Novel Signature Genes for Human Left Ventricle Cardiomyopathies Identified by Weighted Co-expression Network Analysis (WGCNA)

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Research

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Abstract

Background

Cardiomyopathy, a heart disease that arises from different etiologies, brings a huge healthcare burden to the global society. Identification of biomarkers can be very useful for early diagnosis of cardiomyopathy, interruption of the disease procession to heart failure, and decrement of the mortality.

Methods

Clinical cases of cardiomyopathy were screened out of independently investigations from the genomic database. Exploration of its whole transcription disorder pattern by WGCNA (Weighted Gene Co-expression Network Analysis) to discover the signature genes for different subtypes of cardiomyopathy. The hub genes and key pathways, which are correlated to cardiomyopathy traits, were identified through co-expression and protein-protein interaction (PPI) networks enrichment analysis. Discovered hub genes were blast through the Cardiovascular Disease Portal to verify functions related to human cardiomyopathies.

Results

Three common axes of signature genes were discovered for five subtypes of cardiomyopathy: 1) Four genes (MDM4, CFLAR, RPS6KB1, PKD1L2) were common for ischemic and ischemic cardiomyopathy subgroups; 2) Subtypes of cardiomyopathy (ischemic, post. partum, familiar and idiopathic) shared eight genes (MAPK1, MAPK11, MAPK14, LMNA, RAC1, PECAM1, XIAP, CREB1); 3) TFAM and RHEB were the common signature genes for subtypes of cardiomyopathy (viral, post. partum, familiar, and idiopathic). Major pathways enriched were including MAPK signaling pathway, the pathway of protein processing in endoplasmic reticulum, and pathway of regulatory actin cytoskeleton. Aberrant in these pathways caused disorders metabolic process and cellular malfunctions that generally contributes to cardiac dysregulation and functional relapse into cardiomyopathies.

Conclusion

This study identified the key signaling pathways, functions and biological process related to cardiomyopathies and will give a light to better understand the molecular mechanism of processes of cardiomyopathies and figure out the rational clinical interference way to cure the patients. Therein, these novel signature genes may work as potential promising biomarkers for cardiomyopathy diagnosis, and will benefit for the better clinical diagnostics and outcome for patients with cardiomyopathies.

Introduction

Cardiomyopathy is featured with incapacity of heart to pump and/or to fill with blood as body requires, and the worst state lapses into heart failure, a complex pathophysiological condition with left ventricle myocytes dysfunction. Globally, at least 26 million people were suffered from heart failure and consumed
over $30 billion health expenditure in the world \(^1\). The mortality of heart failure is as high as \(\sim 50\%\) in a five-year period \(^2\). Although cardiomyopathy is a primary condition for heart failure, pathogenic damages (abnormal physical structure and dysfunction) have often happened in the tissue of patient’s cardiomyopathy. Identification of biomarkers can be very useful for early diagnosis of cardiomyopathy, interruption of the disease procession to heart failure, and decrement of the mortality.

Etiologies for cardiomyopathy are multiple factors, which make cardiomyopathy a heterogeneous complex cardiovascular disease. The major stimuli include changed physiological conditions (such as pregnancy and delivery), and stressful pathological conditions (for example, ischemia, hypertension, diabetes, viral infection, and so on). According to the distinct etiologies, cardiomyopathy is divided into several subgroups that usually own specific morphological features\(^3\). The most prevalent subtypes are hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), viral cardiomyopathy (VCM), familial cardiomyopathy (FCM), post-partum cardiomyopathy (PCM) and ischemic cardiomyopathy (ISCM)\(^3\). Early clinical investigations recognized cardiomyopathic cases caused by abnormal gene expression, without somatic genetic alteration, suggesting the impact of epigenetics and transcriptional changes on cardiomyopathy \(^4\). However, complex etiologies may lead to a variety of abnormal expression genes, making it difficult to identify the common cardiomyopathy biomarkers with limited number cases.

Computerization methodologies have been applied into the discovery of signature genes as potential biomarkers of diseases. Among many computerization methodologies, the Weighted Gene Co-expression Network Analysis (WGCNA) is a useful approach that analyzes gene expression profiling to find out co-expression network based on their functional features, and to discover the genetic disorders in diseases \(^5\). WGCNA has been widely applied into screen novel biomarkers of cancers, and is demonstrated to be a reliable and powerful tool \(^6\)–\(^8\). In this study, WGCNA was employed to analyze gene expression profiles of several cardiomyopathies, containing 90 human biopsies from GEO databases in NCBI. The genes were discovered that significantly associated with cardiomyopathies. The signature genes and key biological pathways identified by WGCNA were further validated through bioportal database, an independent annotation database of cardiovascular diseases.

**Materials And Methods**

**Screening Gene Expression Datasets of cardiomyopathy from GEO Database**

The gene expression dataset of cardiomyopathy used for data analysis were screened from the Gene Expression Omnibus (GEO) database (NCBI). Numbers of patients (more than 20 cases), variety of subgroups of cardiomyopathy, and clearly annotations with clinical trials were the major metrics for screening. A dataset, with a GEO tracking number GSE1145 and a platform entry number GPL570 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE1145 5), was screened out for further analysis. In this datasets, cardiac transcription profiles were established from cardiomyopathy patients of who
were undergoing heart failure and planned for a cardiac transplantation. Human left ventricle samples were collected from biopsies of cardiomyopathy patients, or from "normal" organ donors whose hearts cannot be used for transplants. The heart failure of these cardiomyopathy patients arises from different etiologies. The transcriptional profiles were measured by Affymetrix Human Genome U133 Plus 2.0 Array. Changes in transcriptional profiles were correlated with the physiologic profile of failure hearts acquired at the time of transplantation. Sample collection and microarray dataset were performed by the cardio-genomics lab, Department of Bauer Center for Genomic Research, Harvard University.

Construction of Weighted Gene Co-expression Network

WGCNA package of R (version 1.63) was downloaded (http://www.Rproject.org) and setup by following the protocol previously. Each gene expression value from the downloaded dataset (GSE1145) was normalized by compared with inter reference genes, and performed a log2 transformation. Microarray quality was tested by sample clustering according to the distance between different samples in Pearson's correlation matrices. Outliers were identified with a height cut of 170000. Outliers and samples with excessive missing values were excluded from next analysis. Data quality was checked by the principal component analysis (PCA). The co-expression network was constructed, setting the soft threshold $\beta = 7$, which means that $R^2$ is equal to 0.98 and indicates the constructed network is close to a scale-free network. Once $\beta$ value was determined, the Topological Overlap Matrix (TOM) and dissTOM = 1 – TOM were obtained automatically. Modules were identified based on TOM and dissTOM. The hierarchical clustering analysis was used to identify gene modules and color to indicate modules, which is a cluster of densely interconnected genes in terms of co-expression. Genes that were not co-expressed were assigned into a grey module. The significant $p$-value of candidate genes was calculated via T-test. The association between the modules and diseases were evaluated by Gene significance (GS) and Module significance (MS); GS was defined as mediated $p$-value of each gene (GS = IgP); MS was defined as the average GS of all the genes involved in the same module. The cut-off significant standard was setup as $p$-value lower than 0.05. Importance of a gene within a module was measured by the module membership (MM). MM was calculated as $\text{MM}(i) = \text{cor}(x_i, \text{ME})$, where $i$ represents gene contained in module; ME (module eigengene) is defined as the first principal component of the module and represents the overall expression level of the module. Modules that significantly associated with the traits of different etiologies were identified by calculation the correlation of MEs with clinical pathological features.

Function & Pathway enrichment analysis for gene significance in module

Significant genes that were related to different pathological phenotype were blast through the web-based GenCLiP 2.0. Correlation analysis that biological functions and molecular networks involved with the genes were performed. The connection strength of a gene to other genes in a global functional pathway and network was measured by gene connectivity. Genes associated with cardiomyopathy were filtered from the identified significant genes by blast in the Cardiovascular Disease Portal, which contains
annotations of 854 genes with verified functions related to human cardiomyopathies\textsuperscript{12,13}. Genes that have been reported to link with pathological features were labelled with purple border.

**Identification of hub genes**

Hub gene in a module, a significant gene that widely connects with other significant genes, is the key interconnected nodes within a functionally network and plays the most important roles in the biological processes\textsuperscript{14}. Hub genes were identified by two methods: co-expression network and PPI network analysis. Potential hub genes among each significant module were selected by co-expression network, using GS (> 0.2), MM (> 0.8, with a threshold of \(p\)-value < 0.05), and correlated to certain clinical traits as the screening criteria. In parallel, protein-protein interaction (PPI) network of the module genes were built in a selected significant module through the STRING database. Interaction between genes was defined as positive, as cutoff > 0.4; potential hub gene was filtered in PPI network analysis, if its connectivity degree of \(\geq 8\) through STRING database. Overlapped potential hub genes in both co-expression network and PPI network analysis were the “real” hub genes.

**Validations of Expression of Hub genes and Signature Genes**

Significant genes were validated by comparison of their expression levels among the subgroups of cardiomyopathy and health group that was used as the benchmark. Individual gene expression in each group was presented as means ± standard error of the mean (SEM). Significance of differences was determined by Student t-test (Prism, GraphPad, San Diego, CA). Differences were significant if \(p < 0.05\) (*). When \(p < 0.05\), The standard of significance was setup as up-expression (Fold change > 1.0, \(p < 0.05\)) or down-expression (Fold change < 1.0, \(p < 0.05\)).

**Results**

**Clinical information of dataset**

From GEO database (NCBI), dataset GSE1145 was screened out and chosen for WGCNA analysis. In this dataset, it contained 90 left ventricle biopsies of patients. These biopsy samples represented 8 subgroups: 1, normal hearts, used as control (Health, \(n = 11\)); 2, idiopathic dilated (ID, \(n = 15\)); 3, ischemic (IS, \(n = 11\)); 4, idiopathic cardiomyopathy (IDCM, \(n = 12\)); 5, familial cardiomyopathy (FCM, \(n = 5\)); 6, post-partum cardiomyopathy (PCM, \(n = 4\)); 7, ischemic cardiomyopathy (ISCM, \(n = 20\)); 8, viral cardiomyopathy (VCM, \(n = 7\)). Using Affymetrix Human Genome U133 Plus 2.0 Array, the dataset contains expression data of 20,283 target genes for each biopsy sample. Each probe-set was linked with gene symbol through the Affymetrix annotation file GLP570. Besides gene symbols, their related function and clinical traits were also annotated. (Figure.1, Table. S1)

**Identified Gene Modules Correlation with Pathological Traits**
The cardiomyopathic and health samples were separated in the PCA plot (Figure.S1). Four normal samples (GSM18444/18445/18447/18448) were detected as outliers and ignored in the subsequent analysis. The total 86 samples were used for next step analysis (Figure.1). Co-expression networks with different clinical traits were built using the Pearson correlation analysis (Figure.2A). Calculated with a dynamic tree cutting algorithm, the distinct co-expression modules were identified that significantly related to different pathological features (Figure.2B & Figure.3A). Twenty-three modules were detected through the dataset (Figure.2B, Table.1). The numbers of significant genes containing in modules were varied from 121 to 14938. The correlation significance of module and pathological features was determined by module significance (MS) correlation and statistics p-value, and significant module varied from different subtype cases. Through calculation of the linear mixed-effects model, significant modules were identified for specific pathological feature (Figure.3B). The higher value of module eigengene (ME) correlation, the module is closer correlated to cardiomyopathies (Figure.S3A-G). In the idiopathic dilated group, six modules were significantly associated with its status, including turquoise module (t-value = 0.75, p-value = 5e-17), light-cyan module (t-value = 0.54, p-value = 7e-08), tan module (t-value = 0.56, p-value = 2e-08), grey module (t-value = 0.7, p-value = 8e-14), green module (t-value = 0.45, p-value = 1e-05) and the blue module (t-value = 0.72, p-value = 8e-15) (Figure.2B, Figure.3B, Table.1). In ischemic group, six significant modules were identified, including light-yellow module (t-value = 0.43, p-value = 3e-05), green-yellow module (t-value = 0.41, p-value = 8e-05), light-green module (t-value = 0.4, p-value = 1e-04), red module (t-value = 0.35, p-value = 8e-04), green module (t-value = 0.24, p-value = 0.03) and the blue module (t-value = 0.44, p-value = 2e-05) (Fig. 2B, Fig. 3B, Table.1). In idiopathic cardiomyopathy (IdCM) group, three modules were significantly linked to pathological trait, listed as module of Magenta (t-value = 0.43, p-value = 4e-05), Purple (t-value = 0.31, p-value = 0.003) and Brown (t-value = 0.25, p-value = 0.02). In familial cardiomyopathy group, only Magenta module (t-value = 0.22, p-value = 0.04) was significantly correlated to this trait (Figure.2B, Figure.3B). For the Hypertrophic cardiomyopathy (HCM) group, none of module was identified and ignored in next step analysis (Figure.2B). In the Post. Partum cardiomyopathy (PCM) group, Magenta module (t-value = 0.24, p-value = 0.03) was correlated to its trait (Figure.2B, Figure.3B, Table.1). In ischemic cardiomyopathy group, seven modules were significantly correlated to pathological trait, including Black (t-value = 0.56, p-value = 2e-08), Midnight-blue (t-value = 0.49, p-value = 2e-06), Dark-red (t-value = 0.43, p-value = 3e-05), Cyan (t-value = 0.34, p-value = 0.02), Yellow (t-value = 0.57, p-value = 1e-08), Pink (t-value = 0.29, p-value = 0.007) and Red (t-value = 0.26, p-value = 0.02) (Figure.3B). For Viral cardiomyopathy (VCM), two significant modules, Cyan (t-value = 0.35, p-value = 0.001) and Magenta (t-value = 0.23, p-value = 0.03), were identified (Figure.2B, Figure.3B).

Enriched Genes Significance related to different cardiomyopathies.

Compared the module memberships (MM) correlation and Genes Significance (GS) among the all significant modules, the module with most significant value was defined as the best candidate for pathological traits correlation analysis (Table 1, Fig. 3B), respectively. These candidates were listed as turquoise module (cor = 0.77, p < 1.0e-200, GS = 0.4244) (Idiopathic dilated group, Figure.S3A), light-yellow module (cor = 0.13, p = 3.0e-05, GS = 0.2733) (Ischemic group, Figure.S3B), magenta module (cor = 0.65, p = 1.5e-114, GS = 0.2783) (Idiopathic cardiomyopathy group, Figure.S3C), magenta module (cor
Hierarchical Clustering of Eigengene Profiles with cardiomyopathies Traits

Based on the ME’s values, the hierarchical clustering was performed between all modules and different cardiomyopathies traits to identify their relationships. Furthermore, the eigengene dendrogram analysis was performed to build the correlation of candidate module with different subtypes of cardiomyopathy feature, respectively (Figure.S4A-G). In the idiopathic dilated group, turquoise module was tightly clustered with idiopathic dilated (Figure. S4A). In the ischemic group, green yellow and light yellow modules were the closest branch clustered with ischemic (Figure.S4B). In previous step analysis, green-yellow module (t-value = 0.41, p-value = 8e − 05, GS = 0.2418) was the secondary higher correlation with ischemic status (Figure.2B, Figure.3B). It suggests that genes containing in green-yellow module would be involve the progress of ischemic. In idiopathic cardiomyopathy group, modules of brown, magenta and purple were clustered with idiopathic cardiomyopathy in a separate branch, and magenta and purple module allocated in same cluster (Figure.S4C). It suggests that module of purple and magenta would be the top two significant associated with cardiomyopathy status (Figure.2B, Table.1). In the familial cardiomyopathy and post. partum cardiomyopathy groups, modules of brown, magenta and purple were tightly clustered with familiar cardiomyopathy, while the magenta module was the most significant associated with disease status (Figure.S4D-4E). In the hypertrophic cardiomyopathy group, no module was associated with its pathological feature. In the ischemic cardiomyopathy group, although module of black and midnightblue were clustered in closer branch, the yellow module was allocated in the adjacent branch (Figure.S4F). Combined with the module-trait relationship correlation and gene significance results, it suggested that module of black, midnightblue and yellow were significant associated with ischemic cardiomyopathy (Figure.2B, Figure. 3B, Table. 1). The module of brown and blue were ignored for next analysis as with higher negative GS values (Table.1, Figure. 2B). The yellow module was the most significant correlation to ischemic cardiomyopathy. In the viral cardiomyopathy group, the modules of magenta, purple and brown were clustered with viral cardiomyopathy in a separate branch, and cyan module had the highest GS value associated with pathological feature (t-value = 0.35, p-value = 1e − 03, GS = 0.2245) (Figure.2B, Figure. 3B, Supporting Figure.4G). It suggested that magenta module containing genes involve the progress of viral cardiomyopathy.

Functions and pathways enrichment analysis

Blast through GenClip2, the enrichments analysis of significance genes contained in the correlated module were summarized as bar chart, including Biological Process, Molecular Function and Cellular Component (Ischemic, Figure.S5A-A; Idiopathic Cardiomyopathy, Figure.S5B-A; Familial Cardiomyopathy,
The Biological Processes were mainly concentrated in subgroups of cellular macromolecular metabolic process, protein metabolic process, organic substance metabolic process and macromolecule modification. The genes significantly enriched in molecular functions were summarized and listed (Table.S4). The molecular functions were linked to endoplasmic reticulum functions, cell functions (migration, death, growth, division), DNA binding, protein-protein interaction, kinases activity and signal transduction, iron binding and nucleotide binding, etc. The cellular components were mainly enriched in membrane-bounded organelle, intracellular organelle part, etc. The pathways concentrated on mitogen-activated protein kinase (MAPK) signaling pathway, protein processing in endoplasmic reticulum, regulation of actin cytoskeleton, etc. These results suggested that dysregulation of cardiac functions would be associated with metabolism abnormal and accelerated progress of cardiomyopathies. Furthermore, through gene network literature mining and clustering analysis, these significance genes were clustered and labelled according to cellular functions and pathological feature keywords literature corresponding (Ischemic, Fig.S5A; Idiopathic Cardiomyopathy, Fig.S5B; Familial Cardiomyopathy, Fig.S5C; Post-Partum Cardiomyopathy, Fig.S5D; Ischemic Cardiomyopathy, Fig.S5E; Viral Cardiomyopathy, Fig.S5F).

Moreover, the network and connectivity of significance genes were identified as node-connection map, which was correlated to different traits (Ischemic, Figure.S5A-B; Idiopathic Cardiomyopathy, Figure.S5B-B; Familial Cardiomyopathy, Figure.S5C-B; Post-Partum Cardiomyopathy, Figure.S5D-B; Ischemic Cardiomyopathy, Figure.S5E-B; Viral Cardiomyopathy, Figure.S5F-B). The key regulatory genes were labelled with purple border, which was reported in pathological cases, and majority of genes involved the pathway were labelled as un-reported. More interesting, no related key regulatory gene was identified in Post-Partum cardiomyopathy group.

Identification of hub genes for cardiomyopathies

Through co-expression network (MM-GS) filtered, the candidates of hub gene were identified in different groups (5 genes in Ischemic group, Figure.4A; 113 genes in Idiopathic Cardiomyopathy group, Figure.4B; 41 genes in Familial Cardiomyopathy group, Figure.4C; 65 genes in Post-Partum Cardiomyopathy group, Figure.4D; 83 genes in Ischemic Cardiomyopathy group, Figure.4E; 60 genes in Viral Cardiomyopathy group, Figure.4F).

Calculated by the PPI network method, the candidates of hub gene were summarized (12 genes in Ischemic group, Figure.4A; 120 genes in idiopathic cardiomyopathy group, Figure.4B; 277 genes in Familial Cardiomyopathy group, Figure.4C; 119 genes in Post-Partum Cardiomyopathy group, Figure.4D; 348 genes in Ischemic Cardiomyopathy group, Figure.4E; 49 genes in Viral Cardiomyopathy group, Figure.4F). These real hub genes were determined as described in method section (Table.3), and the numbers of real hub genes were listed (Figure.4A-F). The Idiopathic Dilated group was dismissed for
further analysis as no identified real hub gene. Through Venn diagrams analysis, three common axes of hub genes were discovered among these cardiomyopathic groups (Figure.4G).

The first axis was PICALM, which shared by Ischemic Cardiomyopathy, Idiopathic Cardiomyopathy and Post. Partum Cardiomyopathy groups (Figure.4G), and significantly up-regulated in Idiopathic Cardiomyopathy and Ischemic Cardiomyopathy groups (Table.3). PICALM is key regulator in iron homeostasis, clathrin-mediated endocytosis. Overexpression of PICALM impaired endocytosis of Transferrin (Tf) Receptor (TfR) and Epidermal Growth Factor Receptor (EGFR) and disturbed the iron homeostasis. Up to now, it is still illusive that the exactly role and deregulatory mechanism of PICALM in cardiomyopathies. It is strongly suggesting that PICALM work as potential novel biomarker and therapy target for these subcases of cardiomyopathies.

The secondary axis, contained genes of PRKACB, MOB1A, CDC40, were shared in Post. Partum Cardiomyopathy and Idiopathic Cardiomyopathy groups. In addition, these genes (PRKACB, MOB1A, CDC40) were significantly overexpressed in Idiopathic cardiomyopathy group, and MOB1A was up-regulated in Post. Partum cardiomyopathy group (Table.3).These genes were linked to the cAMP (cyclic AMP)-dependent protein kinase A (PKA) mediated the exciting-contraction coupling in cardiomyocytes, and regulated microtubule stability, cell cycle and cell proliferation & migration, and restrained cardiomyocyte proliferation and size via Hippo pathway. PRKACB (protein kinase cAMP-activated catalytic subunit β gene) was linked to congenital heart defect with abnormal over-expression. MOB1A (MOB kinase activator 1A) was required for cytokinesis through regulating microtubule stability. It worked as binding partners as well as co-activators of Ndr family protein kinases and mediated phosphor-recognition in core Hippo pathway that restrains cardiomyocyte proliferation during development to control cardiomyocyte size. Overexpression of MOB1A induces centrosomes fail to split and cell size dysregulation. CDC40 (Cell Division Cycle 40), a splicing factor of cell division cycle 40 homolog, regulates cell cycle and cell proliferation and migration. Overexpression of CDC40 causes abnormally cell proliferation and migration, and linked with carcinogenesis.

The third axis consisted of five genes (CREB1, DBT, NCOA2, NUDT21, PIK3C2A) and were overlapped among three groups of Familial / Idiopathic / Post. Partum Cardiomyopathy (Figure.4G). The CREB1 (cAMP-responsive element-binding protein) had been identified as the transcription factor and mediated cAMP stimulation by multiple extracellular signals, such as growth factors and hormones. The CREB1 was the key regulator in heart and linked with heart disease via cAMP-PKA pathway dysregulation. The DBT (dihydrolipoamide branched chain transacylase E2) is an inner-mitochondrial enzyme complex regulated to degrade the branched-chain amino acids isoleucine, leucine, and valine. The DBT was reported as clinical diagnostics biomarker for patients with dilated cardiomyopathy via caused mitochondria dysfunction. NCOA2 (nuclear receptor coactivator 2) is a transcriptional coactivator that functional aid for nuclear hormone receptors, including steroid, thyroid, retinoid, and vitamin D receptors. NCOA2 promotes muscle cells maintenance and growth, eventually regulates in cardiac cTnT levels. Overexpression of NCOA2 regulated cell proliferation in cardiomyopathy. NUDT21 (nudix hydrolase
21) is a novel of cell fate regulator by alternative polyadenylation chromatin signaling, and suppression of NUDT21 will enhance the cell pluripotent, facilitated trans-differentiation into stem cell. NUDT21 regulates cell proliferation through ERK pathway. Up to now, little knows about the function of NUDT21 in cardiomyocytes. PIK3C2A (phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 alpha) is an enzyme belong to phosphorylate the 3'-OH of inositol ring of phosphatidylinositol (PI) superfamily and regulates multiple signaling pathways. PIK3C2A is mainly expressed in endothelial cells, vascular endothelium, and smooth muscle. Lower expression of PIK3C2A in peripheral blood was used as significant biomarker for acute myocardial infarction patients. More interesting, these hub genes indicated different expression pattern. The expression level of DBT, NCOA2, NUDT21 and PIK3C2A were significantly upregulated in Idiopathic cardiomyopathy group, and PIK3C2A was up-regulated in Familial cardiomyopathy group (Table.3). It hints that these hub genes play different regulatory pattern in the progress of these subtype's cardiomyopathies.

The fourth axis of hub genes (HNRNPC, UEVLD) were shared by Familial Cardiomyopathy and Idiopathic Cardiomyopathy groups, and significantly overexpressed in Idiopathic cardiomyopathy group (Table.3). HNRNPC (heterogeneous nuclear ribonucleoprotein C) is RNA binding protein that belong to ubiquitously expressed heterogeneous nuclear ribonucleoproteins subfamily, and mediates pre-mRNAs transport and metabolism between cytoplasm and nucleus and overexpression caused cells multi-nucleation. UEVLD (EV and lactate/malate dehydrogenase domain-containing protein) involves the protein degradation and dysregulated linked with metabolic disease. In this study, the expression level of HNRNPC and UEVLD were significantly up-regulated in Idiopathic cardiomyopathy group (Table.3). Furthermore, through different significant expression analysis, the significant changed hub genes were summarized (Table.3, p < 0.05). Combined these results together, it hints that these significantly expressed Hub genes play dominant role and work as common key regulatory nodes in progress of cardiomyopathies.

**Disease Signature Genes Identification and expression analysis**

The filtered disease signature genes were summarized with the functional annotation of genetic dysregulation correlated to heart diseases phenotypes, including ten signature genes in the ischemic group and viral cardiomyopathy group, forty signature genes among the group of familiar cardiomyopathy, Post-partum cardiomyopathy and Idiopathic cardiomyopathy, and 69 signature genes in the ischemic cardiomyopathy group (Table.4). Through Venn Diagram analysis, the common signature genes were determined among different groups (Figure.5A). Four signature genes (MDM4, CFLAR, RPS6KB1, PKD1L2) were shared by Ischemic and Ischemic Cardiomyopathy group (Table.4, Figure.5A). Ischemic cardiomyopathy group did share eight disease signature genes (MAPK1, MAPK11, MAPK14, LMNA, RAC1, PECAM1, XIAP, CREB1) with Post. Partum/Familiar/Idiopathic Cardiomyopathy groups, which dysregulated in cardiomyopathies, and genes expression level of MAPK1, MAPK11, LMNA, RAC1 were significantly up-regulated in these cardiomyopathies groups (Table.5, Figure.5A). Two
signature genes (TFAM, RHEB) were shared between Viral Cardiomyopathy and Post. Partum / Familiar/
Idiopathic Cardiomyopathy groups, which involved in development of cardiac hypertrophy\textsuperscript{45} and Mitochondrial Cardiomyopathy\textsuperscript{46}.

Furthermore, through different significant expression analysis, the significantly changed signature genes were summarized (Fig. 5D-F, Table.5). In ischemic group, MDM4 gene was significantly upregulated (FC = 1.0495, p = 0.0037) (Table 5, Figure.5B), which genetic deletion associated with cardiomyopathy\textsuperscript{43}. In viral cardiomyopathy group, COA5 was overexpressed (FC = 1.087485, p < 0.0001) (Table 5, Figure.5C), which was upregulated in Ischemia/Reperfusion Injury caused cardiomyopathy\textsuperscript{47}. In idiopathic cardiomyopathy group, seven genes (ADAM10, RAB1A, TFAM, FGF2, ELMOD2, GUF1, ABCC9) were significantly up-regulated, while 8 genes (FHL1, CTNNA3, PDLIM5, LMNA, SIRT4, YME1L1, RHEB, GNB1L) were down-regulated (Figure.5D, Table.5). In idiopathic cardiomyopathy group, four down-regulated genes (NEBL, FHL1, FHL2, SIRT4) and 5 up-regulated genes (GUF1, ELMOD2, ABCC9, FGF2, YME1L1) were significantly changed (Figure.5E, Table. 5). In post-partum cardiomyopathy group, 11 genes (ATL3, ADAM10, ELMOD2, FGF2, GUF1, YME1L1, up-regulated; FHL1, CTNNA3, PTPN11, GNB1L, SIRT4, downregulated) were significantly changed (Figure.5F, Table.5). In Ischemic cardiomyopathy group, 31 genes are significantly changed expression, including 12 genes (RAC1, FKTN, EDNRB, ZBTB33, TXN, RALGAPA1, PSEN1, LAMP2, UBR5, SCN4B, SMAD1, MYO6) down-regulated and 19 genes up-regulated expression (POLRMT, AVP, GATA4, CACNB2, MAPK1, NOS3, LAMA3, SOD2, LMNA, MAP1LC3A, MAPK14, TCAP, LRP4, BAD, DES, AKAP8, CASP9, HSPB1, SNTA1) (Figure.5G, Table.5). These results suggest that these common disease signature genes work as novel biomarker and be potential key regulators of the cardiomyopathy progress.

Discussion

In this study, to discover novel signature genes or biomarkers to accelerate the precise clinical diagnostics and interference for different subtype of cardiomyopathies, the WGCNA pipeline was applied to analyze the gene expression profiling of 86 clinical left ventricle biopsy samples, which represents 8 subtype’s cardiomyopathies. The WGCNA pipeline was widely used for performing various functions in weighted correlation network analysis, including constructing network, detecting module, calculating topological properties, simulating data, visualization, and interfacing with external software\textsuperscript{9}. The whole transcriptome profile contained 20,283 target genes for promise diagnostic assessment and mainly covered variously biological and cellular processes. It is representative of real pathological satiation and valuable to discover the signature gene of cardiomyopathies. First of all, it was reasonable to build the co-expression networks with different clinical cardiomyopathies traits using the Pearson correlation (Figure.2A). To discover the related modules to cardiomyopathies phenotype, the genes significance of the modules was calculated by the linear mixed effects model for testing the association of node to the pathological phenotypes. It was identified that the association significance between individual modules of gene expression profile and different cardiomyopathies feature (Figure.2B). Through the Eigengene dendrogram analysis, the most significantly module was pick out for next analysis (Figure.3B, Figure.S4A-
For the next step, the real hub genes among each significant module were screened by module membership (MM) - Gene Significance and Protein-Protein Interaction Network analysis, and comprised as key interconnected nodes within a functionally network and played important roles in biological functions. Without any real hub genes identified in the Idiopathic Dilated group, it was discarded for next analysis. In addition, the Idiopathic Dilated case was treated as unique physiological state without impaired the normal cardiac function and ignored for analysis. Briefly, the next analysis was mainly concentrated on these five subtype's groups, including idiopathic cardiomyopathy (IdCM), familial cardiomyopathy (FCM), post-partum cardiomyopathy (PCM), Ischemic cardiomyopathy (IsCM) and viral cardiomyopathy (VCM). There were four axes of hub genes shared among these cardiomyopathic groups. It was suggesting that these Hub genes work as common key regulator. It was exception that viral cardiomyopathy group did not share hub gene with the others groups. It was possible unique that the dysregulation expression pattern of viral cardiomyopathy. Furthermore, to deeply dig the correlation of significance genes and different cardiomyopathies, the significance genes were blast through GenCliP2 to mine gene networks and functions connection, biological process, molecular functions, the cellular components and functional pathways. Although the enriched pathways were varied from different subtype's cardiomyopathies, the key pattern was similar. The biological processes were significantly concentrated on cellular metabolic, protein metabolic, organic substance metabolic and macromolecule modification. It suggests that the metabolic process disorder be associated with progress of cardiomyopathies. The molecular functions were mainly involving in protein binding, heterocyclic compound binding, purine ribonucleotide binding, iron binding and nucleotide binding, etc. The cellular components were including membrane-bounded organelle, intracellular organelle part, etc. The pathways were concentrated on MAPK signaling pathway, protein kinase C protein processing in endoplasmic reticulum, regulation of actin cytoskeleton, etc. These related genes were summarized with functions and pathways. Furthermore, most of significance genes were labelled as gene-term association not reported. It represents that the regulatory mechanism of these genes is illusive in progress of cardiomyopathies. Through Literature Gene Networks Mining, the genes were identified in the regulatory network with less published literatures, which were associated with different cardiomyopathies except Post-Partum cardiomyopathy. For subtype of idiopathic cardiomyopathy (IdCM), familial cardiomyopathy (FCM), and Ischemic cardiomyopathy (IsCM), the highlighted genes were mainly concentrated in MAPK signaling pathway, including MAPK1, MAPK14, CREB1, RAC1. The genes YY1, RAPGEF1, SMAD2, JUND, ATF1, and SRA1 linked with viral cardiomyopathy, which are assemble in SMAD signaling pathway and YY1 was overexpressed in heart failure. These results partially matched the key axes of hub genes linked the functions and pathways. These new discovered genes linked to viral cardiomyopathy may give a new view for clinical diagnostics and treatment. It suggests that the dysfunctions of these significance genes would be associated with metabolism disorder and progress of cardiomyopathies.

Limited by hardly accessible to clinical samples, blast through the cardiovascular disease BioPortal database was employed as validation strategy to explore the disease signature genes that associated
with different subtype cardiomyopathies. The Cardiovascular Disease Portal provides easy access to multiple genetic data associated with specific cardiomyopathy types and. The Cardiovascular Disease Portal integrates data for genes, QTLs and strains associated with the disease(s) highlighted and translational research annotation and associated disease information. The filtered genes will be defined as disease signature genes for cardiomyopathies\textsuperscript{12,13}. The varied number of disease signature genes were filtered for different groups (Table.4). There were three axes of common signature genes identified among five subtype's cardiomyopathies groups (Fig. 5A). The first axis contained 4 disease signature genes (MDM4, CFLAR, RPS6KB1, PKD1L2) shared by ischemic and ischemic cardiomyopathy group. Compared with health control, only MDM4 was significantly overexpressed (FC = 1.0495, p = 0.0037) in ischemic group, while the four signature genes did not significantly change in ischemic cardiomyopathy group. It matches the previously reports that the upregulated MDM4 plays cardio-protective effect in ischemia-refuse injury\textsuperscript{50}. Overexpression of MDM4 reflects the self-correction of physiological system in abnormal physiological condition of ischemic. It will generate new strategy to interfere the ischemic lapse into cardiomyopathies by artificial upregulated MDM4 expression. The secondary axis, consisted of eight signature genes (MAPK1, MAPK11, MAPK14, LMNA, RAC1, PECAM1, XIAP, CREB1), was shared by Ischemic Cardiomyopathy with Post. Partum/Familiar/Idiopathic Cardiomyopathy groups. These signature genes (MAPK1, MAPK11 and LMNA down-regulated; RAC1 up-regulated) were significantly changed in ischemic cardiomyopathy group, which played dominant role in progress of cardiomyopathy, and only LMNA was significantly down-regulated in Idiopathic Cardiomyopathy group (Table.5, Fig. 5G). It suggests that the disorder expression pattern of three subtypes cardiomyopathies could be more complex. The signature genes (TFAM, RHEB) were shared by Viral Cardiomyopathy and Post. Partum / Familiar /Idiopathic Cardiomyopathy groups, and only RHEB was significantly down-regulated in Idiopathic cardiomyopathy group. It suggests that signature genes (TFAM, RHEB) play less contribution in pathological progress of viral cardiomyopathy and Post. Partum / Familiar /Idiopathic Cardiomyopathy. Combined these results together, it strongly suggests that these genes could be used as promising biomarkers or therapy targets for cardiomyopathies. This progress will be helpful to integrate precise clinical application for different subtype cardiomyopathies.

There are some limitations in this study. Firstly, lacking of larger number of clinical samples and more detail of disease state information, it was hardly to track the patient's cases with expression profiles and verify these potential biomarkers with original patient's pathological feature. Secondly, due to the nature of bioinformatics analysis, the discovered specific GO pathways and biomarkers do need further investigation. Although these genes were validated significantly associated with cardiomyopathies feature through cardiovascular disease BioPortal database, and it is necessary to verify these potential biomarkers with more clinic patient’s biopsies by immunohistochemistry (IHC) or other genetic detection method, like qPCR or sequencing in coming research work. It is mandatory and rational to investigate the contribution and mechanism of these potential disease signature genes to the progress of cardiomyopathies by animal model and validate with clinical data in the future research. On the other side, this study has several novelties. Firstly, it has applied reverse strategy by using WGCNA approach to discover the genes significantly associated with cardiomyopathies pathological feature in clinical
samples. In parallels, compared these signature genes expression level among health control and disease groups, the results indicated that signature genes have significant expression change in disease groups (Table.5). Secondly, the key functional & GO pathways that genes significance in module involving in the progress of cardiomyopathies were inquired by Genclip enrichment analysis. The results will be a clause for the next step research. Thirdly, exploring through bioportal database, some novel potential signature genes were identified as potential biomarkers of cardiomyopathies in previously independent researches. These results are partially as evidences to support our results and research strategy.

In summary, this study provides new insight to identify the potential novel key regulatory biomarkers or therapy targets for varied cardiomyopathies induced by different etiologies. The disease signature genes associated with cardiomyopathies were identified and listed as the potential therapy targets for clinical application. In the future research, the detail of regulatory mechanism of these disease signature genes will be deeply investigated and develop novel therapy strategy for cardiomyopathies.

**Abbreviations**

| Abbreviation | Description                                      |
|--------------|--------------------------------------------------|
| PCA          | principal component analysis                     |
| GO           | gene ontology                                    |
| TOM          | Topological Overlap Matrix                       |
| GS           | gene significance                                |
| MM           | module membership                                |
| ME           | module eigengene                                  |
| MS           | module significance                               |
| WGCNA        | weighted gene co-expression network analysis      |
| ID           | idiopathic dilated                               |
| IS           | ischemic                                         |
| IDCM         | idiopathic cardiomyopathy                        |

FCM familial cardiomyopathy

PCM post-partum cardiomyopathy

ISCM ischemic cardiomyopathy

VCM viral cardiomyopathy

**Declarations**
Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agree to publish this manuscript.

Availability of supporting data

All supporting data are available from online supplementary.

Competing interests

All authors declare that they have no competing interests.

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Author’s Contributions

SL and YH designed the overall project study; YH collected data, performed data analysis, and drafted the manuscript; SL, YH, JT and ZW interpreted and summarized the results; SL and YH wrote and revised the manuscript; all authors have read and approved the final version of manuscript. JT and ZW contributed equally.

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**Tables**

Due to technical limitations, table 1 to 5 is only available as a download in the Supplemental Files section.