Cardiomyopathy Mechanisms: Meta-analysis of Expression Profiles for Z-disc-associated Genes Across Multiple Microarray Datasets

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Research interests:

- Gene expression in human diseases
- Protein structure and function
- Protein Engineering
- Bioinformatics in Cardiovascular Research

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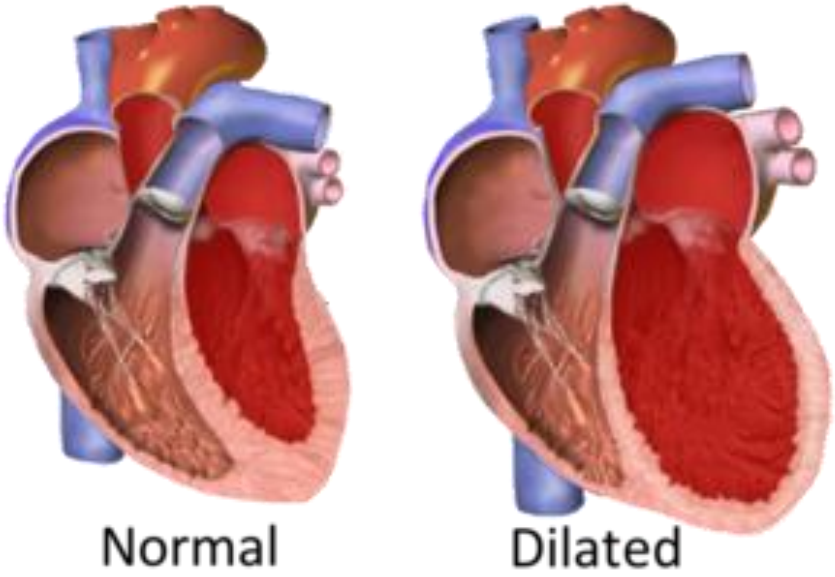
Areas of expertise:

- Generative AI
- Machine Learning
- Algorithm Development
- Research
- Developing state-of-the-art prediction models

Introduction

Cardiomyopathies

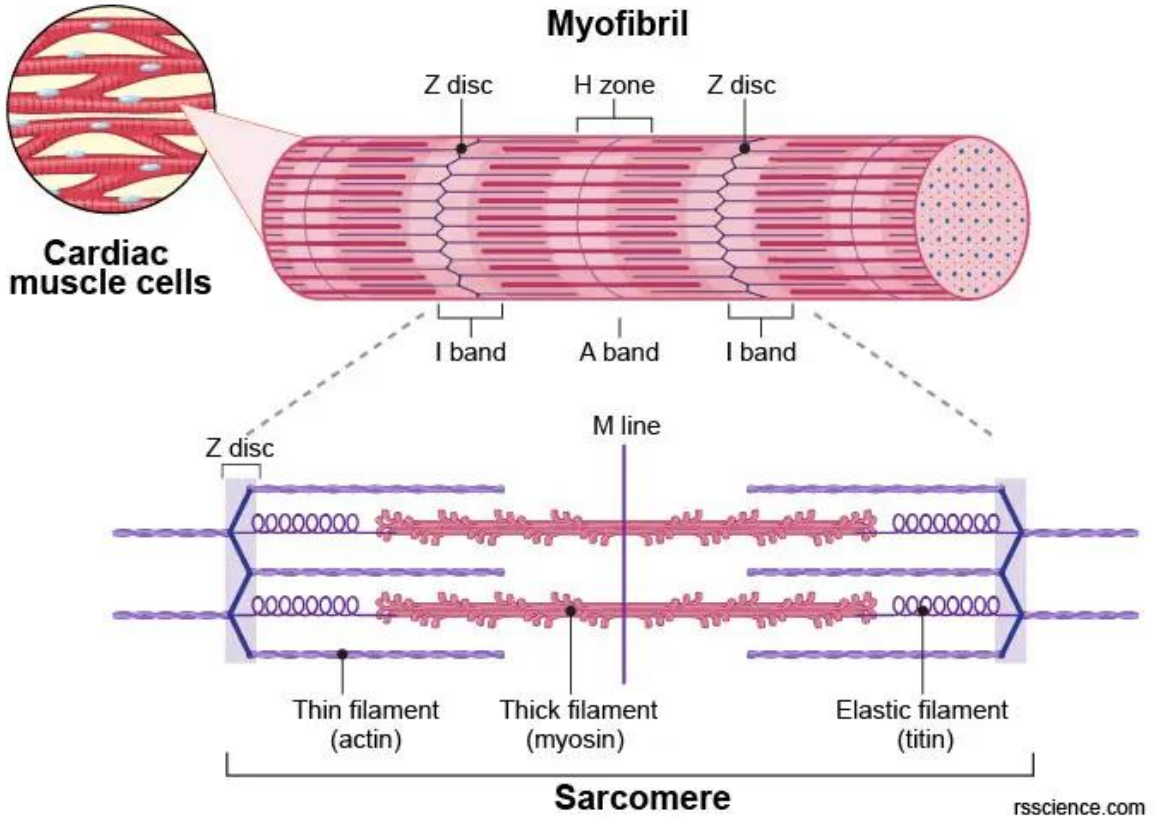
- Diseases of the heart muscle affecting structure and function.
- Ischemic: Caused by reduced blood flow (e.g., coronary artery disease, myocardial infarction).
- Non-ischemic: Linked to genetics, inflammation, metabolic issues, or toxins.



Introduction

Z-Disc in Cardiac Function

- Anchors actin filaments within the sarcomere for efficient contraction.
- Provides mechanical stability and acts as a mechanosensory hub.
- Mutations in Z-disc genes can impair cardiac function and contribute to cardiomyopathies.



Study aims & objectives

- Integrate multiple microarray datasets to assess expression profiles of Z-disc–associated genes in cardiomyopathies.
- Compare gene expression across control, non-ischemic cardiomyopathy, and ischemic cardiomyopathy samples.
- Elucidate the molecular mechanisms underlying cardiomyopathy by focusing on the structural and signaling roles of the Z-disc.

Study Design

1 Target Gene Selection

16 genes associated with cardiomyopathy, involved in Z-disc structure/function

2 Data Acquisition

6 relevant datasets from NCBI GEO database, including sum of 3882 genes across 142 samples

3 Sample Groups

Normal controls, non-ischemic CM, and ischemic CM

Summary of GEO Series and samples in the study

GEO DataSets/ Series, and samples	GEO Series	Control	Non-ischemic CM	Ischemic CM	Number of samples
GDS651	GSE1145	11	15 idiopathic dilated CM	11 ischemic CM	37
GDS1362	GSE1869	6	21 non-ischemic CM	10 ischemic CM	37
GDS2205	GSE3585	5	7 dilated CM		12
GDS2206	GSE3586	15	13 dilated CM		28
GDS3115	GSE9128	3	4 non-ischemic CM	4 ischemic DCM	11
GDS4772	GSE42955	5	12 dilated CM		17

Gene Expression Analysis Methods

1

Differential Expression

Using limma package in R, voom function for linear modeling, batch effect correction using ComBat function

2

Statistical Analysis

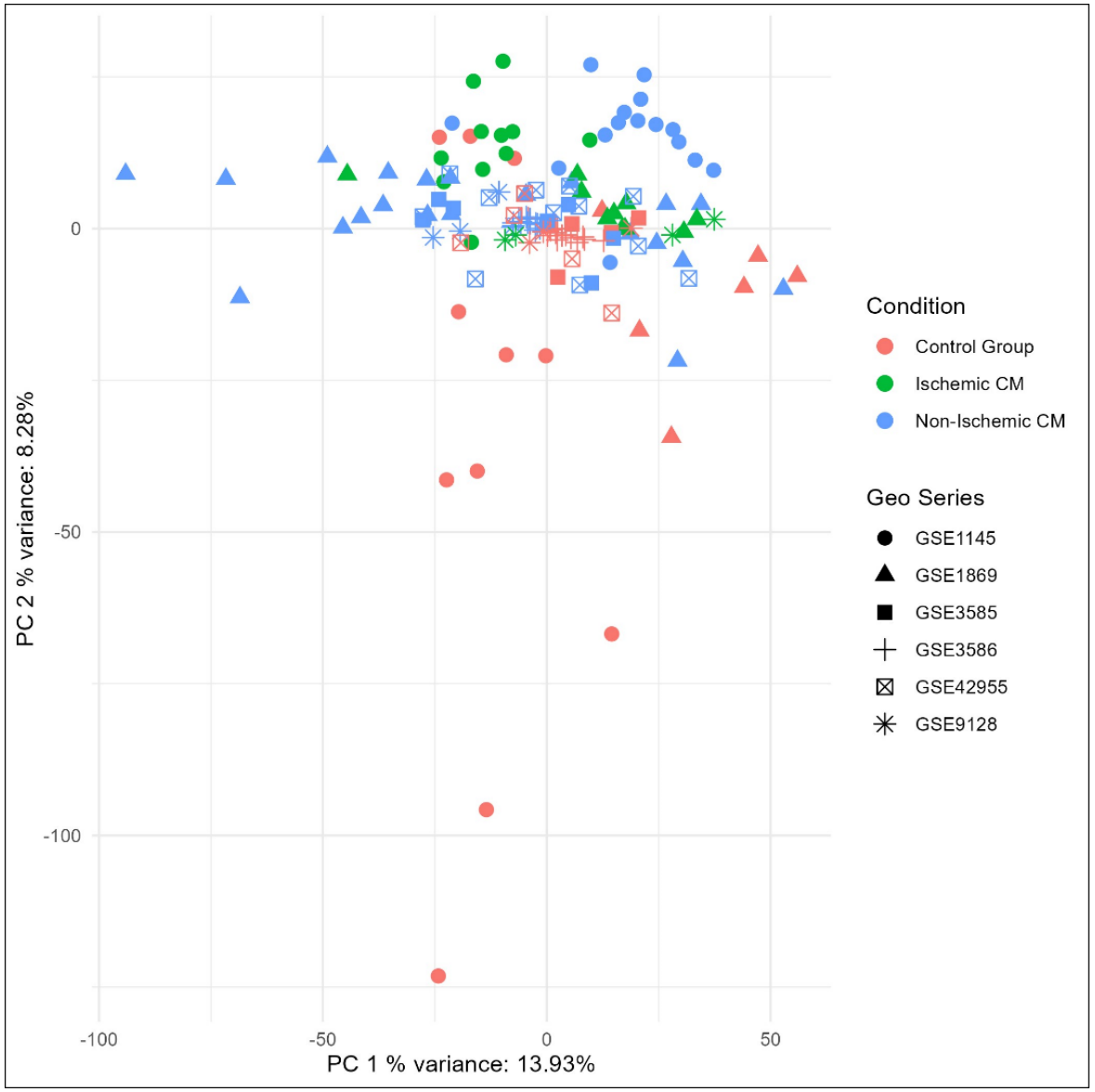
Fligner-Killeen test for variance, Mann-Whitney U test for distribution

3

Visualization

PCA plots, volcano plots, and box plots

Principal Component Analysis



All Groups

13.93% variance for PC1, 8.23% for PC2. Central cluster with distinct but overlapping gene expression profiles.

Non-ischemic CM vs Control

62.84% variance in PC1 and PC2. Clear separation from the control.

Ischemic CM vs Control

72.1% variance in PC1 and PC2. Pronounced separation from the control.

Statistical Analysis of Gene Expression

Variance of Homogeneity

Fligner-Killeen test showed significant differences ($p < 2.2e-16$) between control and both CM types.

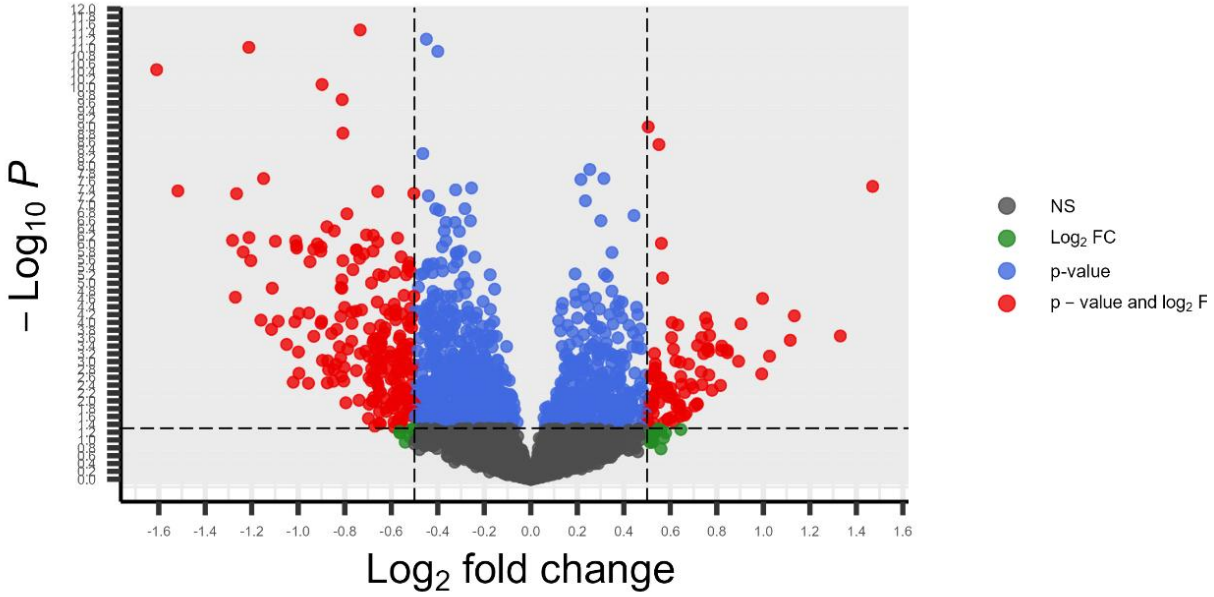
Expression Level Comparison

Mann-Whitney U test revealed significant differences ($p < 2.2e-16$) in median expression levels.

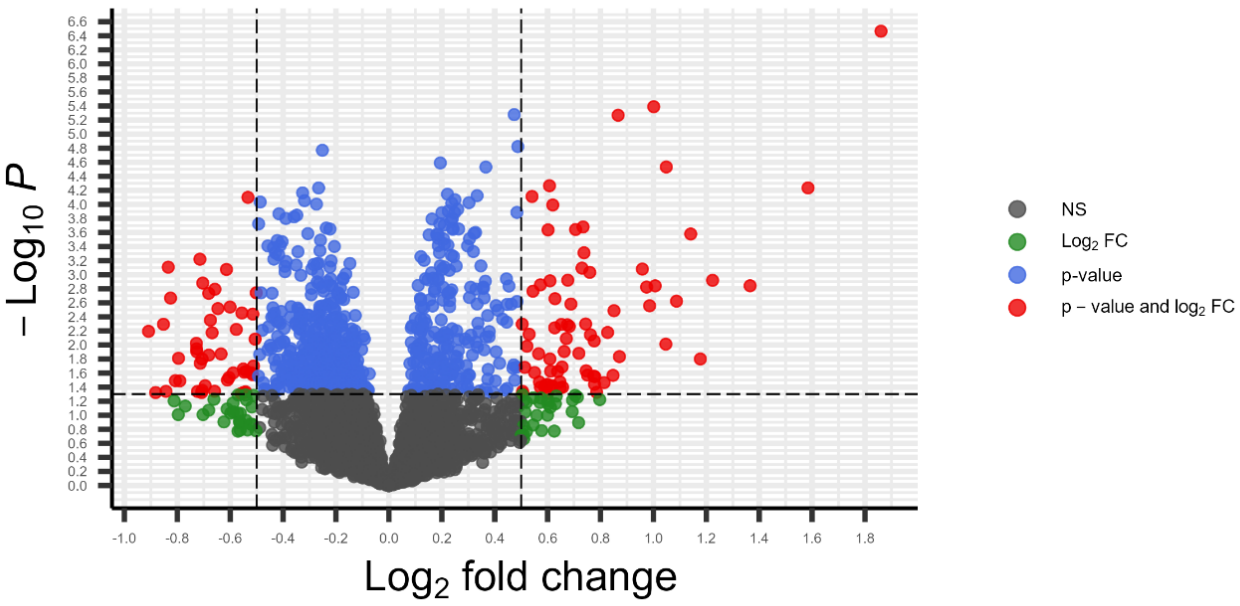
Higher median values in CM groups compared to the control, with ischemic CM showing the most pronounced increase, indicating a general upregulation of gene expression.

Volcano plot analysis

Non-ischemic CM vs. Control group



Ischemic CM vs. Control group



Red – genes meeting criteria $p < 0.05$, $|log2Fc| > 0.5$. Blue - genes meeting criteria of $p < 0.05$ only. Green - genes meeting criteria of $|log2Fc| > 0.5$ only. Gray – non-significant genes.

Differential Gene Expression

Non-ischemic CM vs. Control group

Gene ID	log_2Fc	p-value
ACTN2	-0.0764	0.261315
CRYAB	-0.22213	0.651992
CSRP3	-0.36499	0.631933
FHOD3	0.321436	0.328259
FLNC	-1.28326	0.705306
LDB3	0.349564	0.424669
MYOZ2	-0.1335	0.273395
PDLIM3	0.271241	0.028688
PDLIM5	-0.29905	0.348035
TTN	-0.33427	0.904125

↓ **Downregulated Genes**

FLNC was extremely downregulated but not statistically significant, possibly due to high variability across samples.

↑ **Upregulated Genes**

PDLIM3 showed statistical significance ($p < 0.05$) with slight upregulation.

Red – genes meeting criteria $p < 0.05$, $|log2Fc| > 0.5$. Blue - genes meeting criteria of $p < 0.05$ only. Green - genes meeting criteria of $|log2Fc| > 0.5$ only. Gray – non-significant genes.

Differential Gene Expression

Ischemic CM vs. Control group

Gene ID	log_2Fc	p-value
ACTN2	0.636111	0.07353
CRYAB	0.181341	0.15867
CSRP3	0.212207	0.108346
FHOD3	1.167699	0.002783
FLNC	-0.23117	0.081772
LDB3	1.071716	0.024894
MYOZ2	0.370145	0.264684
PDLIM3	0.988332	0.01423
PDLIM5	0.610629	0.181544
TTN	0.258497	0.105675

Red – genes meeting criteria $p < 0.05$, $|log2Fc| > 0.5$. Blue - genes meeting criteria of $p < 0.05$ only. Green - genes meeting criteria of $|log2Fc| > 0.5$ only. Gray – non-significant genes.

↑ Upregulated Genes

Three Z-disc components met both criteria of $p < 0.05$, $|log2Fc| > 0.5$: FHOD3, LDB3, and PDLIM3 - all three were highly upregulated compared to the control.

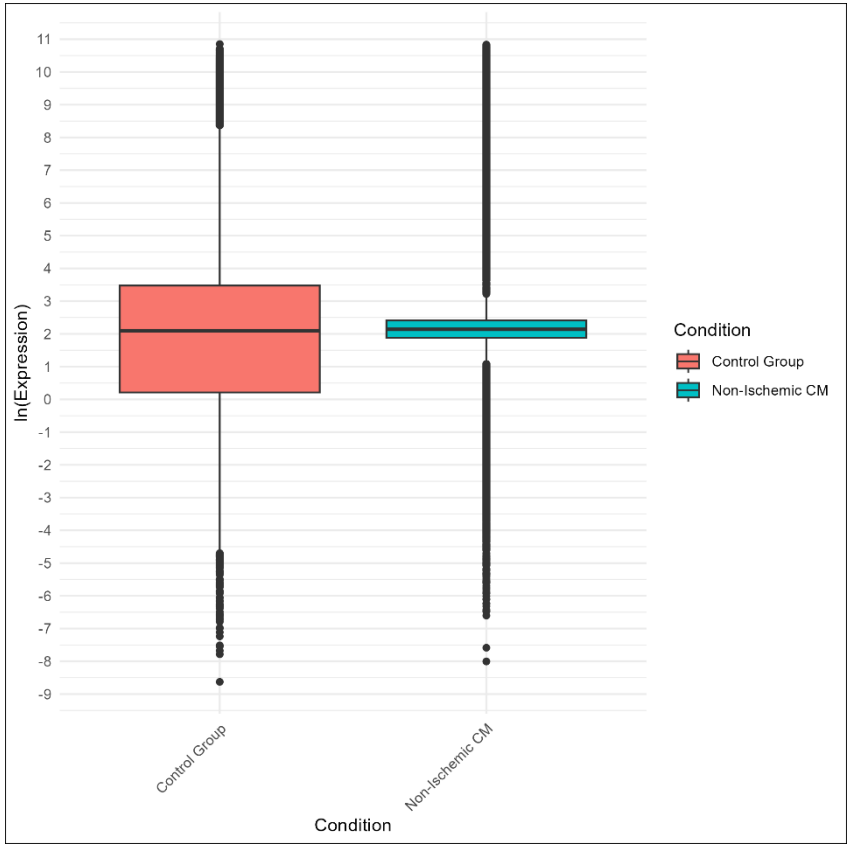
ACTN2 and PDLIM5 were also upregulated above the fold change cutoff, but these findings were not statistically significant, possibly due to high variability across samples.

= Shared Changes

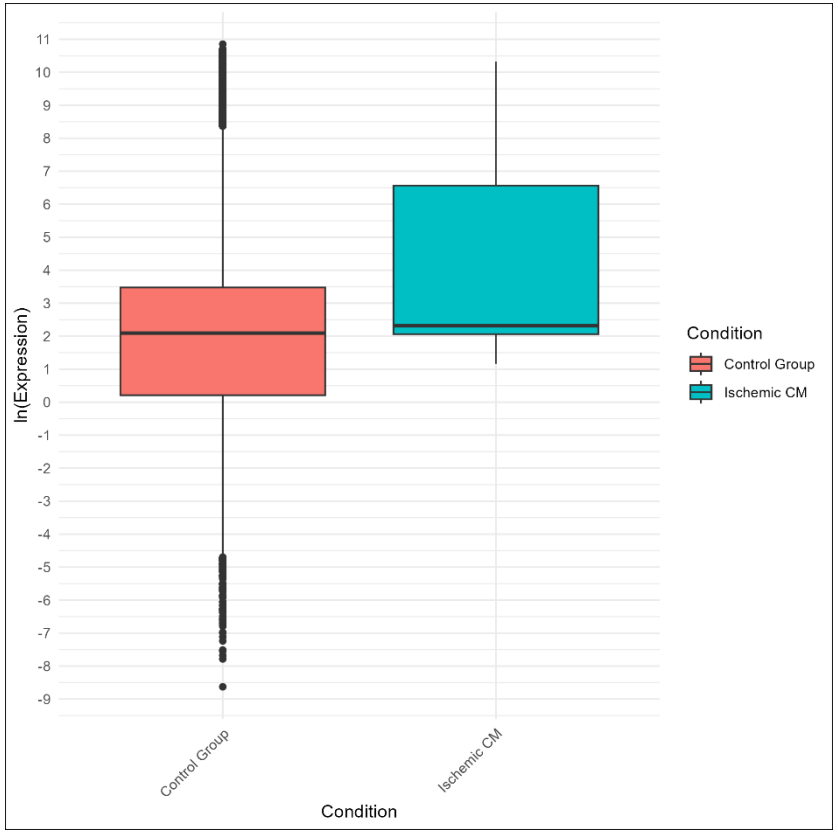
PDLIM3 upregulation shared between both CM types, more pronounced in ischemic CM.

Box plot analysis

Non-ischemic CM vs. Control group



Ischemic CM vs. Control group



Control Group

Moderate variability with both upregulated and downregulated outliers.

Non-ischemic CM

Narrower interquartile range, suggesting tightly regulated gene expression.

Ischemic CM

Broader distribution with fewer outliers, suggesting more uniform clustering.

Gene ID	Condition	Mean	SD	Variance	Median	Q1	Q3	IQR	Min	Max	Range
ACTN2	Control	5.74	4.48	19.63	4.01	2.34	4.11	2.75	2.26	19.63	17.37
ACTN2	Non-ischemic CM	5.69	4.14	16.92	4.00	3.92	4.12	0.20	0.20	16.92	16.72
ACTN2	Ischemic CM	7.98	4.71	21.73	4.35	3.96	13.38	9.42	3.41	21.73	18.31
CRYAB	Control	6.20	5.04	24.90	4.17	2.47	4.28	3.15	2.38	24.90	22.52
CRYAB	Non-ischemic CM	6.08	4.61	21.03	4.18	4.13	4.27	0.14	0.14	21.03	20.89
CRYAB	Ischemic CM	8.53	5.17	25.67	4.66	4.17	14.29	10.12	3.53	25.67	22.14
CSRP3	Control	6.04	5.00	23.83	4.11	2.37	4.23	3.11	2.25	23.83	21.58
CSRP3	Non-ischemic CM	5.91	4.45	19.57	4.13	4.02	4.22	0.21	0.21	19.57	19.36
CSRP3	Ischemic CM	8.33	4.08	24.49	4.58	4.10	13.94	9.82	3.34	24.49	21.16
FHOD3	Control	5.41	4.08	16.33	3.86	2.27	4.07	2.47	2.16	16.33	14.17
FHOD3	Non-ischemic CM	5.48	3.95	15.42	3.94	3.75	4.05	0.30	0.30	15.42	15.12
FHOD3	Ischemic CM	7.78	4.50	19.90	4.26	3.93	12.79	8.85	3.37	19.90	16.53
FLNC	Control	5.99	4.72	21.86	4.01	3.56	4.14	2.79	2.45	21.86	19.41
FLNC	Non-ischemic CM	5.65	3.94	15.38	4.02	3.89	4.11	0.20	0.20	15.38	15.18
FLNC	Ischemic CM	8.00	4.64	21.13	4.35	4.00	13.24	9.24	3.56	21.13	17.58
LDB3	Control	5.50	4.10	16.47	3.94	2.35	4.11	2.45	2.31	16.47	14.17
LDB3	Non-ischemic CM	5.57	4.00	15.91	3.89	3.82	4.07	0.25	0.25	15.81	15.56
LDB3	Ischemic CM	7.80	4.54	20.20	4.25	3.88	12.95	9.06	3.47	20.20	16.73
MYOZ2	Control	5.84	4.70	21.61	4.02	2.34	4.12	2.92	2.20	21.61	19.41
MYOZ2	Non-ischemic CM	5.77	4.32	18.41	4.00	3.95	4.10	0.16	0.16	18.41	18.24
MYOZ2	Ischemic CM	7.80	4.85	22.98	4.42	3.95	13.52	9.57	3.26	22.98	19.72
PDLIM3	Control	5.07	3.72	13.57	3.74	2.15	3.90	2.33	1.99	13.57	11.58
PDLIM3	Non-ischemic CM	5.19	3.55	12.46	3.80	3.66	3.91	0.25	0.25	12.53	12.27
PDLIM3	Ischemic CM	7.14	3.99	15.66	3.89	3.71	11.58	7.86	3.30	18.31	12.36
PDLIM5	Control	5.44	4.11	16.56	3.79	2.32	4.05	2.44	2.24	16.56	14.33
PDLIM5	Non-ischemic CM	5.34	3.72	13.66	3.79	3.69	4.05	0.36	0.36	13.66	13.30
PDLIM5	Ischemic CM	7.46	4.32	18.31	4.04	3.70	12.26	8.55	3.42	18.31	14.89
TTN	Control	5.58	4.21	17.35	4.17	2.23	4.27	2.69	2.08	17.35	15.27

Distribution of expression levels of target genes

1 Non-ischemic CM

Mean expression levels for control and non-ischemic CM conditions were similar across all genes, with differences typically less than 0.1. Non-ischemic CM samples displayed remarkably low IQR values and consistently showed the lowest variability across all parameters.

2 Ischemic CM

Ischemic CM displayed higher mean expression levels compared to both control and non-ischemic CM. Ischemic CM samples exhibited substantially higher IQR values and greater variability in gene expression.

Conclusions – Part 1 – 3882 genes

A comprehensive meta-analysis of expression profiles for Z-disc-associated genes across multiple microarray datasets

1 **Distinct Profiles**

Non-ischemic and ischemic CM show distinct gene expression profiles compared to control

2 **Gene Expression level**

General upregulation in both CM conditions, pronounced mostly in Ischemic CM

3 **Expression Patterns**

Non-ischemic CM shows tightly regulated expression, while ischemic CM displays greater heterogeneity

Conclusions – Part 2 – 10 Z-disc genes

CM subtypes show clear differences from control group with subtype-specific molecular changes

4 Key Genes in Non-ischemic CM

Downregulation of FLNC and upregulation of PDLIM3 in Non-ischemic CM

5 Key Genes in Ischemic CM

ACTN2, FHOD3, LDB3, PDLIM5 and PDLIM3 genes

are significantly upregulated in ischemic CM

Conclusions – Part 2 – 10 Z-disc genes

6

Shared Change

PDLIM3 upregulation is the only shared change, with greater magnitude in ischemic CM

7

Unique Changes

Unique differences in each subtype imply that Ischemic and Non-ischemic cardiomyopathies are driven by distinct molecular mechanisms despite some shared features

Future Perspectives

1. Further exploration of molecular differences between CM subtypes
2. Elucidating the functional roles of these genes and other Z-disc-associated components in CM disease mechanisms
3. Identifying the potential of Z-disc-specific DEGs as biomarkers or therapeutic targets for cardiomyopathies

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