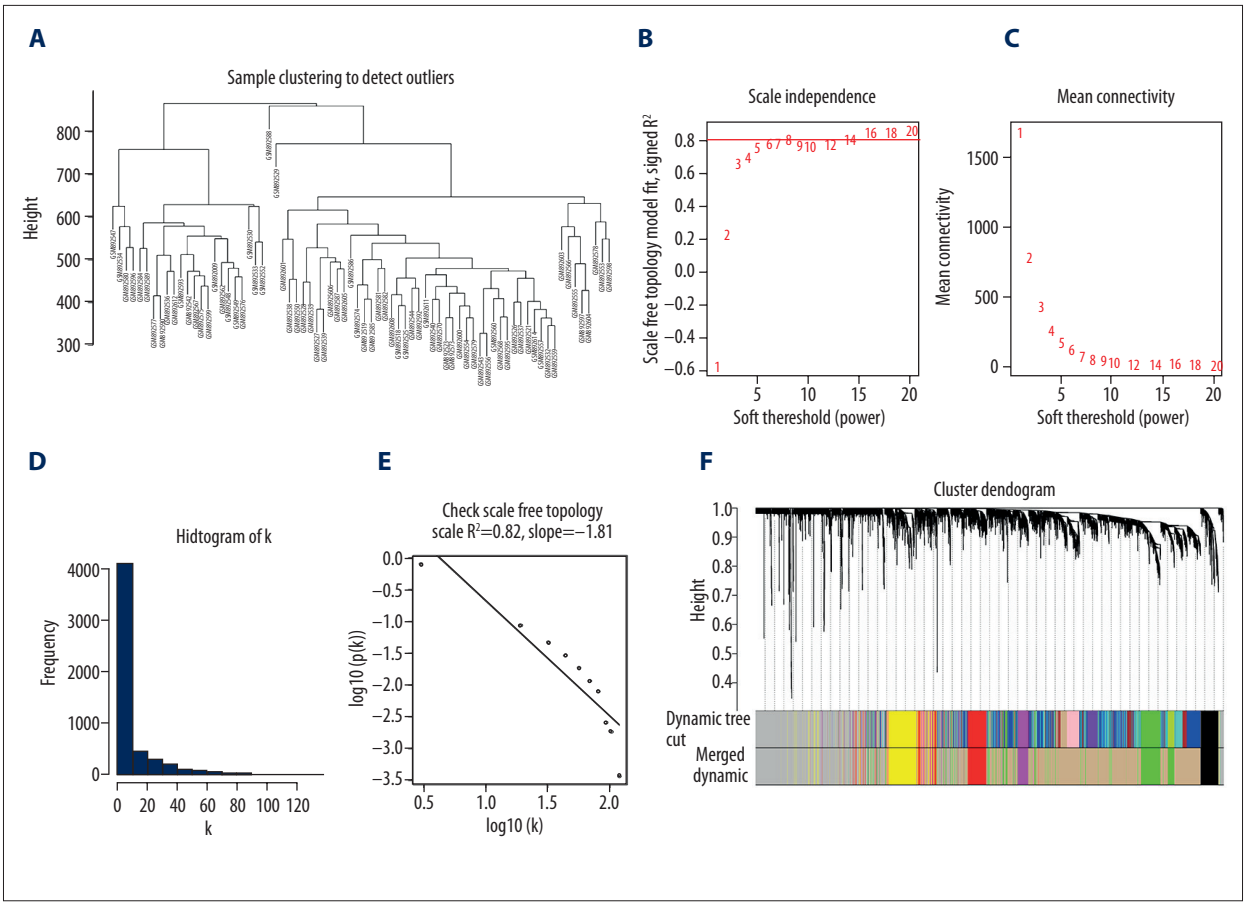
Supplementary Data



Supplementary Figure 1. WGCNA of no-event. (**A**) No outlier was detected by sample clustering. (**B, C**) Selection of soft-threshold β. (**D, E**) Fitness of scale free topology when β-16. (**F**) Cluster dendrogram. Each module was represented by WGCNA.

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Supplementary Table 1. Top10 up-regulated and down-regulated DEGs.

Gene symbol	Official full gene name	log2 (fold-change) (patients with events/patients without events)
Up-regulated		
TNFAIP6	TNF alpha induced protein 6	9.968020902
PTX3	Pentraxin 3	8.826641354
RNASE2	Ribonuclease A family member 2	7.978917622
KCNJ2	Potassium inwardly rectifying channel subfamily J member 2	7.481378497
SERPINB2	Serpin family B member 2	7.18837765
PLA2G7	Phospholipase A2 group VII	6.901998631
BCL2A1	BCL2 related protein A1	6.719719825
CLEC4D	C-type lectin domain family 4 member D	6.272368641
SAMSN1	SAM domain, SH3 domain and nuclear localization signals 1	6.209244405
GPR84	G protein-coupled receptor 84	5.186451434
Down-regulated		
KLRC3	Killer cell lectin like receptor C3	–7.246559422
BTN3A2	Butyrophilin subfamily 3 member A2	–7.003853494
ANKRD20A11P	Ankyrin repeat domain 20 family member A11, pseudogene	–6.02418399
ZNF600	Zinc finger protein 600	–5.985685983
NLRC3	NLR family CARD domain containing 3	–5.579931019
LCK	LCK proto-oncogene, Src family tyrosine kinase	–4.825468373
GOLGA8N	Golgin A8 family member N	–4.799854266
CEP78	Centrosomal protein 78	–4.795917474
SLC9A3R1	SLC9A3 regulator 1	–4.381636674
SEP1	Septin 1	–4.097540674

Supplemenatry Table 2. GO-BP terms for modules of events group and no events group.

Supplementary/raw data available from the corresponding author on request.

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Supplemenatry Table 3. KEGG pathways for modules of events group and no-events group.

Events group	KEGG ID	KEGG pathway	Count	-logP	No-events group	KEGG ID	KEGG pathway	Count	-logP
	Black					**Black**			
	hsa05034	Alcoholism	14	4.58		hsa04611	Platelet activation	11	5.10
	hsa05322	Systemic lupus erythematosus	13	5.13		hsa05322	Systemic lupus erythematosus	11	4.98
	hsa04611	Platelet activation	11	3.80		hsa05034	Alcoholism	11	3.94
	hsa05203	Viral carcinogenesis	10	1.82		hsa04512	ECM-receptor interaction	8	3.83
	hsa05202	Transcriptional misregulation in cancer	9	1.87		hsa05203	Viral carcinogenesis	8	1.71
	hsa04062	Chemokine signaling pathway	9	1.62		hsa04510	Focal adhesion	8	1.70
	hsa04512	ECM-receptor interaction	6	1.62		hsa04810	Regulation of actin cytoskeleton	8	1.66
	hsa04540	Gap junction	6	1.60		hsa04540	Gap junction	6	2.20
	hsa05219	Bladder cancer	4	1.38		hsa04670	Leukocyte transendothelial migration	6	1.73
	Blue					hsa05410	Hypertrophic cardiomyopathy (HCM)	5	1.70
	hsa01100	Metabolic pathways	218	3.72		hsa05414	Dilated cardiomyopathy	5	1.59
	hsa05166	HTLV-I infection	49	1.56		hsa04640	Hematopoietic cell lineage	5	1.54
	hsa04144	Endocytosis	48	1.76		hsa04530	Tight junction	5	1.54
	hsa04141	Protein processing in endoplasmic reticulum	45	4.34		hsa05130	Pathogenic Escherichia coli infection	4	1.52
	hsa01130	Biosynthesis of antibiotics	45	2.15		hsa00590	Arachidonic acid metabolism	4	1.32
	hsa05016	Huntington's disease	38	1.42		**Green**			
	hsa04932	Non-alcoholic fatty liver disease (NAFLD)	36	2.60		hsa04151	PI3K-Akt signaling pathway	16	1.30
	hsa05168	Herpes simplex infection	36	1.33		hsa04144	Endocytosis	15	2.22
	hsa00190	Oxidative phosphorylation	31	2.13		hsa04141	Protein processing in endoplasmic reticulum	13	2.66
	hsa04110	Cell cycle	30	2.30		hsa05166	HTLV-I infection	13	1.35
	hsa04380	Osteoclast differentiation	30	1.96		hsa04510	Focal adhesion	12	1.60
	hsa03040	Spliceosome	30	1.87		hsa04380	Osteoclast differentiation	11	2.52
	hsa05012	Parkinson's disease	30	1.51		hsa04640	Hematopoietic cell lineage	9	2.61
	hsa05161	Hepatitis B	30	1.40		hsa04722	Neurotrophin signaling pathway	9	1.78
	hsa04142	Lysosome	27	1.65		hsa05220	Chronic myeloid leukemia	8	2.48
	hsa00240	Pyrimidine metabolism	25	2.09		hsa05230	Central carbon metabolism in cancer	7	2.11
	hsa01200	Carbon metabolism	25	1.51		hsa05212	Pancreatic cancer	7	2.08
	hsa04660	T cell receptor signaling pathway	23	1.58		hsa05100	Bacterial invasion of epithelial cells	7	1.71
	hsa05132	Salmonella infection	22	2.21		hsa05132	Salmonella infection	7	1.59
	hsa05323	Rheumatoid arthritis	20	1.36		hsa04210	Apoptosis	6	1.57
	hsa03018	RNA degradation	19	1.62		hsa04662	B cell receptor signaling pathway	6	1.40
	hsa04210	Apoptosis	18	2.26		hsa04962	Vasopressin-regulated water reabsorption	5	1.50
	hsa05131	Shigellosis	18	2.11		hsa00510	N-Glycan biosynthesis	5	1.36
	hsa00510	N-Glycan biosynthesis	16	2.54		magenta			

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Events group	KEGG ID	KEGG pathway	Count	-logP	No-events group	KEGG ID	KEGG pathway	Count	-logP
	hsa05221	Acute myeloid leukemia	15	1.60		hsa04670	Leukocyte transendothelial migration	7	2.60
	hsa05134	Legionellosis	14	1.39		hsa04142	Lysosome	7	2.48
	hsa00280	Valine, leucine and isoleucine degradation	13	1.50		hsa04810	Regulation of actin cytoskeleton	7	1.39
	hsa00520	Amino sugar and nucleotide sugar metabolism	13	1.43		hsa04015	Rap1 signaling pathway	7	1.39
	hsa05340	Primary immunodeficiency	12	2.19		hsa03008	Ribosome biogenesis in eukaryotes	6	2.41
	hsa00071	Fatty acid degradation	12	1.49		hsa05131	Shigellosis	5	2.14
	hsa00640	Propanoate metabolism	10	1.86		hsa04520	Adherens junction	5	1.98
	hsa03060	Protein export	9	1.92		hsa05100	Bacterial invasion of epithelial cells	5	1.84
	Green					hsa05132	Salmonella infection	5	1.75
	hsa05166	HTLV-I infection	9	1.36		hsa01200	Carbon metabolism	5	1.33
	hsa03040	Spliceosome	8	2.34		hsa04710	Circadian rhythm	4	2.22
	hsa05010	Alzheimer's disease	8	1.81		hsa05130	Pathogenic Escherichia coli infection	4	1.63
	hsa04110	Cell cycle	6	1.36		hsa04621	NOD-like receptor signaling pathway	4	1.53
	hsa00310	Lysine degradation	5	2.07		**Red**			
	hsa04115	p53 signaling pathway	5	1.70		hsa01100	Metabolic pathways	36	3.34
	Greenyellow					hsa05010	Alzheimer's disease	13	4.71
	hsa04650	Natural killer cell mediated cytotoxicity	19	14.53		hsa05016	Huntington's disease	13	4.14
	hsa04612	Antigen processing and presentation	15	12.70		hsa00190	Oxidative phosphorylation	12	4.96
	hsa04060	Cytokine-cytokine receptor interaction	11	3.16		hsa05012	Parkinson's disease	12	4.69
	hsa04062	Chemokine signaling pathway	7	1.59		hsa04932	Non-alcoholic fatty liver disease (NAFLD)	9	2.46
	hsa05142	Chagas disease (American trypanosomiasis)	5	1.42		hsa03010	Ribosome	8	2.14
	hsa05332	Graft-versus-host disease	4	2.13		hsa03050	Proteasome	4	1.44
	hsa05330	Allograft rejection	4	1.99		hsa00520	Amino sugar and nucleotide sugar metabolism	4	1.35
	hsa04940	Type I diabetes mellitus	4	1.84		**Tan**			
	hsa05321	Inflammatory bowel disease (IBD)	4	1.37		hsa01100	Metabolic pathways	147	2.23
	Magenta					hsa04120	Ubiquitin mediated proteolysis	36	7.03
	hsa04010	MAPK signaling pathway	7	1.45		hsa03013	RNA transport	33	3.48
	hsa04664	Fc epsilon RI signaling pathway	4	1.55		hsa04141	Protein processing in endoplasmic reticulum	30	2.64
	Pink					hsa03040	Spliceosome	25	2.57
	hsa05168	Herpes simplex infection	8	1.91		hsa04110	Cell cycle	22	1.99
	hsa04931	Insulin resistance	6	1,80		hsa03018	RNA degradation	21	4,38
	hsa04145	Phagosome	7	1,78		hsa05161	Hepatitis B	21	1,09
	hsa00190	Oxidative phosphorylation	6	1,46		hsa04114	Oocyte meiosis	18	1,33
	Red					hsa03015	mRNA surveillance pathway	17	1,78

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Events group	KEGG ID	KEGG pathway	Count	-logP	No-events group	KEGG ID	KEGG pathway	Count	-logP
	hsa01100	Metabolic pathways	37	2.07		hsa04070	Phosphatidylinositol signaling system	17	1.50
	hsa04114	Oocyte meiosis	8	2.17		hsa04668	TNF signaling pathway	17	1.20
	hsa04120	Ubiquitin mediated proteolysis	8	1.70		hsa04066	HIF-1 signaling pathway	15	1.04
	hsa04668	TNF signaling pathway	7	1.69		hsa04720	Long-term potentiation	14	1.93
	hsa01200	Carbon metabolism	7	1.59		hsa04115	p53 signaling pathway	13	1.51
	hsa04722	Neurotrophin signaling pathway	7	1.48		hsa05120	Epithelial cell signaling in Helicobacter pylori infection	13	1.51
	hsa04666	Fc gamma R-mediated phagocytosis	6	1.58		hsa04210	Apoptosis	12	1.40
	hsa05230	Central carbon metabolism in cancer	5	1.41		hsa00562	Inositol phosphate metabolism	12	1.04
	hsa05211	Renal cell carcinoma	5	1.37		hsa00520	Amino sugar and nucleotide sugar metabolism	11	1.75
	hsa00010	Glycolysis / Gluconeogenesis	5	1.35		hsa05130	Pathogenic Escherichia coli infection	11	1.58
	hsa04662	B cell receptor signaling pathway	5	1.31		hsa05110	Vibrio cholerae infection	11	1.52
	hsa00512	Mucin type O-Glycan biosynthesis	4	1.63		hsa00510	N-Glycan biosynthesis	10	1.30
	hsa00620	Pyruvate metabolism	4	1.34		hsa00280	Valine, leucine and isoleucine degradation	9	1.05
	Yellow					hsa03420	Nucleotide excision repair	9	1.05
	hsa05152	Tuberculosis	21	4.97		hsa03060	Protein export	8	2.26
	hsa04142	Lysosome	19	6.25		hsa03430	Mismatch repair	6	1.15
	hsa04145	Phagosome	19	4.88		**Yellow**			
	hsa05164	Influenza A	17	3.05		hsa05166	HTLV-I infection	16	2.96
	hsa05166	HTLV-I infection	17	1.50		hsa05152	Tuberculosis	11	2.00
	hsa04380	Osteoclast differentiation	16	3.92		hsa04010	MAPK signaling pathway	11	1.08
	hsa05162	Measles	15	3.31		hsa04145	Phagosome	10	2.01
	hsa01130	Biosynthesis of antibiotics	15	1.52		hsa05168	Herpes simplex infection	10	1.50
	hsa04640	Hematopoietic cell lineage	14	4.68		hsa05203	Viral carcinogenesis	10	1.24
	hsa05140	Leishmaniasis	13	4.92		hsa05161	Hepatitis B	9	1.64
	hsa05323	Rheumatoid arthritis	13	3.96		hsa05164	Influenza A	9	1.24
	hsa05145	Toxoplasmosis	12	2.54		hsa05140	Leishmaniasis	8	2.83
	hsa04064	NF-kappa B signaling pathway	11	2.80		hsa04660	T cell receptor signaling pathway	8	2.00
	hsa05150	*Staphylococcus aureus* infection	10	3.77		hsa05169	Epstein-Barr virus infection	8	1.57
	hsa04066	HIF-1 signaling pathway	10	1.99		hsa05162	Measles	8	1.39
	hsa04620	Toll-like receptor signaling pathway	10	1.72		hsa04612	Antigen processing and presentation	7	2.02
	hsa04612	Antigen processing and presentation	9	2.11		hsa05145	Toxoplasmosis	7	1.32
	hsa04666	Fc gamma R-mediated phagocytosis	9	1.86		hsa03040	Spliceosome	7	1.00
	hsa04660	T cell receptor signaling pathway	9	1.45		hsa05332	Graft-versus-host disease	6	2.99
	hsa04672	Intestinal immune network for IgA production	8	2.75		hsa05330	Allograft rejection	6	2.76

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Events group	KEGG ID	KEGG pathway	Count	-logP	No-events group	KEGG ID	KEGG pathway	Count	-logP
	hsa05134	Legionellosis	8	2.40		hsa04940	Type I diabetes mellitus	6	2.51
	hsa05321	Inflammatory bowel disease (IBD)	8	1.99		hsa03050	Proteasome	6	2.42
	hsa01230	Biosynthesis of amino acids	8	1.73		hsa05320	Autoimmune thyroid disease	6	2.11
	hsa05133	Pertussis	8	1.64		hsa05416	Viral myocarditis	6	1.94
	hsa05204	Chemical carcinogenesis	8	1.50		hsa05323	Rheumatoid arthritis	6	1.22
	hsa00480	Glutathione metabolism	7	1.92		hsa05310	Asthma	5	2.27
	hsa05416	Viral myocarditis	7	1.69		hsa04672	Intestinal immune network for IgA production	5	1.59
	hsa05310	Asthma	6	2.31		hsa05223	Non-small cell lung cancer	5	1.35
	hsa05332	Graft-versus-host disease	5	1.46		hsa05321	Inflammatory bowel disease (IBD)	5	1.17
	hsa00920	Sulfur metabolism	4	2.42		hsa04662	B cell receptor signaling pathway	5	1.08
	hsa00511	Other glycan degradation	4	1.54		hsa01230	Biosynthesis of amino acids	5	1.03
						hsa03022	Basal transcription factors	4	1.03

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