Dysfunctional network and mutation genes of hypertrophic cardiomyopathy

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Research

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Abstract

Background

Hypertrophic cardiomyopathy (HCM) is a group of heterogeneous diseases that affect the myocardium. It is also a common familial disease. The symptoms are not common and easy to find.

Methods

In this study, gene expression profiles of 37 samples (GSE130036) were downloaded from GEO database. Differential analysis was used to identify the related dysregulated genes in patients with HCM. Enrichment analysis identified the biological function and signal pathway of these differentially expressed genes. Then, we build PPI network and verify it in GSE36961 dataset. Finally, the gene of single nuclear variants (SNVs) in HCM samples was screened by means of maftools.

Results

Herein, we obtained 920 differentially expressed genes, and found that these genes are mainly related to metabolic related signaling pathways. 187 interacting genes were identified by PPI network analysis, and the expression trends of C1QB, F13A1, CD163, FCN3, PLA2G2A and CHRDL2 were verified by another dataset. ROC curve analysis showed that they had certain clinical diagnostic ability, and they were the potential key dysfunctional genes of HCM. In addition, we found that PRMT5 mutation was the most frequent in HCM samples, which may affect the pathogenesis of HCM.

Conclusions

Therefore, the key genes and enrichment results identified by our analysis may provide a reference for the occurrence and development mechanism of HCM. In addition, mutations in PRMT5 may be a useful therapeutic and diagnostic target for HCM.

Background

Hypertrophic cardiomyopathy (HCM) is a common inherited cardiovascular disease, which exists in one patient in every 500 people (1). Since the first anatomical description for HCM in 1958, people's understanding of the disease has been greatly improved (2). Recently, great progress has been made in diagnosis and treatment selection, and the awareness of the disease has been improved in clinical practice (3). However, the diagnosis of HCM is often misdiagnosed as asthma, anxiety, mitral valve prolapse and coronary artery disease (4). HCM is easy to cause angina, heart failure and arrhythmia, which is the most terrible complication of HCM and the most common cause of sudden cardiac death in young people (5, 6). The clinical manifestations of hypertrophic cardiomyopathy are various, with a natural history (7). However, once correctly diagnosed, patients with hypertrophic cardiomyopathy can be effectively managed to improve symptoms and survival.
The pathophysiological manifestations of HCM include left ventricular hypertrophy with or without right ventricular hypertrophy, mitral regurgitation, diastolic dysfunction, myocardial ischemia and fibrosis (8, 9). At present, echocardiography, cardiac magnetic resonance and other imaging methods are important imaging methods for the diagnosis, treatment and risk stratification of HCM, but the accuracy of detection is relatively low (10). Patients with hypertrophic cardiomyopathy are relieved by various physical exercise methods, which have made significant contributions to disease management and are carried out without increasing risk (11). Surgical treatment is the first choice for HCM, but due to the high morbidity and mortality in the early stage of the disease, some clinicians are still hesitant (12, 13).

In recent years, research on complex molecular pathophysiology of hypertrophic cardiomyopathy has increased rapidly (14). Gene testing has become more accessible and is increasingly being included in the care of patients with hypertrophic cardiomyopathy (15). It is worth noting that HCM has many genotypes and phenotypic variations (16, 17). Hypertrophic cardiomyopathy is associated with more than 1400 mutations in 11 or more genes encoding cardiac sarcomal protein (5). In most cases, the disease is caused by a single heterozygous mutation, with few (3–5%) multiple mutations leading to particularly severe hypertrophy and more adverse events (18).

In this paper, the dysfunctional gene network related to hypertrophic cardiomyopathy was analyzed, and the key target genes with diagnostic and therapeutic significance for HCM were screened. The results of gene mutation analysis may be helpful to the genetic detection of the subjects and their families. The results of this study can help to improve the diagnosis and distinguish between relatives at risk and those without risk.

Materials And Methods

GEO dataset and differentially expressed genes (DEGs)

The gene expression dataset GSE130036 and GSE36961 were downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/) (19). The GSE130036 series (GPL20795 platform) contained a total of 37 samples (28 HCM patients and 9 healthy donors). The GSE36961 series (GPL15389 platform) contained a total of 145 samples (106 HCM patients and 39 healthy donors).

We used the limma package (20) in R to screen DEGs between HCM and healthy control samples. A threshold of twofold change and P-value <0.05 were set for DEGs in this study.

Functional and pathway enrichment analyses of DEGs

To explore the biological characteristics of these DEGs, we performed Gene Ontology (GO) with clusterProfiler R package (21). Then, we used ClueGO plug-in of Cytoscape to performe Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway for DEGs (22). Significant results were determined with a P-value <0.05.
Construction of the PPI network

To establish the PPI network of all identified DEGs, the STRING (version 10.5) (http://string-db.org/) online database was used (23). The parameter was set as medium confidence >0.4. To visualise the PPI network, Cytoscape software (http://www.cytoscape.org/) was used to draw their interactions (24). ROC curve was plotted and AUC was calculated with "ROCR" package (25).

Single-nucleotide variants (SNVs)

We used Maftools R package (26) to analysis the SNVs in HCM samples. Maftools is implemented as an open source R package and available as a part of the Bioconductor project. Visualization module in Maftools are generated using ComplexHeatmap Bioconductor package (27).

Results

Differentially expressed genes in hypertrophic cardiomyopathy

The difference of gene expression between patients in disease state and healthy people may be closely related to the disease. We analyzed the data of GSE130036 in geo database to explore the genes related to hypertrophic cardiomyopathy. By setting the screening threshold twofold, we identified 920 differentially expressed genes (DEGs) between the hypertrophic cardiomyopathy and the healthy controls (Table S1). There are 636 up-regulated genes with high expression level and 284 down regulated genes with low expression level (Fig. 1A, 1B). It is important to compare these differentially expressed genes with those of HCM and control groups in gse36961 data. We found 18 common differentially expressed genes (Table 1). We suggest that the differentially expressed genes between HCM and control may be dysfunctional genes related to hypertrophic cardiomyopathy.
Table 1
The common differentially expressed genes in the GSE130036 and GSE36961.

| GSE130036 | GSE36961 |
|-----------|----------|
| symbol    | log2FoldChange | pvalue | padj | logFC | P.Value | adj.P.Val |
| CENPA     | 2.428995 | 1.71E-06 | 2.99E-05 | 1.699106 | 2.22E-18 | 1.62E-16 |
| TUBA3E    | -3.20702 | 8.72E-23 | 4.96E-20 | -2.17305 | 3.12E-47 | 1.18E-42 |
| TUBA3D    | -3.1646 | 6.82E-26 | 6.25E-23 | -2.20486 | 2.59E-44 | 1.63E-40 |
| CORIN     | -3.2704 | 8.28E-13 | 6.96E-11 | -1.56596 | 5.15E-11 | 1.18E-09 |
| SLITRK4   | 3.644176 | 1.91E-19 | 6.24E-17 | 1.260442 | 1.38E-14 | 5.43E-13 |
| CA3       | 2.876428 | 5.99E-08 | 1.54E-06 | 1.362298 | 9.87E-13 | 2.93E-11 |
| LYVE1     | -2.0283 | 9.92E-28 | 1.06E-24 | -1.87319 | 2.62E-39 | 7.08E-36 |
| METTL7B   | -2.17555 | 6.86E-20 | 2.45E-17 | -1.35577 | 6E-23 | 9.23E-21 |
| SERPINA3  | -3.81907 | 4.52E-09 | 1.53E-07 | -3.56141 | 3.7E-42 | 1.75E-38 |
| RASD1     | -2.08726 | 6.49E-12 | 4.42E-10 | -3.51434 | 2.45E-45 | 1.85E-41 |
| SLCO4A1   | -2.59057 | 1.24E-24 | 9.11E-22 | -1.00128 | 1.37E-32 | 1.2E-29 |
| COMP      | 2.861793 | 8.22E-07 | 1.56E-05 | 1.099086 | 7.8E-09 | 1.24E-07 |
| C1QB      | -2.15322 | 1.30E-30 | 2.13E-27 | -1.46863 | 4.93E-28 | 1.94E-25 |
| F13A1     | -2.29595 | 1.12E-38 | 7.01E-35 | -1.5787 | 3.76E-30 | 2.26E-27 |
| CD163     | -2.76903 | 4.77E-33 | 1.19E-29 | -2.13651 | 1.05E-37 | 2.34E-34 |
| FCN3      | -2.50434 | 2.72E-17 | 6.00E-15 | -2.03805 | 4.98E-40 | 1.57E-36 |
| PLA2G2A   | -2.93842 | 3.35E-08 | 9.14E-07 | -1.35137 | 2.51E-20 | 2.58E-18 |
| CHRDL2    | -3.62053 | 2.89E-10 | 1.33E-08 | -1.17134 | 1.47E-29 | 7.62E-27 |

**Mechanism of dysregulation related to differentially expressed genes**

In order to explore the biological functions of these dysfunctional genes in HCM, we analyzed the enrichment of DEG with GO and KEGG. From the results of biological process (BP) enrichment, we found that the maladjusted genes are mainly involved in the biological process related to epidermis development (Figure 2A, 2B). In addition, in the enrichment results of cell component (CC), the maladjusted gene is mainly related to hemoglobin complex (Figure 2C, 2D). In the molecular function...
(MF), the maladjusted gene is mainly enriched in the molecular activity related molecular function (Figure 2E, 2F). On the other hand, we found that the signal pathways involved in the maladjusted genes mainly include arachidonic acid metabolism, aldosterone synthesis and secret, and drug metabolism (Figure 2G, 2H). The results showed that the maladjusted genes were mainly related to metabolism related signaling pathway in the course of HCM.

**PPI network identification of key dysregulated molecules**

In order to provide the contents and ways of DEG participating in cell biological activities, we constructed a full view of their interacting proteins to elucidate their functional networks. After mapping up-regulated and down-regulated genes into the network, the PPI network of 187 dysregulated genes (Figure 3A) was screened. Currently, we map 18 common differentially expressed genes into PPI network. Six genes were screened, including C1QB, F13A1, CD163, FCN3, PLA2G2A and CHRDL2. Their expression in the two groups of data is similar, with consistent down-regulation behavior (Figure 3B). In addition, ROC analysis showed that the AUC values of the six genes were all over 91%, indicating that they had a certain clinical diagnostic ability (Figure 3C). Therefore, we think these six genes may be potential biological target genes of HCM.

**Regulatory mechanism of HCM influenced by mutation**

Using gene cohorts in GSE130036 data, we identified mutations in key genes generated using maftools (Table S2, figure 4A). The SNVs in the sample can be divided into six categories (Figure 4B). Thus, we present the mutation types and frequencies of the first 10 mutations (Figure 4C). Including PDE11A, PRMT5−AS1, TSPAN9, MTR−RPL35P1, RBM23, NFKBIZ, PRMT5, RP11−14N7.2, NOTCH2 and RP5−857K21.4. In addition, we found that the total number of samples with PRMT5 mutation was the largest (Figure 4D). It is suggested that the mutation frequency of HCM is the highest, which may have a wider impact. The above results show that the phenomenon of gene mutation often occurs in HCM patients, which means that gene mutation has a significant impact on the disease.

**Discussion**

Most HCM patients have no obvious symptoms, and their life span is close to normal (28). However, a small number but important patients will have a wide range of clinical adverse phenomena, including the development of late heart failure symptoms, atrial and ventricular arrhythmias, thromboembolism events, and even sudden death (29–31). To clarify the exact genetic cause of hypertrophic cardiomyopathy can improve the level of clinical management. This study is based on the genes expressed in HCM patients, combined with network analysis to identify the key genes. We also analyzed the single nucleus variants of genes expressed in HCM patients to explore the molecular mechanism of HCM disease.
Increasingly, genetic testing reports provide an assessment of the pathogenicity of all identified genes (32). By identifying the differentially expressed genes between HCM and control, candidate target genes of HCM can be screened. Among the differentially expressed genes, the mRNA KRT1 with the largest up-regulation multiple and the mRNA CYP1A1 with the largest down-regulation multiple may play an important role in the disease. Note that KRT1 expression increased in patients with heart failure and cardiac pressure overload (33, 34). It has been reported that severe cytochrome P450 (P450) enzymes have been altered during cardiac hypertrophy (35). However, contrary to our results, 3-methylholanthrene and benzo (a) pyrene induced cardiac hypertrophy increased the expression of CYP1A1 (36).

Further, enrichment analysis showed that HCM related dysregulated genes were mainly involved in epidermis development and other related biological functions and metabolism related signal pathways. Similar to our analysis results, the differentially expressed genes are also related to epidermis development in the dilated cardiomyopathy (37). In addition, the mechanism of metabolism has an important influence on the pathophysiological process of HCM (38). Studies have shown that the decrease of left ventricular systolic function in female HCM mice is related to the decrease of activities of fatty acid transporter (CD 36) and AMP activated protein kinase (AMPK) (39).

On the other hand, through PPI network of differentially expressed genes, we identified the dysregulated genes with interaction. Among them, we found six genes verified by GSE36961 data, and their expression levels were down regulated. In recent research results, F13A1 expression changes in the myocardial infection model of pigs (40). HCM is the result of cardiac remodeling caused by myocardial cell injury. Macrophages participate in this process by maintaining the inflammatory and fibrogenic environment (41). CD163 promotes the transformation of macrophages from M1 to M2 phenotype and releases anti-inflammatory factors to solve inflammation (42). In our analysis, CD163 was down regulated in HCM, which may be a key factor in the persistent inflammatory environment of HCM. However, the expression of PLA2G2A decreased in patients with dilated cardiac pathway (43). It is reported that the occurrence of cardiovascular disease is closely related to PLA2G2A (44).

Most HCM cases are caused by mutations in the oncoprotein coding gene in Mendel’s autosomal dominant genetic pattern (45). In HCM patients, gene detection of these genes is of great value for diagnosis and early recognition of individuals at risk (46, 47). However, new HCM related mutations are being discovered, and more genes need to be found. PRMT 5 is known as type II PRMT, which can produce mono methylarginine and symmetric dimethylarginine (48). PRMT 5 directly methylated GATA 4 transcription factor and inhibited phenylephrine induced hypertrophy of neonatal rat ventricular myocytes (49). Therefore, the HCM samples used in this study may be related to PRMT 5 mutations. PDE11A has been proved to be an important gene which contains clinically significant variants (50). The identification of pathogenic mutation gene is helpful to the determination of clinical diagnosis and eliminate the ambiguity related to phenotype variation. Gene expression data may also help guide the use of new therapies. Therefore, the results of this study may be able to guide the treatment of hypertrophic cardiomyopathy and provide an independent quantitative assessment of functional limitations in patients with unknown history.
Conclusion

In this study, the key dysfunctional genes C1QB, F13A1, CD163, FCN3, PLA2G2A and CHRDL2 were identified by studying the network of differentially expressed genes between HCM and healthy controls. Enrichment analysis showed that the molecular mechanism of HCM was related to metabolic related signaling pathway. Interestingly, we found that PRMT 5 is the gene with the highest mutation rate in HCM samples, indicating that PRMT 5 may have an important impact on the pathogenesis of HCM.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All the authors have read and agreed to the publication of this paper.

Availability of data and materials

Not applicable

Competing interests

All authors declare no competing interests.

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Authors’ contributions

Yunwen Cui and Cheng Liu contributed equally to the manuscript preparation, supervised the data analysis and interpretation. Jian Luo contributed to data and manuscript preparation. Jie Liang conceived and designed the study, supervised the experimental work, data analysis and interpretation, and manuscript critical review.

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**Figures**
The differentially expressed genes between hypertrophic cardiomyopathy patients and controls. A. Volcano plot of differentially expressed genes. Red dots represent up-regulated genes and blue dots represent down-regulated genes. B. Thermogram of differentially expressed genes. The node color changes from blue to red, indicating that the gene expression level changes from low to high.
Figure 2

GO and KEGG enrichment of differentially expressed genes. A, B. The biological process which differentially expressed genes participate in. C, D. The cellular component which differentially expressed genes participate in. E, F. The molecular function which differentially expressed genes participate in. The larger the dot is, the more genes are involved. G, H. The KEGG pathway enrichment of differentially expressed genes.
Figure 3

PPI network construction and identification of hub genes. A. PPI network of DEGs. B. The expression of six common differentially expressed genes in PPI network in two groups of data. C. ROC curves of six genes with common differential expression.

Figure 4

Mutations of the expressed genes in HCM samples. A. The summary of the mutations in all samples. B. Mutations in the top 10 genes. Genes are ordered by their mutation frequency, and the mutation rate in the samples. C. Transition and transversion plot displaying distribution of SNVs in HCM classified into six transition and transversion events. Stacked bar plot shows distribution of mutation spectra for every sample. D. Word cloud plot for mutated genes. Size of each gene is proportional to the total number of samples in which it is mutated.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS2.xlsx
- TableS1.xlsx