Original Article

Selection of key genes for dilated cardiomyopathy based on machine learning algorithms and assessment of diagnostic accuracy

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Background: The mechanisms of the occurrence and progression of dilated cardiomyopathy are still unclear and further exploration is needed. The upgrading of programming languages and the improvement of biological databases have created conditions for us to explore the structural and functional information of biological molecules at the nucleic acid and protein levels, screen key pathogenic genes, and elucidate pathogenic mechanisms. This study aimed to screen key pathogenic genes using machine learning algorithms and explore the correlation between key genes and immune microenvironment through transcriptome sequencing data sets of myocardial samples from patients with dilated cardiomyopathy, providing new ideas for elucidating the pathogenesis of the disease.

Methods: The transcriptome sequencing data sets of heart tissue from patients with dilated cardiomyopathy were downloaded from the Gene Expression Omnibus (GEO) database (GSE29819 and GSE21610). Differentially expressed genes (DEGs) were screened between pathological and normal tissues. The key genes were screened using least absolute shrinkage and selection operator (LASSO) regression analysis and random forest tree algorithms. The diagnostic efficiency of the key genes for the disease was evaluated using the receiver operating characteristic (ROC) curve.

Results: Compared with the normal heart tissue (control group) samples, there were 213 DEGs in the heart tissue samples of patients with dilated cardiomyopathy (treat group), including 101 upregulated and 102 downregulated genes. *CCL5* and *CTGF* were highly expressed in the treat group compared to the control group. The ROC curve showed that the areas under the curve (AUCs) of *CCL5* and *CTGF* were 0.821 and 0.902, respectively (P<0.05). In the treat group samples, *CCL5* was positively correlated with the infiltration content of most immune cell subtypes.

Conclusions: *CCL5* and *CTGF* are key disease-causing genes in dilated cardiomyopathy and have good diagnostic efficiency for the disease. *CCL5* and *CTGF* may be related to immune cell enrichment and myocardial fibrosis, respectively.

Keywords: Dilated cardiomyopathy; machine learning algorithms; immune microenvironment

Introduction

Dilated cardiomyopathy is a common heart disease that is mainly caused by cardiac injury and apoptosis and increased ventricular wall thickness (1). Its etiology is unclear, but it may be related to genetic and environmental factors (2,3). It is estimated that there are approximately 25 million new cases each year (4). The incidence of dilated cardiomyopathy increases with age (5). The pathological and physiological mechanisms of dilated cardiomyopathy mainly include 2 parts: one is cardiac injury and apoptosis, and the other is increased ventricular wall thickness. Dilated cardiomyopathy is irreversible and usually leads to heart failure (6). Research has pointed out that hypertensive cardiomyopathy and dilated cardiomyopathy are the most common causes of heart failure (7). One of the main therapeutic goals of dilated cardiomyopathy is to prevent the occurrence of heart failure (8). For dilated cardiomyopathy with end-stage heart failure, heart transplantation is currently the only feasible treatment method, and new treatment methods and pathophysiological mechanisms need to be further explored.

Dilated cardiomyopathy has complex genetic mutations. A variety of gene mutations and abnormal expressions may lead to the occurrence and progression of the disease. With the upgrading of programming languages and the improvement of biological databases, bioinformatics analysis has become an increasingly rich means of clinical disease research. Conducting analysis at the nucleic acid and protein levels to explore the structural and functional information of biological macromolecules provides us with more relevant pathway annotation information on the role of differential genes in disease progression. The *MYH7* and *TNNT2* genes are the most common gene mutations in dilated cardiomyopathy, accounting for 20–35% and 7–30% of dilated cardiomyopathy patients, respectively (9-12). In addition, many other genes (such as *TNNI3*, *LMNA*, *ACTC1*, etc.) may also cause dilated cardiomyopathy (10,13). Currently, the mechanisms of the occurrence and progression of dilated cardiomyopathy are still unclear and further exploration is needed.

This study aimed to further explore the differentially expressed genes (DEGs) and disease-related signaling pathways in dilated cardiomyopathy using bioinformatics analysis methods, using transcriptome sequencing data sets of myocardial samples from patients with dilated cardiomyopathy. Screen key pathogenic genes through machine learning algorithms and explore the correlation between key genes and immune microenvironment, in order to predict relevant genes that may play an important role in the progression of dilated cardiomyopathy at the molecular level. We present this article in accordance with the STREGA reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1086/rc).

Methods

Data download

The transcriptome sequencing data sets of heart tissues from patients with dilated cardiomyopathy (GSE29819 and GSE21610) were downloaded from the Gene Expression Omnibus (GEO) database. The corresponding platform file is GPL570. GSE29819 contains 26 samples of diseased heart tissues and 12 samples of normal heart tissues. The GSE21610 chip dataset includes 21 myocardial tissue samples of dilated cardiomyopathy with heart failure before and after ventricular assist device support treatment, and 8 samples of normal heart tissues. Our inclusion criteria include selecting myocardial tissue samples from all dilated cardiomyopathy patients who have only received standard anti heart failure medication treatment without special treatment or treatment, as well as samples from all normal cardiac function patients. The 2 data sets were merged into 1 data matrix, and batch correction was performed. The normal tissues were set as the Control group, and the diseased tissues were set as the treat group. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Highlight box

Key findings
- The purpose of this study is to screen key pathogenic genes and clarify the pathogenesis of the disease through the transcriptome sequencing data set of myocardial samples from patients with dilated cardiomyopathy.

What is known and what is new?
- At present, the mechanism of the occurrence and progression of Dilated cardiomyopathy is still unclear, and further exploration is still needed.
- This study found *CCL5* may be related to immune cell enrichment and *CTGF* may be related to myocardial fibrosis.

What is the implication, and what should change now?
- *CCL5* and *CTGF* are critical pathogenic genes in dilated cardiomyopathy, and they possess good diagnostic efficacy.

Differential gene expression

R software (R Foundation for statistical Computing, Vienna, Austria) and the limma package were used to screen for DEGs between the control and treat groups. The screening criteria were fold change (FC) >2 and false discovery rate (FDR) <0.05.

DEG enrichment analysis

The Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using the online database (https://david.ncifcrf.gov/) with background of *Homo sapiens*. The enriched functions and pathways of the DEGs were screened with a q-value <0.05 threshold.

Key gene screening

Key genes were screened using both least absolute shrinkage and selection operator (LASSO) regression analysis and random forest tree algorithms. LASSO regression analysis removes redundant gene information by constructing a penalty function. The lambda value and gene set that represented the overall features were determined when the binomial deviance value was minimum. In the random forest tree algorithm, the number of trees was determined when the cross-validation error was minimum and genes with a mean decrease Gini value greater than 2 were retained. The intersection of the gene sets obtained from the 2 algorithms was considered to contain the key genes.

Immune cell subtype infiltration content calculation

The immune cell subtype infiltration content in heart tissues from patients with dilated cardiomyopathy was calculated using single-sample gene set enrichment analysis (ssGSEA). Firstly, rank normalization is performed on the gene expression values of a given sample, and then the enrichment score is calculated using an empirical cumulative distribution function. The enrichment score generated by ssGSEA in a gene set is related to the phenotypic differences in the sample set in a dataset. In this way, ssGSEA converts the gene expression profile into a gene set enrichment profile. Through the gene set related to immune cell markers, we can calculate the immune cell infiltration score.

Diagnostic performance evaluation

The diagnostic performance was assessed by the receiver operating characteristic (ROC) curve. The area under the curve (AUC) represented the diagnostic performance.

Statistical analysis

This study used R software (V3.5.1) for statistical analysis. Group differences were compared, and correlation analysis was performed with a 2-tailed P<0.05 indicating statistical significance.

Results

DEG screening

Compared to normal heart tissue samples (control group), the heart tissue samples of dilated cardiomyopathy with congestive heart failure (treat group) showed differential expression of 213 genes, including 101 upregulated genes and 102 downregulated genes (*Figure 1*).

DEG enrichment analysis

The DAVID online database was used to perform enrichment analysis of the commonly DEGs obtained, using a background of *Homo sapiens*. Enrichment information was obtained for GO. The biological processes enriched by the DEGs included granulocyte chemotaxis, neutrophil chemotaxis, leukocyte chemotaxis, granulocyte migration, and myeloid leukocyte migration. In terms of cell composition, the DEGs were mainly enriched in collagen-containing extracellular matrix, tertiary granule, secretory granule lumen, cytoplasmic vesicle lumen, and vesicle lumen. In terms of molecular function, the DEGs were primarily associated with cytokine activity, receptor ligand activity, signaling receptor activator activity, glycosaminoglycan binding, and chemokine activity (*Figure 2*). In the KEGG signaling pathway enrichment analysis, the DEGs were enriched in cytokine-cytokine receptor interaction, the interleukin 17 (IL-17) signaling pathway, viral protein interaction with cytokine and cytokine receptor, the tumor necrosis factor (TNF) signaling pathway, FoxO signaling pathway, Hippo signaling pathway, chemokine signaling pathway, lipid and atherosclerosis, and Toll-like receptor signaling pathway (*Figure 3*).
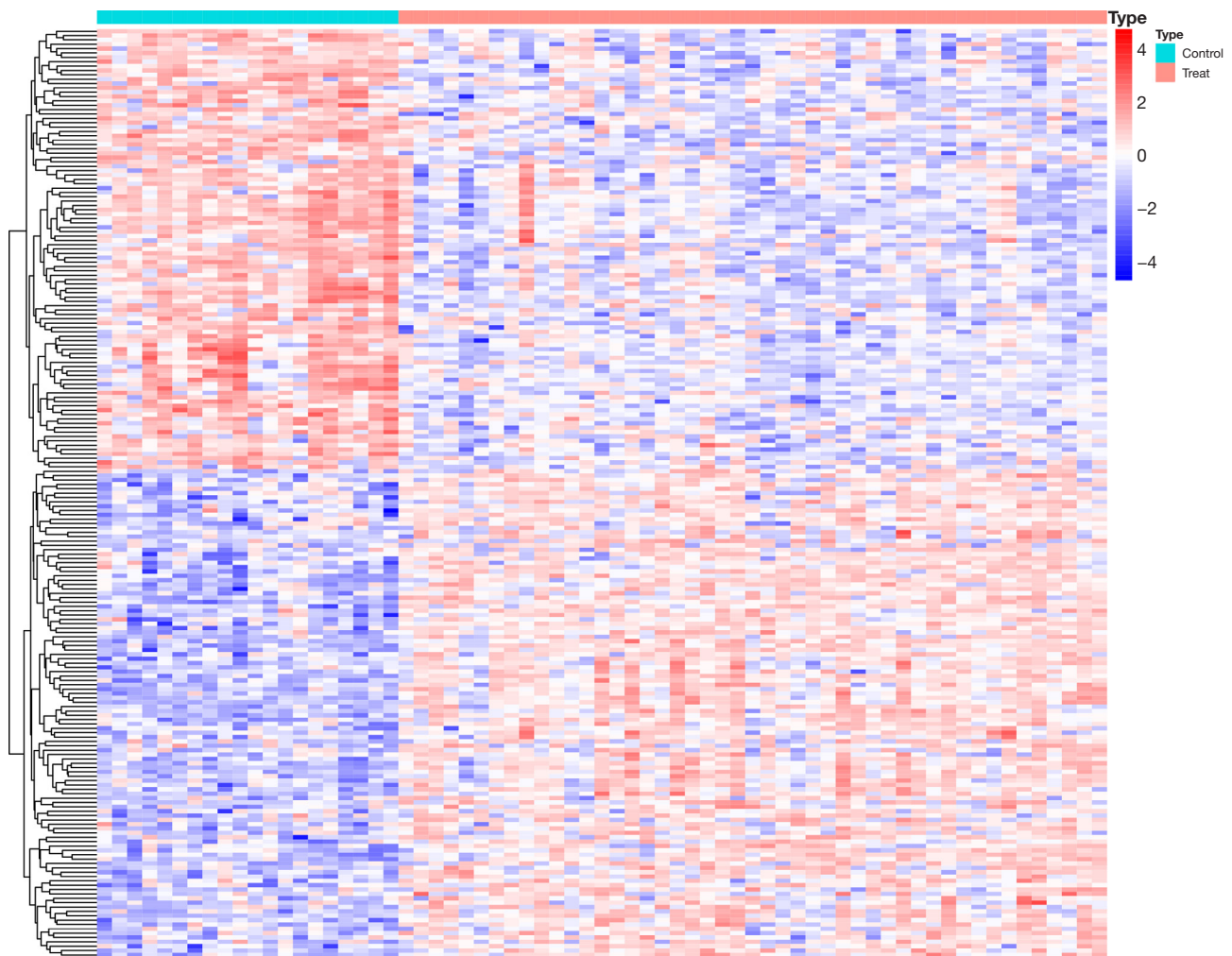
Figure 1 Heatmap of DEGs. The x-axis represents samples, and the y-axis represents genes. Red indicates upregulation, and blue indicates downregulation. DEGs, differentially expressed genes.

Identification of key genes

In this study, based on the messenger RNA (mRNA) levels of DEGs, the key genes were screened using LASSO regression analysis and random forest tree algorithm. The LASSO regression analysis showed that when lg(lambda) =−4.46 and the binomial deviance value was minimum, a total of 21 key genes were screened (*Figure 4*). The random forest tree algorithm showed that when the number of trees was 200, the cross-validation error was minimum (*Figure 5*). Under this condition, there were 9 genes with mean decrease Gini values greater than 2 (*Figure 6*). The gene sets obtained from the 2 algorithms had 2 overlapping genes, namely, *CCL5* and *CTGF*.

Assessment of diagnostic performance of key genes

The expression of *CCL5* and *CTGF* was shown to be upregulated in the treat group compared to the control group (*Figure 7A,7B*). The ROC curve showed that the AUCs for *CCL5* and *CTGF* were 0.821 and 0.902, respectively (*Figure 7C,7D*, P<0.05).

Differentiation in immune cell subtypes

Compared to the control group samples, activated CD8 T cells, effector memory CD4 T cells, central memory CD4 T cells, central memory CD8 T cells, and effector memory CD8 T cells were highly expressed in the Treat
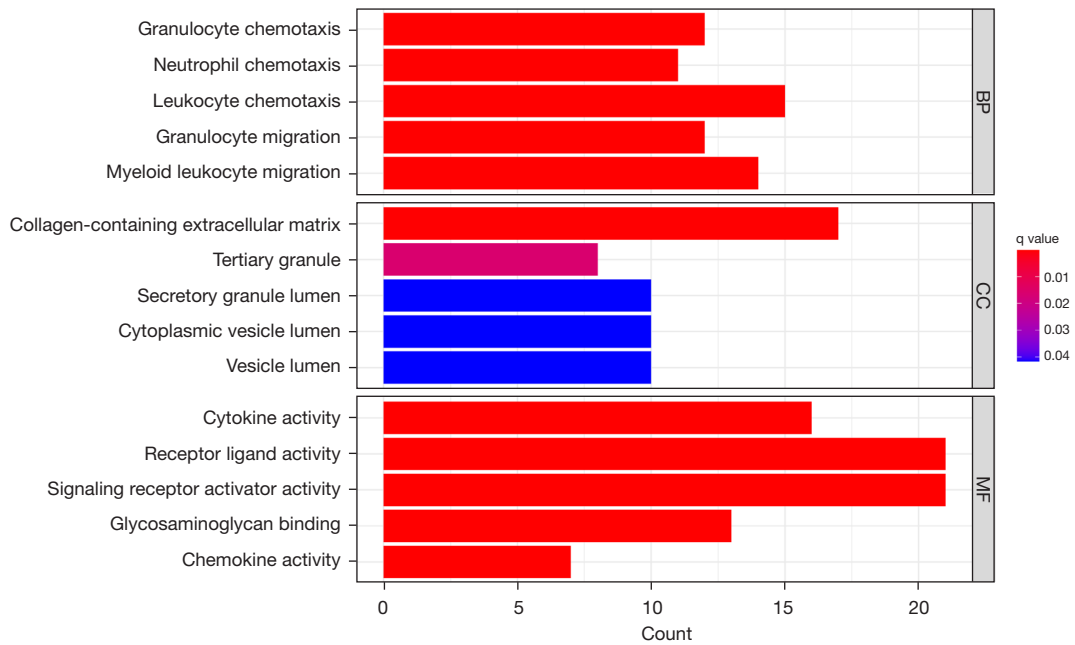
Figure 2 Gene Ontology enrichment analysis. BP, biological progress; CC, cellular component; MF, molecular function.
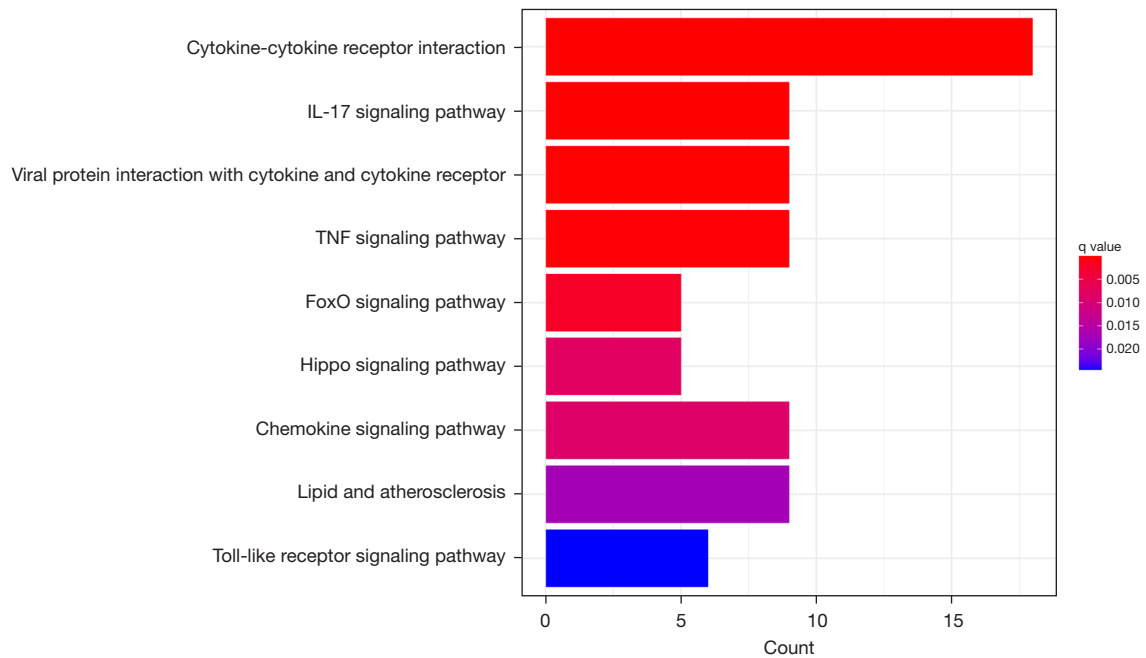

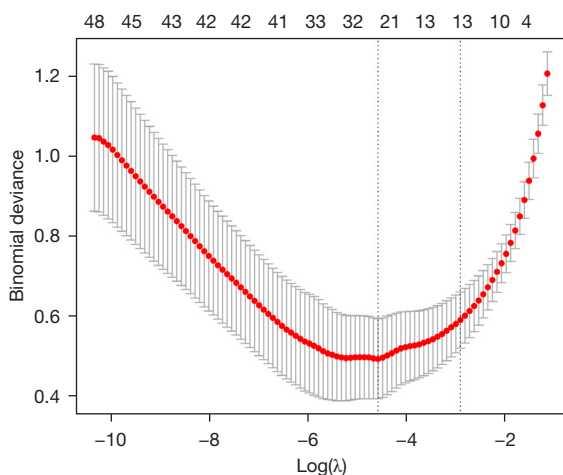**Figure 3** Kyoto Encyclopedia of Genes and Genomes enrichment analysis.

4450

Chen et al. Key genes of dilated cardiomyopathy

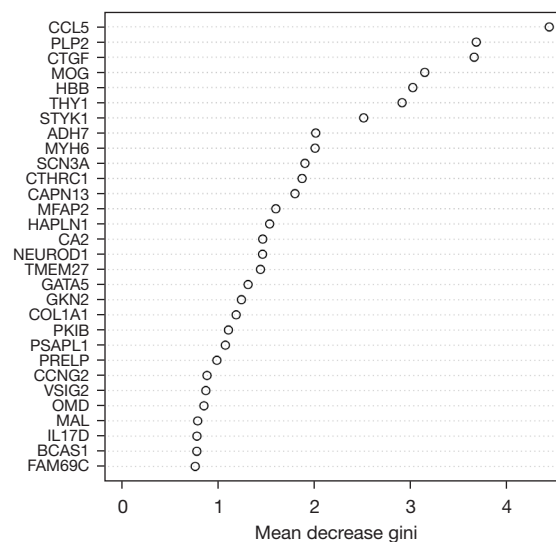**Figure 4** Key gene selection by LASSO regression analysis. LASSO, least absolute shrinkage and selection operator.
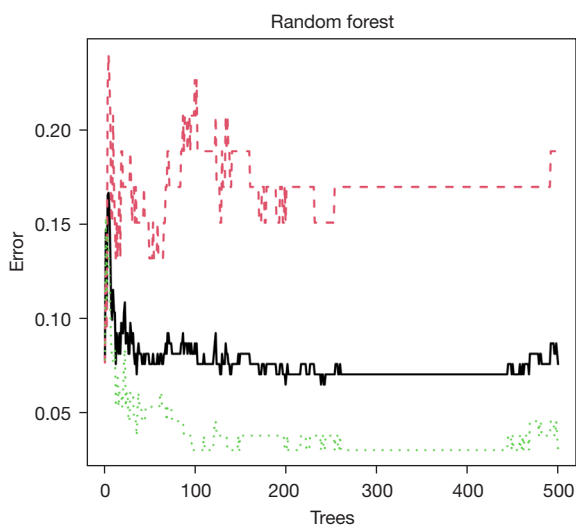

Figure 5 Key gene selection using random forest tree algorithm. The red line represents the control group samples, the green line represents the treat group samples, and the black line represents all samples.

group samples. Meanwhile, activated dendritic cells (DCs), CD56dim natural killer (NK) cells, eosinophils, gamma delta T cells, immature DCs, myeloid-derived suppressor cells (MDSCs), macrophages, mast cells, NK T cells, plasmacytoid DCs, and Regulatory T helper type 1 Cells were lowly expressed in the treat group samples compared to the control group samples (*Figure 8*).

Figure 6 Average decrease Gini value of the key genes selected by the random forest tree algorithm.

Correlation between key genes and immune cell subtypes

In the treat group samples, *CCL5* was positively correlated with the infiltration levels of many immune cell subtypes (*Figure 9*).

Discussion

Over the past several decades, using genomic analysis data from patients, researchers have discovered several genes that play a crucial role in heart disease, many of which regulate or participate in the regulation of the synchronous contractions of the heart muscle. These genes, by regulating the mechanism of heart contractions, affect the synchronous contractions of the heart muscle, ultimately leading to heart failure. Some gene expression abnormalities may also affect the development of heart muscle, mediate cardiac remodeling, and cause abnormal cardiac electrophysiology, leading to a decline in cardiac function. Key pathogenic genes can explain the specific pathological changes of the disease to some extent. Abnormal gene expression may lead to dilated cardiomyopathy and ultimately heart failure. Xu *et al.* (14) reported that genes such as *CYP2J2*, *ETNPPL*, and *FGF1* lead to the occurrence of dilated cardiomyopathy by affecting fatty acid metabolism. Zheng *et al.* (15) proposed that the IL-17 signaling pathway may be an important pathogenic mechanism for dilated cardiomyopathy. Li
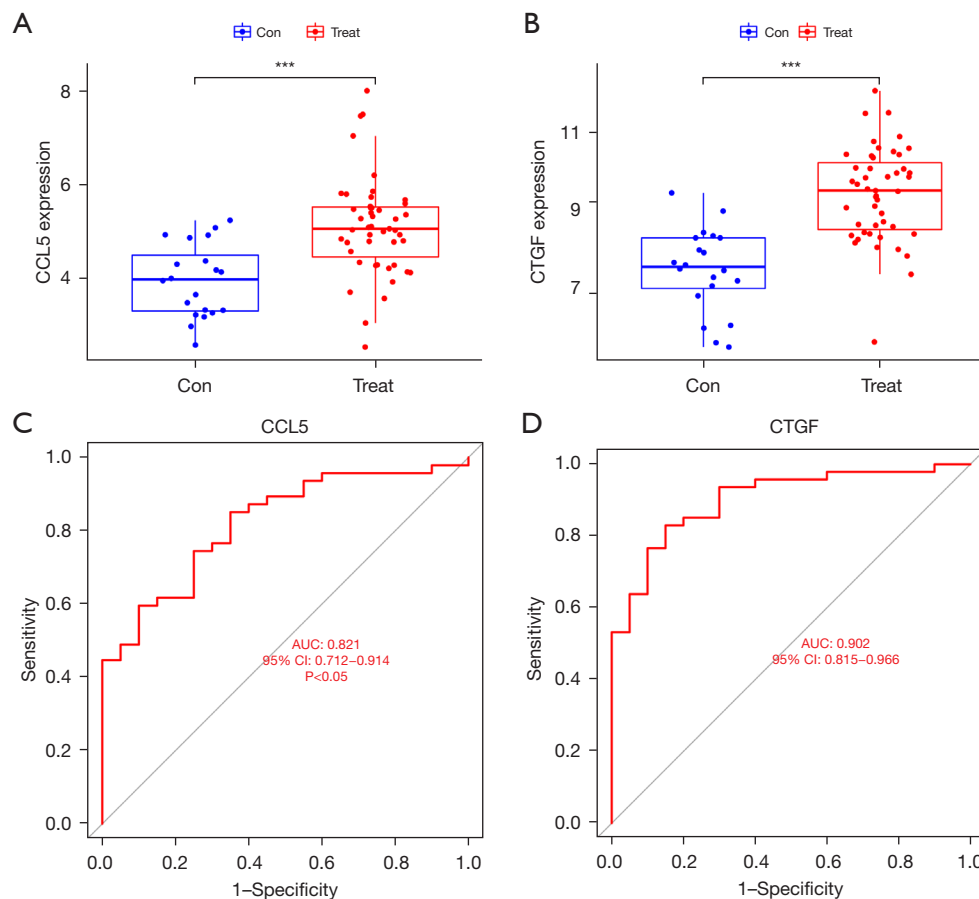
Figure 7 Diagnostic performance of key genes. (A) Comparison of *CCL5* expression levels between the control group samples and the treat group samples. (B) Comparison of *CTGF* expression levels in the control and the treat samples. (C) ROC curve for *CCL5*. (D) ROC curve of *CTGF*. ***, P<0.05. AUC, area under the curve; CI, confidence interval; ROC, receiver operating characteristic.

et al. (16) suggested that the downregulation of *FGF1* expression led to pathological cardiac remodeling. Commonly found key pathogenic genes for heart failure include mutated cardiac actin, anticoagulant plasminogen activator inhibitor-1, alkaline phosphatase A2, threonine kinase 2, and transcription factor 4 (17-19). These genes play crucial roles in the development and repair of heart muscle tissue, and their hereditary mutations may cause functional disorders of heart muscle cells and eventually lead to heart failure. Additionally, pathways associated with cardiac disturbance such as extracellular regulated kinase (ERK), janus kinase (JAK)- signal transducer and activator of transcription (STAT), phosphatidylinositol-3-kinase/ protein kinase B (PI3K/Akt), and P38-mitogen-activated protein kinase (MAPK) may also play an important role in the pathogenesis of heart failure (20-22). The abnormal activities of these pathways may increase cardiac cell death,

thus leading to heart failure. But we need to note that heart failure is an inevitable outcome of the progression of all cardiomyopathy, a description of the state of cardiac function, and not a strict pathological diagnosis. Dilated cardiomyopathy with concomitant heart failure belongs to the late stage of the disease. Comparing with normal myocardial samples can help identify key genes involved in disease occurrence and progression. Su *et al.* identified carbonic anhydrase 2 and 3 as risk biomarkers for dilated cardiomyopathy related heart failure in heart tissue samples of patients with concomitant heart failure and asymptomatic dilated cardiomyopathy (23).

In this study, the results of transcriptome sequencing of normal heart tissue and heart tissue with dilated cardiomyopathy were compared, and DEGs were obtained. These DEGs were significantly enriched in pathways related to inflammation response, activation of chemokines,
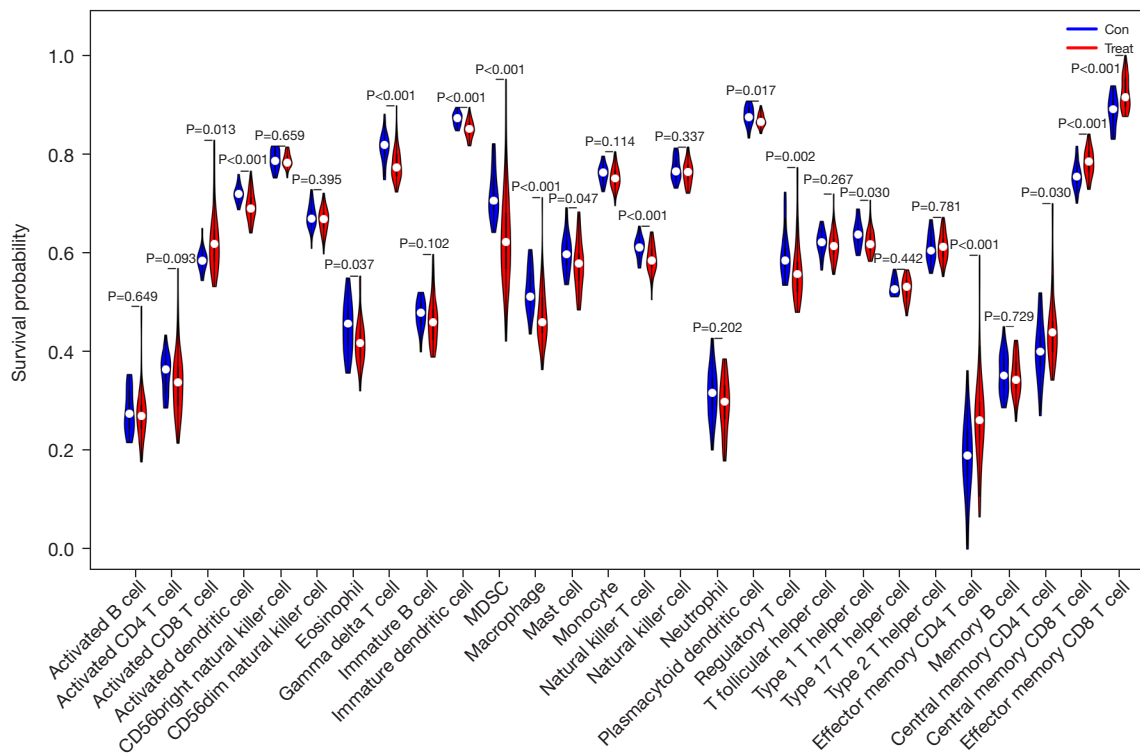
Figure 8 Comparison of the immune cell subtypes in the control and the treat samples. MDSC, myeloid-derived suppressor cell.

or migration of inflammatory cells. This indicates that inflammation is an important pathogenic mechanism in dilated cardiomyopathy. This is consistent with some previous results. Liang *et al.* (24) reported that dilated cardiomyopathy is related to the occurrence of immune response. In dilated cardiomyopathy, the transcription factor ETS1 regulates the expression of inflammation-related genes. Sun *et al.* (25) suggested that inhibiting the activation of inflammatory phagocytes could inhibit heart remodeling and improve dilated cardiomyopathy. Harding *et al.* (26) pointed out that although clinical trials of immune suppression therapy have not achieved the expected results in dilated cardiomyopathy, the evidence of abnormal immune response in dilated cardiomyopathy is sufficient. They also highlighted that in subsequent studies, further subtyping of immune cell subtypes is needed (26). Yang *et al.* suggested that macrophage infiltration may participate in myocardial remodeling and ultimately lead to dilated cardiomyopathy (27). In our study, the infiltration of immune cell subtypes in normal and diseased samples was displayed. We found that CD8+ T cells and CD4+ T cells were enriched in diseased tissues. This may provide reference for the clustering and subtyping of immune cell subtypes.

Through the use of LASSO regression analysis and the random forest tree algorithm, 2 key pathogenic genes, *CCL5* and *CTGF*, were identified. Both *CCL5* and *CTGF* possess good diagnostic efficacy for dilated cardiomyopathy. Our findings also revealed that *CCL5* is positively correlated with the infiltration of various immune cell subtypes, which may be one of the driving factors for immune cell enrichment. Lu *et al.* (28) found that *CCL5* is associated with the development of dilated cardiomyopathy. Zhu *et al.* (29) reported that *CCL2*, *CCL5*, and *TLR2* can serve as characteristic biological markers for dilated cardiomyopathy. *CTGF* has also been confirmed to be related to dilated cardiomyopathy in previous studies. Tan *et al.* (30) argued that downregulation of *CTGF* can inhibit myocardial fibrosis and improve dilated cardiomyopathy. Additionally, some studies have indicated that *CTGF* can act as a marker of myocardial fibrosis in dilated cardiomyopathy, with its serum level declining as the ejection fraction decreases (31).

In this study, through the RNA sequencing data sets of myocardial samples from patients with dilated cardiomyopathy, bioinformatics analysis was used to show the DEGs of dilated cardiomyopathy and disease-related signaling pathways. The key pathogenic genes were
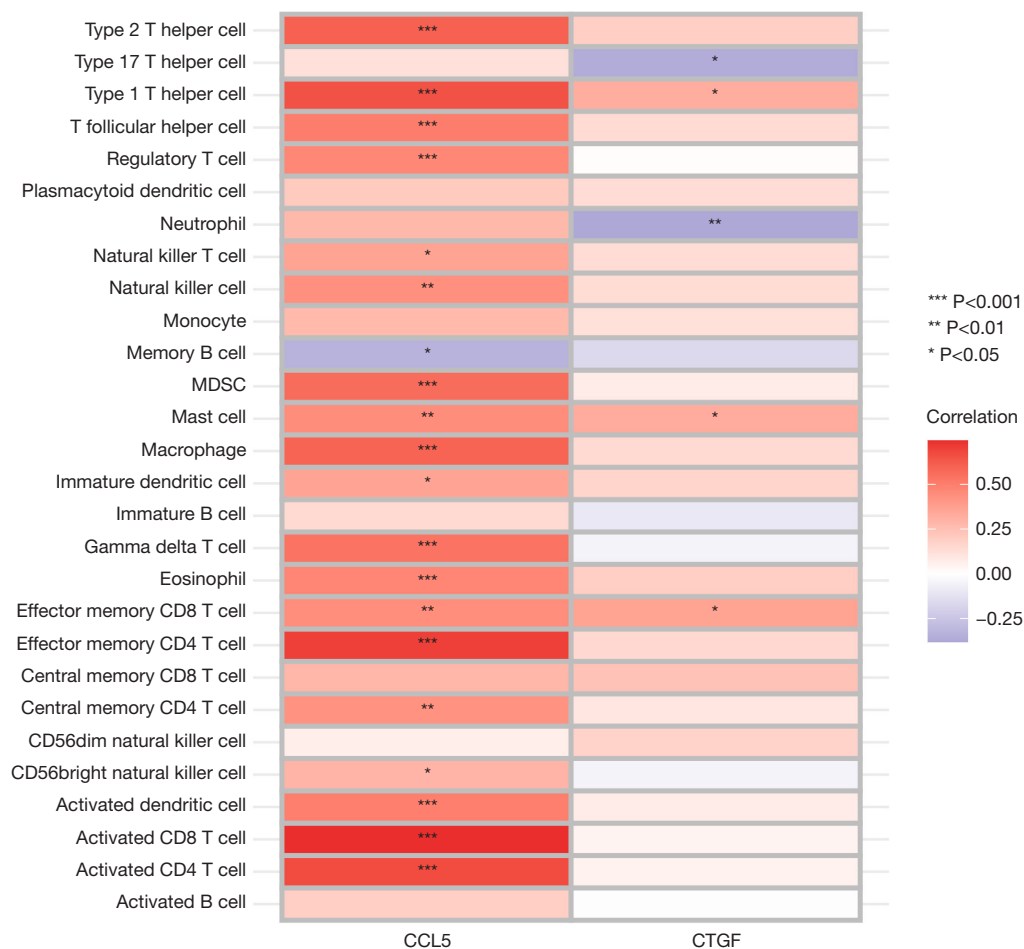
Figure 9 Correlation between key genes *CCL5* and *CTGF* and immune cell subtypes. MDSC, myeloid-derived suppressor cell.

screened by machine learning algorithm, the correlation between which and immune microenvironment was explored. However, there are still some shortcomings in this study. Firstly, this study lacks *in vivo* and *in vitro* experimental validation, and further experiments are needed to confirm the conclusions we have obtained; Secondly, this study only compared normal myocardial samples with dilated cardiomyopathy samples with concomitant heart failure, and did not set up a set of asymptomatic dilated cardiomyopathy samples as groups at different stages of disease development. Finally, there is insufficient depth in exploring gene function.

Conclusions

CCL5 and *CTGF* are critical pathogenic genes in dilated cardiomyopathy, and they possess good diagnostic efficacy.

CCL5 may be related to immune cell enrichment and *CTGF* may be related to myocardial fibrosis.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1086/rc

Peer Review File: Available at https://jtd.amegroups.com/

article/view/10.21037/jtd-23-1086/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups. com/article/view/10.21037/jtd-23-1086/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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