A pathway-based network analysis of hypertension-related genes

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Abstract

Complex network approach has become an effective way to describe interrelationships among large amounts of biological data, which is especially useful in finding core functions and global behavior of biological systems. Hypertension is a complex disease caused by many reasons including genetic, physiological, psychological and even social factors. In this paper, based on the information of biological pathways, we construct a network model of hypertension-related genes of the salt-sensitive rat to explore the interrelationship between genes. Statistical and topological characteristics show that the network has the small-world but not scale-free property, and exhibits a modular structure, revealing compact and complex connections among these genes. By the threshold of integrated centrality larger than 0.71, seven key hub genes are found: \textit{Jun}, \textit{Rps6kb1}, \textit{Cycs}, \textit{Creb3l2}, \textit{Cdk4}, \textit{Actg1} and \textit{RT1-Da}. These genes should play an important role in hypertension, suggesting that the treatment of hypertension should focus on the combination of drugs on multiple genes.

Keywords: Complex network; Hypertension; Hub gene; Pathway; Modular structure

1. Introduction

The study of complex systems clearly shows that the global behavior of systems is determined by their structure rather than by the properties of their individual parts. The complex network approach has become a powerful tool for studying complex systems, and the global properties of systems are usually studied by abstracting individual elements of systems into nodes and reducing interactions between elements to edges between nodes \{1, 2, 3, 4, 5\}. Such an approach has been widely applied to understanding gene functions in biological and medical research.\[6, 7, 8, 9, 10.\]

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Essential hypertension, which accounts for about 90%–95% of all cases of hypertension [11], is a disease caused by long-term interaction between genetic and environmental factors, and salt is one of the important environmental factors [12]. The blood pressure response to salt loading or salt restriction is heterogeneous among individuals, which is known as salt sensitivity [13, 14, 15]. Salt sensitivity is the genetic susceptibility of individual blood pressure response to salt, and is an intermediate phenotype of essential hypertension [16, 17]. The people who suffer the salt-sensitive (SS) hypertension account for about 50% of hypertensive patients [15]. Although the clinical research and treatment of hypertension have improved dramatically [18, 19, 20], its molecular mechanisms and pathologies involved are still difficult to ascertain.

Many omic data have been obtained and become available through advanced high-throughput technologies, which provide the basis for studying the relationship of biological data by network approach [7, 10]. Various biomolecular networks have been constructed to discover essential functions and mechanisms of biological phenomena [6, 8, 9]. For instance, Censi et al. studied the gene regulatory networks induced in heart tissue by atrial fibrillation [21]. Demicheli and Coradini analyzed breast cancer behavior using gene regulatory networks [22]. Therefore, it is of significance to understand hypertension disease at system level using the complex network approach.

In our previous study [23], we constructed a hypertension-related gene co-expression network by focusing on the analysis of gene expression data (GED) [24] among the Dahl SS rat [25, 26] and two consomic rat strains [27, 28], where the 335 nodes are individual genes and the connections are derived from the expression correlations. This is a theoretical analysis based on GED to determine the key hub genes (nodes) and explore the relationship between these hub genes and hypertension. However, to get more biologically relevant information about hypertension, a pathway-based gene network should also be constructed using the actual biological correlations.

In this paper, we will construct the network model of hypertension-related genes according to whether these genes are involved in the same pathways in the KEGG database. Network approach will be employed to investigate the possible relations between network structure and hypertension-related genes based on these data. Through calculating several statistical indices and analyzing topological characteristics of the network, we find that the pathway-based gene network exhibits the small-world but not scale-free property. Meanwhile, the network also exhibits a modular structure: the nodes of the network can be properly divided into groups within which the nodes are highly connected, but between which they are much less connected. The modular structure analysis can visualize the weak connections of the network, and thus help us to study drug targets of hypertension. The results from this paper and the analysis in Ref. [23] would complement each other.

The rest of this paper is organized as follows. In Section 2, we introduce the data source and construct the pathway-based gene network model of hypertension. In Section 3, we analyze the statistical and topological characteristics of the gene network. The modular structure of the network is presented in Section 4, while Section 5 presents summary and concluding remarks.

2. Data source and network construction

The Dahl SS rat, proposed by Dahl et al. in the early 1960s [25, 26], is a widely used genetic model of human hypertension. The consomic rat strains, used as the normotensive control for the Dahl SS rat, are generated by substituting a chromosome or a part of a chromosome from a normal rat strain for the corresponding genomic region of the SS rat [24, 27, 28]. Previous research has shown that substitution of chromosome 13 or 18 can attenuate hypertension [31, 24]. Our study will focus on the hypertension-related genes listed in Ref. [24] by analysis of biological pathways.

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1 KEGG (Kyoto Encyclopedia of Genes and Genomes) website: http://www.kegg.jp/ or http://www.genome.jp/kegg/.
Table 1: Examples of the correspondence between genes and pathways.

| Gene | Gene ID | Pathway(s)          |
|------|--------|---------------------|
| Timp1| 116310 | rno04066            |
| Casp6| 83584  | rno04210            |
| Ank3 | 361833 | rno05205            |
| Aqp1 | 25240  | rno04964, rno04976  |
| Kcnj1| 171293 | rno04142, rno05152  |
| Hist1h2ai| 502129 | rno05034, rno05322  |
| Sdc1 | 25216  | rno04512, rno04514, rno05144, rno05205 |
| Col4a1| 290905 | rno04151, rno04510, rno04512, rno04974, rno05146, rno05200, rno05222 |
| Fzd2 | 64512  | rno04310, rno04390, rno04916, rno05166, rno05200, rno05205, rno05217 |

Let us consider an undirected network $G_H = (V_H, E_H)$, where $V_H = \{v_i\}$ $(i = 1, 2, \ldots, N)$ denotes the set of $N$ nodes, and $E_H = \{v_i, v_j\}$ the set of edges or connections between nodes. We will use the following notation: $A_{ij} = 1$ indicates that there is an edge between nodes $v_i$ and $v_j$; and $A_{ij} = 0$ otherwise. Our pathway-based gene network model is constructed in two steps.

**Step 1. Selection of genes according to pathways**

We first extract nodes from the 335 different hypertension-related genes given in Ref. [24] based on the KEGG PATHWAY Database. From this Database, we can easily obtain the information of whether a gene is involved in single or multiple pathways. Here, we only consider the pathways including hypertension-related genes, and the genes not involved in any pathway are excluded from our study. Thus, the $N = 90$ hypertension-related genes, which are involved in 157 pathways in the KEGG Database [32], are extracted to serve as nodes of the network model, where each node represents an individual gene shown as gene symbol or CloneID. Examples of ten genes involved in one or more pathways are shown in Table 1, a pathway is represented by the entry name of the Database, called the KEGG object identifier consisting of a database-dependent prefix and a five-digit number (such as rno04066, where the prefix “rno” designates the species to be rat).

**Step 2. Establishment of connections**

We now consider the correlations between any two genes according to the information of pathways. If two genes $i$ and $j$ are involved in the same pathway(s), then a connection is made between such two genes (nodes):

$$A_{ij} = \begin{cases} 
1 & \text{if genes } i \text{ and } j \text{ are in the same pathway(s)}; \\
0 & \text{otherwise}. 
\end{cases}$$

Here $i, j = 1, 2, \ldots, 90$ and $i \neq j$ (note that $A_{ii} = 0$ because we do not consider self-connections of nodes, i.e., self-interactions of genes). Consequently, there are biological regulatory relationships between genes $i$ and $j$ when $A_{ij} = 1$. In such a way, we have constructed the pathway-based network of hypertension-related genes, which contains 90 nodes (genes) and 482 edges (connections), as shown in Fig. 1.

### 3. Statistical and topological characteristics of the gene network

In this section, we analyze the pathway-based gene network of hypertension by calculating the following indices: degree and average degree, degree distribution, average path length, clustering coefficient, assortativity coefficient, and four centrality indices (degree centrality, betweenness centrality, closeness centrality and integrated centrality), which can provide us with statistical and topological characteristics of the gene network.

**3.1. Degree and degree distribution**

The degree $k_i$ of a node $i$ is the number of edges connecting to the node. The average of $k_i$ over all nodes is called the average degree of the network, and is denoted as $\langle k \rangle = \frac{1}{N} \sum_{i=1}^{N} k_i$. The degree distribution function $P(k)$ gives the probability that a randomly selected node has exactly $k$ edges [3, 4]. The degree distribution is one of the most basic quantitative properties of a network.
For the pathway-based gene network of hypertension, the degree $k_i$ of a node $i$ is just the number of other genes which are involved in the same pathway(s) as gene $i$. Fig. 2 plots the degree and degree distribution of the gene network. Here, the values of the degree of 90 nodes are ranked in a descending order (with $n_g(k)$ denoting the rank of genes in degree values), and it is found that the gene Jun of $n_g = 1$ has the highest degree 30. The average degree $\langle k \rangle$ of this network is about 10.71. The illustration of degree distribution shows that the probability that a gene can link with $k$ other genes does not decay as a power-law, suggesting that the gene network does not have a scale-free topology.
3.2. Average path length and clustering coefficient

In a network, a path from node $i$ to node $j$ is a sequence of adjacent nodes starting at $i$ and ending at $j$. The path with the smallest number of edges between the two selected nodes is called the shortest path. The distance $d_{ij}$ between two nodes $i$ and $j$ is defined as the number of edges along the shortest path connecting them. The diameter $D$ is the maximum distance between any pair of nodes in the network, i.e., $D = \max\{d_{ij}\}$. The average path length $L$ is defined as the mean distance between two nodes, averaged over all pairs of nodes, i.e.

$$L = \frac{1}{N(N-1)} \sum_{i < j} d_{ij},$$

which offers a measure of the overall navigability of a network \[33\].

A clustering coefficient can be used to describe the cohesiveness of the neighborhood of a node \[1, 34\]. In a network, the clustering coefficient $c_i$ is defined as the ratio between the number $e_i$ of edges that actually link the $k_i$ neighbors of node $i$ to each other and the total possible number of edges among them, i.e.

$$c_i = \frac{2e_i}{k_i(k_i-1)} \quad (k_i \geq 2).$$

The clustering coefficient $C$ of the whole network is the average of $c_i$ over all $i$, $C = \frac{1}{N} \sum_{i=1}^{N} c_i$, which characterizes the overall tendency of nodes to form clusters, clearly, $C \leq 1$.

The pathway-based gene network has a very short average path length: $L$ is about 2.331, and $\log(\log N) < L < \log N$. The diameter $D$ is only 5 (i.e., the distance between genes $Ge^r$ and $Gn^p$), which implies at most five hops separate any two genes in the 482 connections of the network. The clustering coefficient is calculated to be $C = 0.6403$, which is relatively high. Therefore, the pathway-based gene network has the small-world property (short $L$ and high $C$).

3.3. Assortativity

The concept of assortativity is introduced to describe degree correlations between neighboring nodes in a network \[34\]. A network is assortative if high (low) degree nodes tend to be connected to other high (low) degree nodes; otherwise, it is disassortative if high (low) degree nodes tend to be connected to other low (high) degree nodes. The assortativity can be described by the correlation between the degrees of neighboring nodes in terms of the mean Pearson correlation coefficient. Let $x_i$ and $y_i$ be the degrees of the end nodes of the $i$th edge, with $i = 1, 2, \ldots, E$ ($E$ is the number of edges in the network), then the assortativity coefficient of the network is given by \[34\]:

$$r = \frac{E^{-1} \sum_i x_i y_i - \left(E^{-1} \sum_i \frac{1}{2}(x_i + y_i)\right)^2}{E^{-1} \sum_i \frac{1}{2}(x_i^2 + y_i^2) - \left(E^{-1} \sum_i \frac{1}{2}(x_i + y_i)\right)^2} \quad (4)$$

The network is assortative if $r > 0$, and disassortative if $r < 0$. The assortativity coefficient of the pathway-based gene network is calculated to be $r = 0.2178$, exhibiting an assortative behavior. This is different from most biological networks which show negative $r$.

3.4. Centrality

3.4.1. Definitions of four centrality indices

The degree centrality $C_{d}$, betweenness centrality $C_{b}$ and closeness centrality $C_{c}$ are three centrality indices commonly used in finding out the centralization nodes of the network \[35, 36\]. Recently, we also introduced an integrated centrality $C_{mig}$ to fully reflect the contribution of three centrality indices $[C_{d}, C_{b}, C_{c}]$ \[23\].

The degree centrality of a given node $i$ is the proportion of other nodes that are adjacent to node $i$ \[36\], i.e.

$$C_d(i) = \frac{k_i}{N-1} \quad (5)$$
Table 2: Top 25 values of degree centrality $C_d$, betweenness centrality $C_b$, closeness centrality $C_c$ and integrated centrality $C_{intgr}$ in the pathway-based gene network of hypertension. In this paper, nine genes are expressed as abbreviations (cf. Fig. 6 and its caption).

| Gene        | $C_d$   | $C_b$   | $C_c$   | $C_{intgr}$ |
|-------------|---------|---------|---------|-------------|
| Jun         | 0.3271  | 0.0627  | 0.5387  | 0.9280      |
| Cdk4        | 0.3146  | 0.0731  | 0.4800  | 0.8359      |
| RT1-Da      | 0.3034  | 0.0690  | 0.4653  | 0.8156      |
| Pdgfra      | 0.3034  | 0.0605  | 0.4653  | 0.8027      |
| Prolb1      | 0.2921  | 0.1579  | 0.4446  | 0.7547      |
| Crol32      | 0.2809  | 0.0574  | 0.4446  | 0.7281      |
| Actg1       | 0.2809  | 0.1487  | 0.4446  | 0.7128      |
| Cftr        | 0.2697  | 0.0406  | 0.4443  | 0.6662      |
| Fasl        | 0.2697  | 0.0364  | 0.4443  | 0.6598      |
| Col4a1      | 0.2584  | 0.0363  | 0.4381  | 0.6462      |
| Col4a2      | 0.2584  | 0.0308  | 0.4381  | 0.6390      |
| Fzd2        | 0.2472  | 0.0274  | 0.4318  | 0.6293      |
| Cycs        | 0.2360  | 0.0259  | 0.4287  | 0.6206      |
| Shc1        | 0.2360  | 0.0213  | 0.4227  | 0.6041      |
| Tgfb1       | 0.2360  | 0.0213  | 0.4227  | 0.5956      |
| Fgfr1       | 0.2247  | 0.0197  | 0.4277  | 0.5841      |
| Col2a1      | 0.2135  | 0.0195  | 0.4197  | 0.5678      |
| Col5a1      | 0.2135  | 0.0184  | 0.4111  | 0.5678      |
| Col5a2      | 0.2135  | 0.0183  | 0.4083  | 0.5640      |
| Col6a1      | 0.2135  | 0.0145  | 0.4056  | 0.5388      |
| Shh         | 0.2022  | 0.0125  | 0.4056  | 0.4980      |
| Mmp2        | 0.2022  | 0.0108  | 0.4056  | 0.4980      |
| Ctsl        | 0.2022  | 0.0101  | 0.4056  | 0.4980      |
| Col6a1      | 0.1798  | 0.0098  | 0.4056  | 0.4980      |
| Pchth1      | 0.1798  | 0.0098  | 0.3949  | 0.4796      |

here $N - 1$ is the maximum possible degree of the network.

The betweenness centrality of a node $i$ is defined as the proportion of all shortest paths (geodesics) between pairs of other nodes that include this node $i$ [36]:

$$C_b(i) = \frac{N - 1}{\sum_{j \neq i} \sum_{k \neq j} g_{jk}(i)} = \frac{N - 1}{\sum_{j \neq i} d_{ij}},$$

where $g_{jk}$ is the number of shortest paths between nodes $j$ and $k$, and $g_{jk}(i)$ the number of shortest paths containing node $i$ between nodes $j$ and $k$.

The closeness centrality of a node $i$ is the number of other nodes divided by the sum of the distances between node $i$ and all others [35, 36]:

$$C_c(i) = (L_i)^{-1} = \frac{N - 1}{\sum_{j=1}^{N} d_{ij}},$$

where $L_i$ is the average distance between node $i$ and all other nodes.

To comprehensively and quantitatively reflect the contribution of the above three centrality indices $[C_d, C_b, C_c]$, we can also introduce the integrated centrality $C_{intgr}$ of node $i$, defined as follows [23]:

$$C_{intgr}(i) = \frac{1}{3} \left[ \frac{C_d(i)}{C_{d,max}} + \frac{C_b(i)}{C_{b,max}} + \frac{C_c(i)}{C_{c,max}} \right],$$

where $C_{d,max}$, $C_{b,max}$ and $C_{c,max}$ are the maximums of $[C_d]$, $[C_b]$ and $[C_c]$, respectively. Obviously, $C_{intgr}(i)$ has a value between 0 and 1.

### 3.4.2. Centrality analysis and hub genes

For the pathway-based gene network of hypertension, the four centrality indices are calculated based on the above definitions, and the top 25 values of each centrality index are listed in Table 2. In the following, we will determine hub genes in the network through centrality analysis.

Fig. 5 describes the correspondence among degree centrality $C_d$, betweenness centrality $C_b$ and closeness centrality $C_c$ of nodes in the pathway-based gene network. We can see in Fig. 5 that $C_c$ of most of the nodes is distributed
in the range of (0.25, 0.51). In this range of $C_c$, we also observe that in Fig. 3 (left) the distribution of $C_d$ is roughly uniform in the range of (0.01, 0.34); while in Fig. 3 (right) $C_b$ is non-uniformly distributed in the range of [0, 0.094), here most of the nodes have very small $C_b$, and only a few nodes have large $C_b$ which also have relatively large $C_c$. Fig. 4 combines the three centrality indices $[C_d, C_b, C_c]$ in the three-dimensional space. It can be seen from Fig. 4 that a small number of nodes have high values of three centrality indices, which can be viewed as hubs of the network. Generally speaking, each of these three centrality indices has its own focus on an influence of a node on other nodes in the network, thus one can identify hubs according to that focus. However, in order to fully reflect the contribution of all these three centrality indices, we will simply determine key hub genes using integrated centrality $C_{intgr}$, as in Ref. [23].
Figure 5: The values of integrated centrality $C_{\text{intrag}}$ of 90 nodes in descending order for the pathway-based gene network. The red circles represent the seven hub genes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3: Biological descriptions of seven hub genes in the pathway-based gene network.

| Gene   | Official full name       | Gene ID | Description                                           |
|--------|--------------------------|---------|-------------------------------------------------------|
| Jun    | jun proto-oncogene       | 24 516  | Encodes a protein that exhibits double-stranded DNA binding |
| Rps6kb1| ribosomal protein S6 kinase, polypeptide 1 | 83 840  | Encodes a protein that exhibits ATP binding; peptide binding |
| Cycs   | cytochrome c. somatic    | 25 309  | Encodes a protein that exhibits electron carrier activity |
| Creb3l2| cAMP responsive element binding protein 3-like 2 | 362 339 | Encodes a protein that exhibits cAMP response element binding |
| Cdk4   | cyclin-dependent kinase-4 | 94 201  | Related to circadian rhythm; organ regeneration       |
| Actg1  | actin, gamma-1           | 287 876 | Involved in response to calcium ion                    |
| RT1-Da | RT1 class II, locus Da   | 294 269 | Involved in antigen processing                        |

As calculated and shown above, the pathway-based gene network is not scale-free. Fig. 5 illustrates the integrated centrality $C_{\text{intrag}}$ of 90 nodes in a descending order (with $n_i(C_{\text{intrag}})$ denoting the rank of genes in $C_{\text{intrag}}$) for the network, which shows that the top seven nodes (red) have high values of $C_{\text{intrag}}$. From Table 2 and Fig. 5, seven nodes are determined as important hub genes: Jun, Rps6kb1, Cycs, Creb3l2, Cdk4, Actg1 and RT1-Da. These seven genes have the integrated centrality of $C_{\text{intrag}} > 0.71$, which is about 77% of its maximum (0.9280). However, it should be mentioned that there is no strict significance threshold for $C_{\text{intrag}}$, and one can also lower the threshold to enable more genes to be included in hub genes.

In Table 3 we list the official full names, gene IDs and biological descriptions of seven hub genes. Here, we also provide a brief biological description of the first hub gene. The gene Jun is ranked first in $C_{\text{intrag}}$ because it has the highest $C_g$ and $C_r$, as well as the second highest $C_b$. Jun encodes a protein that exhibits double-stranded DNA binding, involved in aging, angiogenesis, endothelin and Rho/Rac/Cdc42 mediated signaling pathways. It is associated with kidney neoplasms and spinal cord injuries. Recent studies have confirmed that Jun is closely related to low potassium reaction and cell renal cell carcinoma. A low potassium diet might induce hypertension, which is always accompanied by hypokalemia. The incidence of renal cell carcinoma coupled with hypertension is up to 14%–40% [17].

Among the seven hub genes, we note that the gene Cdk4 is the only common hub gene in both the pathway-based gene network here and the gene co-expression network of our previous study [23]. Besides Cdk4, we also see in Table 2 that the three genes Shc1, Fzd2 and Col4a1, which have relatively high integrated centrality $C_{\text{intrag}} > 0.60$, are the hub genes identified in the gene co-expression network of Ref. [23]. These four genes have been confirmed by biological and medical research to play important roles in hypertension. Moreover, we also observe that although the gene Sdhb is ranked only joint 17th in $C_{\text{intrag}} (= 0.5678)$, it has the highest betweenness centrality $C_b$. Since $C_b$ is based on the shortest paths and reflects the ability of a node to influence other related nodes in the network, Sdhb should also be a key gene in hypertension. If we lower the threshold of integrated centrality to $C_{\text{intrag}} > 0.50$, then these four genes (Shc1, Fzd2, Col4a1 and Sdhb) can also be included in hub genes. In this paper, we do not take this
Table 4: Dissimilarity scores $d(i,j)$ of ten nodes (genes) in the pathway-based gene network. The specific modules, which are identified after the completion of the modular decomposition, are indicated in parentheses in the first column.

|       | Jun (I) | RT1-Da (I) | Col4a1 (I) | Fzd2 (I) | Sdhb (II) | Gda (II) | Sec13l1 (III) | Sumol1 (III) | Aqp1 (IV) | Kcnj1 (V) |
|-------|---------|-------------|------------|----------|-----------|----------|----------------|-------------|-----------|-----------|
| Jun   | 0.0000  | 0.4310      | 0.2931     | 0.2414   | 0.7241    | 0.6897   | 0.6034         | 0.5517      | 0.5345    | 0.5172    |
| RT1-Da| 0.4310  | 0.0000      | 0.5172     | 0.3621   | 0.7069    | 0.7069   | 0.5517         | 0.5000      | 0.4828    | 0.4655    |
| Col4a1| 0.2931  | 0.5172      | 0.0000     | 0.2931   | 0.6034    | 0.6379   | 0.4828         | 0.4310      | 0.4138    | 0.3966    |
| Fzd2  | 0.2414  | 0.3621      | 0.2931     | 0.0000   | 0.5862    | 0.6207   | 0.4655         | 0.4138      | 0.3966    | 0.3793    |
| Sdhb  | 0.7241  | 0.7069      | 0.6034     | 0.5862   | 0.0000    | 0.1034   | 0.3966         | 0.3448      | 0.3276    | 0.3103    |
| Gda   | 0.6897  | 0.7069      | 0.6379     | 0.6207   | 0.1034    | 0.0000   | 0.3276         | 0.2759      | 0.2386    | 0.2414    |
| Sec13l| 0.6034  | 0.5517      | 0.4828     | 0.4655   | 0.3966    | 0.3276   | 0.0000         | 0.0517      | 0.1034    | 0.0862    |
| Sumol1| 0.5517  | 0.5000      | 0.4310     | 0.4138   | 0.3448    | 0.2759   | 0.0000         | 0.0517      | 0.0345    | 0.0345    |
| Aqp1  | 0.5345  | 0.4828      | 0.4138     | 0.3966   | 0.2756    | 0.2586   | 0.1034         | 0.0517      | 0.0000    | 0.0172    |
| Kcnj1 | 0.5172  | 0.4655      | 0.3966     | 0.3793   | 0.3103    | 0.2414   | 0.0862         | 0.0345      | 0.0172    | 0.0000    |

lower threshold of $C_{Ingr} > 0.50$ because, in view of the relatively large range of variation of $C_b$, we do not want the genes of small $C_b$ to be included in hub genes. In a wider view, however, these four genes ($Shc1$, $Fzd2$, $Col4a1$ and $Sdhb$), together with the above seven hub genes, are worthy of further study in the future.

4. Modular structure of the gene network

Many networks are found to divide naturally into modules or communities, i.e., groups of nodes within which the connections are relatively dense but between which they are sparser [38, 39]. In this section, we explore the modular structure of the pathway-based gene network of hypertension.

4.1. Structural equivalence of nodes

Two nodes are structurally equivalent if they have identical connections with all other nodes. We can use a dissimilarity index $d_s$ to measure the equivalence of two nodes $i$ and $j$ as follows [40]:

$$d_s(i,j) = \frac{|V(i) + V(j)|}{k_1 + k_2}. \quad (9)$$

Here $i, j = 1, 2, \ldots, 90$, $V(i)$ are all neighbors of node $i$, $|\cdot|$ stands for set cardinality, $k_1$ and $k_2$ stand for the largest and the second largest degree in the network, respectively. Obviously, $d_s(i,j)$ has a value between 0 (completely similar) and 1 (completely different). In Table 4, we list the dissimilarity scores of ten genes distributed in different modules (cf. Fig. 7).

The dissimilarity scores allow us to cluster nodes in accordance with the structural equivalence into the corresponding positions by the hierarchical clustering technique. First, the nodes that are most similar are grouped into a cluster. Then, the next pair of nodes or clusters that are most similar are grouped, and this process continues until all nodes have been joined. The dendrogram in Fig. 6 is obtained with Pajek software [40, 41], which visualizes the above clustering process.

4.2. Modular decomposition of the network

Based on the above dendrogram, we can obtain the modular structure of the gene network, which is shown in Fig. 7. The network consists of five modules:

(I) the largest module with 58 nodes (red and green);
(II) the second largest module with 16 nodes (blue);
(III) a small module with the highest clustering coefficient including 6 nodes (brown);
(IV) two pairs of adjacent nodes (each joined by a single connection) (purple);
(V) six isolated nodes (yellow).
Figure 6: Dendrogram of similarities. It shows the hierarchical clustering of the pathway-based gene network. In this paper, the following nine genes (as recorded in Ref. [24]), Rhod predicted, Polr3e predicted, Hist1h2ai predicted // Hist1h4a predicted, Sdhb predicted, Actg1 // LOC2955810, Col6a1 predicted, Col4a2 predicted, Prcp predicted, and Cd36 // RGD1562323 predicted, are abbreviated as Rhod, Polr3e, Hist1h2ai, Sdhb, Actg1, Col6a1, Col4a2, Prcp, and Cd36, respectively.
We can observe that modules I and II constitute the largest connected part of the network, containing 74 nodes and 471 edges; and the nodes are highly connected within a module but much less connected between modules.

In Table 5 we list several statistical characteristics of every module, including the number $N_m$ of nodes, node proportion $N_m/N$, average degree $\langle k \rangle_m$ and clustering coefficient $C_m$ ($m = I, II, III, IV, V$). Here $C_m$ is the average of $c_i$ over all nodes in module $m$. From Table 5 we can see that the average degree of each of the two modules I and II ($\langle k \rangle_I = 12.72, \langle k \rangle_{II} = 12.75$) is greater than the average degree $\langle k \rangle = 10.71$ of the whole network. Except modules IV and V of $C_{IV} = C_{V} = 0$, the clustering coefficient of each of other three modules (I–III) exceeds that of the whole network ($C = 0.6403$), showing that there are more connections within each of these three modules, which justifies the modular decomposition of the whole network.

### 4.3. Characteristics of modular structure

The connections between nodes in the gene network are created based on whether genes are involved in the same pathway(s). Having examined the number of pathways in which each gene is involved, we find that there are nine genes involved in more than 10 pathways: Jun (30), RT1-Da (21), Cdk4 (17), Cycs (17), Creb3l2 (16), Actg1 (15), Pdgfra (14), Tgfbr1 (14) and Shc1 (13), here the number of pathways involved is shown in parentheses. These nine genes (red nodes in Fig. 7) are a basis of dense connections within the largest module I. We also note that among these nine genes, there are six hub genes (large nodes marked red); another hub gene Rps6kb1 (large node marked green) is involved in nine pathways.

We can examine the robustness of the network based on modular structure [39, 42]. The nodes within a module (except IV and V in this paper) are relatively robust against mutation because there are multiple paths between any two nodes and thus the network will not be easily broken when mutation occurs. Nevertheless, the parts of fewer

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**Table 5: Statistical characteristics of five modules in the pathway-based gene network.**

| Module | $N_m$ | $N_m/N$ | $\langle k \rangle_m$ | $C_m$ |
|--------|-------|---------|-----------------------|-------|
| I      | 58    | 64.44%  | 12.72                 | 0.6545|
| II     | 16    | 17.78%  | 12.75                 | 0.8917|
| III    | 6     | 6.67%   | 3                     | 0.9000|
| IV     | 4     | 4.44%   | 1                     | 0     |
| V      | 6     | 6.67%   | 0                     | 0     |

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**Figure 7: Modular structure of the pathway-based gene network.** Modules I–V contain 58, 16, 6, 4 and 6 nodes, respectively. The nine nodes marked red in module I correspond to the top nine genes involved in the largest number of pathways. The seven large nodes (six red and one green) indicate the hub genes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
connections between modules should be the weaknesses of the network system. Fig. 7 visualizes the weak links of
the network. The removal of these weak connections and relevant nodes would result in the breaking of the network.
Thus, the modular structure analysis can facilitate the exploration of the relationship between weak connections of the
network and drug targets of hypertension.

The genes in the largest connected part (i.e., core modules I and II) are involved in multiple pathways, and
contribute to a variety of biological functions. It is difficult to assign each of these genes to a single biological function
because of pleiotropy, namely, one gene might influence many different biological processes in organisms [43]. How-
ever, the genes in the small non-core module III with six nodes are involved in only two specific pathways, rno03013
(RNA transport) and rno04141 (protein processing in endoplasmic reticulum (ER)), which indicates that the non-core
module III corresponds to clearly identified pathways and functions relatively independently.

In the pathway rno03013, the different RNA species produced in the nucleus are exported through the nuclear pore
complexes (NPCs) to the cytoplasm via mobile export receptors, which is fundamental for gene expression. In the
pathway rno04141, newly synthesized peptides enter the ER via the sec61 pore and are glycosylated; correctly folded
proteins are packaged into transport vesicles and misfolded proteins are retained within the ER lumen (cf. KEGG
PATHWAY Database).

5. Summary and concluding remarks

Hypertension is a cardiovascular disease associated with long-term interaction between genetic and environmental
factors. In this study, we use pathways data to obtain backwards the relationships between the hypertension-related
genes, try to extract the complex interactions between genes through calculating statistical characteristics and analyzing
modular structure of the network.

The pathway-based gene network has the following characteristics: (i) The network does not obey a power-law
degree distribution and thus is not of a scale-free property. The seven hub genes that are identified by integrated
centrality $C_{intgr} > 0.71$ are: $Jun$, $Rps6kb1$, $Cycs$, $Creb3l2$, $Cdk4$, $Actg1$ and $RT1-Da$; they are key (feature) genes
involved in the formation of hypertension. (ii) The network shows the small-world property (i.e., a small $L$ and a large
$C$), which reveals the direct influence of these hub genes on hypertension from another perspective. (iii) The network
has a modular structure. The weak connections of the network can be visualized by its modular structure, which can
help to screen out key hypertension-related genes or pathways.

In this paper, we construct the network model of hypertension-related genes based on biological pathways. Among
the seven hub genes identified in this network, only $Cdk4$ is also a hub gene in the gene co-expression network of our
previous study [23]. Besides $Cdk4$, the three genes $Shc1$, $Fzd2$ and $Col4a1$ with $C_{intgr} > 0.60$ in the pathway-based
gene network are identified as the hub genes in the gene co-expression network of Ref. [23]. Although we can see that
more nodes will become hub genes and thus there will be more hub genes overlapped in the both networks if we
lower the threshold of $C_{intgr}$, the hub genes in the two networks are impossible to be completely overlapped because
the two networks are constructed from the different perspectives, i.e., based on the different characters of the genes.
The results from this paper and the theoretical analysis in Ref. [23] would complement each other. The seven hub
genes in the pathway-based gene network, together with the above-mentioned $Shc1$, $Fzd2$ and $Col4a1$, as well as $Sdhb$
of the highest $C_p$, can be regarded as candidate genes or drug targets for further biological and medical research on
their functions; in particular, the common hub gene $Cdk4$ of the both networks would be worth more attention.

Moreover, the network may also be analyzed based on other functional correlation methods, such as GO analy-

sis [21], to get more molecular mechanisms about hypertension. In the next study, we will develop weighted network
models and explore the mutual regulatory relationships between genes of complex diseases using dynamical analy-

sis. These studies will provide another perspective on expounding the differentially expressed genes and finding new
drug targets for other serious diseases. Finally, we expect that the complex network approach can provide clues for
exploring the pathogenesis of critical illness from molecular perspective.

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