

Prediction of marker genes associated with hypertension by bioinformatics analyses

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Received December 22, 2015; Accepted May 9, 2017

DOI: 10.3892/ijmm.2017.3000

Abstract. This study aimed to explore the underlying marker genes associated with hypertension by bioinformatics analyses. A gene expression profile (GSE54015) was downloaded. The differentially expressed genes (DEGs) between the normotensive female (NF) and hypertensive female (HF), and between the normotensive male (NM) and hypertensive male (HM) groups were analyzed. Gene Ontology (GO) and pathway enrichment analyses were performed, followed by protein-protein interaction (PPI) network construction. The transcription factors (TFs), and the common DEGs between the HF and HM groups were then analyzed. In total, 411 DEGs were identified between the HF and NF groups, and 418 DEGs were identified between the HM and NM groups. The upregulated DEGs in the HF and HM groups were enriched in 9 GO terms, including oxidation reduction, such as cytochrome P450, family 4, subfamily b, polypeptide 1 (*Cyp4b1*) and cytochrome P450, family 4, subfamily a, polypeptide 31 (*Cyp4a31*). The downregulated DEGs were mainly enriched in GO terms related to hormone metabolic processes. In the PPI network, cytochrome P450, family 2, subfamily e, polypeptide 1 (*Cyp2e1*) had the highest degree in all 3 analysis methods in the HF group. Additionally, 4 TFs were identified from the 2 groups of data, including sterol regulatory element binding transcription factor 1 (*Srebf1*), estrogen receptor 1 (*Esr1*), retinoid X receptor gamma (*Rxrg*) and peroxisome proliferator-activated receptor gamma (*Pparg*). The intersection genes were mainly enriched in GO terms related to the extracellular region. On the whole, our data indicate that the DEGs, *Cyp4b1*, *Cyp4a31* and *Loxl2*, and the TFs, *Esr1*, *Pparg* and *Rxrg*, are associated with the progression of hypertension, and may thus serve as potential therapeutic targets in this disease.

Introduction

Hypertension, also known as arterial hypertension, is a chronic medical condition in which the blood pressure in the arteries is elevated (1). Hypertension is classified as either primary or secondary hypertension, and primary hypertension accounts for approximately 90-95% of cases (2). In primary hypertension, the cause of the hypertension is not clearly defined, whereas in secondary hypertension, the cause of the high blood pressure is clearly determined. Hypertension can lead to cardiac hypertrophy, aortic dissection and heart failure, due to structural changes in blood vessel walls and in the heart muscle, which has become a major burden to healthcare systems (3). Thus, hypertension is the leading risk factor for cardiovascular disease (4). According to a report of the World Health Organization in 2011, cardiovascular diseases are the leading cause of morbidity and mortality worldwide (5).

As a multifactorial disease, hypertension results from a complex interaction of environmental factors and genetic factors (6). Numerous common genetic variants, as well as some rare genetic variants have been identified to affect blood pressure (7,8). For instance, bone morphogenetic protein receptor type 2, which belongs to the transforming growth factor (TGF)- β superfamily of receptors, has been found to be mutated in the majority of cases of hypertension (9). Interleukin (IL)-17 has also been thought to be involved in hypertension due to its role in increasing the production of some other immune system chemical signals, such as IL-1, IL-6 and tumor necrosis factor (TNF)- α (10). Additionally, Austin *et al* (11) suggested that mutations in caveolin-1 are associated with pulmonary arterial hypertension. Despite advances in understanding the etiology of hypertension, the genetic basis responsible for this disease remains poorly elucidated.

Presently, there are many species of animals used as models of hypertension. In our study, the microarray dataset, GSE54015, which contained data from Schlager BPH/2J and BPN/3J mouse models was downloaded for analysis. Of note, Puig *et al* (12) also used the same mouse models to identify potential pharmacological targets of hypertension. In this study, we identified the differentially expressed genes (DEGs) between normotensive mice and hypertensive mice of both genders. Additionally, we performed functional enrichment analyses and protein-protein interaction (PPI) networks analysis to study and identify the pathways and DEGs associated with hypertension. Furthermore, transcription factors (TFs)

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Key words: hypertension, differentially expressed genes, protein-protein interaction network, transcription factor

and DEGs in both hypertensive female (HF) and male (HM) groups were analyzed. The results of this study may aid in the diagnosis and treatment of hypertension.

Data collection methods

Affymetrix microarray data. The microarray data of GSE54015 were downloaded, which was deposited in the National Center of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) (<http://www.ncbi.nlm.nih.gov/geo/>) database by Chiu *et al* (2). The platform is [MoGene-1_1-st] Affymetrix Mouse Gene 1.1 ST array [transcript (gene) version]. In the study by Chiu *et al* (2), 12- to 13-week-old Schlager hypertensive male and hypertensive female BPH/2J, and normotensive male and normotensive female BPN/3J mice (n=6/group) were used, and their kidney tissues were collected for the microarrays. In the present study, 4 types of mouse samples were used for analysis: normotensive female (NF) BPN/3J, hypertensive female (HF) BPH/2J, normotensive male (NM) BPN/3J and hypertensive male (HM) BPH/2J mice.

Data pre-processing and analysis of DEGs. The CEL raw data were downloaded and preprocessed using oligo (13) in R of Bioconductor (<http://www.bioconductor.org/packages/release/bioc/html/>) with the method of robust multiarray average (RMA) (14). The preprocessing processes included background correction, quartile data normalization and probe summarization.

The paired t-test based on the limma package (15) (no. 1406, Ritchie, 2015) in Bioconductor was used to identify the DEGs between the HF and NF groups, and between the HM and NM groups. The false discovery rate (FDR) was calculated to adjust the P-value with the Benjamini-Hochberg method (16). Only genes with $\log_2FCI \geq 0.58$ and an adjusted P-value < 0.05 were regarded as DEGs.

Gene ontology (GO) and pathway enrichment analyses. The GO (<http://www.geneontology.org>) project is used to provide consistent descriptors for gene products in different databases, and to standardize classifications for sequences (17). Kyoto Encyclopedia of Genes and Genomes (KEGG) (<http://www.genome.ad.jp/kegg/>) is a knowledge base used to analyze gene functions, and to link genomic information with higher order functional information (18). In this study, the obtained DEGs were performed GO and KEGG pathway enrichment analyses to obtain the biological process (BP), molecular function (MF), cellular component (CC) terms and pathways. The analysis tool was the Database for Annotation, Visualization and Integrated Discovery (DAVID) (<http://david.niaid.nih.gov>) (19), which is an integrated biological knowledgebase used for the systematic and integrative analysis of large gene protein lists. The P-value < 0.05 and enrichment count ≥ 2 were considered as thresholds.

PPI network construction. The Search Tool for the Retrieval of Interacting Genes (STRING) (<http://string-db.org/>) database (20) is a precomputed global resource used for evaluating PPI information. In the present study, the STRING online tool was applied to predict the PPI pairs of DEGs with a combined score of > 0.4 . The PPI network was constructed

using cytoscape (21). Additionally, 3 methods including degree centrality, betweenness centrality and subgraph centrality were used to analyze the hub gene in the PPI network using the CytoNCA cytoscape plugin (<http://apps.cytoscape.org/apps/cytonca>) (22).

TF analysis. In order to predict the role of TFs in hypertension, we selected the TFs from the DEG corresponding proteins, and predicted the target associations between the TFs and the DEGs based on the TRANSFAC database in the iRegulon cytoscape plugin (<http://iregulon.aertslab.org>) (23), as previously described. FDR on motif similarity < 0.05 was regarded as the threshold.

Analysis of DEGs in the HF and HM groups. In the present study, we screened the common DEGs between the HF and HM groups in order to analyze the similarity of hypertension in females and males. Subsequently, these selected DEGs were subjected to GO functional enrichment analysis using DAVID. Furthermore, the enriched GO terms were subjected to correlation analysis using the enrichmentmap cytoscape plugin (<http://baderlab.org/Software/EnrichmentMap/>) (24) ($P < 0.05$).

Results

Identification of DEGs. In total, 201 upregulated and 210 downregulated DEGs were identified between the HF and NF groups. Moreover, 204 upregulated and 214 downregulated DEGs were identified between the HM and NM groups. The results are presented in the heatmaps in Fig. 1A and B.

GO and pathway enrichment analyses of DEGs. The upregulated DEGs in the HF group were mainly enriched in GO terms related to ion transport and oxidation reduction, and in the pathway of histidine metabolism. The downregulated DEGs were mainly enriched in the GO terms related to steroid metabolic process and microsome, and in the pathway of arachidonic acid metabolism (Table I).

In the HM group, the upregulated DEGs were mainly enriched in GO terms related to co-factor metabolic process and oxidation reduction, and in the pathway of PPAR signaling. The downregulated DEGs were mainly enriched in GO terms related to hormone metabolic process and endoplasmic reticulum (Table II).

PPI network construction. The constructed PPI network based on the DEGs between the HF and NF groups is shown in Fig. 2A. The PPI network consisted of 193 nodes and 301 interaction pairs. The top 10 hub genes were selected based on the analysis of degree centrality, betweenness centrality and subgraph centrality. Among the 10 genes, cytochrome P450, family 2, subfamily E, polypeptide 1 (*Cyp2e1*) had the highest degree in all the 3 analysis methods (Table III).

The PPI network constructed by the DEGs between HM and NM included 182 nodes and 248 PPI interaction pairs (Fig. 2B). The top 10 hub genes respectively analyzed by 3 methods are shown in Table IV, name UDP glucuronosyltransferase 2 family, polypeptide B37 (*Ugt2b37*), formimidoyltransferase cyclodeaminase (*Ftcd*) and cytochrome P450, family 4, subfamily a, polypeptide 14 (*Cyp4a14*).

Table I. Functional enrichment analysis for the DEGs of the HF group.

		Term	Count	P-value
Up	BP	GO:0006811-ion transport	19	5.68E-05
		GO:0019751-polyol metabolic process	4	6.56E-03
		GO:0055114-oxidation reduction	13	1.66E-02
	CC	GO:0005576-extracellular region	32	4.98E-04
		GO:0042383-sarcolemma	4	1.17E-02
		GO:0005886-plasma membrane	41	1.75E-02
	MF	GO:0022836-gated channel activity	10	1.00E-03
		GO:0005216-ion channel activity	10	4.35E-03
		GO:0022838-substrate specific channel activity	10	5.32E-03
	KEGG	mmu00340:Histidine metabolism	3	1.96E-02
mmu03320:PPAR signaling pathway		4	3.10E-02	
Down	BP	GO:0008202-steroid metabolic process	9	7.92E-05
		GO:0055114-oxidation reduction	17	2.25E-04
		GO:0010565-regulation of cellular ketone metabolic process	4	9.96E-04
	CC	GO:0005792-microsome	9	2.16E-04
		GO:0042598-vesicular fraction	9	2.71E-04
		GO:0005783-endoplasmic reticulum	19	6.14E-04
	MF	GO:0030414-peptidase inhibitor activity	11	2.89E-06
		GO:0004866-endorpeptidase inhibitor activity	10	1.06E-05
		GO:0004857-enzyme inhibitor activity	11	4.83E-05
	KEGG	mmu00590:Arachidonic acid metabolism	5	3.56E-03
		mmu00511:Other glycan degradation	3	6.44E-03
		mmu00830:Retinol metabolism	4	1.49E-02
		mmu04610:Complement and coagulation cascades	4	1.94E-02

DEGs, differentially expressed genes; HF, hypertensive female; up, upregulated DEGs; down, downregulated DEGs; BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

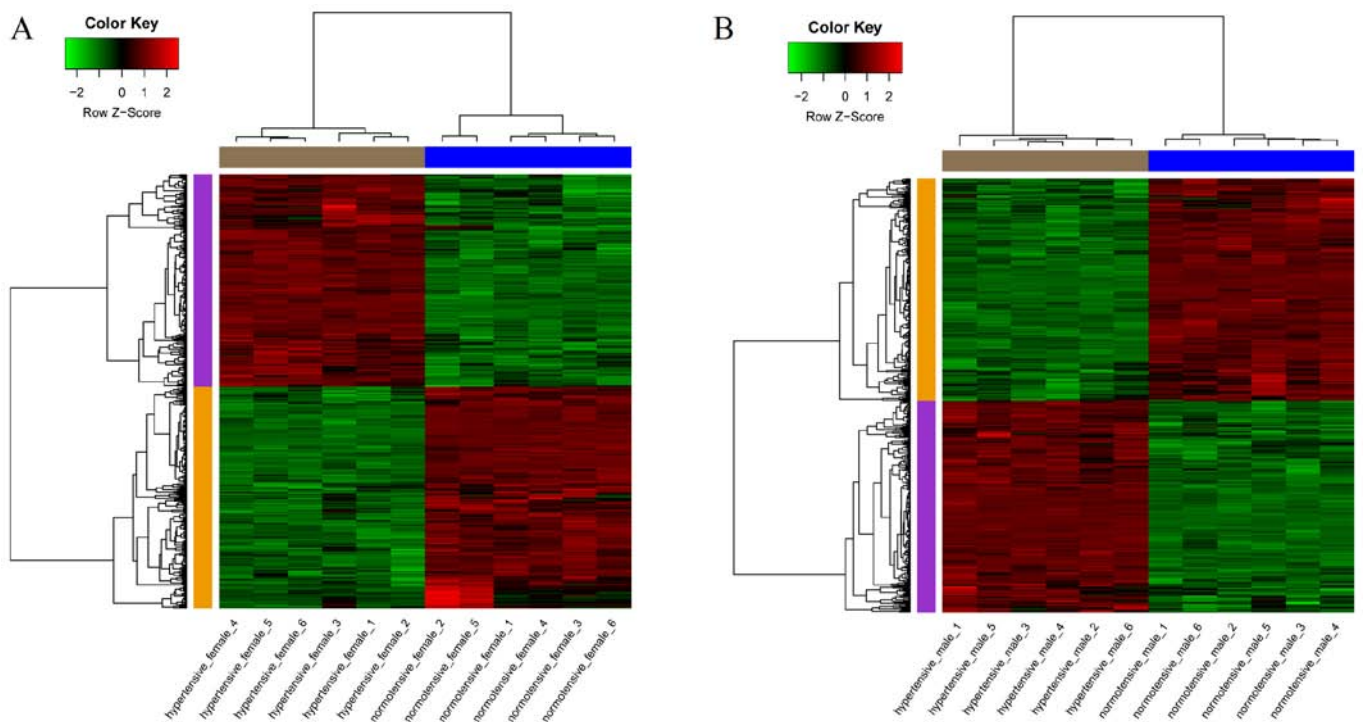


Figure 1. Heatmap plot of differentially expressed genes across all samples. (A) Hypertensive female (HF) vs. normotensive female (NF); (B) hypertensive male (HM) vs. normotensive male (NM). Red stands for high expression value and green stands for low expression value. The changes of color from green to red stands for the changes of expression value from low to high.

Table II. Functional enrichment analysis for the DEGs of the HM group.

		Term	Count	P-value
Up	BP	GO:0051186-cofactor metabolic process	8	1.04E-03
		GO:0055114-oxidation reduction	15	2.04E-03
		GO:0019748-secondary metabolic process	5	3.02E-03
	CC	GO:0005576-extracellular region	37	4.68E-06
		GO:0005777-peroxisome	5	2.03E-02
		GO:0042579-microbody	5	2.03E-02
	MF	GO:0019842-vitamin binding	10	1.64E-06
		GO:0016290-palmitoyl-CoA hydrolase activity	3	8.12E-03
		GO:0030170-pyridoxal phosphate binding	4	1.33E-02
	KEGG	mmu03320:PPAR signaling pathway	5	6.25E-03
mmu00590:Arachidonic acid metabolism		5	7.43E-03	
Down	BP	GO:0042445-hormone metabolic process	7	1.63E-04
		GO:0010817-regulation of hormone levels	7	1.07E-03
		GO:0042398-cellular amino acid derivative biosynthetic process	4	9.31E-03
	CC	GO:0005783-endoplasmic reticulum	15	1.29E-02
	MF	GO:0004866-endorpeptidase inhibitor activity	7	3.52E-03
		GO:0030414-peptidase inhibitor activity	7	5.43E-03
		GO:0004867-serine-type endopeptidase inhibitor activity	5	2.09E-02

DEGs, differentially expressed genes; HM, hypertensive male; up, upregulated DEGs; down, downregulated DEGs; BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table III. The top 10 network nodes in the HF group based on the analysis of degree centrality, betweenness centrality and subgraph centrality.

Gene	Degree		Betweenness		Subgraph
<i>Cyp2e1</i>	18	<i>Cyp2e1</i>	7915.4395	<i>Cyp2e1</i>	295.83835
<i>Cyp4a12a</i>	16	<i>Aldh1a1</i>	4667.3003	<i>Cyp4a12a</i>	279.15607
<i>Cyp2b10</i>	11	<i>Pla2g5</i>	4572.402	<i>Cyp2b10</i>	179.15228
<i>Pla2g5</i>	9	<i>Srebf1</i>	4370.598	<i>Slc22a30</i>	100.235176
<i>Srebf1</i>	9	<i>Dkk1</i>	4108.5645	<i>Pla2g5</i>	99.77931
<i>Serpina1d</i>	9	<i>Cyp4a12a</i>	3427.9216	<i>Slco1a1</i>	99.73588
<i>Cyp7b1</i>	9	<i>Pld1</i>	3171.1284	<i>Cyp4f18</i>	81.0327
<i>Slc22a30</i>	9	<i>Serpina1d</i>	3132.0713	<i>Cyp4f14</i>	81.03269
<i>Anxa1</i>	8	<i>Prok1</i>	3062.5642	<i>Aldh1a1</i>	73.60265
<i>Gm266</i>	8	<i>Plcl2</i>	2823.1924	<i>Aldh1a7</i>	69.66662

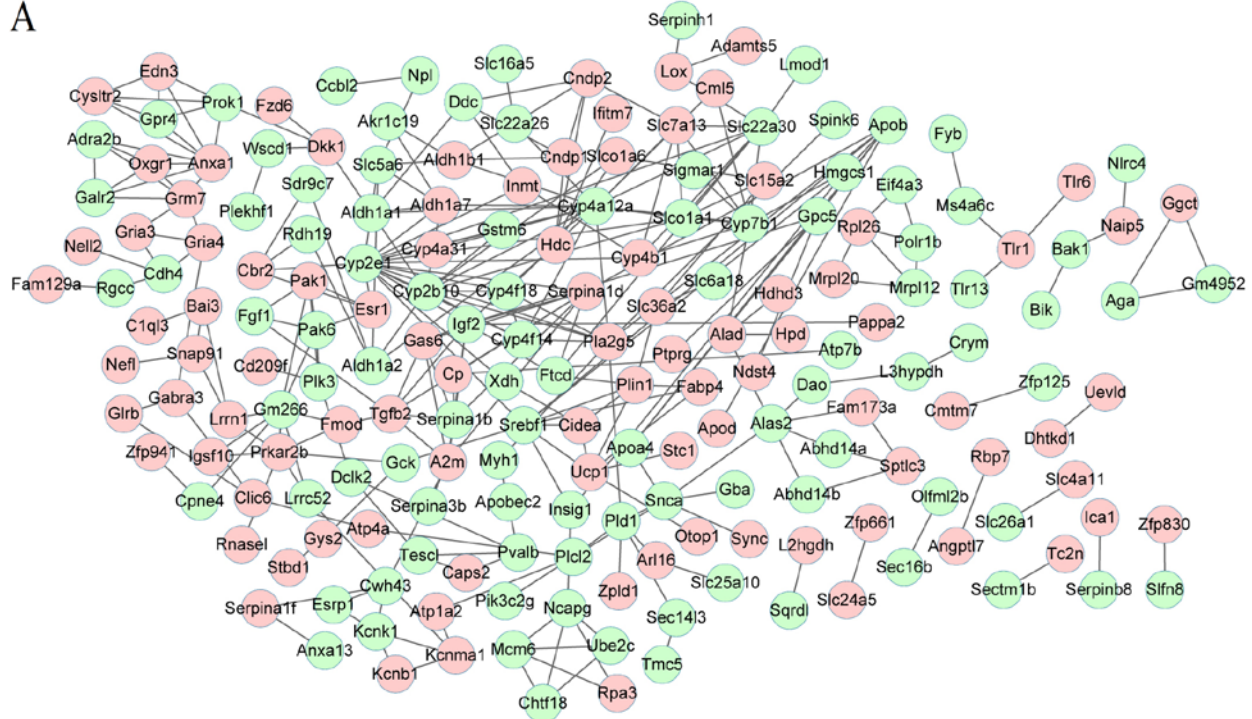
HF, hypertensive female.

TF analysis. Based on the TRANSFAC database in iRegulon, the TFs in the HF and HM samples were predicted and the transcriptional regulatory networks were constructed (Fig. 3A and B). In the HF group, the TFs were sterol regulatory element binding transcription factor 1 (*Srebf1*) and estrogen receptor 1 (*Esr1*). In the HM group, the TFs were retinoid X receptor gamma (*Rxrg*) and peroxisome proliferator-activated receptor gamma (*Pparg*).

Analysis of DEGs in the HF and HM groups. There were 118 intersection genes among the upregulated DEGs in the

HF and HM samples; they were mainly distributed in chromosomes 4 and 7. Among the downregulated DEGs, 112 were intersection genes and they were mainly distributed in chromosomes 11 and 14. Moreover, these intersection genes were mainly enriched in BP terms related to catecholamine biosynthetic process, CC terms related to extracellular region and MF terms related to endopeptidase inhibitor activity (Table V). For instance, lysyl oxidase-like 2 (*Loxl2*) was enriched in the CC term related to extracellular region. The interaction network for these terms are shown in Fig. 4.

A



B

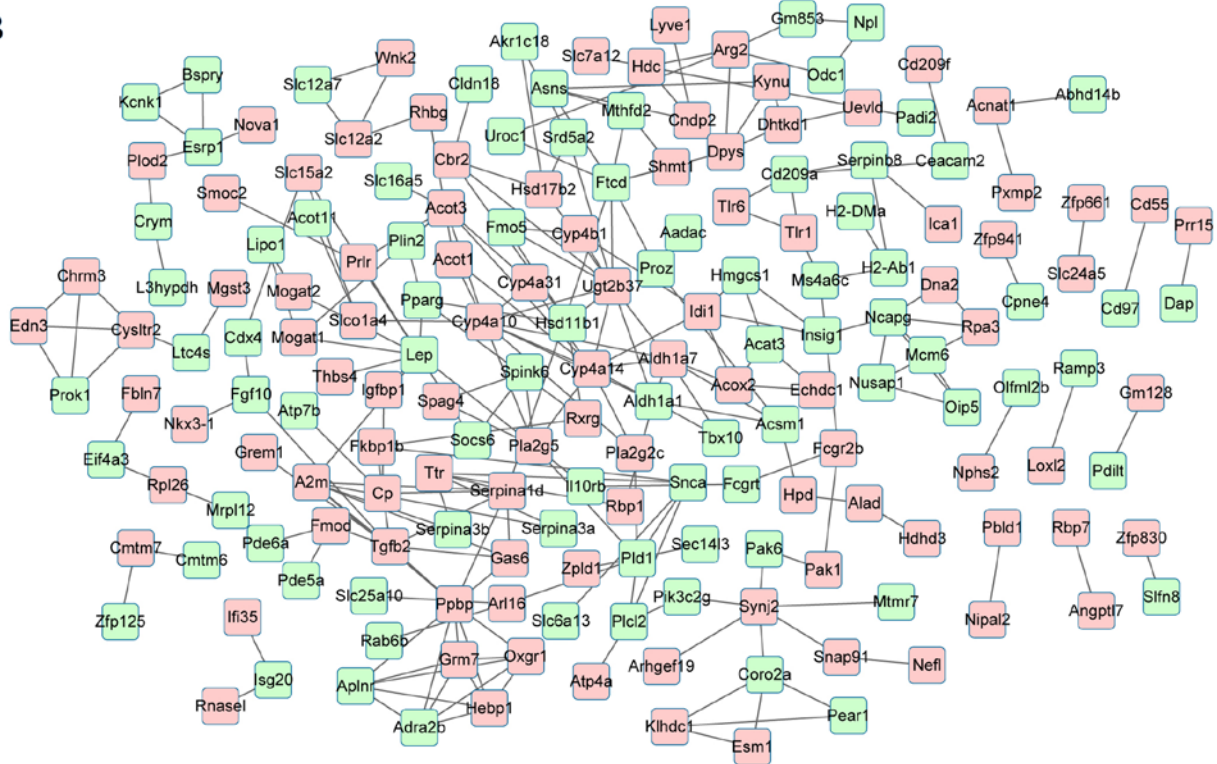


Figure 2. The constructed protein-protein interaction (PPI) network for differentially expressed genes (DEGs). Pink stands for upregulated DEGs and green stands for downregulated DEGs. (A) Hypertensive female (HF) vs. normotensive female (NF); (B) hypertensive male (HM) vs. normotensive male (NM).

Discussion

In the present study, 411 DEGs were identified between the HF and NF samples, and 418 DEGs were identified between

the HM and NM samples. The upregulated DEGs, *Cyp4b1* and *Cyp4a31*, were mainly enriched in BP terms related to oxidation reduction in both the HF and HM samples. In addition, *Esr1*, *Pparg* and *Rxrg* were the key TFs involved in the transcriptional

Table IV. The top 10 network nodes in the HM group based on the analysis of degree centrality, betweenness centrality and subgraph centrality.

Gene	Degree	Betweenness	Subgraph
<i>Ugt2b37</i>	11	5603.764	57.048008
<i>Cyp4a10</i>	10	4363.4756	55.98465
<i>Cyp4a14</i>	10	4147.9277	52.882313
<i>Lep</i>	10	4005.5542	50.838398
<i>Ppbp</i>	9	3500.8042	34.876118
<i>Tgfb2</i>	8	2844.4468	34.272568
<i>Ftcd</i>	8	2784.8557	28.300505
<i>Aldh1a7</i>	7	2771.22	28.300499
<i>Aldh1a1</i>	7	2728.78	28.300493
<i>A2m</i>	7	2569.02	28.300491

HM, hypertensive male.

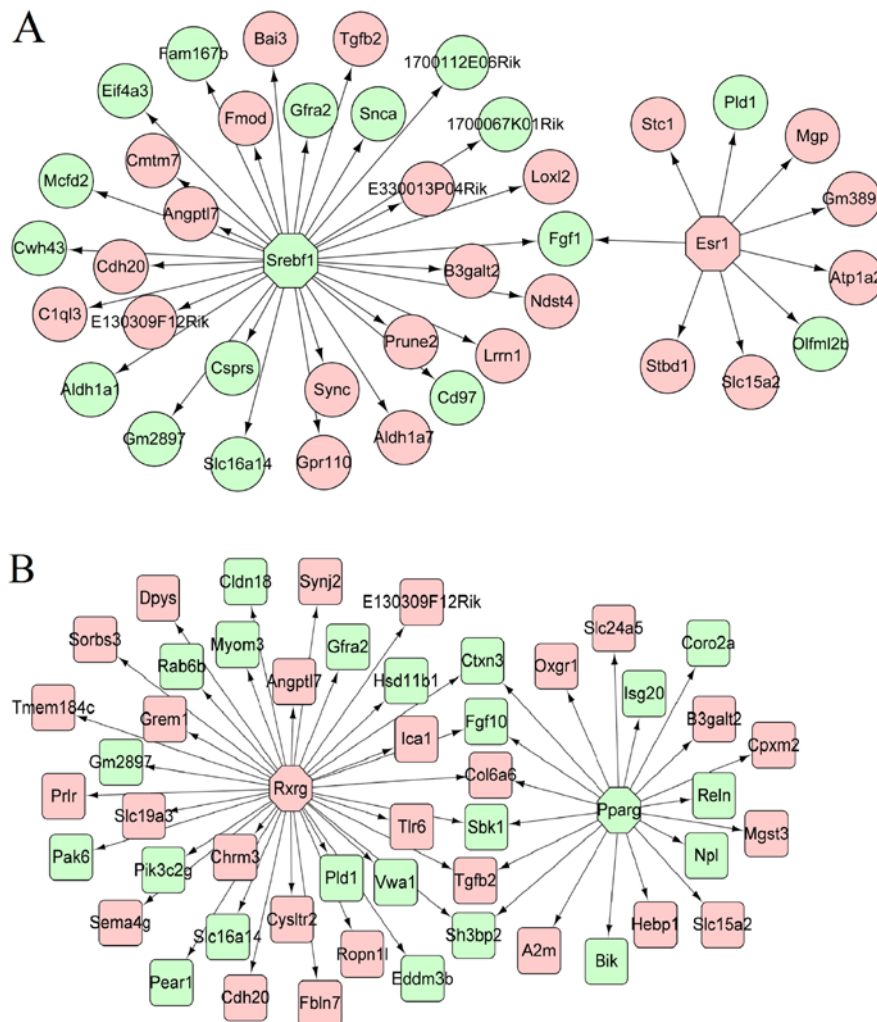


Figure 3. The constructed transcriptional regulatory network. Octagon stands for transcription factor; circle stands for differentially expressed genes (DEGs) in hypertensive female (HF); square stands for DEGs in hypertensive male (HM). Pink stands for upregulated DEGs and green stands for downregulated DEGs. (A) HF group; (B) HM group.

regulatory networks. Following the analysis of DEGs in the HF and HM samples, many intersection DEGs were identified

between the 2 groups, such as *Loxl2*. These genes and TFs may play important roles in the progression of hypertension.

menopausal women (35). In our study, the TF, *Esr1*, was also identified in female hypertensive mice, which indicated that *Esr1* may play an important role in the progression of hypertension in females.

Furthermore, the TFs, *Pparg* and *Rxrg*, were involved in the transcriptional regulatory networks in the HM samples. *Pparg* is a member of PPARs which, as mentioned above, play an important role in the regulation of cardiovascular circadian rhythms. *Pparg* has been implicated in atherosclerosis (36), coronary heart disease (37) and hypertension (38). RXRs are members of the NR2B family of nuclear receptors and are major heterodimerization partners of PPARs (39). RXRs are involved in mediating the anti-proliferative effects of retinoic acid, which can comprise a collection of molecules with retinoids to maintain homeostasis in adults (40). A growing number of studies have reported the role of RXRs and PPARs in controlling cellular metabolism in tissues, including the myocardium (40,41). Nohara *et al* (39) suggested that a variant of *Rxrg* contributes to human genetic dyslipidemia. Taken together, the TFs, *Pparg* and *Rxrg*, may have utility in the diagnose and treatment of hypertension.

Following the analysis of DEGs in the HF and HM samples, we found that there were many intersection DEGs between the 2 groups, such as *Loxl2*. This DEG was found enriched in the BP terms related to oxidation reduction and CC terms related to extracellular region. *Loxl2* encodes a member of the lysyl oxidase gene family, which are extracellular copper-dependent amine oxidases initiating the formation of crosslinks in collagen and elastin (42). The collagen and elastin are important components of the extracellular matrix (43). It has been found that the activity of lysyl oxidase is essential to maintain the features of connective tissues, including cardiovascular systems (44). The deregulation of lysyl oxidase is associated with aneurysms, aortic rupture and different stages of the atherosclerotic process (45,46). Presently, there are few studies on the association between lysyl oxidase and hypertension. Thus, we speculated that *Loxl2* may be a potential biomarker for the progression of hypertension.

In conclusion, our study provides a comprehensive bioinformatics analysis of biomarkers in hypertension, which may provide new insight into the mechanisms responsible for this disease. The DEGs, *Cyp4b1*, *Cyp4a31* and *Loxl2*, and the TFs, *Esr1*, *Pparg* and *Rxrg*, may be associated with the progression of hypertension, and may serve as potential therapeutic targets in this disease.

However, there was a limitation to this study. The present study was implemented by means of bioinformatics; therefore, the screened genes and TFs have not been validated by actual experiments. As a result, further experimental studies with a larger sample size are warranted to confirm the results of our study.

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