



Published in final edited form as:

Nurs Res. 2023 ; 72(3): 175–184. doi:10.1097/NNR.0000000000000648.

Differential Gene Expression Among Patients With Heart Failure Experiencing Pain

Asa B. Smith¹, Miyeon Jung¹, Susan J. Pressler¹, Evelina Mocci², Susan G. Dorsey²

¹Indiana University School of Nursing, Indianapolis, IN

²University of Maryland School of Nursing, Baltimore, MD

Abstract

Background: Chronic pain is frequently experienced by patients with heart failure (HF) and is associated with higher mortality, higher symptom burden, and worsened health-related quality of life. However, the genomic mechanisms underlying chronic pain in HF are understudied. Building an understanding of the mechanistic underpinnings of pain may inform novel interventions.

Objective: The objective was to identify genes associated with pain from mRNA sequence data collected from patients with HF with and without pain.

Methods: The current study analyzed data from 40 patients with HF previously enrolled in a clinical trial. Pain presence was measured using the Health Utilities Index Mark-3. Genes were tested for differential expression using DESeq2, and differentially expressed genes were analyzed for protein–protein interaction (PPI) and relevant ontological pathways using Metascape. Genes located within the core of the PPI network were considered key in disease-relevant biological pathways. Differentially expressed genes within this PPI network were reviewed in existing literature to narrow down candidate genes of interest. These target genes of interest were reanalyzed in a second sample of 24 patients with HF using validation quantitative polymerase chain reaction.

Results: A total of 334 genes (279 upregulated, 55 downregulated) were differentially expressed between patients with and without pain in the primary sample of 40. These genes were largely aligned with neutrophil degranulation pathways. Seven genes of interest were identified from a core network of 15 co-expressed genes in the PPI network and existing literature. Three of these seven genes: matrix metalloproteinase 8 (*MMP8*), proprotein convertase subtilisin/kexin type 9 (*PCSK9*), and neutrophil defensin 3 (*DEFA3*) were upregulated in patients with pain versus without pain in both the primary and validation samples. All seven genes of interest are involved in immune, inflammatory, and atherosclerotic processes.

Corresponding author: Asa Smith, School of Nursing, Indiana University, 600 Barnhill Drive Suite E421, Indianapolis, IN 46202. asasmit@iu.edu.

The authors would like to thank the lab of Gerald Wilson, PhD, Professor, University of Maryland for assisting with the validation qPCR analysis; Bruno Giordani PhD, Professor, University of Michigan; Marita Titler PhD, RN, Professor, University of Michigan; Irmina Gradus-Pizlo MD, Associate Professor, Krannert Institute; Dean Smith, PhD, Professor, Louisiana State University; Sujuan Gao, PhD, Professor, University of Indiana; and Heather Burney, staff member, University of Indiana for their involvement in the parent study.

The authors have no conflicts of interest to report.

This study was conducted as part of a parent clinical trial registered on [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT 03035565).

Discussion: These results identify potential genes that may play a mechanistic role in chronic pain in HF. Further research is needed to evaluate these potential genes among clearly delineated pain phenotypes.

Keywords

genetics; heart failure; pain; protein-protein interaction

Heart failure (HF) is a debilitating chronic illness that affects 6 million patients in the United States (Tsao et al., 2022), and 64.3 million worldwide (Lippi & Sanchis-Gomar, 2020). Patients with HF often experience multiple co-occurring symptoms that increase symptom burden (Tsao et al., 2022). Chronic pain is frequent among patients with HF (Alemzadeh-Ansari et al., 2017), but it is poorly characterized and infrequently mentioned in national practice guidelines (Riegel et al., 2017; Yancy et al., 2017). In a literature review conducted to evaluate chronic pain in HF, pain prevalence was 23% to 85% across 65 studies with a cumulative total of 4,692 patients (Alemzadeh-Ansari et al., 2017). In the review, chronic pain in HF was associated with numerous mechanisms, including inflammation, edema, impaired circulation, visceral ischemia, sensitization of peripheral and central neurons (neuropathic pain), and musculoskeletal fatigue (Alemzadeh-Ansari et al., 2017; Haedtke et al., 2017; McDonald et al., 2015). In other studies, chronic pain in HF was associated with increased sleep disturbances (Conley et al., 2019), worsened depression (Haedtke et al., 2017), impaired mobility (Goodlin et al., 2012), and decreased health-related quality of life (Pantilat et al., 2016). Given the observed heterogeneity and complexity of chronic pain in patients with HF, a better understanding of the mechanisms underlying chronic pain is needed to design efficacious interventions.

Applying omics methodologies can build understanding of the biologic underpinnings of chronic pain in HF. For example, transcriptomic methods have not been applied to study chronic pain in HF, which is the analysis of a patient's complete messenger RNA (mRNA) transcriptome. Characterization of the mRNA transcriptome has been applied to differentiate vulnerability to pain conditions in other samples without HF, including chronic low-back pain and complex regional pain syndrome (Dorsey et al., 2019; Jin et al., 2013). These studies identified novel potential mechanisms for pain through gene expression in antigen presentation pathways (Dorsey et al., 2019) and neuroinflammatory mediators (Jin et al., 2013). As such, transcriptomics methods may represent an innovative method to improve understanding of pain in HF.

Studies of pain in HF using transcriptomics methods are needed to better characterize the mechanistic foundations of pain as a complex symptom. Identifying genes associated with pain mechanisms by testing differentially expressed mRNA among patients with HF who are experiencing pain will inform development of novel interventions to prevent or decrease chronic pain. Inadequate characterization of pain mechanisms in HF can lead to ineffective treatment of pain (Haedtke et al., 2017), increased hospitalizations and morbidity (Badar et al., 2014), and diminished health-related quality of life (Pantilat et al., 2016). Therefore, the overall objective of this study was to identify differentially expressed genes associated with pain mechanisms. This study aimed to identify differentially expressed genes in patients

with HF and pain compared to patients with HF without pain. The research question guiding this study was, “What genes are differentially expressed between patients with HF who are experiencing pain compared to patients with HF who are not experiencing pain?”

Methods

Design and Procedures

A cross-sectional retrospective case-control design was used for this study. Data were obtained as part of a three-arm randomized controlled trial. The parent study evaluated the efficacy of a computerized cognitive training intervention to improve memory among patients with HF [R01 NR016116; [Clinical Trials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03035565) identifier: NCT 03035565]. Detailed information regarding the methods of the parent study has been previously published (Pressler et al., 2018). The university institutional review board approved the parent study, and this current study did not require separate approval. All patients provided written informed consent in the parent study prior to data collection.

Sample

Inclusion criteria for the parent study were adults age 21 years or older who understood English with a diagnosis of chronic HF with a New York Heart Association (NYHA) HF class of I, II, or III, supported by echocardiography or comparable method; in addition, they prescribed NYHA guideline-derived medical therapy (Pressler et al., 2018). Exclusion criteria for the parent study were a history of major substance abuse; diagnosis of Alzheimer, other dementia, central nervous system degenerative disorders, cancer, or a Montreal Cognitive Assessment score of less than 19 (Pressler et al., 2018).

Additional exclusion criteria for the current study were those without baseline mRNA biospecimens. These criteria led to a total of 115 eligible patients with HF from the parent study (36 with pain and 79 without pain), from which two independent groups of 20 patients with pain and 20 without pain were selected. This sample of 40 was analyzed as the primary sample. A second validation sample of participants from the parent study was analyzed to verify the findings observed in the primary sample (Dorsey et al., 2019; Jin et al., 2013). This validation sample was selected to be comparable to the primary sample by age (within 10 years) and gender. Applying these criteria to the 75 remaining patients from the parent study who met eligibility criteria (16 with pain, 59 without pain) produced a validation sample of 24 patients; 10 with pain and 14 without pain.

Measures

Pain presence was measured at baseline in the parent study using the Health Utilities Index (HUI) Mark-3 questionnaire. The HUI Mark-3 evaluates health status and health-related quality of life with a total possible score ranging from 0.0 = dead to 1.0 = perfect health (Horsman et al., 2003). The question used for pain presence was, “Have you had any trouble with pain or discomfort during the past 4 weeks?” Based on the HUI Mark-3 instructions, patients were assigned a score of 0 if they responded “no” to the question about pain presence and 1 if they answered “yes.” The total HUI Mark-3 has satisfactory reliability, validity, and responsiveness across various health conditions, including HF (Horsman et al.,

2003; Pressler et al., 2011). The pain items of the HUI Mark-3 have validity and reliability and acceptable sensitivity to pain conditions in HF (Pressler et al., 2011). The 40 patients in the primary sample and 24 in the validation sample were divided into two mutually exclusive groups based on their answers to the baseline HUI Mark-3 pain question: (a) no pain present, (b) pain present.

Other variables from the parent study were examined for significant differences that might explain the observed findings in gene expression between groups with and without pain. These included depressive symptoms, health-related quality of life, functional mobility, and daytime sleepiness (Alemzadeh-Ansari et al., 2017; Conley et al., 2019; Goodlin et al., 2012; Haedtke et al., 2017). Self-reported demographic variables included age, gender, self-reported race (White, Black, Asian/Pacific Islander, or more than one race), ethnicity (Hispanic/Latino, non-Hispanic/Latino), years of education, marital status, and employment status. Clinical status variables included BMI, left ventricular ejection fraction (LVEF), NYHA HF class, comorbid medical conditions (arthritis, atrial fibrillation, coronary artery disease, coronary artery bypass graft, depression, diabetes, hyperlipidemia, hypertension, myocardial infarction, stroke, or ventricular arrhythmias). Self-report data were obtained from the patient interviews at baseline. Clinical status variables were retrieved from the patients' medical records.

Depressive symptoms were measured using the Patient Health Questionnaire (PHQ-8) (Kroenke et al., 2009). Health-related quality of life was measured using the Minnesota Living with Heart Failure Questionnaire (LHFQ; Rector & Cohn, 1992). The LHFQ includes a physical, emotional, and total score, with a higher score indicating poorer health-related quality of life (Rector & Cohn, 1992). Functional mobility and balance were measured using the Timed Up and Go (TUG) Test (Podsiadlo & Richardson, 1991). Lastly, daytime sleepiness was measured with the Epworth Sleepiness Scale (ESS; Johns, 1991), which consists of a 4-point Likert-type subjective report scale rating a person's tendency to be sleepy or doze off.

RNA-seq—In the current study, mRNA and noncoding RNA (i.e., RNA that does not code into a protein) were extracted and analyzed from blood specimens collected during the parent study. Blood specimens were obtained during the baseline visit in the parent study and stored at -80° until ready for analysis. The mRNA was isolated from whole blood specimens using the PAXgene Blood RNA System and sequenced at the Institute for Genome Sciences at the University of Maryland School of Medicine, Baltimore, MD. Libraries were prepared from 25ng of RNA using the TruSeq RNA Sample Prep kit (Illumina) and according to the manufacturer's instructions—except for an additional polymerase chain reaction cycle. Samples were sequenced on an Illumina HiSeq 4000 platform using a 150bp paired-end read configuration. Read quality was assessed using the FastQC toolkit. The reads were aligned to the human reference genome Homo_sapiens.GRCh38 using the HiSat (version HISAT2-2.0.4) alignment tool (Kim et al., 2015), and the number of reads by gene was determined using HTSeq (Anders et al., 2015).

Analytic Methods

Statistical Analyses—Descriptive statistics were used to examine the demographic and clinical characteristics of the primary and validation samples. Differences in the demographic and clinical variables were compared between patients with and without pain using independent t-tests for continuous variables and Fisher's exact test for categorical variables. Patients who were not self-reported Black or White race were combined into one category (Other), because of sample size limitations. Selected comorbid conditions were compared to control for variables that may explain pain presence between the pain and no-pain groups. A significance level of alpha .05 was applied.

RNA-seq Analysis: Genetic mechanisms of pain were assessed by identifying the differentially expressed genes (DEGs) between HF patients with pain and without pain using the R package DESeq2 (Love et al., 2014). This method applies the negative binomial distribution to model gene expression. Principal component analysis (PCA) was performed to assess for outliers among the analyzed samples and whether demographic variables of age, gender, race, and ethnicity led to variation in the genetic data. Genes were removed from further DEG analysis if their total count across all 40 samples was lower than 10. Since PCA by gender showed samples clustering on the PC1 axis, gender was entered as a covariate in the regression model and applied to test for DEGs in HF samples with pain versus those without pain. A gene was considered differentially expressed if the log fold change (LFC) was ± 0.58 between the pain and no-pain groups and the false discovery rate (FDR) p -value was $< .05$.

Identification of Genes of Interest: From the DEGs, genes of interest (GOIs) were identified through an iterative process. First, all DEGs were evaluated for their alignment into enriched ontology clusters using Metascape (Zhou et al., 2019). Second, all the DEGs were grouped using a protein-protein interaction (PPI) network analysis using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database. Third, GOIs were entered in a subsequent literature search using terms for the gene name, pain, and either up- or downregulation (as appropriate), in non-HF populations and animal models. This literature search was conducted to further narrow down and prioritize target GOIs, given the lack of literature concerning these genes in HF populations. Following this process, seven target GOIs were selected and evaluated in the second validation sample.

Validation of Genes of Interest: The target GOIs were validated using a separate sample of 24 patients with HF from the same parent study. This validation was conducted to ensure replication of the observed differentially expressed genes in the primary sample (Dorsey et al., 2019). Target GOIs were validated using quantitative polymerase chain reaction (qPCR; Dorsey et al., 2019). Custom primers for the target GOIs were developed at the University of Maryland Institute for Genome Sciences. The qPCR data of each GOI was analyzed using the Livak method (Livak & Schmittgen, 2001), also known as double delta Ct, which requires at least a reference gene and a sample or group calibrator. The reference gene *GAPDH* was used, and HF patients without pain were used as the calibrator group. Independent samples t-tests were used to compute the statistical significance of the differences in gene expression between the pain and no-pain groups.

Results

Demographic and clinical characteristics are presented for the primary sample in Table 1. The primary sample consisted of 65% men and 35% women. Self-reported race was 67.5% White, 30% Black, and 2.5% Other. The average age was 64.6 (SD = 10.8) years. The NYHA class of the primary sample was 22.5% class I, 42.5% class II, and 35% class III. Based on medical record review, 5% of patients who reported pain on the HUI Mark-3 measure also had a history of abdominal pain, 30% back pain, 35% chest pain, and 10% joint/limb pain. There were no significant differences in the demographic variables, clinical variables, or comorbid conditions between the pain and no-pain groups in the primary sample.

To obtain a list of DEGs between patients with and without pain, a total of 179,407 million base pair reads per patient sample was obtained. After completing mRNA sequencing, 334 DEGs (279 upregulated and 55 downregulated) were identified between the pain and no-pain groups (see Supplemental Digital Content [SDC] Table 1). The genes with the largest fold change in expression (both upregulated and downregulated) between patients with and without pain are presented in Figure 1.

Figure 2 displays how the 334 DEGs aligned into enriched ontology clusters through Metascape. From this analysis, 57 of the 334 DEGs following completion of RNA sequencing were significantly represented in the Reactome neutrophil degranulation pathway (FDR = 7.9e-17). The second most represented pathway was Reactome extracellular matrix organization (N = 19, FDR = 4.5e-03). Applying co-expression analysis in STRING v10 produced a PPI network of 288 nodes and 497 edges, compared to the expected number of 182 edges (PPI enrichment p-value < 1.0e-16). Figure 3 displays the PPI network of the 334 DEGs identified following completion of RNA sequencing. There was a core within the PPI network consisting of over 15 genes. Genes located in the core of the PPI network were selected for further examination as core genes show the highest number of interactions with the other genes in the network and are likely involved in essential disease-related pathways and biological processes.

From the PPI network, seven genes were identified as target GOIs based on currently existing literature. The seven target GOIs identified following completion of the mRNA sequencing were: (a) matrix metalloproteinase 8 (*MMP8*), (b) matrix metalloproteinase 9 (*MMP9*), (c) proprotein convertase subtilisin/kexin type 9 (*PCSK9*), (d) lipocalin-2 (*LCN2*), (e) neutrophil defensin 3 (*DEFA3*), (f) lactotransferrin (*LTF*), and (g) cathepsin G (*CTSG*). All these genes were upregulated in patients with HF and pain compared to patients with HF but without pain in the primary sample.

The LFC for the total primary and validation samples is presented in Table 2. The seven target genes were analyzed using validation qPCR to assess replication of the results of the RNA sequencing. Demographic and clinical characteristics of the validation sample are presented in Table 1. The average age of the validation sample was 68.6 (SD = 8.5) years. The validation sample consisted of 25% women and 75% men. Self-reported race was 87.5% White, 12.5% Black. The validation sample showed no statistically significant

differences in demographic variables, clinical variables, or comorbid conditions. However, patients without pain in the validation sample reported higher LHFQ scores (25.7, SD = 19.9 vs. 17.4, SD = 14.1) and lower TUG scores (12.2, SD = 3.5 vs. 17.6, SD = 17.3) compared to patients without pain.

A complete list of the primer sets developed for the validation analysis is presented in SDC Table 2. All seven target GOIs were upregulated in pain versus no-pain group in the primary sample; however, only three of these seven genes were upregulated in both the primary and validation samples. These three genes were: (a) *CTSG* (primary sample LFC: 1.44; validation sample LFC: 1.29); (b) *MMP8* (primary sample LFC: 1.37; validation sample LFC: 1.80); and (c) *DEFA3* (primary sample LFC: 1.43; validation sample LFC: 1.30). The differences in gene expression between the pain and no-pain groups were statistically significant for all genes in the primary sample. However, the differences in expression between the pain and no-pain groups were not statistically significant in the validation sample.

Discussion

This exploratory study is one of the first to identify and validate target GOIs from mRNA sequence data among patients with HF and pain. The seven genes identified in this study were all upregulated (i.e., they had higher levels of expression) between patients with HF and pain compared to patients without pain. Three of the seven target genes were upregulated in a second sample of patients using validation qPCR. All seven genes have been previously identified in other studies among patients with a variety of chronic pain conditions, including osteoarthritis (Luo et al., 2021; Näkki et al., 2016), complex regional pain syndrome (Jin et al., 2013), and degenerative disc disease (Shao et al., 2017).

Generally, neuroinflammatory influences of pain are thought to occur through the interaction between nociceptors and cytokines, lipids, and growth factors released from immune cells (Baral et al., 2019). The genes identified in this study code for amino acids and proteins involved in several immune processes, including cytotoxic peptides (*DEFA3*), elimination of foreign pathogens (*CTSG*, *LTF*), and other innate immunity mechanisms (*LCN2*). Inflammation is of particular interest within HF populations, given the observed activation of both innate and adaptive immune systems following myocardial injury, which leads to subsequent elevation of pro-inflammatory cytokines (Frantz et al., 2018). Previously conducted gene expression studies of myocardial tissue have identified that inflammatory and immune pathways are indeed enriched among patients with HF compared to healthy participants (Hahn et al., 2021).

Other researchers have explored the genes and inflammatory mechanisms identified in this study. The gene *DEFA3* was previously identified as a possible synergistic biomarker for inflammation among patients with coronary heart disease, possibly due to the lesions caused by atherogenesis (Maneerat et al., 2017). Upregulation of *MMP9* was observed among patients with complex regional pain syndrome (Jin et al., 2013) and is thought to play a significant role in neuroinflammation by regulating cellular response after injury (Wang et al., 2013). Both *MMP8* and *MMP9* act as notable mediators of osteoarthritis due

to their protective role in remodeling and reducing abnormal collagen (Luo et al., 2021; Näkki et al., 2016). The gene *LCN2* is involved in regulating both neuroinflammation and subsequent chronic inflammatory pain and is thought to contribute to pain hypersensitivity (Jha et al., 2015). In other studies of patients with osteoarthritis, the gene *LTF* may exhibit analgesic properties by reducing activation of neuronal inflammatory signaling pathways (Godínez-Chaparro et al., 2021). Lastly, studies of the gene *CTSG* have reported a mediating role of chronic inflammatory pain in patients with herniated discs (Shao et al., 2017).

The findings in this study indicate that inflammatory processes may constitute a mechanism underlying pain among patients with HF. However, it remains unclear whether the increased inflammation observed among patients with HF occurs because of or is a precursor to the disease process of HF or if inflammation arises because of other comorbid conditions such as arthritis (Adamo et al., 2020). As a result, the prevalence of potential confounding inflammatory pain conditions should be considered. In the current study, 30% of patients with pain in the primary sample and 40% with pain in the validation sample had a history of arthritis, and 10% of patients reporting pain had a history of joint/limb pain. These sample characteristics indicate that arthritis or other inflammatory conditions could explain the observed gene expression findings; nevertheless, it does not explain the findings among the rest of the sample with pain but no history of arthritis. In addition, 10% of the primary sample without pain and 21.4% of the validation sample without pain had a history of arthritis. Given that etiologies of pain were not available in the parent study, additional research is needed on inflammatory conditions in HF.

In addition to the inflammatory response caused by HF progression, another reason for the observed upregulation of genes involved with inflammatory processes may be the high prevalence of comorbid cardiovascular disease. One gene that plays a role in regulating plasma cholesterol homeostasis: *PCSK9*, it was significantly upregulated in this study. Studies of patients with both chronic and acute chest pain-associated cardiac conditions (such as myocardial infarction) have found correlations between increased levels of *PCSK9* with vascular disease (Gao et al., 2018). Alongside *PCSK9*, the other six GOIs identified in this study play a role in both inflammation and cardiovascular disease. The gene *DEFA3* can serve as a synergistic biomarker of inflammation in patients with hyperlipidemia and coronary artery disease (Maneerat et al., 2017). In addition, *LTF* has been proposed as a marker of increased risk for coronary artery disease (Vengen et al., 2010). Some members of the matrix metalloproteinase family may contribute to developing atherosclerosis, which can frequently cause inflammatory responses (Ye, 2015). These findings are of unique relevance given that hyperlipidemia and coronary artery disease are established risk factors for HF (Tsao et al., 2022). Compared to general populations, patients with HF present with higher rates of comorbid coronary artery disease (28.8% vs. 16.9%) and comorbid dyslipidemia (34.6% vs. 29.1%; Loosen et al., 2022). However, neither the primary nor validation sample had statistically significant differences in prevalence of hyperlipidemia or comorbid cardiac conditions. These findings may indicate a different mechanism for pain—particularly for cardiac chest pain—though additional research is needed to understand lipid profiles and cardiac chest pain among patients with HF.

Limitations

First, the parent study did not collect the cause of the pain. In addition, while previous locations of pain were recorded historically from clinic progress notes, the parent study did not actively measure locations of pain. As a result, this secondary analysis study was framed as a preliminary examination of several possible mechanisms underlying pain in HF broadly rather than profile a specific pain phenotype. Second, a single-item pain measure with a binary outcome (pain or no pain) was used to designate groups that lacked specificity compared to continuous pain measures. However, the analyzed pain groups produced a promising list of target GOIs despite these limitations. Third, while the sample consisted of 35% women and 28% Black, additional studies are needed to fully evaluate pain with a deeper appraisal of the different sociocultural and geographic factors that make up race and ethnicity. Fourth, only three of seven genes identified from the 334 DEGs were validated in the second sample of patients; this indicates that these findings should be evaluated in additional studies, particularly stratified across specific pain conditions.

Implications for Research and Practice

Accurate identification of relevant mechanistic pathways underlying pain in HF is necessary before more effective, precision-based interventions can be developed to treat pain. Although findings in this study are preliminary, additional research among patients with HF experiencing pain can uncover the role of transcriptomics in understanding pain expression. Subsequent studies should aim for concurrent deep phenotyping and genotyping of pain in HF among clearly demarcated pain conditions to best understand these potential biologic mechanisms. Additional research is needed, given that many upregulated genes observed in the primary sample were not replicated in the validation sample. This research is necessary because patients with HF frequently suffer from pain that is poorly understood and managed. This is partly due to the clinical complexity of both HF and pain—which often presents alongside multiple comorbid conditions and symptoms (Haedtke et al., 2017)—leaving clinicians without the necessary knowledge to manage pain (Chen et al., 2020). This issue is exacerbated by the lack of clear strategies for pain management within national treatment guidelines (Yancy et al., 2017). These gaps reinforce the need for building a deeper understanding of pain in HF.

Conclusion

Pain is a significant problem among patients with HF and remains inadequately characterized. In this study, differentially expressed genes were identified that may underly pain among patients with HF. Seven genes were identified, which were connected to two possible mechanisms for pain in HF: immune/inflammatory processes and atherosclerotic processes. Additionally, three of these genes (*CTSG*, *MMP8*, and *DEFA3*) were upregulated in a second validation sample. This study is novel in that the mechanisms underlying pain in this population are understudied. Identifying target genes and biologic mechanisms will be crucial for future novel interventions to reduce pain among patients with HF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The parent study was funded by the National Institute of Nursing Research (NR016116). The current study was funded by an Advancing Early Research Opportunities (AERO) Grant from the Rita and Alex Hillman Foundation. This study was supported by a T32 postdoctoral Fellowship from the National Institute of Nursing Research (T32 NR018407; Program Director, Miller & Robb); Advanced Training in Self-Management Interventions for Serious Chronic Conditions.

Institutional review board approval was granted for the parent study.

References

- Adamo L, Rocha-Resende C, Prabhu SD, & Mann DL (2020). Reappraising the role of inflammation in heart failure. *Nature Reviews Cardiology*, 17, 269–285. 10.1038/s41569-019-0315-x [PubMed: 31969688]
- Alemzadeh-Ansari MJ, Ansari-Ramandi MM, & Naderi N (2017). Chronic pain in chronic heart failure: A review article. *Journal of Tehran University Heart Center*, 12, 49–56. [PubMed: 28828019]
- Anders S, Pyl PT, & Huber W (2015). HTSeq—A Python framework to work with high-throughput sequencing data. *Bioinformatics*, 31, 166–169. 10.1093/bioinformatics/btu638 [PubMed: 25260700]
- Badar AA, Perez-Moreno AC, Jhund PS, Wong CM, Hawkins NM, Cleland JGF, van Veldhuisen DJ, Wikstrand J, Kjekshus J, Wedel H, Watkins S, Gardner RS, Petrie MC, & McMurray JJV (2014). Relationship between angina pectoris and outcomes in patients with heart failure and reduced ejection fraction: An analysis of the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA). *European Heart Journal*, 35, 3426–3433. 10.1093/eurheartj/ehu342 [PubMed: 25265976]
- Baral P, Udit S, & Chiu IM (2019). Pain and immunity: Implications for host defence. *Nature Reviews Immunology*, 19, 433–447. 10.1038/s41577-019-0147-2
- Chen J, Walsh S, Delaney C, & Cong X (2020). Pain management in patients with heart failure: A survey of nurses' perception. *Pain Management Nursing*, 21, 365–370. 10.1016/j.pmn.2019.09.004 [PubMed: 31623989]
- Conley S, Feder SL, Jeon S, & Redeker NS (2019). Daytime and nighttime sleep characteristics and pain among adults with stable heart failure. *Journal of Cardiovascular Nursing*, 34, 390–398. 10.1097/JCN.0000000000000593 [PubMed: 31365442]
- Dorsey SG, Renn CL, Griffioen M, Lassiter CB, Zhu S, Huot-Creasy H, McCracken C, Mahurkar A, Shetty AC, Jackson-Cook CK, Kim H, Henderson WA, Saligan L, Gill J, Colloca L, Lyon DE, & Starkweather AR (2019). Whole blood transcriptomic profiles can differentiate vulnerability to chronic low back pain. *PLoS ONE*, 14, e0216539. 10.1371/journal.pone.0216539 [PubMed: 31095601]
- Frantz S, Falcao-Pires I, Balligand J-L, Bauersachs J, Brutsaert D, Ciccarelli M, Dawson D, de Windt LJ, Giacca M, Hamdani N, Hilfiker-Kleiner D, Hirsch E, Leite-Moreira A, Mayr M, Thum T, Tocchetti CG, van der Velden J, Varricchi G, & Heymans S (2018). The innate immune system in chronic cardiomyopathy: A European Society of Cardiology (ESC) scientific statement from the Working Group on Myocardial Function of the ESC. *European Journal of Heart Failure*, 20, 445–459. 10.1002/ejhf.1138 [PubMed: 29333691]
- Gao Y, Qiu Y, Wu J, Diao W, Zhang H, Wang S, Du Z, Dong J, Zhang M, & Jiang L (2018). Acute-phase plasma PCSK9 levels and recurrent cardiovascular events in a Chinese acute myocardial infarction cohort. *Cardiology*, 141, 88–97. 10.1159/000493785 [PubMed: 30423567]
- Godínez-Chaparro B, Guzmán-Mejía F, & Drago-Serrano ME (2021). Lactoferrin and its potential impact for the relief of pain: A preclinical approach. *Pharmaceuticals*, 14, 868. 10.3390/ph14090868 [PubMed: 34577568]

- Goodlin SJ, Wingate S, Albert NM, Pressler SJ, Houser J, Kwon J, Chiong J, Storey CP, Quill T, Teerlink JR, & PAIN-HF Investigators. (2012). Investigating pain in heart failure patients: The pain assessment, incidence, and nature in heart failure (PAIN-HF) study. *Journal of Cardiac Failure*, 18, 776–783. 10.1016/j.cardfail.2012.07.007 [PubMed: 23040113]
- Haedtke C, Smith M, VanBuren J, Klein D, & Turvey C (2017). The characteristics of pain in patients diagnosed with depression and heart failure. *Pain Management Nursing*, 18, 353–362. 10.1016/j.pmn.2017.05.005 [PubMed: 28843637]
- Hahn VS, Knutsdottir H, Luo X, Bedi K, Margulies KB, Haldar SM, Stolina M, Yin J, Khakoo AY, Vaishnav J, Bader JS, Kass DA, & Sharma K (2021). Myocardial gene expression signatures in human heart failure with preserved ejection fraction. *Circulation*, 143, 120–134 10.1161/CIRCULATIONAHA.120.050498 [PubMed: 33118835]
- Horsman J, Furlong W, Feeny D, & Torrance G (2003). The Health Utilities Index (HUI®): Concepts, measurement properties and applications. *Health and Quality of Life Outcomes*, 1, 54. 10.1186/1477-7525-1-54 [PubMed: 14613568]
- Jha MK, Lee S, Park DH, Kook H, Park K-G, Lee I-K, & Suk K (2015). Diverse functional roles of lipocalin-2 in the central nervous system. *Neuroscience & Biobehavioral Reviews*, 49, 135–156. 10.1016/j.neubiorev.2014.12.006 [PubMed: 25511817]
- Jin E-H, Zhang E, Ko Y, Sim WS, Moon DE, Yoon KJ, Hong JH, & Lee WH (2013). Genome-wide expression profiling of complex regional pain syndrome. *PLoS ONE*, 8, e79435. 10.1371/journal.pone.0079435 [PubMed: 24244504]
- Johns MW (1991). A new method for measuring daytime sleepiness: The Epworth sleepiness scale. *Sleep*, 14, 540–545. 10.1093/sleep/14.6.540 [PubMed: 1798888]
- Kim D, Langmead B, & Salzberg SL (2015). HISAT: A fast spliced aligner with low memory requirements. *Nature Methods*, 12, 357–360. 10.1038/nmeth.3317 [PubMed: 25751142]
- Kroenke K, Strine TW, Spitzer RL, Williams JBW, Berry JT, & Mokdad AH (2009). The PHQ-8 as a measure of current depression in the general population. *Journal of Affective Disorders*, 114, 163–173. 10.1016/j.jad.2008.06.026 [PubMed: 18752852]
- Lippi G, & Sanchis-Gomar F (2020). Global epidemiology and future trends of heart failure. *AME Medical Journal*, 5, 1–6. 10.21037/amj.2020.03.03
- Livak KJ, & Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻CT method. *Methods*, 25, 402–408. 10.1006/meth.2001.1262 [PubMed: 11846609]
- Loosen SH, Roderburg C, Curth O, Gaensbacher J, Joerdens M, Luedde T, Konrad M, Kostev K, & Luedde M (2022). The spectrum of comorbidities at the initial diagnosis of heart failure a case control study. *Scientific Reports*, 12, 2670. 10.1038/s41598-022-06618-5 [PubMed: 35177698]
- Love MI, Huber W, & Anders S (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15, 550. 10.1186/s13059-014-0550-8 [PubMed: 25516281]
- Luo S, Li W, Wu W, & Shi Q (2021). Elevated expression of MMP8 and MMP9 contributes to diabetic osteoarthritis progression in a rat model. *Journal of Orthopaedic Surgery and Research*, 16, 64. 10.1186/s13018-021-02208-9 [PubMed: 33468174]
- Maneerat Y, Prasongsukarn K, Benjathummarak S, & Dechkhajorn W (2017). PPBP and DEFA1/DEFA3 genes in hyperlipidaemia as feasible synergistic inflammatory biomarkers for coronary heart disease. *Lipids in Health and Disease*, 16, 80. 10.1186/s12944-017-0471-0 [PubMed: 28420383]
- McDonald DD, Soutar C, Chan MA, & Afriyie A (2015). A closer look: Alternative pain management practices by heart failure patients with chronic pain. *Heart & Lung*, 44, 395–399. 10.1016/j.hrtlng.2015.06.001 [PubMed: 26088386]
- Näkki A, Rodriguez-Fontenla C, Gonzalez A, Harilainen A, Leino-Arjas P, Heliövaara M, Eriksson JG, Tallroth K, Videman T, Kaprio J, Saarela J, & Kujala UM (2016). Association study of MMP8 gene in osteoarthritis. *Connective Tissue Research*, 57, 44–52. 10.3109/03008207.2015.1099636 [PubMed: 26577236]

- Pantilat SZ, O’Riordan DL, Rathfon MA, Dracup KA, & De Marco T (2016). Etiology of pain and its association with quality of life among patients with heart failure. *Journal of Palliative Medicine*, 19, 1254–1259. 10.1089/jpm.2016.0095 [PubMed: 27494139]
- Podsiadlo D, & Richardson S (1991). The timed “Up & Go”: A test of basic functional mobility for frail elderly persons. *Journal of the American Geriatrics Society*, 39, 142–148. 10.1111/j.1532-5415.1991.tb01616.x [PubMed: 1991946]
- Pressler SJ, Eckert GJ, Morrison GC, Murray MD, & Oldridge NB (2011). Evaluation of the Health Utilities Index Mark-3 in heart failure. *Journal of Cardiac Failure*, 17, 143–150. 10.1016/j.cardfail.2010.08.014 [PubMed: 21300304]
- Pressler SJ, Giordani B, Titler M, Gradus-Pizlo I, Smith D, Dorsey SG, Gao S, Jung M Design and rationale of the Cognitive Intervention to Improve Memory in Heart Failure Patients study. *Journal of Cardiovascular Nursing*, 2018, 33, 344–355. 10.1097/JCN.0000000000000463 [PubMed: 29601367]
- Rector TS, & Cohn JN (1992). Assessment of patient outcome with the Minnesota Living with Heart Failure questionnaire: Reliability and validity during a randomized, double-blind, placebo-controlled trial of pimobendan. *American Heart Journal*, 124, 1017–1025. 10.1016/0002-8703(92)90986-6 [PubMed: 1529875]
- Riegel B, Moser DK, Buck HG, Dickson VV, Dunbar SB, Lee CS, Lennie TA, Lindenfeld J, Mitchell JE, Treat-Jacobson DJ, Webber DE, & American Heart Association Council on Cardiovascular and Stroke Nursing; Council on Peripheral Vascular Disease; and Council on Quality of Care and Outcomes Research. (2017). Self-care for the prevention and management of cardiovascular disease and stroke: A scientific statement for healthcare professionals from the American Heart Association. *Journal of the American Heart Association*, 6, e006997. 10.1161/JAHA.117.006997 [PubMed: 28860232]
- Shao J, Yu M, Jiang L, Wu F, & Liu X (2017). Sequencing and bioinformatics analysis of the differentially expressed genes in herniated discs with or without calcification. *International Journal of Molecular Medicine*, 39, 81–90. 10.3892/ijmm.2016.2821 [PubMed: 27959380]
- Tsao CW, Aday AW, Almarzooq ZI, Alonso A, Beaton AZ, Bittencourt MS, Boehme AK, Buxton AE, Carson AP, Commodore-Mensah Y, Elkind MSV, Evenson KR, Eze-Nliam C, Ferguson JF, Generoso G, Ho JE, Kalani R, Khan SS, Kissela BM, American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. (2022). Heart disease and stroke statistics—2022 update: A report from the American Heart Association. *Circulation*, 145, e153–e639. 10.1161/CIR.0000000000001052 [PubMed: 35078371]
- Vengen IT, Dale AC, Wiseth R, Midthjell K, & Videm V (2010). Lactoferrin is a novel predictor of fatal ischemic heart disease in diabetes mellitus type 2: Long-term follow-up of the HUNT 1 study. *Atherosclerosis*, 212, 614–620. 10.1016/j.atherosclerosis.2010.06.008 [PubMed: 20598696]
- Wang X, Yu YY, Lieu S, Yang F, Lang J, Lu C, Werb Z, Hu D, Miclau T, Marcucio R, & Colnot C (2013). MMP9 regulates the cellular response to inflammation after skeletal injury. *Bone*, 52, 111–119. 10.1016/j.bone.2012.09.018 [PubMed: 23010105]
- Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr., Colvin MM, Drazner MH, Filippatos GS, Fonarow GC, Givertz MM, Hollenberg SM, Lindenfeld J, Masoudi FA, McBride PE, Peterson PN, Stevenson LW, & Westlake C (2017). 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA guideline for the management of heart failure: A report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. *Circulation*, 136, e137–e161. 10.1161/CIR.0000000000000509 [PubMed: 28455343]
- Ye S (2015). Putative targeting of matrix metalloproteinase-8 in atherosclerosis. *Pharmacology & Therapeutics*, 147, 111–122. 10.1016/j.pharmthera.2014.11.007 [PubMed: 25448039]
- Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, Benner C, & Chanda SK (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nature Communications*, 10, 1523. 10.1038/s41467-019-09234-6

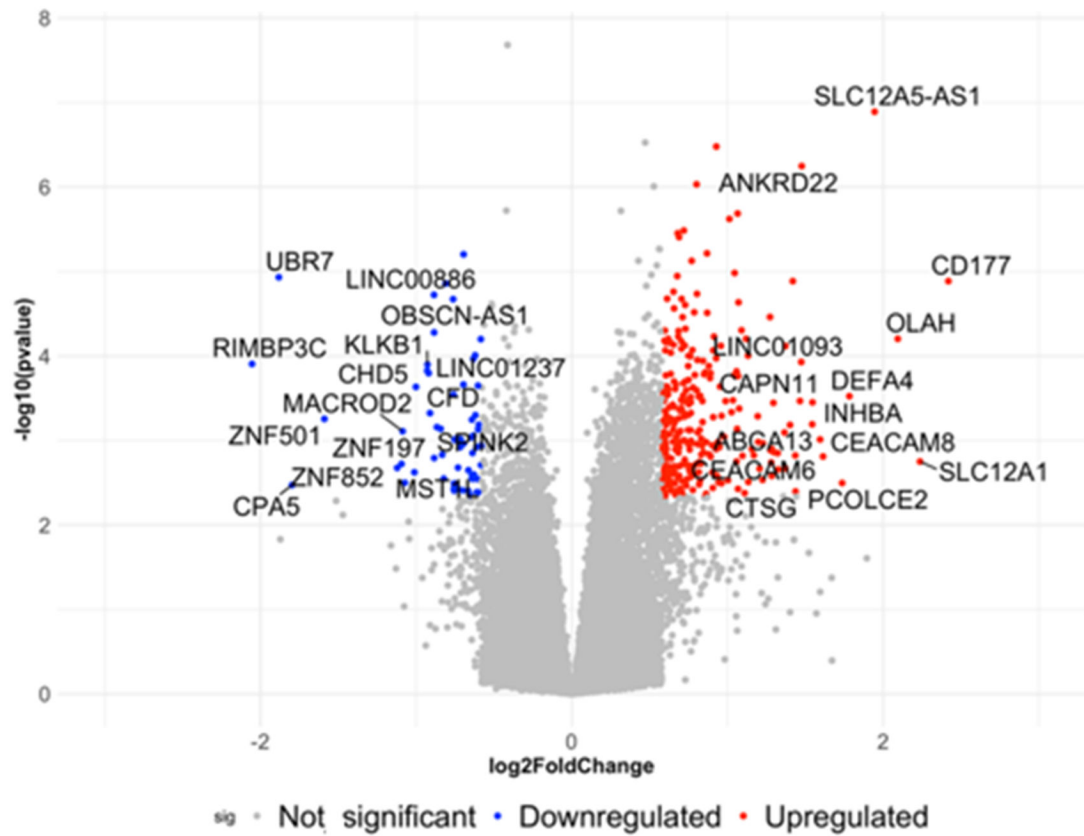


Figure 1 –.
Volcano plot showing the significantly differentially expressed genes with the highest log fold change ($\text{LFC} \pm 0.58$; $\text{FDR p-value} < 0.05$; $N = 334$) among the pain and no-pain groups in the primary sample ($N=40$).

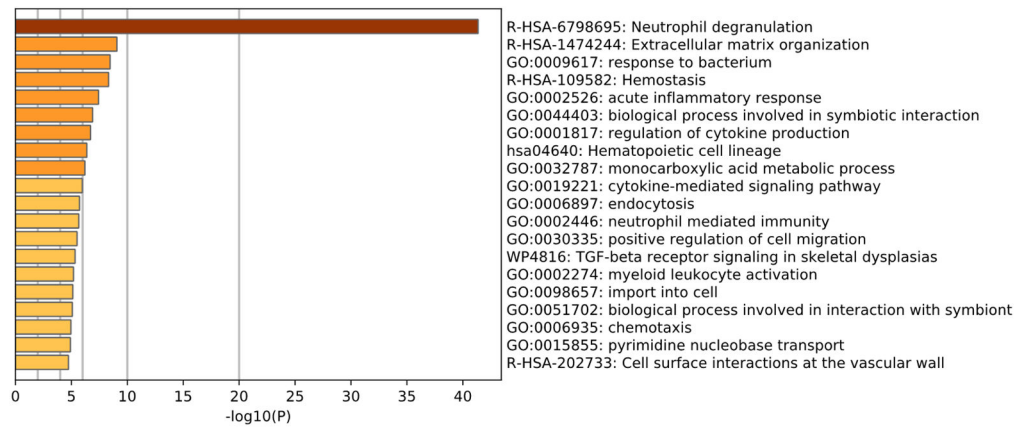


Figure 2 –.
Enrichment ontology clusters using Metascape

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

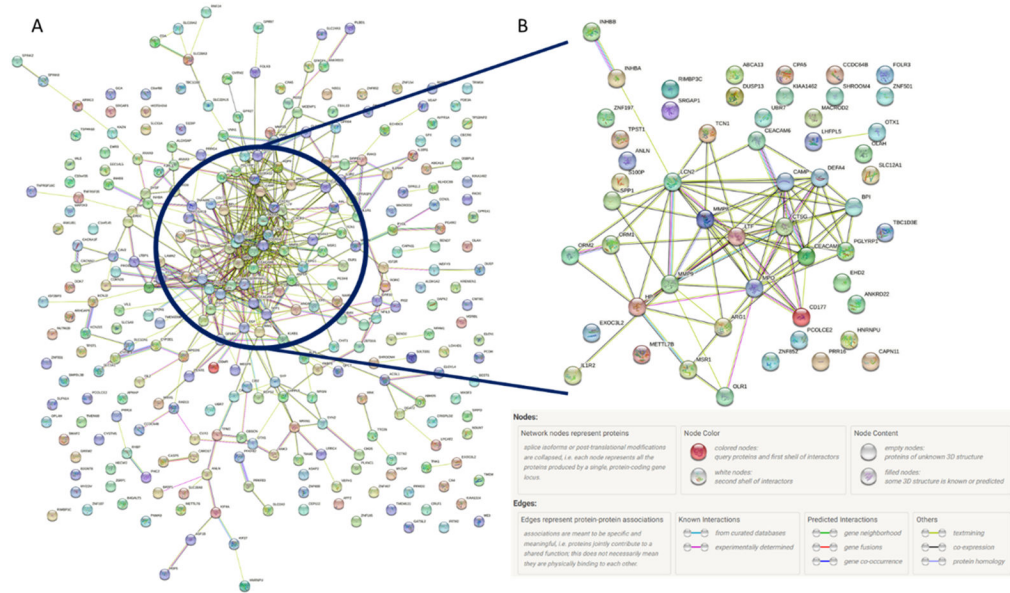


Figure 3 –. Protein-protein interaction network analysis. A. representing all 334 DEGs, B. zoomed on the DEGs with absolute log₂ fold-change>1

Table 1

Demographics of Primary and Validation Sample

Variable	Primary sample (N= 40)			Validation sample (N = 24)			p-value
	Total sample	No pain (n = 20)	Pain (n = 20)	Total sample	No pain (n = 14)	Pain (n = 10)	
Age, years	64.55 ± 10.78	62.65 ± 13.87	66.45 ± 6.21	68.58 ± 8.52	70.21 ± 9.82	66.30 ± 6.00	.274 ^a
Gender, n (%)							.741 ^b
Women	14 (35)	6 (30)	8 (40)	6 (25)	3 (21.43)	3 (30)	
Men	26 (65)	14 (70)	12 (60)	18 (75)	11 (78.57)	7 (70)	
Race, n (%)							.501 ^b
White	27 (67.50)	12 (60)	15 (75)	21 (87.50)	11 (78.57)	10 (100)	
Black	12 (30)	7 (35)	5 (25)	3 (12.50)	3 (21.43)	0	
Other	1 (2.50)	1 (5)	0	0	0	0	
Ethnicity, n (%)							< .999 ^b
Not Hispanic/Latino	39 (97.50)	19 (95)	20 (100)	24 (100)	14 (100)	10 (100)	
Hispanic/Latino	1 (2.50)	1 (5)	0	0	0	0	
Education, years	15.20 ± 3.47	15.10 ± 3.78	15.30 ± 3.23	14.44 ± 2.13	14.67 ± 2.60	14.14 ± 1.46	.858 ^a
Marital status, n (%)							.751 ^b
Married	18 (45)	8 (40)	10 (50)	15 (62.50)	7 (50)	8 (80)	
Not married	22 (55)	12 (60)	10 (50)	9 (37.50)	7 (50)	2 (20)	
Employment status, n (%)							.838 ^b
Employed	9 (22.50)	5 (25)	4 (20)	5 (20.83)	3 (21.43)	2 (20)	
Unemployed	9 (22.50)	5 (25)	4 (20)	1 (4.17)	1 (7.14)	0	
Retired	22 (55)	10 (50)	12 (60)	18 (75)	10 (71.43)	8 (80)	
BMI*	33 ± 6.78	32.06 ± 6.11	34.00 ± 7.46	32.19 ± 7.13	30.30 ± 6.63	34.85 ± 7.27	.382 ^a
NYHA Class							.134 ^a
I	9 (22.50)	7 (35)	2 (10)	1 (4.17)	1 (7.14)	0	
II	17 (42.50)	8 (40)	9 (45)	11 (45.83)	8 (57.14)	3 (30)	
III	14 (35)	5 (25)	9 (45)	12 (50)	5 (35.71)	7 (70)	

Variable	Primary sample (N= 40)			Validation sample (N= 24)			
	Total sample	No pain (n = 20)	Pain (n = 20)	Total sample	No pain (n = 14)	Pain (n = 10)	
LVEF, %	42.56 ± 13.58	43.05 ± 14.04	42.08 ± 13.44	49.92 ± 11.33	48.79 ± 11.82	51.5 ± 11.03	.570 ^a
Comorbid conditions, n (%)							
Arthritis	8 (20)	2 (10)	6 (30)	7 (29.17)	3 (21.4)	4 (40)	.393 ^b
Atrial fibrillation	12 (30)	5 (25)	7 (35)	8 (33.33)	5 (35.71)	3 (30)	<.999 ^b
Coronary artery disease	14 (35)	6 (30)	8 (40)	15 (62.50)	11 (78.57)	4 (40)	.092 ^b
Coronary artery bypass graft	7 (17.50)	2 (10)	5 (25)	8 (33.3)	7 (50)	1 (10)	.040 ^b
Depression	4 (10)	0	4 (20)	1 (4.17)	0	1 (10)	.417 ^b
Diabetes	14 (35)	8 (40)	6 (30)	13 (54.17)	6 (42.86)	7 (70)	.240 ^b
Hypertlipidemia	29 (72.50)	15 (75)	14 (70)	16 (66.7)	7 (50)	9 (90)	.079 ^b
Hypertension	28 (70)	16 (80)	12 (60)	22 (91.67)	12 (85.71)	10 (100)	.493 ^b
Myocardial infarction	7 (17.50)	3 (15)	4 (20)	7 (29.17)	5 (35.71)	2 (20)	.653 ^b
Stroke	3 (7.50)	2 (10)	1 (5)	4 (16.67)	3 (21.43)	1 (10)	.615 ^b
Ventricular arrhythmia	11 (27.5)	5 (25)	6 (30)	2 (8.33)	1 (7.14)	1 (10)	<.999 ^b
Depressive symptoms, PHQ-8	4.23 ± 5.05	3.95 ± 5.82	4.50 ± 4.27	4.38 ± 3.66	4.43 ± 3.27	4.30 ± 4.32	.938 ^a
Health-related quality of life, LHFQ	16.4 ± 17.35	15.85 ± 18.09	16.95 ± 17.02	22.25 ± 17.85	25.71 ± 19.89	17.40 ± 14.06	.243 ^a
Mobility, TUG Score*	8.74 ± 1.96	8.65 ± 1.81	8.84 ± 2.14	14.46 ± 11.48	12.21 ± 3.47	17.60 ± 17.34	.357 ^a
Daytime sleepiness, ESS	8.3 ± 4.24	8.95 ± 4.27	7.65 ± 4.21	8.25 ± 3.78	8.14 ± 4.28	8.40 ± 3.17	.867 ^a

Note: BMI = body mass index; LVEF = left ventricular ejection fraction; PHQ = Patient Health Questionnaire; LHFQ = Living with Heart Failure; TUG = Timed Up and Go; ESS = Epworth Sleepiness Scale

^a independent samples t-test

^b Fisher's Exact

* sample size for BMI, TUG score in the primary sample = 39

Table 2
Log Fold Change of Gene Expression From RNA Sequence and Validation qPCR of Patients With Heart Failure and Pain Compared to Patients With Heart Failure and No Pain

Gene name (symbol)	RNAseq (N = 40)			qPCR (N = 24)		
	LFC	p-value	padj	LFC	p-value	
Cathepsin G (CTSG)	1.44	4.02E-03	4.74E-02	1.29	4.91E-01	
Neutrophil defensin 3 (DEFA3)	1.43	1.51E-02	8.95E-02	1.30	5.64E-01	
lipocalin-2 (LCN2)	1.28	1.26E-03	2.84E-02	0.96	4.96E-01	
Lactotransferrin (LTF)	1.33	2.20E-03	3.63E-02	0.77	5.32E-01	
Matrix metalloproteinase 8 (MMP8)	1.37	2.14E-03	3.60E-02	1.80	2.14E-01	
Matrix metalloproteinase 9 (MMP9)	1.42	1.30E-05	7.01E-03	0.78	2.66E-01	
Protein convertase subtilisin/kexin type 9 (PCSK9)	2.95	1.45E-02	N/A	0.98	9.19E-01	

Note. padj= adjusted p-value; LFC = log fold change; qPCR = quantitative polymerase chain reaction