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Cytochrome c oxidase subunit VIIb as a potential target in familial hypercholesterolemia by bioinformatical analysis

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Abstract. – OBJECTIVE: The aim of the study is to explore the potential familial hypercholesterolemia markers by comparing with healthy controls.

MATERIAL AND METHODS: We downloaded the gene expression profile GSE13985 from Gene Expression Omnibus database including five patients diagnosed with familial hypercholesterolemia (FH) and five age, sex, status matched controls. We applied *t*-test, Wilcox test and Fisher test in Multtest package of R language to identify the differentially expressed genes (DEGs) with $p < 0.05$ and $|logFC| > 1$, and constructed the interaction network of the top 3 up- and down-regulated genes using STRING. Besides, the modules of network were analyzed with Cytoscape and screened out with Mcode plugin, and the functional annotation of the genes involved in the modules was analyzed with BiNGO (Biological Networks Gene Ontology).

RESULTS: Firstly, totally 101 differentially expressed genes were identified in FH samples compared with control samples, the genes ranked in top 3 up- and down-regulated genes were selected. Then, basing on the interaction network of these selected genes, ribosomal L24 domain containing 1 (RSL24D1) and cytochrome c oxidase subunit VIIb (COX7B) showed a central position in the interaction network, and also exited in the modules of the network. The functional annotation of the genes in modules showed that COX7B was associated with oxidative phosphorylation.

CONCLUSIONS: COX7B might play vital roles in FH via oxidative phosphorylation system, and might be potential target in the treatment of FH.

Key Words:
Potential familial hypercholesterolemia markers, COX7B, Oxidative phosphorylation system.

Introduction

Hypercholesterolemia, which is a higher serum total cholesterol level, causes sclerotic changes in blood vessels and might result in colorectal cancer (CRC)[1]. Familial hypercholesterolemia (FH) is an autosomal disorder characterized by increased levels of total cholesterol and low density lipoprotein cholesterol, and increased risk of premature coronary heart disease[2,3]. Nowadays, with a prevalence of about one in 500 individuals, FH remains the most common monogenic disorder of lipoprotein metabolism[4]. In the United States, only 34% of patients with familial hypercholesterolemia were diagnosed[5]. And in Dutch, the incidence of FH among children is every 400 births[6].

It is known that FH can result from mutations in the low density lipoprotein receptor gene (LDLR), apolipoprotein B-100 gene (APOB), and proprotein convertase subtilisin/kexin type 9 gene (PCSK9)[7]. LDLR mutation databases currently list more than 800 different mutations[8,9]. Many different types of LDLR mutation have been identified in patients with FH worldwide, such as large rearrangements and mutations in the promoter region that affect gene transcription[10]. For the therapy of FH, some drugs are widely used, for example, ezetimibe as a cholesterol-absorption inhibitor[11], torcetrapib as an inhibitor of cholesteryl ester transfer protein[12], and pravastatin[13]. However, the using of these drugs in treating for FM can reduce the levels of LDL cholesterol, but it cannot eliminate the disease and prevent the occurrence of carotid atherosclerosis completely.

In present study, in order to detect the potential key genes in FH and their possible functions, we analyzed the differentially expressed genes (DEGs) in FH samples with t-test, Wilcox test and Fisher test by comparing with control samples, and the top genes were selected to construct the interaction network with their potential target genes. Then, the

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modules in the network were analyzed, and the functional annotation of the genes involved in the modules were performed. We expected this research would provide more understanding and useful information for the treatment of FH.

Materials and Methods

Data Sources

The expression profile of GSE13985 was downloaded form Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/), including five patients diagnosed with FH and five age, sex, BMI and smoking status matched controls. The corresponding platform was GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array.

Pre-processing of Data and Screening the DEGs

Affy package[14] in R language was used to transform the raw data into the recognizable expression profile data. Then, the missing parts of data were imputed[15], and the complete data were standardized with Median standardization[16]. Next, we applied *t*-test, Wilcox test and Fisher test in Multtest package of R language[17] to identify the differentially expressed genes (DEGs) between the familial hypercholesterolemia blood samples and control samples, with the p value < 0.05 and $|\log FC| > 1$. And among the DEGs, the up-regulated genes and down-regulated genes, whose fold-change ranked within top 3, were screened out for further study.

Constructing the Interaction Network

Single gene does not often play roles in organism alone. In fact, the gene can interact with other genes to accomplish several function[18]. In this study, the up- and down-regulated genes stood in the front of the rank were screened, and the interaction network of these genes were constructed with STRING (Search for the Retrieval of Interacting Genes/Proteins) software[19] to mine their target genes. This software provides uniquely comprehensive coverage, all interactions are provided with a probabilistic confidence score. Then, we counted the node degree of the genes in the interaction network, and selected the genes with high connectivity.

Analyzing the Modules in the Network

The modules of the whole network we obtained was analyzed with Cytoscape software[20], the modules were mined with MCODE (Molecular Complex Detection)[21], and the functional annotation of the modules were performed with Biological Networks Gene Ontology tool (BiNGO) plugin[22]. The threshold of hypergeometric distribution of functional annotation was 0.05.

Results

Data Pre-Treatment

The primary data of chips exist some problems, such as background and probe design, so there are great differences between data of chips, and normalization is necessary for analysis. After normalization, the data had better correlation shown in Figure 1-A, the spots with different color represented the sample data of various groups. Besides, the fitting curves to data was almost near to standard diagonal, and the correlation coefficient of data in different samples was equal to 0.97.

Identification of the Differentially Expressed Genes

T-test, Wilcox test and Fisher test were applied to test the expression data of genes, the genes with $p < 0.05$ and $|\log FC| > 1$ were identified as the DEGs, including up- and down-regulated genes. We selected top 3 gene expression values listed in Table I. In order to prove the p value and $|\log FC|$ whether conform to logic with different test, the VOLCANO plot was drawn (Figure 1-B). We could see that smaller p value was corresponding to larger $|\log FC|$, this was logical according to the previous studies[23,24].

Interaction Network

The interaction network was constructed by combining with STRING database, according to the top 3 up- and down-regulated genes we obtained before, the result was shown in Figure 2. Then, the node degree was counted, and the genes ribosomal L24 domain containing 1 (RSL24D1) and cytochrome c oxidase subunit VIIb (COX7B) located on the top of the genes involved in the network, this suggested that the connective function of RSL24D1 and COX7B were important in the network.

Modules Analysis in Network and Functional Annotation

Cytoscape software was applied to analyze the modules of the network we obtained before. After being mined with MCODE and annotated

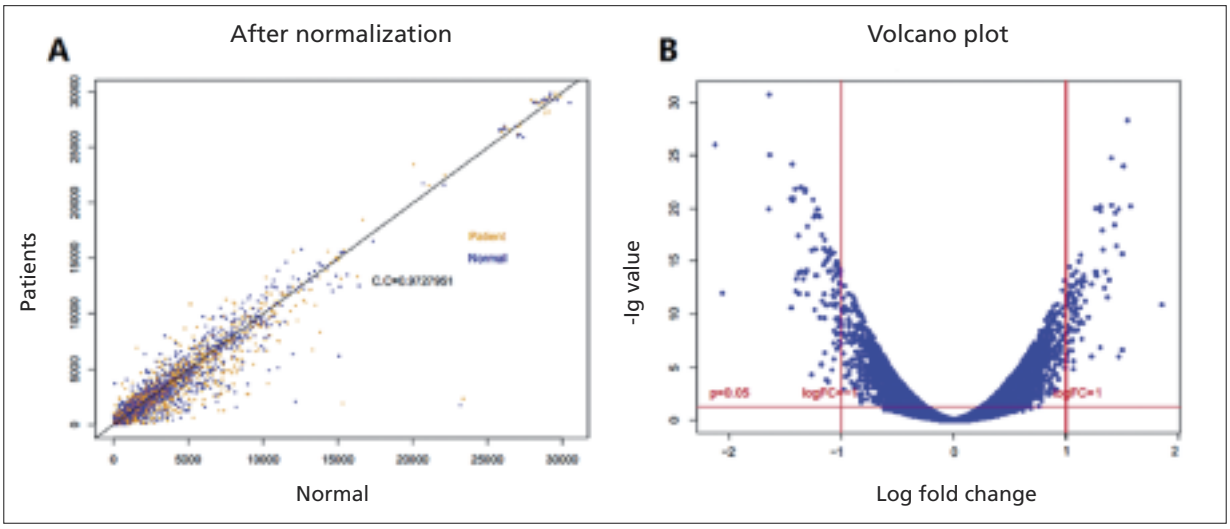

Figure 1. The expression association graph of the differentially expressed genes ***(A)*** and the Volcano plot of the differentially expressed genes ***(B)***.

Table I. The top 3 up- and down-regulated genes among the differentially expressed genes (DEGs).

Gene symbol	ID_REF	*t*-test	Wilcox	Fisher	logFC
RSL24D1	222465_at	0.012339	0.036145	2.76E-17	-1.65193
COX7B	202110_at	0.038268	0.036145	1.28E-21	-1.64387
FLCN	235250_at	0.017768	0.021177	4.45E-18	-1.45301
IGHD	230877_at	0.034049	0.011925	5.60E-14	1.451809
TCL1A	39318_at	0.02652	0.036145	2.76E-17	1.471415
IGHD	213674_x_at	0.026028	0.036145	1.90E-17	1.57356

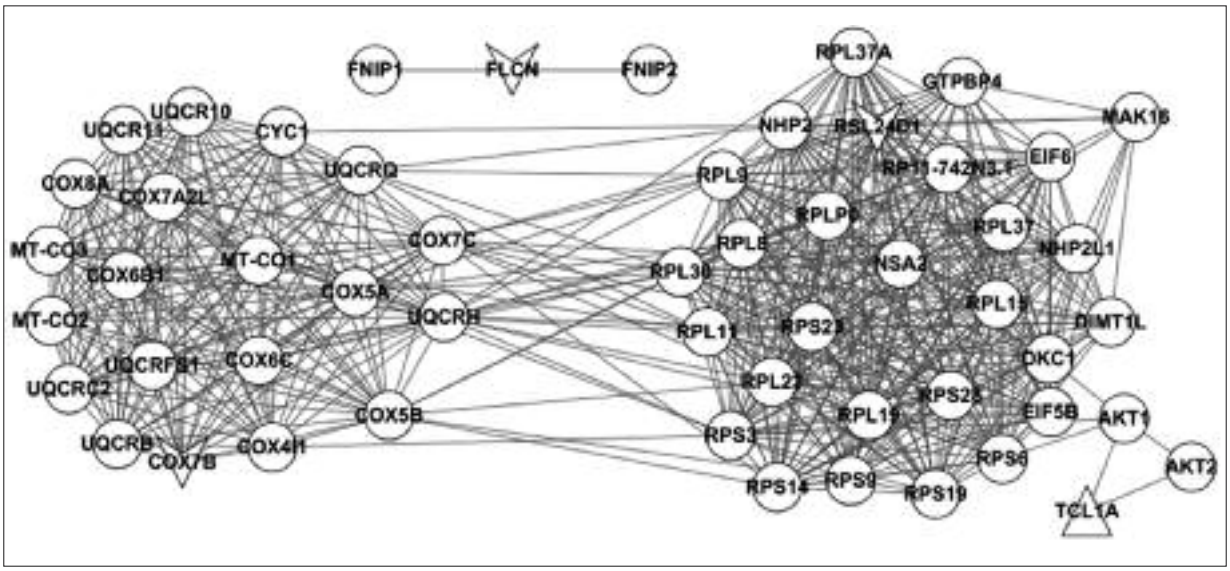

Figure 2. The interaction network of the selected top genes with their potential target genes. Inverse triangular represented the down-regulated genes, and regular triangle displayed the up-regulated genes.

with BiNGO, the modules we mined were seen in Figure 3, and the functional annotation of the genes involved in the modules were analyzed shown in Table II. In Figure 2, the genes RSL24D1 and COX7B were also included in the modules. The result of functional annotation showed that oxidative phosphorylation (p = 1.80E-07), respiratory electron transport chain (p = 3.47E-07), ribosomal small subunit biogenesis (p = 6.89E-07), energy derivation by oxidation of organic compounds (p = 1.72E-06) and cellular protein metabolic process (p = 2.60E-06) were significantly enriched (Table II). COX7B was also enriched in the function of oxidative phosphorylation.

Discussion

In present study, the data were downloaded from GEO database (http://www.ncbi.nlm.nih.gov/geo/), and the DEGs of the FH samples were identified by comparing with control samples, including up- and down-regulated genes. Meanwhile, the top 3 genes were selected, and the interaction network of these genes was constructed with their potential target genes. The two genes RSL24D1 and COX7B were the top of the genes involved in the network according to the node degree of the genes. In addition, the modules were mined and the functional annotation of genes in modules was analyzed, the node degree of RSL24D1 and COX7B also stood at the top of the genes in the modules. Moreover, COX7B was associated with the function of oxidative phosphorylation, which was the most significant function in the modules.

Cytochrome c oxidase (COX), which is a fundamental mitochondrial respiratory chain MRC complex (CIV), comprises three mitochondrial-encoded proteins (COX1, COX2, and COX3) that combine with ten nuclear-encoded proteins, including COX4, COX5A, COX5B, COX6A, COX6B, COX6C, COX7A, COX7B, COX7C, and COX8, to form the mature holocomplex in humans[25,26]. COX7B as the only X-linked gene encodes a poorly characterized structural subunit of cytochrome c oxidase[27], has been reported its down-regulation in Medaka result in microcephaly and microphthalmia[28]. The over-expression of COX7B and high positive rate were observed in nasopharyngeal carcinoma (NPC), suggesting COX7B might be the putative molecular markers of NPC[29]. In glioma cells treated with temozolomide and the combination of Y15 and

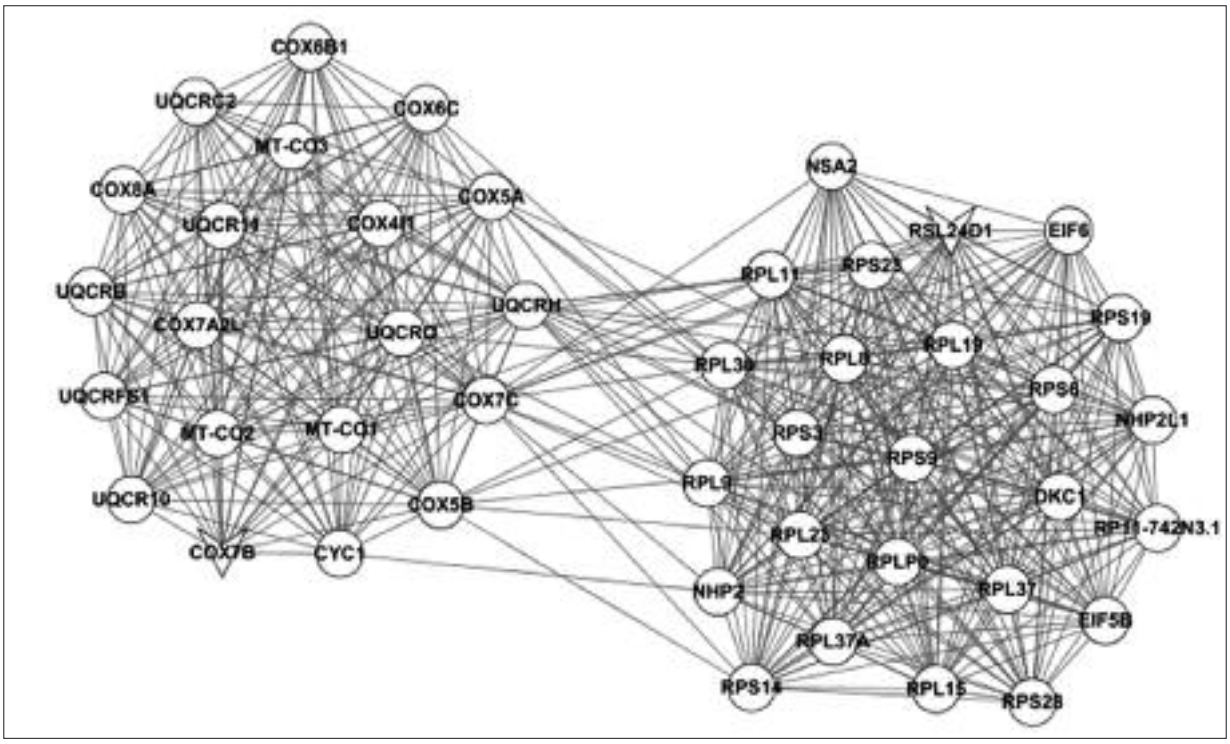

Figure 3. The modules of the network included the top genes among all the differentially expressed genes. Inverse triangular represented the down-regulated genes, and regular triangle displayed the up-regulated genes.

Table II. Functional annotation of the genes included in the nodules.

GO-ID	corr *p*-value	x	Description
6119	1.80E-07	7	Oxidative phosphorylation
22904	3.47E-07	6	Respiratory electron transport chain
42274	6.89E-07	4	Ribosomal small subunit biogenesis
34470	6.89E-07	8	ncRNA processing
15980	1.72E-06	7	Energy derivation by oxidation of organic compounds
44267	2.60E-06	20	Cellular protein metabolic process
34660	2.82E-06	8	ncRNA metabolic process
55114	1.14E-05	11	Oxidation reduction
19538	1.14E-05	21	Protein metabolic process
44260	1.99E-05	24	Cellular macromolecule metabolic process
44085	2.71E-05	13	Cellular component biogenesis
42255	4.35E-05	3	Ribosome assembly
43170	5.51E-05	25	Macromolecule metabolic process
9987	9.71E-05	38	Cellular process
6396	1.90E-04	9	RNA processing
28	2.01E-04	2	Ribosomal small subunit assembly
42257	3.90E-04	2	Ribosomal subunit assembly
6123	6.31E-04	2	Mitochondrial electron transport, cytochrome c to oxygen
9060	9.25E-04	3	Aerobic respiration
30218	1.83E-03	3	Erythrocyte differentiation
34101	2.63E-03	3	Erythrocyte homeostasis
70925	3.61E-03	3	Organelle assembly
1522	4.98E-03	2	Pseudouridine synthesis
44238	6.45E-03	25	Primary metabolic process
16070	8.03E-03	9	RNA metabolic process
22618	9.12E-03	3	Ribonucleoprotein complex assembly
30099	1.54E-02	3	Myeloid cell differentiation
48872	1.54E-02	3	Homeostasis of number of cells
31118	1.76E-02	1	rRNA pseudouridine synthesis
45738	1.76E-02	1	Negative regulation of DNA repair
42256	3.39E-02	1	Mature ribosome assembly
16310	3.39E-02	7	Phosphorylation
6610	4.77E-02	1	Ribosomal protein import into nucleus
30490	4.77E-02	1	maturation of SSU-rRNA
2309	4.77E-02	1	T cell proliferation involved in immune response
9451	4.81E-02	2	RNA modification

temozolomide, COX7B was also up-regulated significantly[30]. Meanwhile, COX7B as a differentially expressed gene was identified in female residents in a Cd-polluted area by comparing with matched residents in a non-Cd-polluted area[31]. And our study revealed a potential function in familial hypercholesterolemia. Thus, these evidences suggested that COX7B might be a key gene not only in microcephaly, microphthalmia and cancer, but also in FH and controlling oxidative stress response.

As we all know, oxidative phosphorylation is the mainly biological process and function in mitochondria of organism, and molecular defects in oxidative phosphorylation system can cause mitochondrial dysfunction[32]. Defects in the oxidative phosphorylation system can result in devastating multisystem diseases, and recent years have been detect genetic mutations in mitochondrial and nuclear genes[33]. In previous studies, mtDNA, which encodes genes essential for oxidative phosphorylation, was one of the risk factors of cardiovascular disease, basing on the generation of increased reactive oxygen and nitrogen species[34]. Furthermore, increased expression of cytochrome oxidase gene might be related to early Alzheimer's disease[35], and Parkinson's disease was reported as a systemic disorder of oxidative phosphorylation[36]. In fact, regulating mammalian cytochrome c oxidase and its substrate cytochrome c is representative activity for the entire oxidative phosphorylation system[37]. Our research also indicated that COX7B significantly enriched in oxidative phosphorylation. Hence, we speculated that COX7B might play roles in FH via oxidative phosphorylation system.

Conclusions

We identified the DEGs in the FH samples by contrasting with control samples, and COX7B as the core gene in the interaction network of the top 3 up- and down-regulated genes. In addition, COX7B also existed in the modules of the network we analyzed, and was significantly enriched in the function of oxidative phosphorylation. Therefore, COX7B was supposed to be key gene in the FH, it might play vital roles via oxidative phosphorylation system and may be a new target for the treatment of FH. However, the specific function of COX7B in FH needs to further study in future.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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