A comprehensive bioinformatics analysis Young-Aged Coronary Heart Disease

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Abstract

Background Coronary heart disease (CHD) is a leading cause of morbidity and mortality worldwide[1]. Although effective primary and secondary prevention successfully reduced the mortality of CHD, morbidity and mortality of young aged coronary heart disease (YA-CHD) didn't decrease. However, little is known about the prevalence and mechanism of YA-CHD. **Methods** Dataset GSE 12288 from Gene Expression Omnibus was imported and performed comprehensive bioinformatics analysis, including gene ontology analysis (GO analysis), pathway analysis, protein-protein interaction network (PPI) analysis and core network analysis. **Results** RAP1A, which regulates platelet integrin activation and has a critical role in platelet production, was significantly up regulated, while TNKS2, which keeps the integrity of the leukocyte telomere structure and shows a significant association with longevity, was significantly downregulated. Biological process analysis showed "phagosome" pathway was mostly significant related to YA-CHD. Innate immune response module and type I interferon signaling module, interacts with IRF1, may major in the regulation of YA-CHD progression and maybe the potential therapeutic target of YA-CHD. **Conclusions** RAP1A and TNKS2 in peripheral leukocytes may serve as novel biomarkers in predicting the onset of YA-CHD. Further studies about whether IRF1 influence YA-CHD through regulating innate immune type I interferon signaling pathway was needed.

Background

Coronary heart disease (CHD) remains one of the major causes of death around the world[2]. Although excellent primary and secondary prevention significantly reduced the mortality of CHD[3], death caused by CHD didn't decreased among young aged patients[4-6]. What's more, the incidence of young aged coronary heart disease (YA-CHD) still increases steadily[7, 8]. Epidemiological studies shows premature onset CHD was associated with a 2 to 3 times increased risk of CHD in first-degree relatives, which indicates the genetic predisposition of CHD[9]. Present studies on YA-CHD patients (CHD patients younger than 45 years old) showed obviously different characteristics compared with CAD in old patients (elder than 65 years old)[10]. Based on these evidence, YA-CHD seems to have stronger genetic predisposition, grater differences in sex ratio, better outcomes with early intervention, less complications and worse long-term outcome. Early screening and intervention seem to be essential to solve the problem. However, the mechanism hiding behind YA-CHD and suitable serum biomarker in screening YA-CHD patients remains unclear.

Next-generation sequencing technologies had successfully helped in identifying a variety of genes that are involved in the onset and progression of multiple diseases, and screening high risk populations in the past few decades. With the help of gene profiling studies, we may identify novel biomarkers and therapeutic targets of YA-CHD and demonstrated the roles of specific metabolic pathways. In this study, we carried out a comprehensive bioinformatics analysis between normal young-adults and YA-CHD patients' blood samples on Gene Expression Omnibus (GEO) database. we tried to identify key differentially expressed genes (DEGs), biological processes, pathways and PPI networks closely associated with YA-CHD.

Methods

Gene Expression Profiles

GEO (<https://www.ncbi.nlm.nih.gov/gds>) is a public repository at the National Center of Biotechnology Information for storing high-throughput gene expression datasets[11]. We use “CAD” or “CHD” AND “Homo sapiens” [Organism] in the GEO Database to identify potential datasets. We further screened the datasets according to the following inclusion criteria: 1. clearly diagnosed of CAD by coronary angiography (CAG), 2. patients younger than 45 years old. 3. healthy young aged adults has similar age with patients (less than 2 years) were taken as controls. Finally, 1 dataset GSE12288 based on the GPL96 platform (Affymetrix Human Genome U133A Array) was included in our study, which contains 7 “cases-control” pairs. GSE12288 tested Gene expression profile in circulating leukocytes in patients with coronary artery disease. Baseline conditions are shown in Table 1.

Differentially expressed genes screening:

The average expression value of different probes corresponded to the same gene was used to represent the expression value of the gene. DEGs (Differentiated Expressed Genes) between YA-CHD group and healthy control group were screened and selected by the cut-off point $P < 0.05$ and $|\text{fold change (FC)}| > 1.5$. The expression data were processed using limma package in R software[12].

Functional enrichment analysis

The selected DEGs were deposited to the Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.8 Beta (<https://david-d.ncifcrf.gov/>) for further analysis. DAVID bioinformatics resources consists of an integrated biological knowledgebase and analytic tools aimed at systematically extracting biological meaning from large gene/protein lists[13]. In this study, DAVID database was applied to investigate Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs. $P < 0.05$ was chosen as the threshold.

Protein-protein interaction analysis

Protein-protein interaction (PPI) of DEGs was obtained from Search Tool for the Retrieval of Interacting Genes (STRING, <http://string-db.org/>). It provides uniquely comprehensive coverage and ease of access

to both experimental as well as predicted interaction information[14]. Confidence score>0.4 of PPI was the selection threshold to construct the PPI network. PPI networks were visualized with Cytoscape software[15]. Modules from the PPI network were identified using the Molecular Complex Detection (MCODE) plugin (13) with the following criteria: Degree cutoff, 2; node score cutoff, 0.2; k-core, 2; and max depth, 100. $p < 0.05$ was used as a threshold value.

Statistical analysis was performed using R-studio (0.99.902, R v3.3.0).

Results

Differentially Expressed Genes

We identified 384 DEGs, 293 up-regulated in young aged CHD patients and 91 down-regulated in YA-CHD patients(Figure 1). The top 20 DEGs from GES12288 are shown in Table 2. In the top 20 DEGs, 17 DEGs were upregulated in young aged CHD patients, while 3 DEGs were down regulated in YA-CHD patients.

Gene Oncology (GO) analysis.

GO analysis was applied to investigate the biological function of the 384 DEGs. 293 GO terms ($P < 0.05$) were significantly enriched by up-regulated DEGs, while 91 genes ($P < 0.05$) were significantly enriched by down-regulated DEGs. In upregulated DEGs, generation of a signal involved in immune response (GO:0006955, $p = 5.2e-09$) was most significantly enriched for biological processes, while for molecular functions was protein binding (GO:0005515, $p = 4.53E-04$), and for cellular component were cell periphery (GO:0071944, $p = 5.8e-07$) and plasma membrane (GO:0005886, $p = 6.83e-07$). (Table 3)

KEGG pathway analysis

To further research the functions of upregulated DEGs, we represented them to the KEGG database. We identified 17 significant pathways based on KEGG database analysis. The most significant pathway in our KEGG analysis was phagosome with a P-value of $3.2E-08$. In down regulated DEGs, macromolecular complex disassembly (GO:0032984, $p = 0.00216$) was involved in biological processes, while large ribosomal subunit (GO:0015934, $p = 0.00667$) was involved in Cellular Component. No KEGG pathway was found in downregulated DEGs. (Table 4)

PPI network and module selection.

In 293 upregulated DEGs, the PPI network consisted of 261 nodes and 401 edges. Using the MCODE Cytoscape plugin, 6 significant modules were identified. The most significant module contains 13 nodes: TLR1, CLEC7A, SLC11A1, NCF2, HCK, TLR4, MYD88, FCGR2A, ITGAM, LYN, STAT3, FCGR1B, DDX58, and the second significant module contains 5 nodes: IRF2, IRF9, MX2, IFITM3, GBP2 (Figure2). There were 3 intermedia genes between these two modules, which were associated with both modules. The intermedia genes are IRF1, TRIM8, STAT5A. KEGG pathway analysis demonstrated 13 genes in first module associated with leishmaniasis ($5.7\text{e-}10$), tuberculosis ($5.71\text{e-}10$) and phagosome ($1.59\text{e-}08$). Biological Process analysis demonstrated 5 genes in second module associated with type I interferon signaling pathway ($1.49\text{e-}09$) and cellular response to type I interferon ($1.49\text{e-}09$). Network formed by 91 downregulated genes does not have more interactions than expected.

Discussion

Gene may play an important role in the onset and progression in YA-CHD. This is the first time approaching comprehensive bioinformatics analysis between normal young-adults and YA-CHD patients' blood samples. Among 384 DEGs, RAP1A, a novel regulator of significant processes in the cardiovascular system ranging from blood vessel formation and permeability, platelet aggregation, was mostly up regulated in YA-CHD, while TNKS2, which keeps the integrity of the leukocyte telomere structure, was obviously down regulated. Phagosome pathway is important in the progression in YA-CHD. The GO term "immune response" and "protein binding" was significant enriched in YA-CHD, which indicates immune response and PPI network is important. Phagosome pathway enrichment indicated the importance of macrophage. Innate immune response module and type I interferon signaling module, interacts with IRF1, may major in the regulation of YA-CHD progression and maybe the potential therapeutic target of YA-CHD.

RAP1A and TNKS2 are important in the progression in YA-CHD. In the top 20 DEGs, the top 3 DEG up regulated in YA-CHD patients were RAP1A, BACH1, ERV3-2, while top 3 down regulated 3 DEGs were HLA-DQA1, TNKS2, ACYP1. RAP1A (Ras-Related Protein Rap-1A) expressed mostly in YA-CHD patients and owns the greatest difference. RAP1A encodes a member of the Ras family of small GTPases. It was reported as an novel regulators of significant processes in the cardiovascular system ranging from blood vessel formation and permeability, platelet aggregation, to cardiac myocyte growth and survival[16]. Nearly study demonstrate an essential role for RAP1 signaling in platelet integrin activation and a critical role in platelet production[17]. The expressive abundance of RAP1A may serve as a prediction factor in identifying the high risk group young aged adults more susceptible to CAD. BACH1 (BTB Domain and CNC Homolog 1) was associated with tumor metastasis, especially in breast cancer. ERV3-2(Endogenous Retrovirus Group 3 Member 2) belongs to uncategorized gene, no enough present studies about this gene is available. HLA-DQA1 (MHC Class II HLA-DQ-Alpha-1), belongs to the HLA class II alpha chain paralogues. Gene Ontology (GO) annotations related to this gene include peptide antigen binding and MHC class II receptor activity. The association between HLA-DQA1 and YA-CHD remains unclear. TNKS2

(Tankyrase 2) is a protein coding gene, which involved in various processes such as Wnt signaling pathway, telomere length and vesicle trafficking. Further studies found its importance for keeping the integrity of the leukocyte telomere structure, shows a significant association with longevity [18]. Leukocyte telomere length (LTL) was found associated with CAD since 2001[19], and has been regarded as a potential marker of biologic aging[20]. The down-regulated of TNKS2 in YA-CHD patients reflects the biological aging of cardiovascular system. Thus, the expressive abundance may serve as a marker of biological aging of cardiovascular system. ACYP1 (Acylphosphatase 1) is a protein coding gene, related pathways are Pyruvate metabolism and Pyrimidine metabolism. Gene Ontology (GO) annotations related to this gene include acylphosphatase activity, has been reported to possesses two alternative splicing forms that induce apoptosis[21]. GO analysis in 293 up regulated genes suggests the attack of YA-CHD has close relationship with immune response. What's interesting is, RAP1A was not involved in immune response for biological processes, which indicates its influence in YA-CHD was independent from inflammation. The platelet aggregation associated with RAP1A and biological aging associated with TNKS2 are significant in YA-CHD, which may become novel biomarkers in predicting the disease risk.

Macrophage played an important role in YA-CHD. KEGG enrichment analysis indicates the major contribution of phagosome pathway in YA-CHD comparing with other pathways. Present studies showed that coronary atherosclerosis is associated with macrophage polarization in epicardial adipose tissue[22]. What's more, increased circulating C-reactive protein and macrophage-colony stimulating factor are complementary predictors of long-term outcome in patients with chronic coronary artery disease[23]. The onset of YA-CHD and CHD in elder patients may both influenced by the activation of macrophage.

Core network analysis further points out the innate immune response and type I interferon signaling pathway are important in YA-CHD. There are 13 nodes in the most significant module, module1, which formed an immune response network. GO analysis on these 13 nodes shows innate immune response (GO:0045087, $p = 5.16e-12$) was significantly enriched for biological progression. Many of the genes were associated with regulating the function of macrophage, either up regulated or down regulated. Module 2 consists of 5 nodes and regulates type I interferon (IFN) signaling pathway. Present studies show B-cells producing type I IFN modulate macrophage polarization in tuberculosis[24]. The relationship between module 1,2 and macrophage further hint that innate immune system may regulate the polarization of macrophage by type 1 interferon and influence the progression in YA-CHD. Actually, interferons (IFNs) are key regulators of both innate and adaptive immune responses[25]. Myeloid type I interferon signaling was found to promote atherosclerosis by stimulating macrophage recruitment to lesions in mice[26]. Reducing macrophage proteoglycan sulfation will increase atherosclerosis and obesity through enhanced type I interferon signaling[27]. Considering YA-CHD patients owns higher BMI level, whether YA-CHD was caused by the reduction of macrophage proteoglycan sulfation needs further exploration.

Although the findings in this study is encouraging, there are still several limitations can't be ignored in this study. Firstly, considering our laboratory condition, no clinical validation was performed. Secondly, whether the patients included in our study accompanied with familial hypercholesterolemia or Kawasaki

diseases was uncertain, cause the clinical and genetic background of GSE12288 was not available. However, the cluster result in the heatmap of DEGs showed outstanding homogeneity, which indicates a reliable internal validity.

In summary, RAP1A may be a new treatment target in YA-CHD patients. The expression of TNKS2 may be a novel biomarker to predict the biologic age for cardiovascular system. The immune response and macrophage may also play an important role in YA-CHD. Further studies on the role of IRF1 and type I interferon signaling pathway in YA-CHD in needed.

Abbreviations

CHD	Coronary heart disease
YA-CHD	Young-aged coronary heart disease
GO	Gene ontology
PPI	Protein-protein interaction
GEO	Gene Expression Omnibus
DEGs	Differentially expressed genes
DAVID	Database for Annotation, Visualization, and Integrated Discovery
KEGG	Kyoto Encyclopedia of Genes and Genomes
MCODE	Molecular Complex Detection
LTL	Leukocyte telomere length
RAP1A	Ras-Related Protein Rap-1A
ACYP1	Acylphosphatase 1
BACH1	BTB Domain and CNC Homolog 1
ERV3-2	Endogenous Retrovirus Group 3 Member 2
HLADQA1	MHC Class II HLA-DQ-Alpha-1
TNKS2	Tankyrase 2
IFN	Interferon

Declarations

Availability of data and materials:

The datasets generated and/or analysed during the current study are available in the [Gene Expression Omnibus] repository, [<https://www.ncbi.nlm.nih.gov/gds>].

Declaration of interests

The author declares no conflicts of interest.

Individual contributions

Xin-xue Liao and Xiao-dong Zhuang designed the study. Xiang-bin Zhong obtained the funding. Xiang-bin Zhong, Hua-qiang Zhou performed the data analysis. Hua-qiang Zhou provided methodological support. Shao-Zhao Zhang, Hui-min Zhou, Xiu-ting Sun, performed the chemoinformatic studies. Xiang-bin Zhong, Man Huang, Da-ya Yang wrote the paper. Yue Guo, Zhen-yu Xiong performed grammar check. All authors approved the final version of the manuscript.

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Tables

Table 1: Baseline of 7 case-control pairs.

Source Name	Group	Sex	Age	Ethnicity	CADi
GSM308712	Control	Female	43	Caucasian	0
GSM308748	Control	Male	44	Caucasian	0
GSM308763	Control	Female	37	Caucasian	0
GSM308656	Control	Male	42	Caucasian	0
GSM308566	Control	Male	40	Caucasian	0
GSM308661	Control	Male	44	Caucasian	0
GSM308735	Control	Male	42	Caucasian	0
GSM308780	Case	Female	37	Caucasian	32
GSM308623	Case	Male	44	Caucasian	48
GSM308705	Case	Female	43	Caucasian	32
GSM308668	Case	Male	44	Caucasian	63
GSM308674	Case	Male	40	Caucasian	32
GSM308618	Case	Male	42	Caucasian	32
GSM308644	Case	Male	42	Caucasian	37

Duke CAD index (CADi), a validated angiographical measure of the extent of coronary atherosclerosis that correlates with outcome.

Table 2: Top 20 Differentially Expressed Genes

Gene symbol	LogFC	P-Value	Regulation
RAP1A	1.589545	4.70E-08	UP
BACH1	1.354692	2.08E-06	UP
ERV3-2	1.268461	1.16E-06	UP
ORM1 /// ORM2	1.243681	4.79E-05	UP
IGF2R	1.203612	7.85E-08	UP
C1RL	1.137442	6.16E-10	UP
TNFAIP6	1.121772	0.000419	UP
DYSF	1.104497	1.84E-07	UP
KIR2DS2	1.082765	5.94E-08	UP
NPL	1.072599	6.88E-05	UP
MGAM	1.064839	5.43E-06	UP
LOC101928676 /// MAEA	1.05982	1.84E-05	UP
KCNJ15	1.059621	4.43E-05	UP
PLOD1	1.052658	1.77E-08	UP
BSN	1.050959	1.14E-08	UP
NT5C2	1.022595	2.36E-05	UP
DDX58	1.017918	6.61E-08	UP
ACYP1	-1.00612	9.84E-10	DOWN
TNKS2	-1.02483	1.13E-08	DOWN
HLA-DQA1///LOC100509457	-1.24082	0.030303	DOWN

Table 3: GO analysis related to 293 up regulated DEGs in GES 12288

Pathway ID	Pathway description	Count in gene set	False discovery rate
TOP 10 Biological progress GO analysis			
GO:0006955	immune response	49	5.2e-09
GO:0006952	defense response	49	2.98e-08
GO:0006950	response to stress	82	8.08e-08
GO:0045087	innate immune response	38	8.94e-08
GO:0002376	immune system process	57	2.78e-07
GO:0050896	response to stimulus	126	4.88e-07
GO:0044700	single organism signaling	100	8.35e-07
GO:0007154	cell communication	101	1.13e-06
GO:0002252	immune effector process	23	3.6e-06
GO:0007165	signal transduction	93	3.6e-06
Molecular function GO analysis			
GO:0005515	protein binding	93	0.000453
GO:0005488	binding	162	0.000509
GO:0043167	ion binding	103	0.0446
Top 10 Cellular components GO analysis			
GO:0071944	cell periphery	98	5.8e-07
GO:0005886	plasma membrane	96	6.83e-07
GO:0031982	vesicle	80	3.12e-06
GO:0031988	membrane-bounded vesicle	78	3.22e-06
GO:0070062	extracellular exosome	67	3.62e-06
GO:0044444	cytoplasmic part	127	1.09e-05
GO:0005737	cytoplasm	159	1.14e-05
GO:0044464	cell part	202	2.62e-05
GO:0005623	cell	202	3.22e-05
GO:0016020	membrane	139	8.1e-05

Table 4: Top 17 pathways in terms of KEGG pathway analysis related to DEGs in GES 12288

pathway ID	pathway description	count in gene set	false discovery rate
04145	Phagosome	12	2.77e-05
04670	Leukocyte trans endothelial migration	11	2.77e-05
05140	Leishmaniasis	9	2.77e-05
05152	Tuberculosis	12	0.000142
05162	Measles	10	0.000359
04666	Fc gamma R-mediated phagocytosis	8	0.000739
04380	Osteoclast differentiation	9	0.00104
05133	Pertussis	7	0.00104
04062	Chemokine signaling pathway	10	0.0032
04810	Regulation of actin cytoskeleton	10	0.0102
05205	Proteoglycans in cancer	10	0.0134
00770	Pantothenate and CoA biosynthesis	3	0.0184
04640	Hematopoietic cell lineage	6	0.0184
04062	Chemokine signaling pathway	10	0.0257
04612	Antigen processing and presentation	6	0.0154
04144	Endocytosis	12	0.0309
04810	Regulation of actin cytoskeleton	10	0.0257

Figures

Figure 1

384 Differentiated Expression Genes between YA-CHD group and control group.

Figure 2

Core network Module1, Module2 and intermedia genes.

Figure 3

GO analysis in 293 upregulated DEGs.

Figure 4

Top 17 KEGG pathway analysis in 293 upregulated DEGs.