The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 3, Issue 5, 2025

INVESTIGATING THE EXPRESSION OF NOVEL GENES IN HEART CELL

Shadab Izhar[1], Abdul Jabbar[*2], Naheed Bano[3], Dr. Alishbah Roobi[4], Aisha Ambreen[5], Fazeela Zaka[6]

[1]Department of Zoology, Lahore College for Women University, Jail Road, Lahore.
*[*2]Institute of Microbiology, University of Agriculture Faisalabad, Pakistan*
[3]Faculty of Veterinary & Animal Sciences, MNS-University of Agriculture Multan
[4]Department of Physiology, The University of Faisalabad
[5]Department of Biochemistry, Faisalabad Medical University, Pakistan
[6]Department of Animal Breeding and Genetics, Faculty of Animal Husbandry. University of Agriculture Faisalabad, Pakistan

[*1]dr.rabbaz@gmail.com

DOI: https://doi.org/10.5281/zenodo.15395356

Keywords
Myocardin, dilated cardiomyopathy, Pakistani population

Article History
Received on 05 April 2025
Accepted on 05 May 2025
Published on 13 May 2025

Corresponding Author: *
Abdul Jabbar

Abstract
To determine the expression of myocardin in dilated cardiomyopathy, the cross-sectional study was done on dilated cardiomyopathic subjects visiting Fatima Memorial Hospital Lahore during Jan, 2016 – June, 2016. The subjects were divided into two groups as diseased group (n=20) & control group (healthy persons) (n=20). A self-designed questionnaire was filled by each subject to collect data regarding age, exercise and disease history. RNA was isolated from the blood samples of studied subjects. After quantification of RNA by nano drop, cDNA was prepared and RT-PCR was performed for expression of myocardin. Statistical analysis was done by using SPSS. The expression level of Myocardin in DCM i.e. diseased group (n =20) was in the range of 1.6 to 7.5 with a mean value of 3.66± 0.357. While for control group containing healthy persons (n=20), gene expression level was 1.00 as assessed in blood samples of control group. The expression of myocardin gene has been increased in dilated cardiomyopathic subjects.

INTRODUCTION

Cardiomyopathy, a group of myocardial disorders and is emerging worldwide. Cardiomyopathy is a prolonged disease related to heart muscle (myocardium) causing ventricular dysfunction in heart. In this disease, cardiac muscle is abnormally enlarged, stiffened, thickened, or weakened. This weakened heart muscle, in turn, loses the ability to pump blood effectively, leading to irregular heartbeats (arrhythmias) and possibly even heart failure. It is the structural and functional abnormalities of the ventricular myocardium. Cardiomyopathies (disease with various types) are a group of non-inflammatory conditions of myocardium resulting in cardiac-dysfunction (Fazal and Tariq 2009).

Several forms of "hemodynamic stresses, such as myocardial infarction, "hypertension, and aortic "stenosis, lead to in cardiac compensatory remodeling that can "ultimately cause functional disorders and the outset of heart failure (HF). These and few other kinds of stress signals generate cardiac hypertrophy, which may progress to ventricular dilation. Sometimes, ventricular dilation and heart "failure is caused without intermediate hypertrophic stage (Olson, 2003; Sussman, 2002). Hence, these

complications ultimately lead to the situation of cardiomyopathy.

Cardiomyopathies are a group of non-inflammatory conditions of myocardin, resulting in cardiac dysfunction.7 In Europe and North America, 2 to 3% of the population had been affected in 2014. This displayed an increase from 0.4 to 1% of populations from 2005. In the unindustrialized world, its prevalence is about 0.6% for males and 0.4% for females. Cardiomyopathy is the second most common reason of heart failure in children of our country (Rahim, 2003).

It has several subtypes including dilated"cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), peripartum cardiomyopathy (PCM), arrhythmogenic right ventricular dysplasia (ARVD) etc. Dilated Cardiomyopathy (DCM) has been reported to be the most common type of cardiomyopathy in Pakistan (Khan *et al.*, 2010). Structural features of dilated cardiomyopathy include, increased left ventricular mass with increased left ventricular cavity size, having normal or reduced left ventricular wall thickness.

In the "lethal stages, thrombus may develop in the heads of ventricles" (Davies, 2000). The causes for dilated Cardiomyopathy (DCM) are largely not known but generally it is observed that disease represents a final common expression of myocardial damage that could be consequence of multiple insults, such as hemodynamic, infective, immunologic, toxic, nutritional or genetic factors (Wynne and Braunswald, 2001).

At molecular level, cardiac disorders affect genetic program. Prior to the onset of these disorders, cardiac remodeling occurs at molecular level (Oka *et al.*, 2007; Rajabi *et al.*, 2007). Myocardin (MYOCD) is a remarkably powerful transcriptional coactivator exclusively expressed in cardiomyocytes (Parmacek, 2007)." It is a cardiac muscle–specific and transcriptional co-activator of serum response factor (SRF) that trans-activates "CArG-box" comprising "cardiac" and "smooth muscle target genes", such as "atrial natriuretic factor (ANF)", being among the most "sensitive markers" of "hypertrophic signaling" (Wang *et al.*, 2001). It regulates its action by linking directly with serum response factor (SRF), which then attaches to CArG elements located in the "regulatory regions of many muscle cell structural genes (Miano, 2003). Its expression changes in response to stress and diseased condition which is also a cardio-protective mechanism.

Materials & Methods

In this case study, 20 patients with DCM and 20 healthy subjects (controls) of similar age and sex distribution, were enrolled after medical examination from Fatima Memorial Hospital Lahore. The duration of this study was from January 2016 to August 2016.

Fresh blood samples were collected from diseased as well as controls after the consent of hospital's ethical committee, according to inclusion & exclusion criteria.

Inclusion criteria

- patients with suspected heart failure,
- Patients with the presence of left ventricular (LV) dilation and dysfunction.
- LV end-diastolic dimension >55 mm.

Exclusion criteria

- Patients of any genetic disorder
- Inflammatory and infectious, autoimmune diseases

Physical examination, history and routine tests were also used to exclude patients with chronic or acute renal failure or hepatic failure.

Blood Samples were processed immediately after collection for RNA isolation. RNA was isolated by Trizole method. After RNA isolation, quantification of RNA was done by Nanodrop.

Isolated RNA was reverse transcribed for cDNA synthesis. That cDNA was confirmed by gradient polymerase chain reaction (PCR) and gel electrophoresis.

Kits used

- Thermo First Strand cDNA synthesis Kit Fermentas
- PCR Master Mix (2X) Fermentas
- SYBR Green Mix qPCR Master Mix (2X) Fermentas

Primer Designing

Primers were designed by primer designing tool (www.primer3) after retrieving myocardin (MYOCD) gene sequence from NCBI. Only exonic sequences

were used for primer designing to avoid genomic DNA contamination. The sequences were placed into primer designing tool i.e. (www.primer3) for primer designing.

Primer designed for myocardin gene is as follows:

Table-1: Primer designed for myocardin gene

Primer Sequence	Gene Name	Product size
F': 5'-TTCAGAGGTAACACAGCCTCC-3' R': 5'-TGATCCTCTCTAGCGTCTGCT-3'	MYOCARDIN	132 bp

Table-1: Primer designed for myocardin gene

Criteria, kept under consideration during primer designing are as follows:

- GC rich content near to 50%
- Primer length was kept 20-22 nucleotides
- Minimal complementarities within the primers of the same gene in order to avoid primer dimmer formation.

Then primers were optimized for best reaction conditions by PCR and gel electrophoresis. Gene expression of myocardin (MYOCD) was analyzed by real time- polymerase chain reaction (RT-PCR).

Statistical Analysis

Data was expressed as mean ± SE. Difference between groups was determined with help of t-test. While all the statements of significance were made on the probability level of 0.05 and confidence level 95%. Level of significance and standard error was determined by SPSS software for Windows.

Results

This case control study includes twenty patients with DCM (aged 39.1 ± 6.03 years) and 20 healthy subjects (aged 34 ± 3.71 years) (Figure 1)Among these, , 55% (n=12) were females with mean value of age 33.3 ± 0.50 and 45% (n=8) were males with mean value of age 62.37 ± 4.18 (Figure 2). In control group, 80% (n=8) were males with mean value of age 62.375±4.18 and 20% (n=2) were females with mean value of age 39± 5.0 (Figure 3). As both DCM and control group were age matched, there was no statistically significant difference between the groups.

On the basis of physical activity, three categories were formed: vigorous activity, moderate activity and sedentary life style. In DCM group, 5% (n=1) showed the vigorous activity, 20% showed moderate activity and 75% had sedentary lifestyle (Figure 4). In control group, 20 % (n=2) showed vigorous activity, 5% sedentary lifestyle and 70% were moderately active (Figure 5). In DCM group, 15% (n=3) former smokers were recorded (Figure 6) but in control group there were no smokers included. Age groups of the subjects in diseased group had distribution of 20% children (n=4), 5% young and 70% older individuals (Figure 7) because child subjects were all girls so included in female subjects distributing diseased group in male and female.

Systolic and diastolic blood pressure of the diseased group was found with the mean value of 125± 0.7 and 84±0.8 as compared to control group having 103± 0.9 and 79±1.5 respectively. Etiological attributes of the disease were idiopathic 65%, nutritional 15% and multifactorial 15% (Figure 8). Symptomatology of the disease includes 85% breathlessness, palpitation 75% and chest pain in 40% of the patients (Figure 9).

The expression level of myocardin in DCM i.e. diseased group (n =20) was in the range of 1.6 to 7.5 with a mean value of 3.66± 0.357. While for control group containing healthy persons (n=8), gene expression level was one as assessed in blood samples of diseased group as well as control group by RT-PCR. Myocardin level in both the groups was compared. Consequently, myocardin gene expression level was found to be more in diseased group i.e. dilated cardiomyopathy patients in comparison to control group containing healthy persons (Figure 10).

The reactivation of fetal gene program due to any mutation or cause which body encounters at molecular, cellular or genetic level, affects heart and switch towards fetal gene program increased the myocardin level in DCM patients. All the values were found statistically significant after statistical analysis ($P < 0.05$) after student's sample t-test and SPSS version 22.

The values for gene expression analysis as revealed by RT-PCR in case of control and diseased group are as follows in the table.

Table 2: Gene expression values for control and diseased samples

#	Values for gene expression (Control)	Values for gene expression (diseased)
1	1	1.6
2	1	1.8
3	1	2.7
4	1	3.2
5	1	4.5
6	1	2.84
7	1	2.95
8	1	3.3
9	1	7.4
10	1	3.0
11	1	2.7
12	1	2.4
13	1	4.9
14	1	2.4
15	1	4.2
16	1	2.2
17	1	3.7
18	1	6.5
19	1	5.4
20	1	5.7

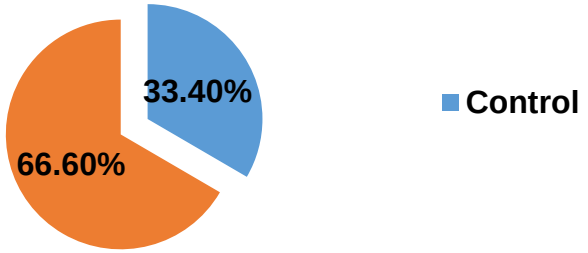

Figure 1: %age distribution of total subjects in diseased (Cardiomyopathy) and control (heathy) group

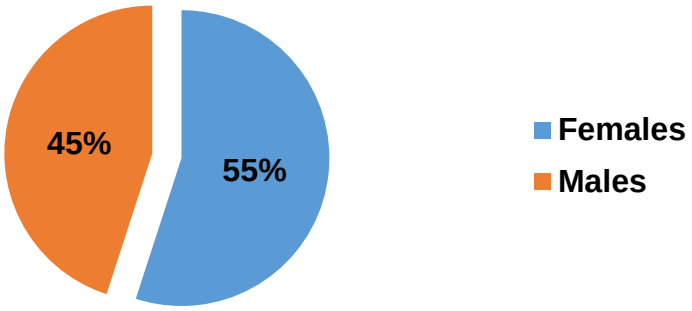

Fig. 2: %age distribution of both male and female subjects in cardiomyopathy

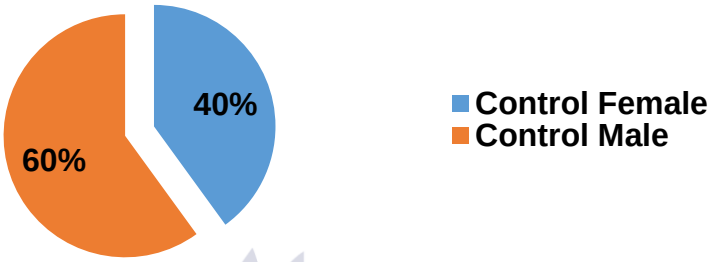

Figure 3: %age distribution of both male and female in Control group

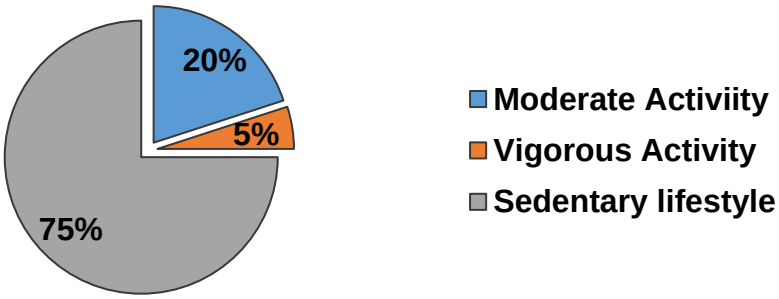

Figure 4: Prevalence %age of physical activity in diseased group (dilated cardiomyopathy)

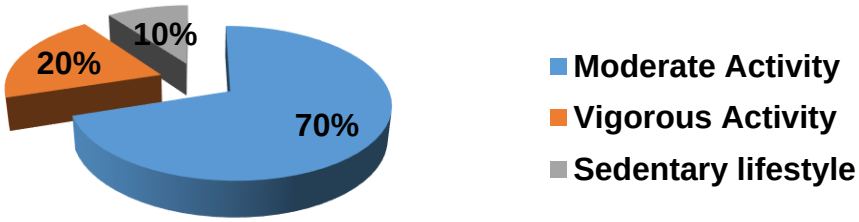

Figure 5: Prevalence %age distribution of physical activity in control group

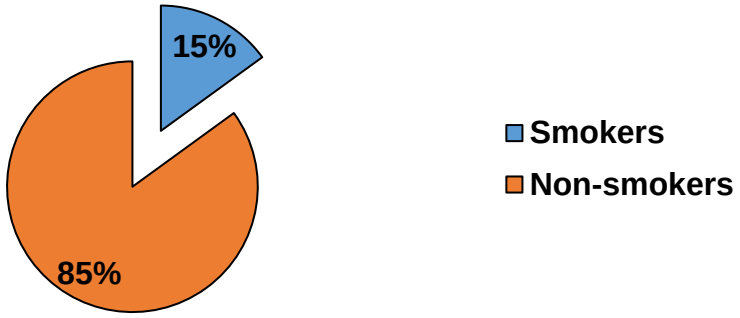

Figure 6: Prevalence %age of smoking in diseased group

Figure 7: Symptomatology of the disease group (dilated cardiomyopathy).

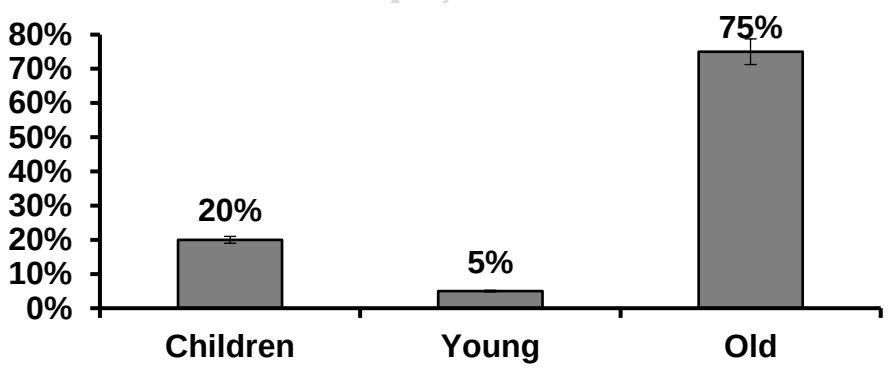

Figure 8: Distribution of age groups in dilated cardiomyopathy subjects

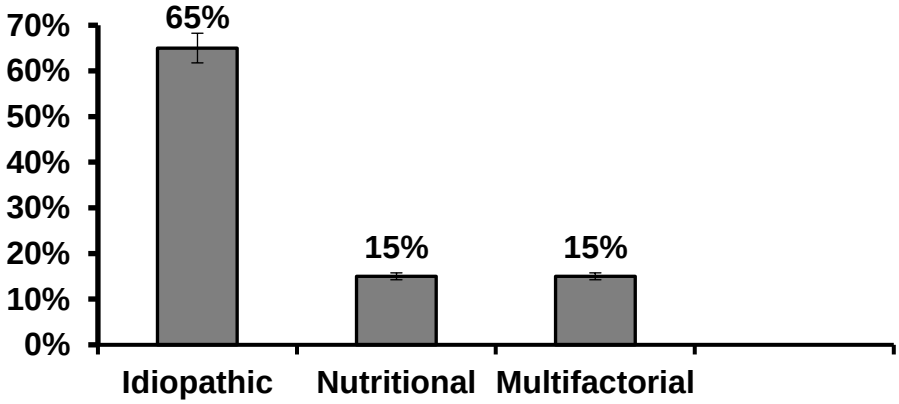

Figure 9: Attributes of disease

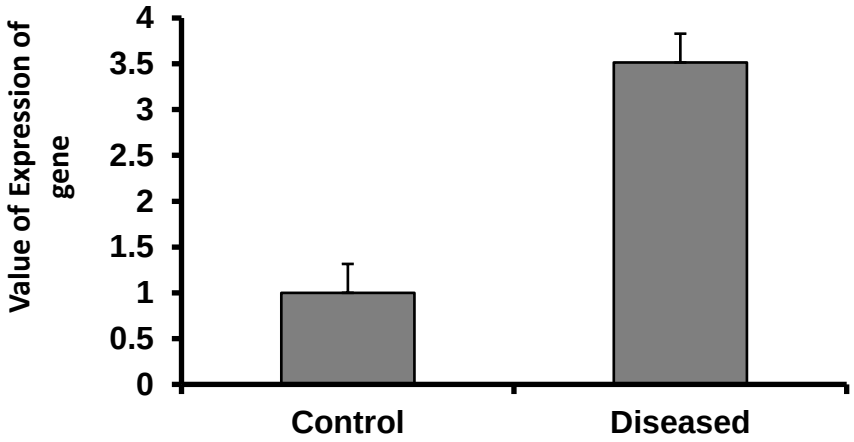

Figure 10: Comparison of Myocardin gene expression (mean ± SE) in control/healthy person and diseased (Dilated Cardiomyopathy) subjects

Discussion

Cardiomyopathy is a prolonged disease related to heart muscle causing ventricular dysfunction. WHO has recognized that cardiac dysfunction may result from a failure to control hypertension or to control volume/pressure burden in valve disease (Davis, 2007). Among cardiovascular problems, cardiomyopathy has originated as prominent cardiac disorder leading to heart transplantation. Among all types of cardiomyopathies, dilated cardiomyopathy occupies significant position. From the last two decades, it has shown increased prevalence. The prevalence of dilated cardiomyopathy in western countries is round about 36.5 lacs with annual incidence of 5 to 8 per lac population (Brawnswald, 1991).

The present study observed the expression of myocardin gene in dilated cardiomyopathy subjects. Among cardiomyopathy, subjects suffering from dilated cardiomyopathy were selected as treatment and for comparison selected healthy persons as control. In comparison to control group, myocardin's overexpression was demonstrated in diseased group i.e. dilated cardiomyopathy subjects. This alteration in "myocardin gene expression" in the blood cells of dilated cardiomyopathic subjects is observed under the influence of diseased condition and defected gene program at molecular level.

Just like in previous studies by Torrado *et al.*, 2003 and Joanna *et al.*, 2010 , myocardin expression has been shown to be augmented in failed hearts compared with non-failed, augmented expression of myocardin in blood cells in patients was found in our study when compared with healthy individuals. Joanna *et al.*, 2010 in a study observed a positive relation between myocardin expression and left ventricular ejection fraction (LVEF). It demonstrated that increased expression of myocardin reflects better left ventricular function. Perhaps, that increased expression of myocardin, at least in the peripheral blood of idiopathic dilated cardiomyopathy subjects, attends a less acute stage of the disease. The result of our study is in according to previous study that showed that before transplantation forced expression of myocardin in was associated with better left ventricular function (Joanna *et al.*, 2010).

In this study, myocardin expression in dilated cardiomyopathy (DCM) suffering subjects was analyzed by RT-PCR. The RT-PCR analysis revealed the overexpression and upregulation of myocardin in DCM from the range of 1.6 to 5.7 having mean value 3.66 ± 0.357 having far more values than controls. The average increase in the myocardin level observed to be 3.305. Another study confirms the overexpression of myocardin level in DCM patients in which levels of myocardin mRNA in failing left ventricular myocardium from patients of dilated cardiomyopathy were assessed by both semi-quantitative RT-PCR and northern blotting. The results showed that all deteriorating left ventricular samples with elevated levels of myocardin mRNA ranging from 2.0 to 7.2 records having enhancement

over non-failing left ventricular myocardium samples. Averaged upsurge of the myocardin mRNA in DCM samples was 4.5 as compared to normal. RT-PCR data was confirmed by northern blotting experiments, showing up-regulated myocardin mRNA levels in failing hearts (Torrado *et al*., 2003).

Present studies showed certain and direct relationship in the myocardin's increased expression and dilation of the left ventricle. It is obvious to accept that early-developmental genes are related to the onset of the disease. Our results for the expression of myocardin gene in DCM patients compared to healthy individuals are in settlement with other animal and human studies using myocardial tissue. Early cardiac genes in peripheral blood might possibly be expressed in circulating stem cell progenitor cells that reside in the bone marrow. This indicates that peripheral blood could feasibly act as radar of gene expression adjustments happening in response to disease.

The results of present study augmented the support for this hypothesis that gene expression in peripheral blood cells may be the reflection of the disease harshness. As proved by Obikili, and Okoye, 2005, there is a strong correlation between the cardiac diameter and body mass index, suggesting that the cardiac diameter is principally dependent on the body build (Obikili and Okoye 2005). This present study also proved the hypothesis that blood could perhaps be a noble alternate for expression enquiry, even in non-endstage cardiovascular disease (Jabbar *et al*., 2023).

Conclusion

In this study, it is concluded that "myocardin (MYOCD)" gene is augmented in dilated cardiomyopathy suffering subjects as compared to healthy subjects. Its expression in dilated cardiomyopathy is recorded as (mean ± SE) 3.66 ± 0357. This altered expression is one of the most significant factors in causing the dilation of the heart ventricle. It is an important factor in the progression of dilated cardiomyopathy. Its expression is Cardiac gene transcript levels changes in the peripheral blood cells of DCM patients by following indices of LV function. It can be suggested here that gene expression levels in peripheral blood cells of IDCM patients may reflect the disease severity. Therefore, peripheral blood could possibly be a good substitute tissue for gene expression analysis in cardiomyopathies.

REFERENCES

Akazawa H, Komuro I. Roles of cardiac transcription factors in cardiac hypertrophy. Circulation Research . 2003;92:1079–1088

Chen F, Kook H, Milewski R, Gitler AD, Lu MM, Li J. Hop is an unusual homeobox gene that modulates cardiac development. Cell 2002;110:713– 23

Davis J, Wen HT, Edwards J, Metzger M. Thin filament disinhibition by restrictive cardiomyopathy mutant R193H troponin I induces Ca2+-independent mechanical tone and acute myocyte remodeling. Circ. Res. 2007;100:1494–1502.

Davis RC, Hobbs FD, Lip GYH. History and epidemiology 2000;320(7226): 39-42.

Epstein ND, Davis JS. Sensing stretch is fundamental. Cell. 2003;112:147-150.

Fazlur Rahim, M. Younas, A.J. Gandapur, A Talat. 2003. Pattern of congenital heart Diseases in children at tertiary care center in Peshawar. *Pakistan Journal of Medical Sciences*. 1682-024X.

Frey N, Olson EN. Cardiac hypertrophy: the good, the bad, and the ugly. Annual Review of Physiology 2003;65:45–79

Jabbar A, Ashraf M, Rahman SU, Sajid MS. Prevalence, molecular characterization and antibiogram of Mycoplasma bovis isolated from milk in Pakistan. Polish Journal of Veterinary Sciences. 2023:461-71.

Joanna KE, Parthenakis FI, Nyktari EG. Myocardial gene expression alterations in peripheral blood mononuclear cells of patients with idiopathic dilated cardiomyopathy. European Journal for Heart Fail 2010;12:541–548.

Kontaraki JE, Parthenakis FI, Patrianakos AP, Karalis IK, Vardas PE. Altered expression of early cardiac marker genes in circulating cells of patients with hypertrophic cardiomyopathy. Cardiovascular Pathology 2007;16(6), 329-335.

Lakdawala NK, Stevenson LW, Loscalzo J, Fauci AS, Hauser SL, Longo DL, Jameson JL, Loscalzo J, *et al.* Harrison's Principles of Internal Medicine (19th ed.). McGraw-Hill. 2015;1553.

Liao XH, Wang N, Liu QX, *et al.* Myocardin-related transcription factor-A induces cardiomyocyte hypertrophy. IUBMB Life. 2011;63(1):54–61.

Miano JM. Serum response factor: toggling between disparate programs of gene expression. Journal of Molecular and Cellular Cardiology. 2003;35:577–593

Oka T, Maillet M, Watt AJ, Schwartz RJ, Aronow BJ, Duncan SA. Cardiac-specific deletion of Gata4 reveals its requirement for hypertrophy, compensation, and myocyte viability. Circulatory Research. 2006;98:837–845.

Oka T, Xu J, Molkentin JD. Re-employment of developmental transcription factors in adult heart disease. Seminars in Cell & Developmental Biology 2007;18:117–131.

Olson EN, Schneider MD. Sizing up the heart: development redux in disease. Genes Development. 2003;17:1937–1956.

Parmacek MS. Myocardin-related transcription factors: critical coactivators regulating cardiovascular development and adaptation. Circulation Research. 2007;100(5):633–644

Rajabi M, Kassiotis C, Razeghi P, Taegtmeyer H. Return to the fetal gene program protects the stressed heart: a strong hypothesis. Heart Fail Review 2007;12:331–343.

Taegtmeyer H, Scaglia F, Graham BH, Kralli A, Shimizu N, Tanaka H, Makela TP, Schneider MD. Menage-a-Trois 1 is critical for the transcriptional function of PPAR gamma coactivator 1. Cell Metabolism 2007;5:129–142

Wang D, Chang PS, Wang Z, Sutherland L, Richardson JA, Small E, Krieg PA, Olson EN. Activation of cardiac gene expression by myocardin, a transcriptional cofactor for serum response factor. Cell. 2001;105: 851–862.

Wang Z, Wang DZ, Hockemeyer D, McAnally J, Nordheim A, Olson EN. Myocardin and ternary complex factors compete for SRF to control smooth muscle gene expression. Nature. 2004;428:185–189.