

Identification of modules of hepatic encephalopathy based on protein-protein network and gene expression data

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Abstract. Hepatic encephalopathy (HE) is regarded as a complication of liver cirrhosis, and 50-75% of patients who have been diagnosed with cirrhosis have HE syndrome. The aim of this study was to identify genes and pathways associated with HE alcoholics. Human protein-protein interactions were downloaded from the STRING database. Gene expression data were downloaded from EMBL-EBI. Combined score and Pearson's correlation coefficient were calculated to construct differential co-expression networks. Graph-theoretical measure was used to calculate the module connectivity dynamic score of multiple differential modules. In total, 11,134 genes were obtained after mapping between probes and genes. Then, 501,736 pairs and 16,496 genes were obtained to form background protein-protein interaction networks, 1,435 edges and 460 nodes were obtained constituting differential co-expression networks. Twenty-three seed genes and 10 significantly differential modules were identified. Four significantly differential modules which had larger connectivity alternation were observed. The identified seed genes and significantly differential modules offer novel understanding and molecular targets for the treatment of HE alcoholics.

Introduction

Hepatic encephalopathy (HE) is a complication of liver cirrhosis (1), and 50-75% of patients who have been diagnosed with cirrhosis have the HE syndrome (2,3). The HE also has other types classified according to the standard constituted at the World Congress of Gastroenterology in 1998, including encephalopathy associated with acute liver disease and encephalopathy without liver disease (4). It is

reported that the damage of astrocytes triggers disturbance of neurotransmission and induces development of HE (5). Astrocytes play an important role in the central nervous system (CNS) and constitute about a third of brain cortical volume (6). Astrocytes act as the main regulators in neurotransmitters, such as transition of amino acids (7). Glutamine synthase, which maintains an ammonia balance in the human body, converts glutamate and ammonia into glutamine. Glutamine synthase is mainly contained in astrocytes (8).

Ammonia is known as a main cause of HE. It is reported that most of HE is associated with high a concentration of ammonia (9). Ammonia is mainly produced in the gut and synthesized by bacteria. High levels of ammonia disturb the neural system function and neurotransmission. Up to now, the main treatment for HE focuses on reducing the production and concentration of ammonia (10). It is reported that sodium benzoate and sodium phenylacetate are used as ammonia cleaners in patients who are diagnosed with hyperammonemia or urea cycle disorder (11). Glycerol phenylbutyrate is used to treat HE through the regulation of ammonia metabolism. Glycerol phenylbutyrate is a pro-drug of phenylacetate and decomposes nitrogen to urinary phenylacetylglutamine (12). Polyethylene glycol 3350 is used to clean the intestine and has proven to be a more effective therapy than lactulose (β -1, 4-galactosido-fructose) (13). Bass *et al* reported rifaximin significantly decreased the risk of HE compared with placebo and approximately 90% of patients were treated with lactulose (14). Lactulose, a disaccharide, cannot be digested by human intestinal disaccharidases (15). Lactulose is digested into small molecular organic acids, such as acetic acid and lactic acid. The osmotic effect produced by these acids induces fermentative diarrhoea (16). Lactulose was first reported to be used as treatment for HE in 1966 (17). Rahimi *et al* reported that lactulose caused more acute electrolyte imbalance and loss than PEG (18). Besides that, hepatic encephalopathy occurs as a complication of alcoholic liver disease mainly found in the most advanced stage (19). And while hepatic encephalopathy occurs in earlier stages, it is usually a consequence of excessive alcohol consumption before the onset of encephalopathy.

In addition, substantial molecular research is associated with HE. Protein-protein interactions (PPIs) processed by bioinformatic algorithms have been used to search for biomarkers and biological pathways in various types of cancer, such as breast cancer (20), lung cancer (21,22), colon cancer (23), ovarian cancer (24) and glioma (25).

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In the present study, we downloaded all human PPI networks and gene expression data associated with HE alcoholics. Differential co-expression networks (DCNs), which comprise 1435 edges and 460 nodes were constructed based on PPI networks and gene expression data. Twenty-three seed genes and 13 multiple differential modules (M-DMs) were identified. Ten differential modules were found when P-values were <0.05. Four differential modules had major connectivity alternation using the graph-theoretical measure method.

Materials and methods

Gene expression data. Gene expression data associated with HE alcoholics E-GEOD-53808 were downloaded from the European Molecular Biology Laboratory at European Bioinformatics Institute (EMBL-EBI). The data contained 9 control samples, 15 non-HE alcoholic samples and 8 HE alcoholic samples.

PPI networks. All human PPI networks were downloaded from the String database. There were 787,896 pairs of PPI networks and 16,730 genes. PPI network pairs were selected when the combined scores were >0.2. Then, 501,736 pairs and 16,496 genes were obtained, and these genes and networks formed background PPI networks. Expression profiling was chosen if they contained genes belonging to background PPI networks. The new expression profiling data contained 9,608 genes.

Construction of DCNs. The PPI networks were selected from background PPI networks if they contained genes belonging to the new expression profiling. The Pearson's correlation coefficients of HE and non-HE alcoholics were calculated respectively. Edges were observed when absolute values of Pearson's correlation coefficient were greater than δ ($\delta=0.9$). P-values of genes in the DCNs of two groups were calculated using one-side t-test algorithm. Weight value between gene *i* and gene *j* was calculated as:

$$w_{i,j} = \begin{cases} \frac{(\log p_i + \log p_j)^{1/2}}{(2 * \max_{j \in V} |\log p_i|)^{1/2}}, & \text{if } \text{cor}(i, j) \geq \delta, \\ 0, & \text{if } \text{cor}(i, j) < \delta, \end{cases}$$

V denotes a node set of DCN.

Identification of M-DMs. M-DMs were identified from DCNs. The process comprises of three steps: i) seed prioritization, ii) module search and iii) refinement of M-modules. Importance value (score value) of each gene in DCNs was calculated as:

$$g(i) = \sum_{j \in N_k(i)} A'_{ijk} g(j)$$

Each network had an adjacent matrix. $N_k(i)$ represents adjacent nodes of gene *i* in G_k network, A'_k represents adjacent matrix which was weighted by normalized degree; $g(i)$ equals z-score. The z-score of each gene in the DCNs was averaged and ranked. One seed gene was regarded as differential module *C*. Then, gene *u*, which was adjacent to gene *v*, was joined into module *C* to form module C' . The entropy of the two modules was calculated as:

$$H_k(C_i) = -p_i^k \log p_i^k - (1 - p_i^k) \log(1 - p_i^k)$$

$$p_i^k = \frac{L_k(i)}{L_k(i) + L_k^-(i)} \quad L_k(i) = \sum_{j \neq i, j \in C} a_{ij}$$

$L_k(i)$ denotes total weight between gene *i* and other nodes in modules *C*. $L_k^-(i)$ represents weight between gene *i* and other nodes in modules *C*.

$$H_k(C) = \sum_{i \in C} H(C_i) \quad H(C) = \frac{\sum_{k=1}^M H_k(C)}{|C|}$$

The candidate modules which had <4 nodes were eliminated. Two modules were merged into one module if the overlapped degree of two modules was >0.05. In total, 13 modules were identified.

Significant statistical test of candidate modules. One thousand, four hundred and thirty-five edges were selected from 178,888 edges and regarded as the random network. Module search processing was done following the above methods. Construction of random networks was repeated 100 times and 3,696 modules were generated. The P-value of the candidate module was calculated as the probability of the module, which has the observed score or smaller by chance. The Benjamini-Hochberg method (26) was used to correct the P-value. Ten modules were identified as significantly differential modules as P-values were ≤ 0.05 .

Quantification of M-DMs connectivity and significance analysis. Graph-theoretical measure method was used to calculate module connectivity dynamic score (MCDS). MCDS between two adjacent modules was calculated as:

$$MCDS = \Delta A_{i,j+1} C = \|A_i C - A_{i+1} C\|_2 / |C|$$

C denotes one differential module, $A_i C$ presents adjacent matrix of *C*. The total MCDS of differential module *C* equals the average of MCDS of all adjacent modules.

$$\tau(A^C) = \sum_{i=1}^{M-1} \Delta A_{i,j+1}^C / (M-1)$$

Differential modules were regarded as significantly differential modules of connectivity alternation at P-value <0.05.

Results

Gene expression data and PPI network processing. Gene expression data E-GEOD-53808 were downloaded from the EMBL-EBI database, and 11,134 genes were obtained following mapping between probes and genes. The human PPI networks were downloaded from the STRING database. Networks comprised of 787,896 pairs of PPI networks and 16,730 genes.

PPI network pairs were selected if their combined scores were >0.2. Pairs (50,1736) and 16,496 genes were obtained to form background PPI networks. The new expression profiling data identified contained 9,608 genes.

Identification of DCNs. Interaction pairs (178,888) which contained genes of new expression profiling were identified. These pairs were selected from background PPIs. The edges were selected if absolute values of Pearson's correlation coefficient were >0.9. One thousand, four hundred and thirty-five edges and 460 nodes were obtained and constituted DCNs. Two DCNs, constituting the non-HE alcoholics and HE alcoholics groups, were identified. Two DCNs had the same node sets and different edge sets.

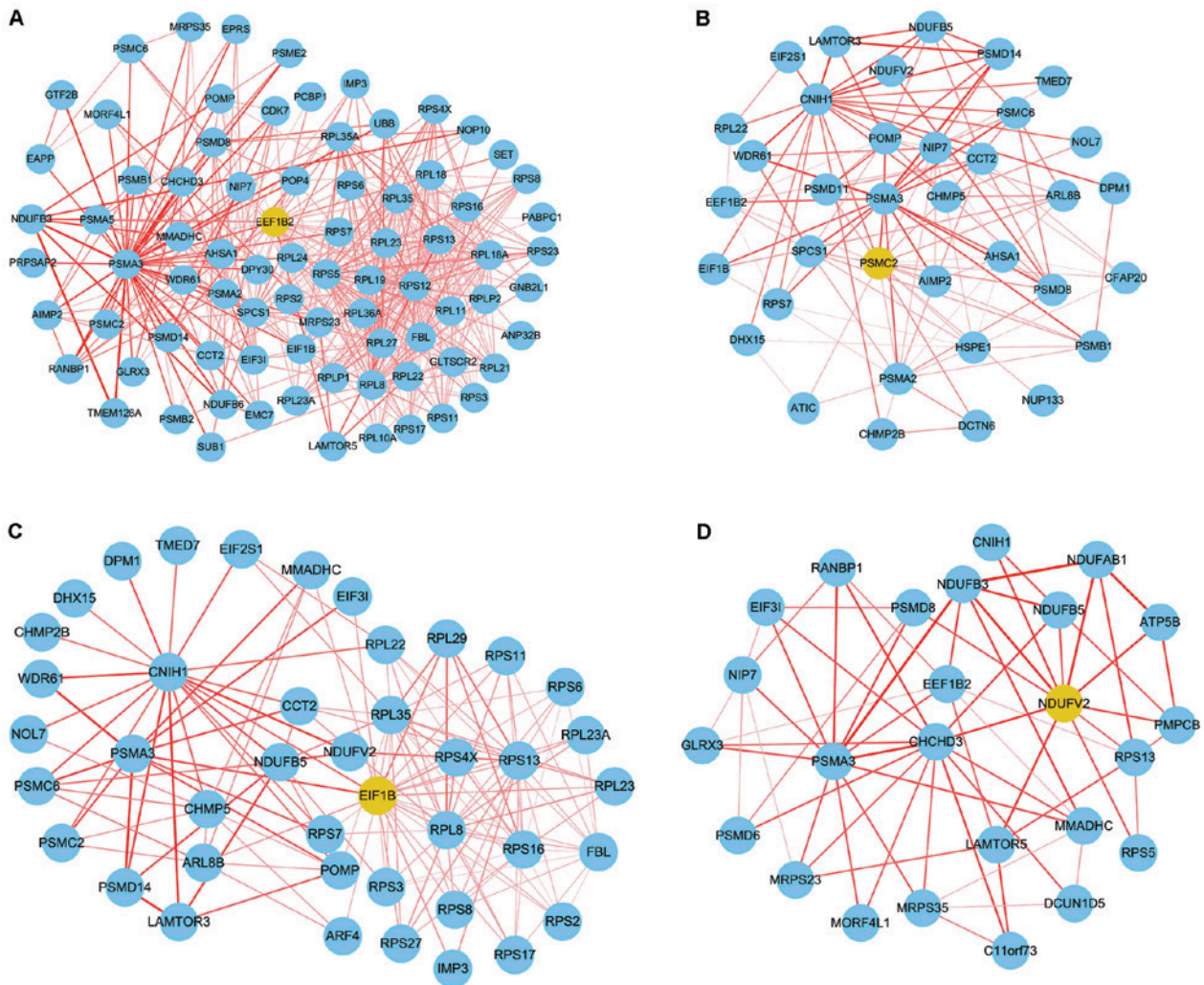


Figure 1. Four significant differential modules with larger connectivity alteration: (A) 80 nodes and 478 edges; (B) 36 nodes and 119 edges; (C) 42 nodes and 145 edges and (D) 25 nodes and 64 edges.

Identification of M-DMs. The important value (z-score) of each gene was calculated. The z-score of each gene in all the DCNs were averaged and ranked. Top 5% genes were selected as seed genes and 23 seed genes were identified (Table I).

Modules were searched based on each gene. Entropy of two modules and change of entropy were calculated. Candidate modules were eliminated if nodes were <5. Two modules were merged into one module if the overlap degree was ≥ 0.5 . Finally, 13 modules were identified.

Significant test of candidate modules. One thousand, four hundred and thirty-five edges were chosen from 178,888 edges randomly to constitute one random network. The random networks were constructed 100 times, and generated 3,696 modules. After P-values were corrected by the Benjamini-Hochberg algorithm, 10 significant differential modules were identified.

Quantification of connectivity dynamics of M-DMs and statistical significant test. Graph-theoretical measure algorithm was used to calculate MCDS. MCDS between two modules and total MCDS were calculated. Four significant differential modules, which had larger connectivity alternation were found

($P < 0.05$) (Fig. 1). Module 1 contains 80 nodes and 478 edges; module 2 comprises 36 nodes and 119 edges; module 3 contains 42 nodes and 145 edges; module 4 comprises of 25 nodes and 64 edges.

Discussion

Alcohol damage on hepatic cells impairs enzyme activities and detoxification of liver. In addition, impairment of hepatic cells disturb brain functions (27). Hepatic encephalopathy (HE) is a serious complication of alcoholic-associated hepatic disease (19). HE has various clinical syndromes, such as nausea, malaise, asterixis and coma (28,29).

In the present study, we obtained all the PPI networks and gene expression data from international databases. We identified 23 seed genes and 10 significant differential modules associated with HE alcoholics. Four differential modules, which had larger connectivity alternations, were obtained by calculating the module connectivity dynamic score of M-DMs.

The 23 genes identified in this study included *PSMA3*, *RPS13*, *RPL8*, *PSMA2*, *AHSA1*, *RPS5*, *EEF1B2*, *PSMC2*, *ATP5B* and *NDUFV2*. *PSMA3* is one subunit of 20S proteasome and interacted with *ROA1*, *PCBP2* and *IREB2*. These

Table I. Twenty-three seed genes identified by z-score.

Name	z-score 1	z-score 2	Average
<i>PSMA3</i>	86.5335583	122.6082221	104.57089
<i>RPS13</i>	34.8732349	120.1981589	77.535697
<i>RPL8</i>	25.1907276	97.03482872	61.112778
<i>PSMA2</i>	57.812847	64.10648625	60.959667
<i>AHSA1</i>	81.0358135	37.6419885	59.338901
<i>RPS5</i>	22.1380737	96.04627227	59.092173
<i>EEF1B2</i>	29.0671882	88.5651997	58.816194
<i>RPS12</i>	20.7253557	95.23469314	57.980024
<i>RPL35</i>	26.199604	89.41832806	57.808966
<i>PSMC2</i>	58.4824325	50.02097225	54.251702
<i>ATP5B</i>	31.4457881	76.47658876	53.961188
<i>SPCS1</i>	37.2801529	69.5430352	53.411594
<i>NDUFAB1</i>	26.0584344	79.74001391	52.899224
<i>EIF1B</i>	43.2034053	62.06634957	52.634877
<i>NDUFB3</i>	25.9831661	78.69291867	52.338042
<i>NDUFV2</i>	33.9425327	69.17172639	51.55713
<i>RPL18A</i>	20.9168676	81.97652628	51.446697
<i>RPL19</i>	25.2334709	76.47090293	50.852187
<i>GAPDH</i>	23.1407005	76.70369969	49.9222
<i>PSMA5</i>	52.2804348	47.28292135	49.781678
<i>RPL18</i>	23.4177997	73.9560672	48.686933
<i>SYP</i>	25.0252229	71.74102033	48.383122
<i>RPS16</i>	23.0946453	73.32578699	48.210216

proteins function in RNA processing, such as splicing (30). *PSMA3* (rs2348071) GG homozygote is related to children with asthma, and children with GG homozygotes are susceptible to disease in Taiwan (31). The potential function of *PSMA3* was analyzed in HE alcoholics.

Proteasome 26S subunit ATPase 2 (*PSMC2*) is an important component of 26S proteasome. The complex processes substrates into 20S pivotal component (32). *PSMC2* expression level is increased in osteosarcoma and silencing of *PSMC2* suppresses cell growth and migration (33). In a further study, we will investigate the function of *PSMC2*. The 26S proteasome may play a role in HE alcoholics.

ATP5b is one subunit of ATP synthase complex. $\alpha 2/\delta 1$ interacts with *ATP5b* and constitute a complex in intracellular membrane. The complex is capable of regulating calcium metabolism and promotes a decreasing rate of calcium transients (34). The *ATP5b* expression level was markedly increased in colorectal tumor tissues compared with normal tissues, but the expression level of *ATP5b* has no association with tumor location (35). ATP synthase complex can generate energy and the energy supply may affect the treatment of HE alcoholics.

Activator of 90KD heat shock protein ATPase homolog 1 (*AHSA1*) is an important factor that can activate ATPase of *HSP90*, which also is a chaperone of *HSP90* (36). In osteosarcoma, *AHSA1* promotes cell proliferation, migration, and invasion by regulating Wnt/ β (37). In a further study, we will clone the *AHSA1* sequence, and investigate its effect on cell proliferation and treatment of HE alcoholics.

In eukaryotic cells, Rps5/uS7 is a subunit of 40S protein complex, which plays an important role in recognition of initi-

ated codon. Substitution in C-terminal residues and β -strand of Rps5 subunit reduces initiation rate and recognition accuracy (38). There is no study of Rsp5 on HE alcoholics.

It is reported that *EEF1B* has four loci, including *EEF1B1*, *EEF1B2*, *EEF1B3* and *EEF1B4*. Three of them are functional (39). *EEF1B2* is mapped to chromosome 2 by PCR method (40). *EEF1B2* is related with retrotransposition function and capable of transcriptional activity (41).

NADH dehydrogenase ubiquinone flavoprotein 2 (*NDUFV2*) encodes one 24 kDa component of NADH-ubiquinone oxidoreductase complex and is involved in electron transportation (42). *NDUFV2* is involved in neuronal mobility and psychiatric dysfunction (43). The 3542G>A polymorphism, which is located at the promoter region of *NDUFV2*, is associated with bipolar disorder (44). Therefore, we suggested that *NDUFV2* may play an important role in HE progression.

In conclusion, we identified 23 seed genes and 10 significantly differential modules associated with HE alcoholics. Four modules were identified as they had relatively larger connectivity alternations. The newly identified seed genes and modules offer understanding of the potential mechanisms and biomarkers for the therapeutic target of HE alcoholics.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

HW conceived and designed the study and wrote the manuscript; ML performed the data analyses; JZ contributed to the conception of the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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