MEDICAL SCIENCE MONITOR

LAB/IN VITRO RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2018; 24: 1533-1539
DOI: 10.12659/MSM.905970

Received: 2017.06.29
Accepted: 2017.08.14
Published: 2018.03.14

Exploring the Molecular Mechanism of Thoracic Aortic Aneurysm via Bioinformatics Analysis

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

ABCDEFG 1 Hongfang Li*
ABCDEFG 2 Yuzhi Zhen*
BCDE 3 Yunshuang Geng
BCDE 1 Junyan Feng
BCDF 1 Jun Wang
ACDEFG 4 Hongsong Zhang

1 Department of Cardiac Surgery, The First Hospital of Hebei Medical University, Hebei, Shijiazhuang, P.R. China
2 Department of Cardiovascular Medicine, The First Hospital of Hebei Medical University, Hebei, Shijiazhuang, P.R. China
3 Department of Pediatrics, Ningjin County Hospital of Hebei, Hebei, Shijiazhuang, P.R. China
4 Department of Vascular Surgery, The First Hospital of Hebei Medical University, Hebei, Shijiazhuang, P.R. China

* Hongfang Li and Yuzhi Zhen contributed to this equally

Corresponding Author: Hongsong Zhang, e-mail: 13903323813@163.com
Source of support: Departmental sources

Background: The aim of this study was to identify some key genes related to the pathogenesis of thoracic aortic aneurysm (TAA) and gain more insights to the molecular mechanism of TAA.

Material/Methods: The expression profile of GSE9106 was downloaded from the Gene Expression Omnibus (GEO) database. The data contained 58 TAA peripheral blood samples and 36 normal peripheral blood samples. The differently expressed genes (DEGs) between the TAA samples and the normal samples were identified via limma package of *R*. Functional enrichment analysis of the DEGs were performed via the Database for Annotation, Visualization and Integrated Discovery (DAVID). The differentially co-expressed genes in TAA samples compared to normal samples were identified via the DCGL package in *R*. The protein-protein interaction (PPI) network of the DEGs was constructed through the Search Tool for the Retrieval of Interacting Proteins (STARING) database and visualized by Cytoscape software.

Results: A total of 407 DEGs were obtained in TAA samples compared with normal samples. The DEGs were enriched in 29 Gene Ontology (GO) terms. There were 1,441 co-expression gene pairs that had significant changes in the co-expression status in TAA samples compared with normal samples and a differential co-expression network was constructed based on them. Moreover, a PPI network of the DEGs was constructed, containing 101 nodes.

Conclusions: Bioinformatics methods could identify significant biological processes and genes related to TAA. *KRTDAP, BICD1*, and genes in the OR family might play an important role in TAA.

MeSH Keywords: Gene Expression Profiling • Molecular Biology • Transcriptome

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/905970

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Background

Thoracic aortic aneurysms (TAAs) are localized dilatations of the supra-diaphragmatic aorta that result from weakening and expansion of the arterial wall [1]. Many cases remain undetected because TAAs are usually asymptomatic and indolent until complications such as aortic dissection or rupture occurs [2]. In the treatment of TAA, pharmacological treatments have not shown consistent and significant improvements in clinical outcomes [3]. In spite of advances in surgical care for TAA, surgical risks remain high. Patients surviving surgery are at risk of significant postoperative morbidity due to myocardial infarction, renal failure, stroke, neurological deficits, and paraplegia [1]. Therefore, it is crucial to have a better understanding of the pathogenesis of TAA to guide early diagnosis, predict prognosis, as well as develop new therapeutic approaches.

Hereditary factors play an important etiologic role in TAA. There has been evidence reported that suggests that patients with specific genetic mutations require earlier interventions at smaller aortic sizes [4]. A number of genes have been proven to be associated with TAA. For example, *FBN1* gene mutation was shown to cause Marfan syndrome [5]. *BRG1* was reported to be overexpressed in the aortic media of TAA, and the interaction between *BRG1* and HIF 1 alpha-antisense RNA 1 played a key role in the proliferation and apoptosis of vascular smooth muscle cells, which may contribute to the pathogenesis of TAA [6]. TGFBR2 mutations were reported to be associated with both syndromal and non-syndromal TAA, and identification of novel TGFBR2 mutations, such as S553T, in non-syndromal TAA might have meaningful clinical implications for patients with TAA [7]. However, the molecular mechanism of pathogenesis is not completely clear.

Gene microarray is an effective technology that allows for detecting gene expression in cells and tissues on a genome-wide scale. Through bioinformatics analysis, new signaling pathways or key genes associated with tumor-genesis can be discovered [8].

In this study, different expressed genes in TAA samples compared with normal samples were identified via bioinformatics methods. Through functional enrichment analysis, as well as the construction of differential co-expression networks and protein-protein interaction (PPI) network, a better understanding of the molecular mechanism of TAA was obtained.

Material and Methods

Microarray data

Gene Expression Omnibus (GEO; *http://www.ncbi.nlm.nih.gov/geo/*) database is an international public repository for high-throughput microarray and next-generation sequence functional genomic data sets. The gene expression profile of GSE9106 was downloaded from the GEO database. Whole Genome gene expression profiles from a total of 94 peripheral blood samples (collected from 58 individuals diagnosed with TAA and 36 normal controls) were analyzed in this study.

Data processing and identification of differentially expressed genes

The raw data with the CEL format was normalized and log2 transformed via the preprocessCore package (*http://www.bioconductor.org/packages/release/bioc/html/preprocessCore.html*), and the expression value matrix was obtained with the gene as the row and the sample as the column. Afterwards, the limma [9] package of *R* was used to identify the differentially expressed genes (DEGs) in TAA samples compared with normal samples (*http://bioconductor.org/packages/release/bioc/html/limma.html*). The DEGs were selected with the threshold of $|\log2(\text{fold change})|>1$ and $p<0.05$.

Functional enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID) is a web-accessible program that integrates functional genomic annotations with intuitive graphical summaries (*https://david.ncifcrf.gov/*) [10]. To further explore the biofunctions of the DEGs, Gene Ontology (GO) enrichment analysis of the DEGs was performed with DAVID according to the criteria: $p<0.05$.

Construction of the differential co-expression gene network

DEGs which had similar expression profiles were included in the differentially co-expressed genes analysis to investigate the potential mechanism of TAA. The differentially co-expressed genes in TAA samples compared to normal samples were identified via the DCGL package in *R*. Afterwards, The differential co-expression gene network was constructed based on the Cytoscape software.

Construction of PPI network

Gene interactions play a key role in disease progression. Search Tool for the Retrieval of Interacting Genes (STRING) (*http://string-db.org/*) was an online database for predicting functional interactions between proteins [11,12]. To further explore the regulatory mechanism of the DEGs in TAA, the interactions of the DEGs were screened out in the STRING database with the threshold of combined score >0.4. The PPI network of the DEGs was constructed based on the STRING database and visualized using Cytoscape package.

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Table 1. The top 20 DEGs in TAA samples compared with normal samples.

Gene name	*P* value	LogFC
IL18R1	3.16×10^{-05}	0.502265
FGB	3.33×10^{-05}	–0.73658
ENPP4	3.37×10^{-05}	0.785024
TGM5	3.45×10^{-05}	0.922215
SYNGAP1	4.30×10^{-05}	0.707436
OR5B21	4.43×10^{-05}	0.659949
RASSF3	5.75×10^{-05}	0.51418
PCDHA5	6.20×10^{-05}	0.615558
R3HDML	6.60×10^{-05}	1.025435
CCL24	7.04×10^{-05}	0.694118
SERPINB10	9.07×10^{-05}	0.84035
NR1I2	9.42×10^{-05}	0.565695
ZDHHC15	9.60×10^{-05}	0.720328
OR4X1	9.92×10^{-05}	0.825982
PIF1	1.00×10^{-04}	0.692451
EPPIN	1.12×10^{-04}	0.812233
IFNK	1.15×10^{-04}	0.792714
ARPIN	1.28×10^{-04}	0.513909
ELF5	1.33×10^{-04}	–0.58356
ELSPBP1	1.45×10^{-04}	0.542693

DEGs – differentially expressed genes; FC – fold change; TAA – thoracic aortic aneurysm.

Results

The DEGs

A total of 407 DEGs were obtained in TAA samples compared with normal samples, including 313 upregulated and 94 downregulated DEGs. The top 20 DEGs are listed in Table 1.

Enriched GO terms

The DEGs were enriched in 29 GO terms. The top 10 enriched GO terms for the DEGs are shown in Figure 1. The top four enriched GO terms were water transporter activity, plasma membrane, neurological system process, and water transport according to *p* values.

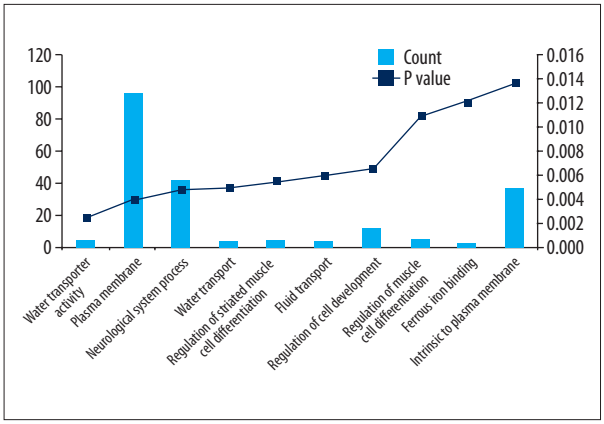

Figure 1. The top 10 enriched Gene Ontology terms for the differently expressed genes.

The differential co-expression gene network

A total of 1,441 co-expression gene pairs had significant changes in the co-expression status in TAA samples compared with normal samples. Based on these pairs, a differential co-expression network was constructed (Figure 2). The top 20 genes in the co-expression network with higher degree are listed in Table 2.

The PPI network

A total of 147 interaction pairs of the DEGs were identified via the STRING database. A PPI network of the DEGs was constructed based on these pairs, containing 101 nodes (Figure 3). The top 10 pairs of the PPI network are listed in Table 3. Table 4 shows the top 20 nodes of the PPI network according to the degree.

Discussion

TAA is a serious threat to human health. The formation and expansion of TAA result from many factors. Hereditary factors play an important etiologic role in TAA [13]. However, the molecular mechanism of TAA is still not completely clear. In this study, bioinformatics methods were used to identify key genes and biological processes involved in TAA. The results might help us have a better understanding of TAA.

The top four enriched GO terms were water transporter activity, plasma membrane, neurological system process, and water transport. These processes play critical roles in cardiovascular disease. The cell membrane (also known as the plasma membrane) is a biological membrane that separates the interior of all cells from the outside environment [14]. Alterations in the plasma membrane fluidity have been associated with various diseases. One research study demonstrated that erythrocyte membrane fluidity was decreased in essential hypertension and

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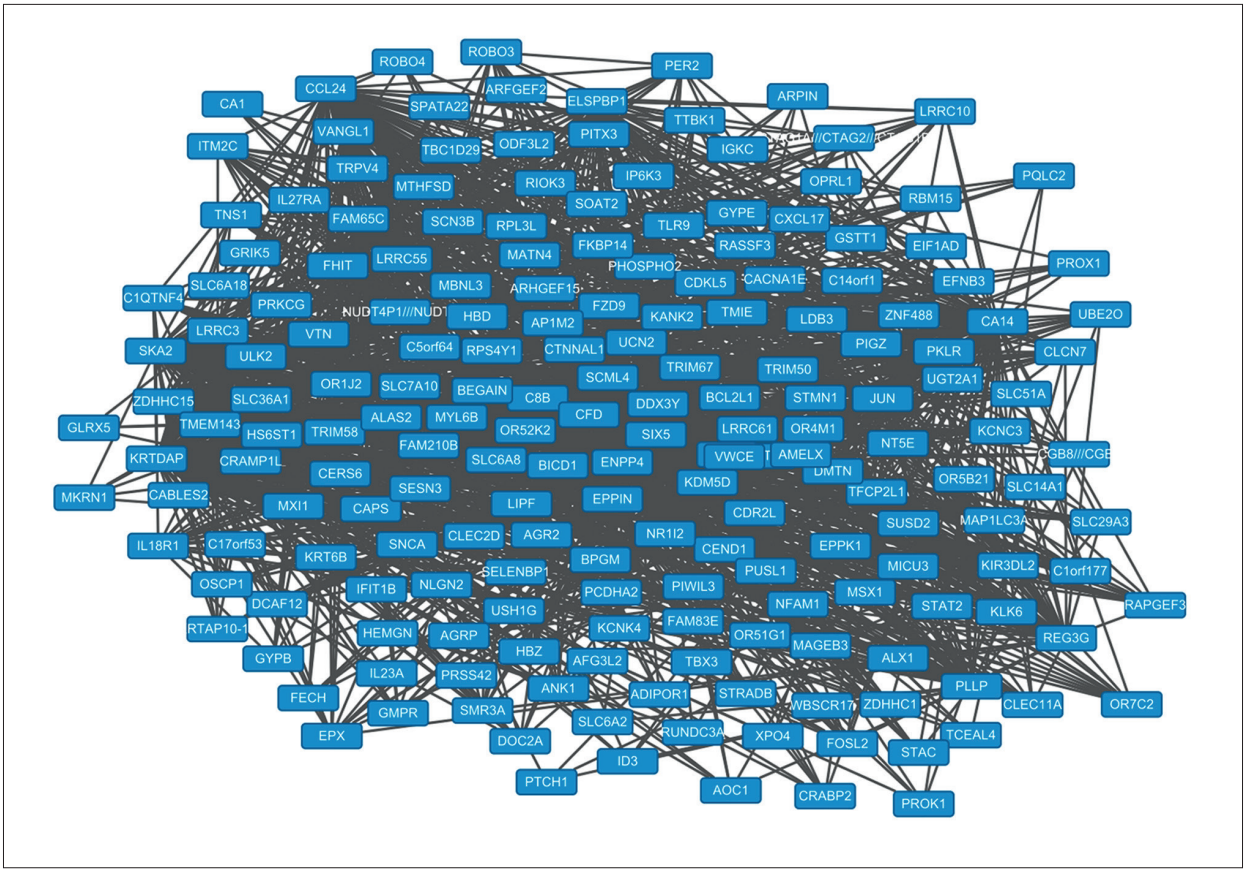

Figure 2. The differential co-expression network for the co-expression gene pairs in thoracic aortic aneurysm samples compared to normal samples.

Table 2. The top 20 genes in the co-expression network.

Gene	Degree	Gene	Degree
KRTDAP	122	JUN	63
CA14	107	FAM65C	59
TMEM143	94	PLLP	58
BICD1	91	REG3G	49
CCL24	90	KIR3DL2	48
SKA2	82	DDX3Y	43
SIX5	80	EPPIN	42
ENPP4	77	TFCP2L1	42
ZDHHC15	71	KDM5D	38
ELSPBP1	63	RPS4Y1	36

coronary artery disease patients [15]. These diseases appeared to be significant risk factors for development of arterial aneurysms [16]. There were also some relationships reported between neurological system process and arterial aneurysm. One study reported that cerebral berry aneurysm was associated with central nervous system lupus [17]. Another study reported that multiple aneurysms usually had manifestations of central nervous system abnormality [18]. Furthermore, neurohumoral systems were reported to be essential in vascular homeostasis. Through the modification of the neuronal discharge

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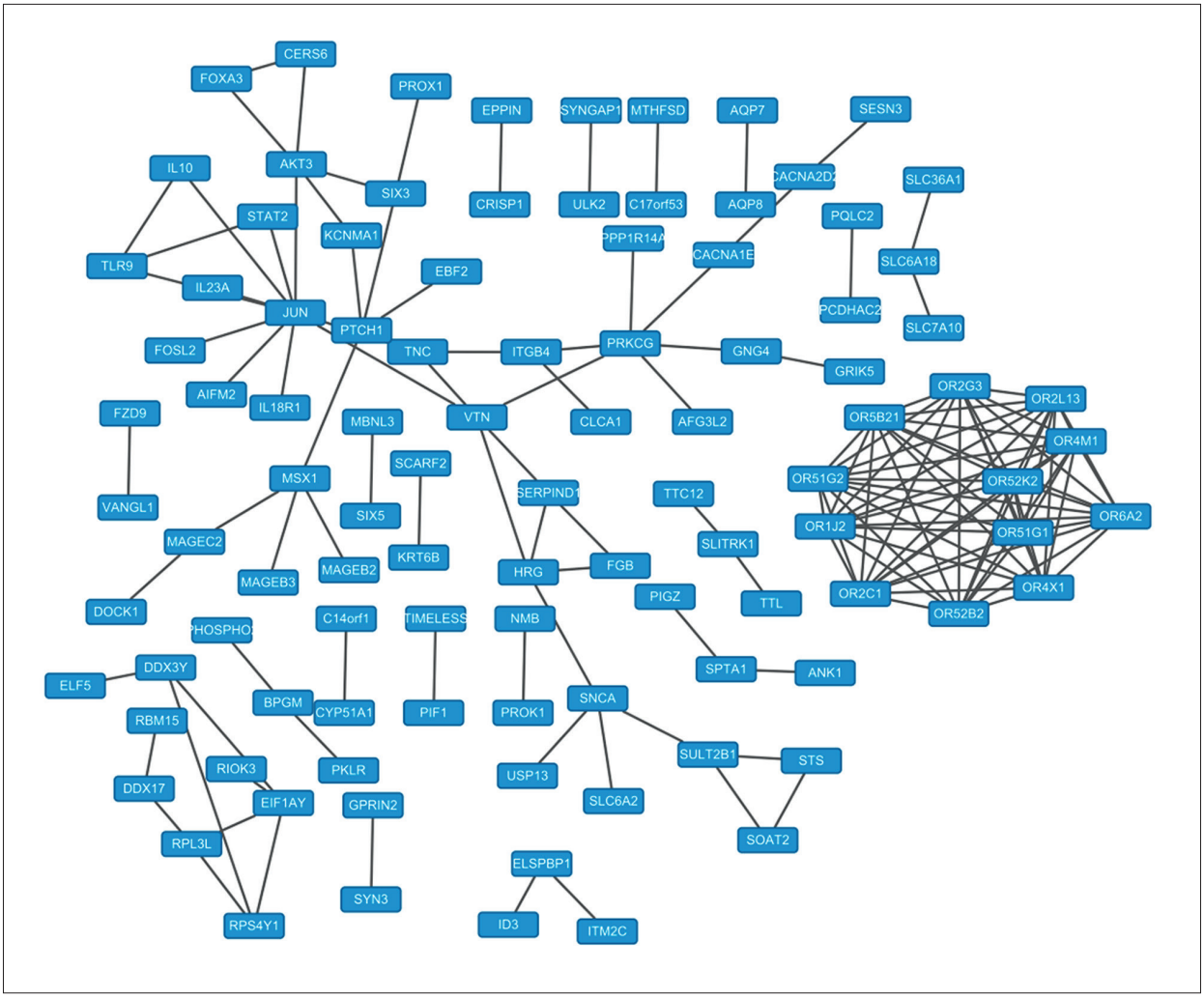

Figure 3. The protein-protein interaction network for the differential expression genes.

Table 3. The top 10 pairs of the PPI network with high combined score.

Gene1	Gene2	Combined score
JUN	FOSL2	0.999
RPL3L	RPS4Y1	0.999
SPTA1	ANK1	0.99
IL10	JUN	0.984
JUN	IL18R1	0.919
JUN	IL23A	0.917
OR6A2	OR2C1	0.904
STS	SULT2B1	0.903
OR52B2	OR2L13	0.903
OR52B2	OR6A2	0.902

PPI – protein-protein interaction.

or changes in circulating catecholamines, the autonomic nervous system induced central or local vasomotor alterations and participated in the control of the internal environment and homeostasis [19]. Dysregulation in vascular homeostasis could induce a series of vascular diseases, such as aortic aneurysm [20]. Water transport and water transporter activity are essential to maintain water balance [21]. The changes in body water could lead to various diseases. Vascular water balance is essential to maintain blood pressure and local blood flow, which plays critical roles in vascular homeostasis [22,23]. The relationship between vascular homeostasis and aortic aneurysm has been discussed above.

In the differential co-expression network, the top four genes according to degree were *KRTDAP, CA14, TMEM143*, and *BICD1*. *KRTDAP* was associated with epithelial differentiation. As a kind of epithelial, the arterial endothelium exquisitely regulates vascular function. Endothelial dysfunction plays a critical role in the development of atherosclerosis, which is one of

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Table 4. The top 20 nodes in the PPI net work.

Gene	Degree
OR1J2	11
OR2C1	11
OR2G3	11
OR2L13	11
OR4M1	11
OR4X1	11
OR51G1	11
OR51G2	11
OR52B2	11
OR52K2	11
OR5B21	11
OR6A2	11
JUN	10
PRKCG	6
AKT3	5
VTN	5
EIF1AY	4
HRG	4
MSX1	4
PTCH1	4

PPI – protein-protein interaction.

the key factors that could lead to arterial aneurysm [24,25]. Bicaudal-D1 (BICD1) is an α-helical coiled-coil protein [26]. Research has demonstrated that regulatory SNP of the *BICD1* gene could contribute to telomere length variation in humans [27]. Telomeres were repetitive sequences of variable length at the ends of chromosomes involved in maintaining their integrity [28,29]. Short relative telomere length was reported to be associated with vascular aging, inflammation, and cardiovascular risk factors [30–32]. Furthermore, the relationship between abdominal aortic aneurysm and short relative telomere length had been reported. One study found a significantly shorter telomere length among aortic aneurysm patients compared to controls [33]. However, few studies reported the relationship between *CA14* and *TMEM143*, and the pathogenesis of arterial aneurysm. Their function in aneurysm development needs to be further explored.

The top 10 nodes of the PPI network were *OR1J2*, *OR2C1*, *OR2LG3*, *OR2L13*, *OR4M1*, *OR4X1*, *OR51G1*, *OR51G2*, *OR52B2*, and *OR52K2*. They all belonged to the olfactory receptor (OR) family. The olfactory receptor proteins were members of a large family of G-protein-coupled receptors (GPCR) arising from single coding-exon genes [34,35]. ORs are expressed not only in the sensory neurons of the olfactory epithelium, but also in various other tissues and play different roles [36]. There were some studies that reported the relationship between OR proteins and vessels, as well as vascular disease. One research demonstrated that both OR proteins and GPCRs were expressed in smooth muscle cells of small resistance vessels, and the expression of OR proteins could also modulate blood pressure [37]. Another research reported that OR proteins and GPCRs were expressed in neutrophils and sympathetic ganglia [38]. The relationship between the neurological system and vascular disease was discussed earlier in this report. There have also been studies that reported that OR proteins could function as receptors for short chain fatty acids (SCFAs), and SCFAs could induce vasodilation in both animals and humans [39,40]. In our study, several genes belonging to the OR family were identified. In consideration of their relationship with vessels and vascular disease, we deduced that they might play a role in the development of TAA.

Conclusions

In conclusion, bioinformatics methods could identify significant biological processes and genes related to diseases. *KRTDAP*, BICD, and genes in the OR family might play an important role in TAA. Our results might help provide a significant reference for the diagnosis, prevention, and treatment of TAA. However, further research is still needed to confirm our conclusion.

Conflict of interest

None.

Acknowledgements

We would like to thank all the members of our research group for their enthusiastic participation in this study.

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