COMPARATIVE TRANSCRIPTOMIC SIGNATURES IN ISCHEMIC, IDIOPATHIC, AND NON-FAILING HUMAN MYOCARDIUM: INSIGHTS FROM GSE5406

Komparatívne transkriptomické signatúry pri ischemickom, idiopatickom a nezlyhávajúcom ľudskom myokarde: poznatky zo súboru GSE5406

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Abstract
Background. Heart failure (HF) remains a leading cause of morbidity and mortality worldwide. Ischemic and idiopathic cardiomyopathies share overlapping clinical manifestations but differ in underlying molecular remodeling. Comparative transcriptomic analysis may reveal distinct biological pathways contributing to these phenotypes.
Methods. Public microarray dataset **GSE5406** was analysed using the GEO2R/limma pipeline to compare left-ventricular tissue from *ischemic*, *idiopathic*, and *non-failing* human hearts. Differential expression was assessed by ANOVA; genes with an adjusted p-value < 0.01 were considered significant. Gene Ontology (GO) and KEGG enrichment analyses identified predominant biological processes.
Results. Out of 22 283 probes, **n ≈ 600** genes were significantly dysregulated. The top signals included *HMGN2*, *TRMT5*, *MYOT*, *ASPN*, *LUM*, *HTRA1*, *SERPINA3*, *HMOX2*, and *IL1RL1*, implicating extracellular-matrix (ECM) remodeling, oxidative stress, inflammatory activation, and cytoskeletal disorganization. Enrichment analysis revealed pathways related to ECM–receptor interaction, TGF-β and cytokine signalling, oxidative phosphorylation, and metabolic adaptation.
Conclusions. Transcriptomic heterogeneity between ischemic and idiopathic failing myocardium reveals converging fibrotic and inflammatory mechanisms, alongside partially distinct metabolic signatures. These findings underscore the molecular complexity of heart failure and may guide the development of aetiology-tailored therapies and diagnostic biomarkers (Fig. 3, Ref. 15). *Text in PDF www.lekarskyobzor.sk.*
KEY WORDS: heart failure, ischemic cardiomyopathy, idiopathic dilated cardiomyopathy, transcriptomics, extracellular matrix remodeling.
Lek Obz 2026, 75 (1): 17-22

Abstrakt
Pozadie problému. Srdcové zlyhávanie (HF) zostáva jednou z hlavných príčin chorobnosti a úmrtnosti na celom svete. Ischemická a idiopatická kardiomyopatia majú podobné klinické prejavy, líšia sa však v molekulovej prestavbe. Komparatívna transkriptomická analýza môže odhaliť rozdielne biologické dráhy, ktoré prispievajú k týmto fenotypom.
Metódy. Verejne dostupný mikroarray dataset GSE5406 bol analyzovaný pomocou pipeline GEO2R/limma na porovnanie tkaniva ľavej komory z ischemických, idiopatických a nepostihnutých (nezlyhávajúcich) myokardov. Diferenciálna expresia bola hodnotená pomocou ANOVA; gény s upravenou hodnotou p < 0,01 boli považované za signifikantné. Analýzy obohatenia Gene Ontology (GO) a KEGG identifikovali dominantné biologické procesy.
Výsledky. Z 22 283 sond bolo približne 600 génov významne deregulovaných. Najvýraznejšie signály zahŕňali *HMGN2, TRMT5, MYOT, ASPN, LUM, HTRA1, SERPINA3, HMOX2* a *IL1RL1*, čo poukazuje na remodeláciu extracelulárneho matrixu (ECM), oxidačný stres, zápalovú aktiváciu a dezorganizáciu cytoskeletu. Analýza obohatenia odhalila dráhy súvisiace s interakciou ECM-receptor, signalizáciou TGF-β a cytokínov, oxidačnou fosforyláciou a metabolickou adaptáciou.
Záver. Transkriptomová heterogenita medzi ischemickým a idiopatickým zlyhávajúcim myokardom odhaľuje zbiehajúce fibrotické a zápalové mechanizmy, popri čiastočne odlišných metabolických vplyvoch. Tieto zistenia zdôrazňujú molekulovú komplexnosť srdcového zlyhávania a môžu napomôcť vývoju etiologicky cielených terapií a diagnostických biomarkerov (obr. 3, lit. 15). *Text v PDF www.lekarskyobzor.sk.*
KĽÚČOVÉ SLOVÁ: srdcové zlyhávanie, ischemická kardiomyopatia, idiopatická dilatačná kardiomyopatia, transkriptomika, remodelácia extracelulárneho matrixu.
Lek Obz 2026, 75 (1): 17-22

Introduction

Heart failure represents a final common phenotype of diverse myocardial injuries, yet its molecular underpinnings vary with etiology, shaping remodeling trajectories and clinical outcomes. Among the leading causes of advanced HF, ischemic cardiomyopathy (ICM) and

idiopathic dilated cardiomyopathy (IDCM) exemplify this tension between convergence and heterogeneity: both culminate in systolic dysfunction, and adverse ECM turnover, but they arise from distinct initiating mechanisms, chronic ischemia and scar formation in ICM versus primary myocardial disease in IDCM, often driven by genetic, cytoskeletal, or inflammatory abnormalities (1, 2). Understanding how these etiologies differ, and where they converge, at the transcriptional level is essential for refining disease taxonomy and identifying biologically grounded targets for diagnosis and therapy.

Public myocardial expression datasets now allow such comparisons to be performed directly in human tissue without additional sampling. The GEO dataset GSE5406 provides a well-characterized collection of left-ventricular samples from patients with advanced ICM and IDCM undergoing transplantation, alongside unused donor hearts serving as non-failing controls, all processed using Robust Multi-array Average (RMA) normalization to ensure cross-sample comparability. This dataset has become a cornerstone for transcriptomic exploration of cardiomyopathy, enabling investigators to interrogate gene-expression networks underlying myocardial remodeling across etiologies.

Recent systems-level and integrative multi-omics analyses have revealed that cardiomyopathies share a core remodeling signature dominated by fibro-inflammatory activation, ECM reorganization, mitochondrial dysfunction, and metabolic reprogramming, yet each etiology retains specific molecular axes reflecting its initiating insult—heightened immune and oxidative-stress signaling in ICM, and dysregulation of cytoskeletal and cell-adhesion machinery in IDCM (2, 3). These findings suggest that, while pathogenesis diverges upstream, the failing myocardium ultimately converges on a limited repertoire of maladaptive pathways – sometimes described as the "final common pathway" of HF progression (4).

Extracellular matrix expansion and collagen remodeling are pivotal in this process, driven by activated fibroblasts, altered matrix metalloproteinase (MMP) activity, and changes in matricellular proteins, all consistently observed at transcript, protein, and histologic levels in failing human myocardium (5). Simultaneously, inflammatory cascades, particularly those mediated by interleukin-33/ST2 and tumor necrosis factor pathways, intertwine with oxidative stress and neurohormonal activation to accelerate structural deterioration, linking molecular remodeling with clinically measurable biomarkers such as soluble ST2 and natriuretic peptides (5, 6).

Against this biological and clinical background, the present study re-examines the GSE5406 dataset using a rigorous multi-group analytical framework encompassing ICM, IDCM, and non-failing myocardium. The objective is to delineate both the shared transcriptional programs that define the common end-stage heart-failure phenotype and the etiology-specific differences that distinguish ischemic from idiopathic remodeling trajectories. By integrating these convergent and divergent molecular patterns, we aim to provide a more comprehensive understanding of myocardial remodeling and to establish a transcriptomic context for translating gene-level findings into clinically interpretable pathways with potential diagnostic and therapeutic implications.

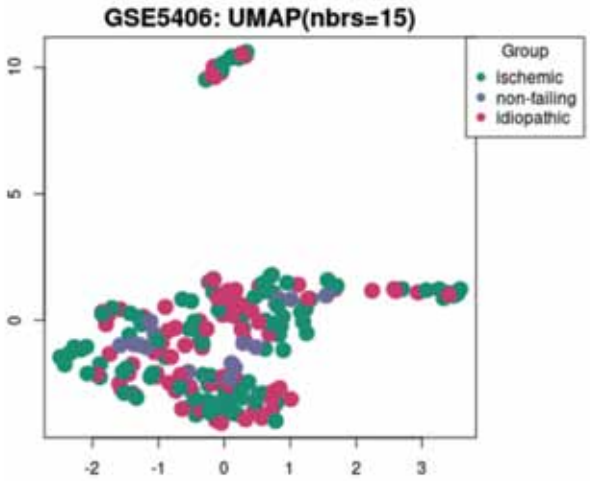
Methods

This study was conducted as an in silico comparative transcriptomic analysis based on publicly available human myocardial microarray data. Gene-expression profiles were obtained from the GEO dataset GSE5406, which includes left-ventricular tissue from patients with ICM and IDCM who underwent heart transplantation, as well as non-failing donor hearts serving as controls. In total, the dataset comprises 210 samples (108 IDCM, 86 ICM, and 16 non-failing) analysed on the Affymetrix Human Genome U133A Array platform. All data were de-identified and accessible through public repositories, therefore ethical approval was not required. Raw CEL files were retrieved and pre-processed using the RMA algorithm within the affy package in R (version 4.3.1), which applies background correction, quantile normalization, and $\log_2$ transformation, followed by summarization of probe-level intensities to gene-level expression values. The resulting distributions were examined visually by boxplot to verify uniformity and to exclude batch-related bias. Data quality and inter-sample comparability were assessed through boxplots and principal-component analysis prior to statistical testing. Differential gene expression across the three diagnostic groups was evaluated using the Linear Models for Microarray Data (limma) framework implemented in the GEO2R analytical environment (Bioconductor 3.17). A one-way analysis of variance (ANOVA) model was fitted to identify transcripts differing among the three conditions, and empirical Bayes moderation was applied to stabilize variance estimates. P-values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) procedure, and genes with an adjusted p-value below 0.01 were considered significantly deregulated. The magnitude of expression difference was summarized by the F-statistic, and genes were ranked accordingly. Functionally significant transcripts were annotated using gene symbols and literature-supported roles in extracellular matrix remodeling, inflammation, metabolism, and cytoskeletal regulation. Enriched terms were subsequently interpreted within the context of heart-failure biology, emphasizing extracellular-matrix remodeling, inflammation, metabolic adaptation, and contractile regulation. Differential-expression results were summarized as volcano plots, which display the distribution of $\log_2$ fold change against statistical significance, and as a Venn diagram highlighting overlap of significant transcripts among group comparisons. These visualizations reflect the normalized, quality-controlled data and constitute the basis for all subsequent interpretation.

Results

Global transcriptomic analysis revealed distinct yet partially overlapping gene expression profiles across non-failing, IDCM, and ICM samples. Dimensionality reduction using Uniform Manifold Approximation and Projection (UMAP) demonstrated that non-failing myocardium segregated from both cardiomyopathy groups, whereas ICM and IDCM samples exhibited substantial transcriptomic overlap, suggesting convergence of gene expression programs in advanced heart failure irrespective of etiology (Fig. 1). This was further supported by differential expression statistics: while numerous transcripts were significantly deregulated in both failing-vs-control comparisons, no genes passed the significance threshold between ICM and IDCM alone, indicating a shared end-stage phenotype.

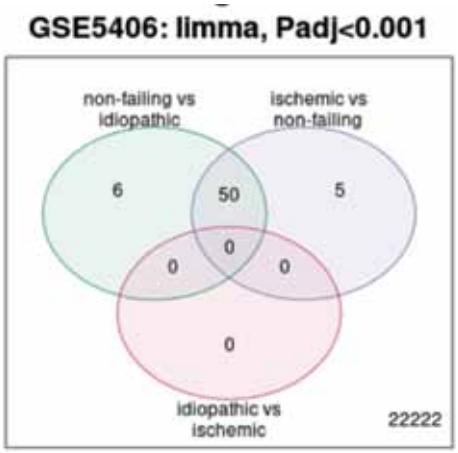
Figure 1. Uniform Manifold Approximation and Projection plot of myocardial transcriptomes, from the GSE5406 dataset, showing partial separation between non-failing controls and failing hearts (ICM and IDCM)**, with substantial transcriptomic overlap between the two cardiomyopathy groups.** X axis demonstrates UMAP dimention 1: first principal structure in the transcriptomic similarity, Y axis represents: UMAP dimention 2: second most relevant dimension for sample separation, Nbrs: number of nearest neighbors, UMAP: Uniform Manifold Approximation and Projection.



Using the limma framework with empirical Bayes moderation, a total of 61 genes were identified as significantly deregulated (adjusted $p < 0.001$) across at least one contrast. Fifty of these transcripts were common to both non-failing vs IDCM and non-failing vs ICM comparisons, reflecting a core remodeling program, whereas six were unique to the IDCM comparison and five to the ICM comparison (Fig. 2). No genes met significance in the direct comparison between IDCM and ICM, reinforcing the hypothesis of downstream transcriptional convergence despite divergent etiologies.

Volcano and mean-difference plots provided further insight into the scale and direction of gene expression changes (Fig. 3). In both failing-vs-control contrasts, clear separation of upregulated (red) and downregulated (blue) genes was observed, with fold changes exceeding ±2 and adjusted p-values well below 0.001, underscoring the robustness of these differences (Fig. 1). In contrast, the IDCM vs ICM comparison yielded a dense cloud centered around the null, with no genes surpassing the significance threshold, consistent with minimal residual etiology-specific signal at the transcriptome level in explanted hearts.

Figure 2. Venn diagram of differentially expressed genes across pairwise comparisons. Overlapping regions represent shared significant transcripts. *Linear Models for Microarray Data*, Padj: adjusted *p* value.



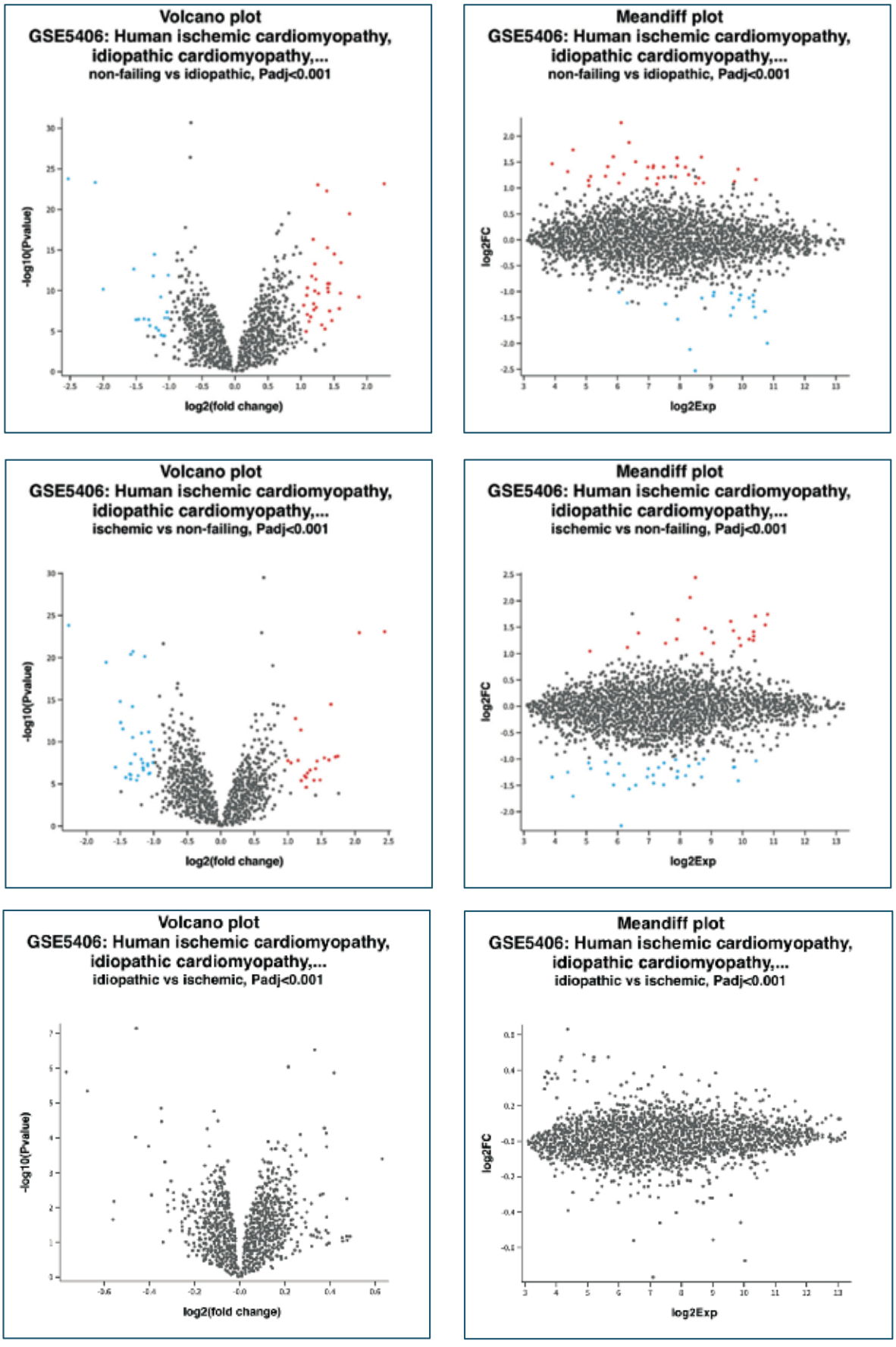
Examination of the most highly deregulated transcripts revealed biologically coherent patterns. Upregulated genes prominently included those involved in extracellular matrix remodeling, such as asporin (ASPN) and lumican (LUM), both members of the small leucine-rich proteoglycan family known to drive fibrosis and collagen cross-linking in heart failure. Other overexpressed transcripts included HTRA1, a serine protease implicated in TGF-β regulation and matrix degradation, and IL1RL1 (also known as ST2), a clinical biomarker of myocardial stress and inflammation. These findings point to a dominant fibro-inflammatory transcriptional signature shared across both ICM and IDCM.

Downregulated transcripts provided complementary insight into the failing myocardial phenotype. MYOT (myotilin), a Z-disc-associated protein essential for sarcomeric structure, was among the most suppressed genes, suggesting disruption of contractile integrity. CNN1 (calponin 1), a smooth muscle marker and regulator of actin–myosin interactions, was similarly downregulated, alongside members of the tubulin family (TUBA3D/C), highlighting cytoskeletal dysregulation. Additional downregulated transcripts included HMOX2 (heme oxygenase 2), a modulator of oxidative stress responses, and HMGN2, a chromatin-binding protein involved in nuclear architecture, indicating perturbation of redox balance and nuclear function.

Discussion

This transcriptomic reanalysis of the GSE5406 dataset reinforces the concept that end-stage heart failure, regardless of initiating etiology, converges on a shared molecular phenotype marked by fibro-inflammatory re-

Figure 3. Summary of differential gene expression analysis. Each panel displays either a volcano plot (left column) or mean-difference plot (right column) generated from the GSE5406 dataset. Red dots represent significantly upregulated genes and blue dots indicate significantly downregulated genes (adjusted p < 0.001). Exp: Gene Expression Level (log_2-transformed), FC: Fold Change, Padj: Adjusted *p*-value (Benjamini–Hochberg correction).



modeling, ECM expansion, and cytoskeletal disarray. While ICM and IDCM originate from fundamentally different pathogenic processes – chronic ischemia and infarct scarring in the former, versus primary myocardial dysfunction in the latter—our results indicate that the downstream transcriptional programs largely overlap at the point of myocardial decompensation.

The absence of significantly deregulated genes between ICM and IDCM samples is consistent with findings from single-nucleus RNA-sequencing studies, which have shown that transcriptional profiles in end-stage ICM closely mirror those of dilated and hypertrophic cardiomyopathies. Simonson et al. demonstrated that although cell-type proportions and signaling pathways may differ subtly, the final transcriptional landscape of failing myocardium converges across etiologies, with shared upregulation of ECM, immune signaling, and endothelial activation pathways (7).

Notably, many of the top deregulated genes in our analysis, ASPN, LUM, HTRA1, and IL1RL1 (ST2), have been independently validated as part of the fibrotic gene expression signature in both ICM and IDCM. Asporin and lumican, members of the small leucine-rich proteoglycan family, have been previously associated with adverse myocardial remodeling and fibrosis, particularly in the setting of pressure or volume overload (8). Similarly, ST2 signaling, particularly via IL1RL1, is a well-established biomarker of adverse prognosis in HF and is upregulated in both ischemic and non-ischemic etiologies (9 – 11).

In contrast, the observed downregulation of contractile proteins such as MYOT and CNN1 aligns with prior findings from experimental and human studies of sarcomeric disintegration in dilated cardiomyopathy. Genetic studies have shown that mutations in sarcomeric components, including ACTC (alpha-cardiac actin) and tropomyosin, can lead to either hypertrophic or dilated cardiomyopathy depending on the nature of the mutation, further underlining the importance of contractile dysfunction in disease pathogenesis (12, 13).

Although we anticipated greater transcriptional divergence between ICM and IDCM based on their distinct etiologies, the convergence we observed is in line with clinical data showing overlapping hemodynamic and structural features in end-stage disease. Histopathologic comparisons have consistently demonstrated that replacement fibrosis is more prominent in ICM, whereas IDCM tends to show diffuse interstitial fibrosis and hypertrophy, yet both ultimately result in similar clinical phenotypes and treatment pathways (14).

Importantly, while the transcriptomic overlap limits the utility of gene expression profiling for distinguishing etiology at late stages, it reinforces the concept of targeting shared maladaptive pathways in therapeutic development. For instance, fibrosis- and inflammation-related genes like HTRA1 and IL1RL1 represent promising candidates for pharmacologic intervention across etiologies.

Finally, our findings align with recent integrated analyses showing that BAG3 and other cytoskeletal regulators are downregulated in both familial and non-familial forms of dilated cardiomyopathy, supporting the role of mechanical stress sensing as a final common pathway in myocardial failure (15).

Our results reinforce the idea that transcriptional remodeling in advanced heart failure converges across ischemic and idiopathic etiologies, highlighting a dominant fibro-inflammatory and cytoskeletal disruption program. While this convergence limits the diagnostic value of transcriptomics at late stages, it offers a unifying framework for identifying shared therapeutic targets applicable across etiologies.

Conclusion

Our results show that, despite differing etiologies, both forms of cardiomyopathy share a largely overlapping pattern of gene regulation at the end stage of heart failure. Key pathways involved in extracellular matrix remodeling, inflammation, and contractile function were commonly affected, with consistent upregulation of genes such as ASPN, LUM, and IL1RL1, and downregulation of structural components like MYOT and CNN1. While no significant differences were observed between ICM and IDCM at the transcript level, these shared molecular features provide insight into common mechanisms of myocardial remodeling. These findings contribute to a growing body of evidence supporting the use of shared biological pathways as potential therapeutic targets in advanced heart failure.*

*__Compliance with Ethics Requirements:__ Authors declare no conflict of interest regarding this article. The authors declare, that all the procedures and experiments of this research respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008 (5), as well as the national law.

__Conflict of interest:__ The authors declare no conflict of interest.

References

1. SWEET ME, COCCIOLO A, SLAVOV D, JONES KL, SWEET JR, GRAW SL, REECE TB, AMBARDEKAR AV, BRISTOW MR, MESTRONI L, et al. Transcriptome analysis of human heart failure reveals dysregulated cell adhesion in dilated cardiomyopathy and activated immune pathways in ischemic heart failure. BMC Genomics 2018, 19: 812. DOI: 10.1186/s12864-018-5213-9.
2. FRANGOGIANNIS NG. The Extracellular Matrix in Ischemic and Nonischemic Heart Failure. Circ Res 2019, 125: 117 – 146. DOI: 10.1161/circresaha.119.311148.
3. RAMIREZ FLORES RO, LANZER JD, HOLLAND CH, LEUSCHNER F, MOST P, SCHULTZ JH, LEVINSON RT, SAEZ-RODRIGUEZ J. Consensus Transcriptional Landscape of Human End-Stage Heart Failure. Journal of the American Heart Association 2021, 10: e019667. DOI: 10.1161/JAHA.120.019667.
4. KUNER R, BARTH AS, RUSCHHAUPT M, BUNESS A, ZWERMANN L, KREUZER E, STEINBECK G, POUSTKA A, SÜLTMANN H, NABAUER M. Genomic analysis reveals poor separation of human cardiomyopathies of ischemic and nonischemic etiologies. Physiological Genomics 2008, 34: 88 – 94. DOI: 10.1152/physiolgenomics.00299.2007.

5. CASELLI C, D'AMICO A, RAGUSA R, CARUSO R, PRESCIMONE T, CABIATI M, NONINI S, MARRACCINI P, DEL RY S, TRIVELLA MG, et al. IL-33/ST2 pathway and classical cytokines in end-stage heart failure patients submitted to left ventricular assist device support: a paradoxic role for inflammatory mediators? Mediators Inflamm 2013, 2013: 498703. DOI: 10.1155/2013/498703.
6. TOMASONI D, ITALIA L, ADAMO M, INCIARDI RM, LOMBARDI CM, SOLOMON SD, METRA M. COVID-19 and heart failure: from infection to inflammation and angiotensin II stimulation. Searching for evidence from a new disease. European Journal of Heart Failure 2020, 22: 957 – 966. DOI: https://doi.org/10.1002/ejhf.1871.
7. SIMONSON B, CHAFFIN M, HILL M, ATWA O, GUEDIRA Y, BHASIN H, HALL A, HAYAT S, BAUMGART S, BEDI K, et al. Single-nucleus RNA sequencing in ischemic cardiomyopathy reveals common transcriptional profile underlying end-stage heart failure. Cell reports 2023, 42: 112086 – 112086. DOI: 10.1016/j.celrep.2023.112086.
8. YANG G, CHEN S, LU J, WANG T. Identification of the difference in the pathogenesis in heart failure arising from different etiologies using a microarray dataset. Clinics 2017, 72: 600 – 608. DOI: 10.6061/clinics/2017(10)03.
9. MILLER W, HARTMAN K, BURRITT M, BURNETT J, JAFFE A. Troponin, B-type natriuretic peptides and outcomes in severe heart failure: Differences between ischemic and dilated cardiomyopathies. Clinical Cardiology 2007, 30. DOI: 10.1002/clc.20075.
10. LJUNGMAN C, JAMIALAHMADI O, BOLLANO E, ANDERSON B, ROMEO S, BERGH N. Sex differences in RNA transcripts in dilated cardiomyopathy. European Heart Journal 2024. DOI: 10.1093/eurheartj/ehae666.764.
11. DEHGHANI K, STANEK A, BAGHERABADI A, ATASHI F, BEYGI M, HOOSHMAND A, HAMEDI P, FARHANG M, BAGHERI S, ZOLGHADRI S. CCND1 Overexpression in Idiopathic Dilated Cardiomyopathy: A Promising Biomarker? Genes 2023, 14. DOI: 10.3390/genes14061243.
12. CAO L, RUSHAKOFF J, ZHANG Z, WILLIAMSON I, KARLSTAEDT A, KITTLESON M, CZER L, KRANSDORF E. Abstract 4136581: Shared Genetic Burden of Ischemic and Non-Ischemic Dilated Cardiomyopathy. Circulation 2024. DOI: 10.1161/circ.150.suppl_1.4136581.
13. BIWOTT F, RAO N, DONG C, WANG G. Weighted gene co-expression network analysis reveals similarities and differences of molecular features between dilated and ischemic cardiomyopathies. Journal of Electronic Science and Technology 2023. DOI: 10.1016/j.jnlest.2023.100193.
14. NIKULINA S, KUZNECOVA O, CHERNOVA A. Results of the association study of the type 3 matrix metalloproteinase gene – 1171 5A/6A (rs35068180) and dilated cardiomyopathy. European Heart Journal, Acute Cardiovascular Care 2021. DOI: 10.1093/ehjacc/zuab020.010.
15. WANG Y, XIE Y, MAHARA G, XIONG Y, XIONG Y, ZHENG Q, CHEN J, ZHANG W, ZHOU H, LI Q. Intestinal microbiota and metabolome perturbations in ischemic and idiopathic dilated cardiomyopathy. Journal of Translational Medicine 2024, 22. DOI: 10.1186/s12967-023-04605-6.

Accepted for publication October 27, 2025.

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