

Exploring the shared genes of hypertension, diabetes and hyperlipidemia based on microarray

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Given their relationship with metabolic syndrome and systematic inflammatory diseases, the pathogenesis of hypertension, hyperglycemia, and hyperlipidemia is closely related. To explore the common genes among these three conditions, spontaneous hypertensive rats (SHR), spontaneous diabetic Goto-Kakizaki rats (GK) and hyperlipidemia rats (HMR) were reared for experiments. Gene array was used to identify the genes of SHR, GK and HMR compared with normal Wistar rats using TBtools software. First, real-time PCR was applied to verify these genes, and Cytoscape software was used to construct networks based on the National Center for Biotechnology Information (NCBI) database. Second, Kyoto Encyclopedia of Genes and Genomes (KEGG) database analysis was performed to classify the genes. Visualization and Integrated Discovery (DAVID) database and Gene Ontology database were used to explore the biological function. Finally, Onto-tools Pathway Express was used to analyze the pathways of shared genes. Importantly, upregulated common genes, such as Bad, Orm1, Arntl and Zbtb7a, were used to construct a network of 150 genes, while downregulated genes, such as Mif and Gpx1, formed a network of 29 genes. Interestingly, the networks were involved in various pathways, such as insulin signal pathway, endometrial cancer pathway, circadian rhythm pathway, and pancreatic cancer pathway. We discovered common genes of SHR, GK and HMR compared with normal Wistar rats, and the association of these genes together with biological function were preliminarily revealed.

Keywords: Hypertension. Diabetes. Hyperlipidemia. Genes. Microarray. Neoplasm.

INTRODUCTION

Hypertension, diabetes and hyperlipidemia are common and frequently encountered chronic noncommunicable diseases that pose a serious risk to human health. Recently, the number of people with hypertension, diabetes and hyperlipidemia has been sharply increasing (Je *et al.*, 2017), and the majority of these patients has a combination of dysarteriotony,

pathoglycemia and dyslipidemia (Chen *et al.*, 2016; Patel *et al.*, 2016).

Moreover, dyslipidemia, hyperglycemia, hypertension, hypercholesterolemia and abdominal obesity are characterized by a cluster of interdependent disorders (Mohammadbeigi *et al.*, 2018), triggering a collection of metabolic abnormalities (Takeyama *et al.*, 2018), such as heart disease, thrombosis, vasomotor dysfunction in microvessels, mitochondrial function disorder, diabetic nephropathy and renal interstitial fibrosis (Rahmatallah *et al.*, 2017) (Hye Khan *et al.*, 2018). In a recent study, Lee *et al.* identified an overexpression of metabolic disease related-gene related to the upregulation of the inflammatory-related factors

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interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha (Lee *et al.*, 2018). Furthermore, Adamts18-knockout mice with increased expression of enhancer binding protein beta exhibited hypertension, blood glucose metabolic disorder and hyperlipidemia (Zhu *et al.*, 2018).

Additionally, previous retrospective trials suggested that hypertension was associated with the development of cancer (Dyer *et al.*, 1975; Buck, Donner, 1987; Wannamethee, Shaper, 1996; Felmeden, Lip, 2001; Jeong *et al.*, 2015). Existing drugs that are used to treat hypertension, such as Angiotensin II receptor antagonists (ARB), exhibit potential in the treatment of endometrial cancer (Choi *et al.*, 2012). In addition, during the treatment process, one study used Kaplan-Meier analysis to reveal that the most common statin monotherapy exhibited a potentially protective effect against colorectal cancer-related mortality (Yokomichi *et al.*, 2017). As an anabolic hormone, insulin promotes cell proliferation and the progression of cancer through a variety of channels (Parekh *et al.*, 2009). Based on the abovementioned studies, a potential relationship was noted among hypertension, diabetes and hyperlipidemia that was also associated with other systematic inflammatory diseases regardless of the disease development stage or treatment period.

However, previous studies have explored the key genes or the significant pathways that impact the diseases. At present, microarray technology and high-throughput sequencing have been widely used to screen for differential gene expression in diseases (C. Wu *et al.*, 2018). Thus, we proposed that an intrinsic genetic relationship potentially exists among hypertension, diabetes and hyperlipidemia. The aim of the present study is to explore the shared genes of SHR, GK and HMR compared with normal Wistar rats based on microarray technology and analyze the biological function and critical biological pathways based on bioinformatics analysis.

MATERIAL AND METHODS

Animals

Spontaneous hypertensive rats (SHR) (n=6), spontaneous diabetic Goto-Kakizaki rats (GK) (n=6) and hyperlipidemia rats (HMR) (n=6) were selected

to serve as experimental groups. These rats were compared with normal Wistar rats. All of the rats were obtained from the Chinese National Rodent Laboratory Animal Resources Shanghai Branch. HMR were duplicated by feeding Wistar rats with a high fat diet that contained 60% general feed, 12% lard, 5% sucrose, 5% milk powder, 5% peanut, 5% hen eggs, 1% sesame oil and 2% salt for 90 days. The serum triglyceride levels were increased compared with that of the general feed groups ($p < 0.05$). This work was performed in accordance with the EU Directive 2010/63/EU for animal experiments.

RNA preparation

Livers were isolated from SHR, GK, HMR and normal Wistar rats that were anesthetized with inactin (80 mg/kg, intraperitoneally). Total RNA was isolated from liver tissues using TRI Reagent (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturer's protocol. Potential impurities were removed (RNeasy Mini Kit, Qiagen, Valencia, CA).

Gene expression analysis

Probe preparation and hybridization were performed according to protocols recommended by Shanghai BioChip. A software package was used to collect fluorescent intensity signals from Agilent scanner with 10- μ m resolution, and expression data were normalized with Genespring and summarized using a multiarray analysis. Comparison analyses were used to discriminate changes in gene expression levels between the experimental group and normal group. cDNA direct labeling was adopted in this experiment. Cy3 fluorescent labeling was applied in experimental group, and Cy5 fluorescent labeling was used in control group. The ratio value could be calculated (Cy3/Cy5) in the same microarray. The screening standard of differentially expressed genes was employed. Specifically, a ratio ≥ 2 indicates upregulation, while a ratio ≤ 0.5 indicates downregulation.

Then, downregulated genes and upregulated genes from the SHR, GK and HMR microarrays were identified by comparison with normal Wistar rats, and shared downregulated and upregulated genes were analyzed using TBtools v 0.5 software.

Confirmation of changes in gene expression by SYBR Green RT-PCR

SYBR Green real-time polymerase chain reaction (RT-PCR) was performed for several genes to confirm the hybridization results. Using samples from the array experiments, 5 µg of total RNA was reverse transcribed into cDNA using the Superscript First Strand System for RT-PCR (Invitrogen). Then, each cDNA sample was used as a template for real-time PCR amplification by applying SYBR green PCR Master Mix (Applied Biosystems) and forward/reverse primers for the various target genes (Table I). Amplification and detection were performed on an ABI Prism 7000 Real-Time PCR system according to the manufacturer's instructions using a two-stage cycle of 95 °C for 15 s and 59 °C for 1 min that was repeated for 40 cycles and followed by a dissociation stage. Relative fold mRNA expression levels were determined via the $2^{-\Delta\Delta Ct}$ method using GAPDH as housekeeping control.

Network analysis

Cytoscape v 3.5.1 software was used to construct networks by importing networks from NCBI Entrez Eutilities Web Service Client.

Analysis of differentially expressed genes

The Kyoto Encyclopedia of Genes and Genomes (KEGG) (<http://www.kegg.jp/>) is a collection of

online databases dealing with genomes, enzymatic pathways, and biological chemicals. KEGG considers genes and expression information as a whole to study systematically the function of genes. We applied KEGG analysis to classify the differentially expressed genes.

The Database for Annotation, Visualization and Integrated Discovery (DAVID) provides a comprehensive set of functional annotation tools for investigators to understand the biological meaning of large lists of genes (<https://david.ncifcrf.gov/>) to identify enriched biological themes, particularly GO terms, and to explore gene names in batch and display related many-genes-to-many-terms by 2-D view. We applied DAVID to analyze The Biological Process and The Cell Component.

The Gene Ontology platform (<http://geneontology.org/>) analyzes many functions of genes, such as cellular component organization, molecular activities of gene products, pathways and larger processes composed of the activities of multiple gene products. We applied DAVID to analyze The Protein Class and The Molecular Function.

GO enrichment and DAVID database analyses were performed using the 8 genes with significantly increased expression and the 13 genes with significantly decreased expression. Significant GO term enrichment and DAVID data were defined according to the P-value using a cut-off value of 0.05.

Pathway analysis

Analysis of the most biologically relevant disease pathways shared genes was performed by using the web-

TABLE I - Forward and reverse primer

GenBank ID	Forward primer 5'-3'	Reverse primer 5'-3'	Length
<i>AI012209</i>	CACCTCATTGAAGCCATCCA	GAAGGAAGGCGGAGAGAACAT	133
<i>NM_022698</i>	AGTTGGACGCGCATTATCCA	TTCCGCCACACCCAATAT	135
<i>AY011147</i>	CAATTCTCCTAGCACAAAGTGTTGAC	AAAGCACGCCTGTGTTGG	122
<i>NM_144739</i>	TGACTGTGCGTGTCTCATGCT	TAGATGGCGCTTTCCAGTGGA	107
<i>NM_053607</i>	AGCAAAGCAACGTGGACGA	TGGCAGCAAATACTGCAGG	112
<i>NM_031051</i>	CCAGAACCGCAACTACAGCAA	TGGCTGCGTTCATGTCGTAA	104
<i>GAPDH</i>	TCCTGCACCACCAACTGCTTAG	AGTGCCAGTGATGGCATGGACT	102

based tools Onto-tools Pathway Express (<http://vortex.cs.wayne.edu>). An impact factor (IF) was calculated for each pathway by incorporating parameters, such as the normalized fold change of the differentially expressed genes, the statistical significance of the set of pathway genes, and the topology of the signaling pathway. IF \geq 2 was considered significant.

RESULTS

Differentially expressed genes

To unveil the potential relations among hypertension, diabetes and hyperlipidemia based on pathogenesis, SHR, GK and HMR were selected to serve as experimental groups, and normal Wistar rats served as control. Total RNAs were isolated from their liver tissues, and gene expression analysis was performed using cDNA microarray. Compared with normal Wistar rats, significant up or down regulated common genes were identified from SHR, GK and HMR groups using TBtools software (Figure 1, Table II and Table III) and were confirmed by real-time PCR (Table IV). These results revealed that the pathogenesis of hypertension, diabetes and hyperlipidemia shared common genes. The results hinted that hypertension, diabetes and hyperlipidemia interact during onset, which suggests

that the genetic origins of hypertension, diabetes and hyperlipidemia might be shared.

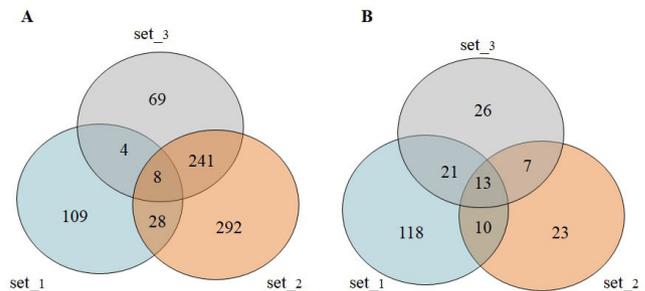


FIGURE 1 - Upregulated and downregulated common genes were identified from SHR, GK and HMR microarrays using TBtools software. (A) Set_1 represents 149 upregulated genes from the HMR group compared with the normal group; Set_2 represents 569 upregulated genes from the GK group compared with the normal group; Set_3 represents 322 upregulated genes from the SHR group compared with the normal group; The intersection of the three regions in the center represents 8 common upregulated genes in SHR, GK and HMR groups compared with the normal group; (B) Set_1 represents 162 downregulated genes in the HMR group compared with the normal group; Set_2 represents 53 downregulated genes in the GK group compared with the normal group; Set_3 represents 67 downregulated genes in the SHR group compared with the normal group; The intersection of the three regions in the center represents 13 common downregulated genes in SHR, GK and HMR groups compared with the normal group.

TABLE II - Genes upregulated in hyperlipidemia, diabetes and hypertension groups

Locus	Gene	Ratio			Gene definition
		HMR	GK	SHR	
<i>AI012209</i>		4.56	2.7	2.02	Similar to WW domain-containing protein 2, mRNA.
<i>XM_131329</i>		6.54	4.0	2.29	Mitogen-activated protein kinase 7, mRNA.
<i>XM_135927</i>		19.64	5.0	2.14	Mus musculus RIKEN cDNA, mRNA.
<i>NM_053288</i>	<i>Orm1</i>	34.63	2.3	2.93	Rattus norvegicus Orosomuroid 1 (Orm), mRNA.
<i>NM_022698</i>	<i>Bad</i>	2.68	5.1	3.46	Rattus norvegicus bcl-2-associated death agonist, mRNA.
<i>NM_054002</i>	<i>Zbtb7a</i>	2.19	2.3	2.01	Rattus norvegicus zinc finger and BTB domain containing 7a, mRNA.

(continuing)

TABLE II - Genes upregulated in hyperlipidemia, diabetes and hypertension groups

Locus	Gene	Ratio			Gene definition
		HMR	GK	SHR	
<i>NM_024362</i>	<i>Arntl</i>	2.33	2.9	3.56	Rattus norvegicus aryl hydrocarbon receptor nuclear translocator-like, mRNA
<i>L22654</i>		9.75	4.5	2.28	Rat anti-acetylcholine receptor antibody gene, rearranged Ig gamma-2a chain, mRNA.

Ratio value was calculated (Cy3/Cy5). A ratio ≥ 2 indicates upregulation, which served as the screening standard of differential gene expression.

TABLE III - Genes downregulated in hyperlipidemia, diabetes and hypertension groups

Locus	Gene	Ratio			Gene definition
		HMR	GK	SHR	
<i>M91235</i>		0.32	0.44	0.29	Rattus norvegicus transposon VL30, complete sequence.
<i>AI236753</i>		0.45	0.28	0.18	EST233315 Rattus norvegicus cDNA.
<i>AY011147</i>		0.21	0.41	0.36	Rattus norvegicus 16S ribosomal RNA gene, partial sequence. mitochondrial.
<i>NM_144739</i>	<i>Fmo5</i>	0.31	0.34	0.27	Rattus norvegicus flavin containing monooxygenase 5 (Fmo5), mRNA.
<i>NM_019286</i>	<i>Adh1</i>	0.31	0.34	0.19	Rattus norvegicus alcohol dehydrogenase 1 (class I) (Adh1), mRNA.
<i>NM_030826</i>	<i>Gpx1</i>	0.38	0.50	0.47	Rattus norvegicus glutathione peroxidase 1 (Gpx1), mRNA.
<i>BG665142</i>		0.35	0.43	0.32	DRABZA08 Rattus norvegicus cDNA.
<i>NM_031835</i>	<i>AGT2</i>	0.31	0.32	0.39	Rattus norvegicus beta-alanine-pyruvate aminotransferase (AGT2), mRNA.
<i>NM_053607</i>	<i>Facl5</i>	0.39	0.47	0.38	Rattus norvegicus long-chain fatty acid coenzyme A ligase 5, mRNA.
<i>NM_02254777</i>	<i>Fthfd</i>	0.37	0.44	0.45	Rattus norvegicus 10-formyltetrahydrofolate dehydrogenase. (Fthfd).mRNA.
<i>AI535168</i>		0.18	0.44	0.32	UI-R-C3-sq-d-05-0-UI. s1 Rattus norvegicus cDNA.
<i>BF284744</i>		0.37	0.38	0.37	EST449335 Rattus norvegicus cDNA.
<i>NM_031051</i>	<i>Mif</i>	0.13	0.36	0.28	Rattus norvegicus macrophage migration inhibitory factor (Mif) mRNA.

Ratio value could be calculated (Cy3/Cy5). A ratio ≤ 0.5 indicates down regulation which served as the screening standard of differential gene expression.

TABLE IV - Real-time PCR confirmation of array results

Locus	Array Ratio			PCR($2^{-\Delta\Delta Ct}$)		
	HMR	GK	SHR	HMR	GK	SHR
<i>AI012209</i>	4.56	2.78	2.02	1.85	1.19	1.93
<i>NM_022698</i>	2.68	5.13	3.46	50.59	16.43	34.85
<i>AY011147</i>	0.21	0.41	0.36	0.35	0.24	0.30
<i>NM_144739</i>	0.31	0.34	0.27	0.38	0.18	0.33
<i>NM_053607</i>	0.39	0.47	0.38	0.22	0.28	0.24
<i>NM_031051</i>	0.13	0.36	0.28	0.22	0.39	0.35

Relative expression levels of the six genes were consistent with microarray data.

Network analysis of the shared genes

Cytoscape was applied to further explore the network of those shared genes, upregulated shared genes such as Bad, Orm1, Arntl and Zbtb7a, etc. were found constructing network with 150 genes in Figure 2, while common downregulated shared, such as Fmo5, AGT2, Mif and Gpx1, formed a network with 29 genes in Figure 3.

Biological function analysis of the shared genes

These shared genes were classified by KEGG to explore potential correlations among the genes. We found that the genes covered five main functions, including inflammation, apoptosis, metabolism, oxidative stress and signal transduction (Table V). This finding implied that the development of hypertension, diabetes and hyperlipidemia was anfractuous. The pathogenesis of these conditions may involve common pathways.

Shared genes were analyzed using the DAVID data bank to explore the categories of Biological Processes and Cellular Component. Biological Processes involved in hypertension, diabetes, hyperlipidemia included response to glucose (25%), cell proliferation (25%), oxidation-reduction process (25%), cellular response to hypoxia (16.7%), and organ regeneration 16.7% in Figure 4A.

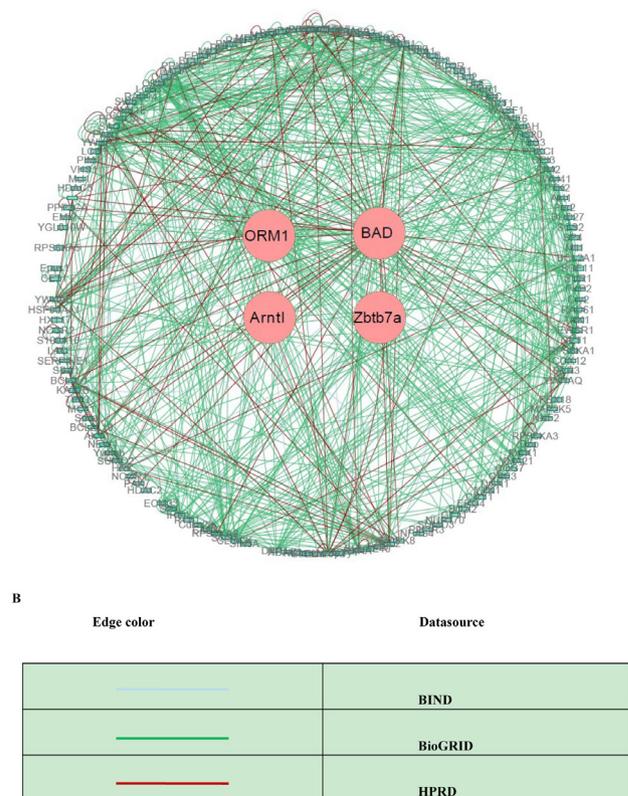


FIGURE 2 - Network of upregulated shared genes. (A) Network of upregulated shared genes based on Cytoscape v 3.5.1 software; (B) Red circular nodes in the center of network represent upregulated genes common to hypertension, diabetes and hyperlipidemia. The outer ring was constructed by green rectangular nodes of first neighbor genes of those in center.

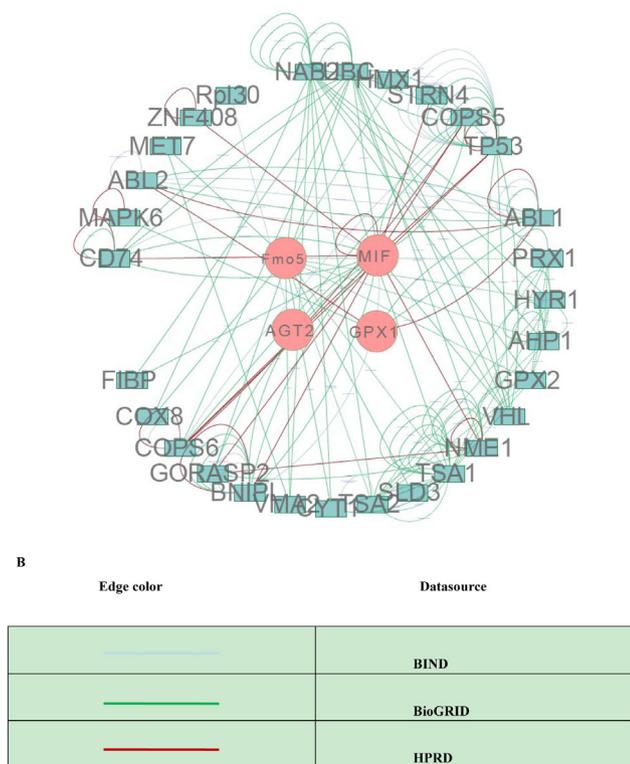


FIGURE 3 - Network of downregulated shared genes. (A) Network of downregulated shared genes based on Cytoscape v 3.5.1 software; (B) Red circular nodes in the center of the network represent downregulated genes common to hypertension, diabetes and hyperlipidemia. The outer ring was constructed by green rectangular nodes of first neighbor genes of those in center.

Shared genes were analyzed using the Gene Ontology data bank to explore the categories of Protein Class and Molecular Functions. Protein Class analysis revealed the following functions, which were generally consistent with Gene Ontology data: oxidoreductase (44.4%), transcription factor (22.2%), ligase (11.1%), transferase (11.1%), and nucleic acid binding (11.1%) in Figure 4B. Molecular Functions included catalytic activity 60%, transporter activity (10%), antioxidant activity (10%) and binding (20%) in Figure 4C. The Cellular Component corresponding to these genes included mitochondrion (50%), cytosol (33.3%), blood microparticle (16.70%), and mitochondrial outer membrane (16.70%) in Figure 4D.

Pathways of the shared genes

In the final step, we analyzed the shared pathways using the Onto-tools Pathway Express to verify close

relationships between genes. This program processed a list of input genes to identify KEGG pathways based on fold-change, pathway position and pathway topology. One novel discovery was that the genes shared among the SHR, GK and HMR groups exhibited close relationships with cancers pathway, such as the endometrial cancer pathway, circadian rhythm pathway, pancreatic cancer pathway, colorectal cancer pathway, and prostate cancer pathway in Figure 5. The higher the impact factor is, the greater the probability that the pathway is involved in the disease.

DISCUSSION

The cause of hypertension, diabetes and hyperlipidemia has not been fully clarified. Gender, age, drinking, environmental and genetic factors are involved in the pathogenesis of these diseases. However, genetic factors play critical roles in the pathogenesis of cardiovascular diseases (Zhao *et al.*, 2017). In this study, the shared genes of SHR, GK and HMR via comparison with normal Wistar rats were determined via microarray analysis.

In a previous study, Hindle, AK *et al.* conducted a microarray analysis using in the morbidly obese diabetic and nondiabetic patients (Hindle *et al.*, 2010). However, the study by Hindle, AK *et al.* did not incorporate gene expression differences for other cardiovascular diseases. Similarly, in the study by You, Z *et al.*, obesity, hyperlipidemia, diabetes mellitus, and hypertension were associated with Uii gene deletion (You *et al.*, 2012). This study also demonstrated that a single causal gene had an impact on metabolic diseases. In contrast, multiple related risk factors and susceptible genes were involved in hypertension, diabetes and hyperlipidemia (Sun *et al.*, 2012). Thus, microarrays can be performed to analyze the expression levels of copious genes simultaneously combined with bioinformatics analysis to identify associated pathways and key genes within complex diseases (Wu *et al.*, 2018).

The principal finding of this study was that Bad, Orml, Arntl, Zbtb7a, Mif and Gpx1 were identified from the hypertension, diabetes and hyperlipidemia rats. In addition, AI236753, AY011147, XM_131329, and XM_135927, which are not well known, were identified from multi-experiment microarray comparisons (Table II and Table III). In this sense, these identified genes may collectively regulate the biological process, signal pathways and molecular mechanisms of these three diseases.

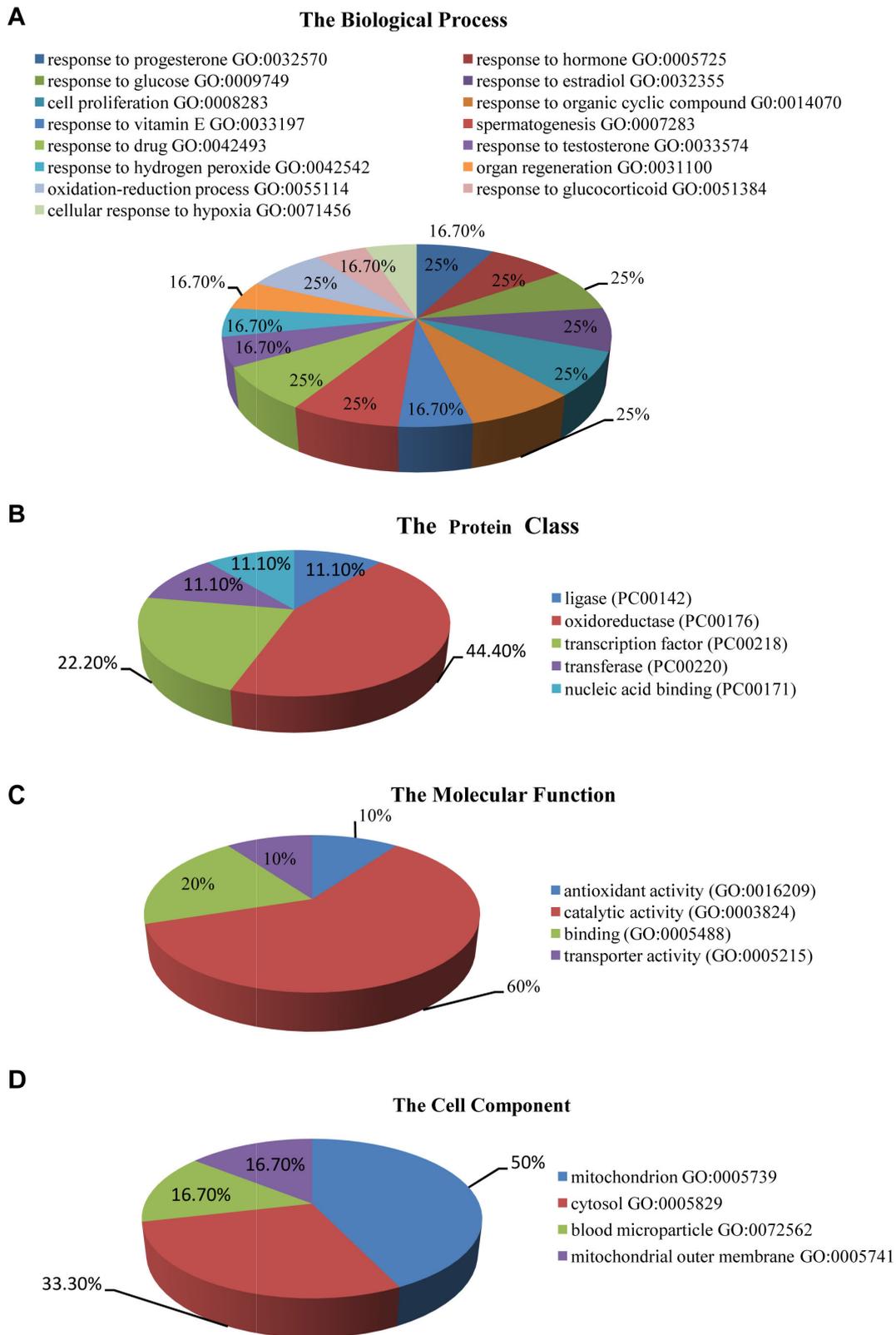


FIGURE 4 - Biological Process analysis based on DAVID data bank and GO enrichment analysis of differentially expressed genes. (A) Biological Process enrichment; (B) Protein Class analysis; (C) Molecular Function enrichment; (D) Cell Component enrichment.

TABLE V- Gene classification based on functions

Inflammation	Apoptosis	Metabolism	Oxidative stress	Signal transduction
NM_144739	NM_030826	NM_031835	NM_054002	XM_131329
NM_053288	NM_031835	A1236753	NM_022698	NM_031835
NM_031051	NM_144739	NM_019286	NM_053607	XM_135927
BF284744	NM_024362	NM_022547		
M91235				

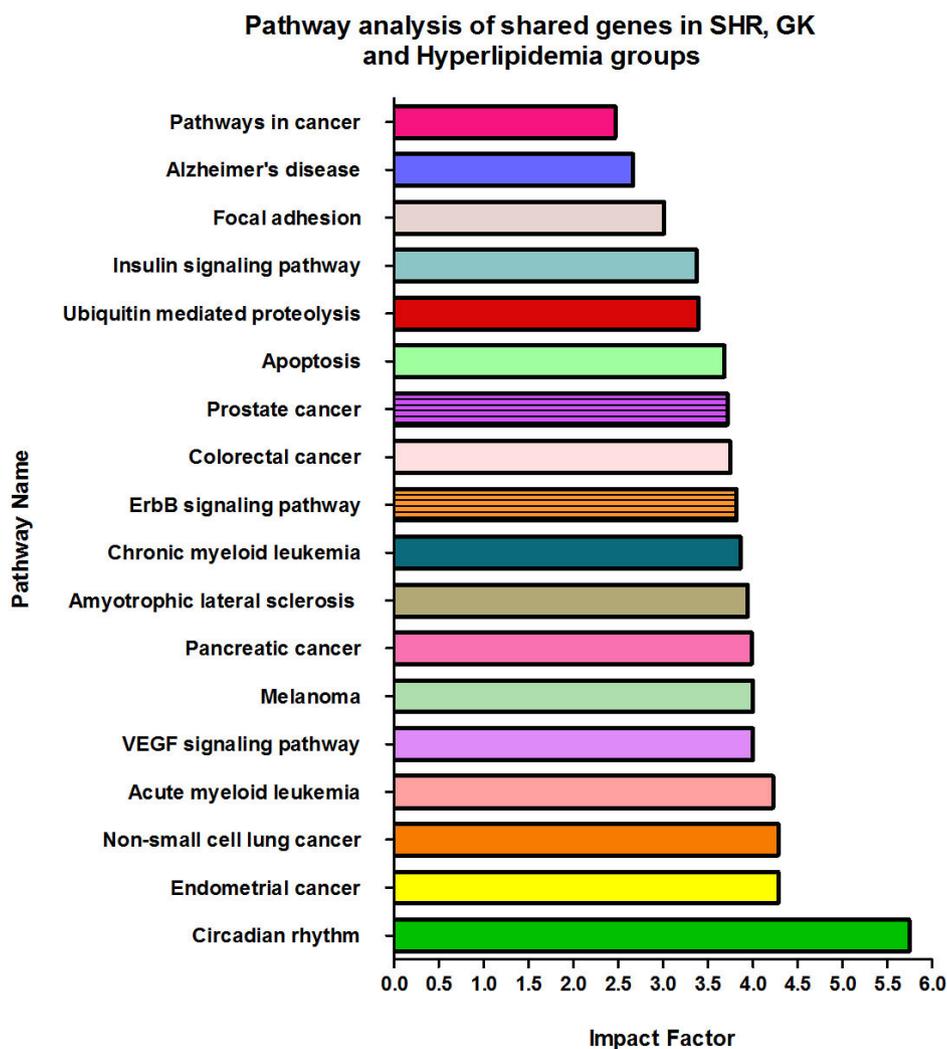


FIGURE 5 - Pathway analysis of shared genes in SHR, GK and HMR using Onto-tools Pathway Express. Pathways were associated with the circadian rhythm pathway, endometrial cancer pathway, pancreatic cancer pathway, etc.

Some points should be considered in the interpretation of the results of the present study. Valencia *et al.* and Y. Wu *et al.* reported that the GPx1 (*Rattus norvegicus* Glutathione peroxidase 1) gene could reduce the expression of the p53 gene (Y. Wu *et al.*, 2014), endothelial damage, mitochondrial dysfunction and reactive oxygen species accumulation in metabolic syndrome (Valencia *et al.*, 2016). More specifically, the function of GPx1 in most aortic regions could also reduce diabetes-associated atherosclerosis and protect mice from renal structural and functional disorders (Chew *et al.*, 2010). These findings were consistent with those presented in Figure (4A, 4B, 4C, 4D). Specifically, the oxidation-reduction process separately accounted for Go:0055114 (25%), oxidoreductase (44.4%), antioxidant activity (10%), and mitochondrion (50%).

To demonstrate adequate agreement and validate microarray results, a previous study reported that ARNTL (Aryl Hydrocarbon Receptor Nuclear Translocator-Like) was characterized as the crucial gene involved in energy homeostasis, Type 2 diabetes, hypertension, and circadian rhythm (Shimba *et al.*, 2011). Additionally, a genetic association study of 1,304 individuals from 424 families primarily selected for type 2 diabetes revealed that ARNTL was associated with type 2 diabetes and hypertension (Woon *et al.*, 2007). Crucially, recent studies have validated ARNTL as a novel anti-oncogene to control progression by activating the p53 tumor suppressor pathway in pancreatic cancer (Jiang *et al.*, 2016; Kiessling *et al.*, 2017). Interestingly, these findings were consistent with the biological pathways identified in this study, which were associated with the circadian rhythm pathway, endometrial cancer pathway, and pancreatic cancer pathway in Figure 5.

To date, previous studies exclusively investigated some key genes and significant pathways to identify the effects on the disease of hypertension, diabetes and hyperlipidemia. However, in the present study, the shared genes identified from these three diseases via gene microarray analysis were first conducted to assess the relationship with disease through bioinformatics analysis. Moreover, more studies are needed to identify the roles in the hypertension, diabetes and hyperlipidemia, leading to new breakthroughs in etiopathogenesis, prevention and treatment.

CONCLUSION

In summary, the network genes that we constructed by Cytoscape revealed the complex interaction of the shared genes from SHR, GK and HMR compared with normal Wistar rats, such as Bad, Orm1, Arntl, Zbtb7a, Mif and Gpx1, which could affect the related pathways by influencing inflammatory factors, apoptosis factors, metabolic factors, oxidative factors and signal transduction factors, such as the circadian rhythm pathway, endometrial cancer pathway, apoptotic pathway, and insulin signaling pathway.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

- Buck C, Donner A. Cancer incidence in hypertensives. *Cancer*.1987;59(7):1386-1390.
- Chen QC, Xiao J, Zhang PP, Chen LL, Chen XX, Wang SM. Longitudinal Changes in Liver Aminotransferases Predict Metabolic Syndrome in Chinese Patients with Nonviral Hepatitis. *Biomed Environ Sci*. 2016;29(4):254-266.
- Chew P, Yuen DY, Stefanovic N, Pete J, Coughlan MT, Jandeleit-Dahm KA, et al. Antiatherosclerotic and renoprotective effects of ebselen in the diabetic apolipoprotein E/GPx1-double knockout mouse. *Diabetes*. 2010;59(12):3198-3207.
- Choi CH, Park YA, Choi JJ, Song T, Song SY, Lee YY, et al. Angiotensin II type I receptor and miR-155 in endometrial cancers: synergistic antiproliferative effects of anti-miR-155 and losartan on endometrial cancer cells. *Gynecol Oncol*. 2012;126(1):124-131.
- Dyer AR, Stamler J, Berkson DM, Lindberg HA, Stevens E. High blood-pressure: a risk factor for cancer mortality? *Lancet*. 1975;1(7915):1051-1056.
- Felmeden DC, Lip GY. Antihypertensive therapy and cancer risk. *Drug Saf*. 2001;24(10):727-739.

- Hindle AK, Edwards C, McCaffrey T, Fu S, Brody F. Identification of cardiovascular genes in omentum from morbidly obese patients with type 2 diabetes. *Int J Obes (Lond)*. 2010;34(6):1020-1027.
- Hye Khan MA, Kolb L, Skibba M, Hartmann M, Blocher R, Proschak E, et al. A novel dual PPAR-gamma agonist/sEH inhibitor treats diabetic complications in a rat model of type 2 diabetes. *Diabetologia*. 2018;61(10):2235-2246.
- Je Y, Kim Y, Park T. Development of a self-assessment score for metabolic syndrome risk in non-obese Korean adults. *Asia Pac J Clin Nutr*. 2017;26(2):220-226.
- Jeong JR, Kim S, Jo SR, Joh JY, Kim YP. Health Behaviors of Breast Cancer Survivors with Hypertension: A Propensity Analysis of KNHANES III-V (2005-2012). *PLoS One*. 2015;10(5):e0127346.
- Jiang W, Zhao S, Jiang X, Zhang E, Hu G, Hu B, et al. The circadian clock gene *Bmal1* acts as a potential anti-oncogene in pancreatic cancer by activating the p53 tumor suppressor pathway. *Cancer Lett*. 2016;371(2):314-325.
- Kiessling S, Beaulieu-Laroche L, Blum ID, Landgraf D, Welsh DK, Storch KF, et al. Enhancing circadian clock function in cancer cells inhibits tumor growth. *BMC Biol*. 2017;15(1):13.
- Lee YS, Yang WK, Kim HY, Min B, Caturla N, Jones J, et al. *Metabolaidd(R)* Combination of Lemon Verbena and Hibiscus Flower Extract Prevents High-Fat Diet-Induced Obesity through AMP-Activated Protein Kinase Activation. *Nutrients*. 2018;10(9).
- Parekh N, Okada T, Lu-Yao GL. Obesity, insulin resistance, and cancer prognosis: implications for practice for providing care among cancer survivors. *J Am Diet Assoc*. 2009;109(8):1346-1353.
- Patel TP, Rawal K, Bagchi AK, Akolkar G, Bernardes N, Dias Dda S, et al. Insulin resistance: an additional risk factor in the pathogenesis of cardiovascular disease in type 2 diabetes. *Heart Fail Rev*. 2016;21(1):11-23.
- Rahmatallah Y, Khaidakov M, Lai KK, Goyne HE, Lamps LW, Hagedorn CH, et al. Platform-independent gene expression signature differentiates sessile serrated adenomas/polyps and hyperplastic polyps of the colon. *BMC Med Genomics*. 2017;10(1):81.
- Shimba S, Ogawa T, Hitosugi S, Ichihashi Y, Nakadaira Y, Kobayashi M, et al. Deficient of a clock gene, brain and muscle *Arnt*-like protein-1 (*BMAL1*), induces dyslipidemia and ectopic fat formation. *PLoS One*. 2011; 6(9):e25231.
- Sun YF, Cao J, Li XL, Fan L, Wang Q, Wang H, et al. [Correlation of coronary heart disease with multiple genes, gene polymorphisms and multiple risk factors in old Chinese Han patients]. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*. 2012;28(5):411-417.
- Takeyama A, Nagata Y, Shirouchi B, Nonaka C, Aoki H, Haraguchi T, et al. Dietary *Sparassis crispa* Reduces Body Fat Mass and Hepatic Lipid Levels by Enhancing Energy Expenditure and Suppressing Lipogenesis in Rats. *J Oleo Sci*. 2018;67(9):1137-1147.
- Valencia AP, Schappal AE, Morris EM, Thyfault JP, Lowe DA, Spangenburg EE. The presence of the ovary prevents hepatic mitochondrial oxidative stress in young and aged female mice through glutathione peroxidase 1. *Exp Gerontol*. 2016;73:14-22.
- Wannamethee G, Shaper AG. Blood pressure and cancer in middle-aged British men. *Int J Epidemiol*. 1996;25(1):22-31.
- Woon PY, Kaisaki PJ, Braganca J, Bihoreau MT, Levy JC, Farrall M, et al. Aryl hydrocarbon receptor nuclear translocator-like (*BMAL1*) is associated with susceptibility to hypertension and type 2 diabetes. *Proc Natl Acad Sci U S A*. 2007;104(36):14412-14417.
- Wu C, Zhao Y, Lin Y, Yang X, Yan M, Min Y, et al. Bioinformatics analysis of differentially expressed gene profiles associated with systemic lupus erythematosus. *Mol Med Rep*. 2018;17(3):3591-3598.
- Wu Y, Zhou H, Wu K, Lee S, Li R, Liu X. *PTEN* phosphorylation and nuclear export mediate free fatty acid-induced oxidative stress. *Antioxid Redox Signal*. 2014;20(9):1382-1395.
- Yokomichi H, Nagai A, Hirata M, Tamakoshi A, Kiyohara Y, Kamatani Y, et al. Statin use and all-cause and cancer mortality: BioBank Japan cohort. *J Epidemiol*. 2017;27(3S):S84-S91.
- You Z, Genest J, Jr Barrette PO, Hafiane A, Behm DJ, D'Orleans-Juste P, et al. Genetic and pharmacological manipulation of urotensin II ameliorate the metabolic and atherosclerosis sequelae in mice. *Arterioscler Thromb Vasc Biol*. 2012;32(8):1809-1816.
- Zhao Y, Yu Y, Shi M, Yang X, Li X, Jiang F, et al. Association study to evaluate *TFPI* gene in CAD in Han Chinese. *BMC Cardiovasc Disord*. 2017;17(1):188.



Wenzhu Dong, Hangping Chen, Lu Wang, Xiaoqian Cao, Xiawei Bu, Yan Peng, Aiqing Dong, Mengjiang Ying, Xu Chen², Xin Zhang, Li Yao

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Associated Metabolic Syndrome in Mice. *Am J Pathol.* 2018;188(2):461-473.

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