RESEARCH ARTICLE Indian Journal of Animal Research, Volume 57 Issue 6: 692-697 (June 2023)

Investigation of Mutations in Exon 12 Of *β-MYH7*, 16 Of *MYBPC3* and 2 Of *TCAP* Gene in Dogs with Dilated Cardiomyopathy using PCR-SSCP Technique

R.B. Vishnurahav[1], S. Ajithkumar[2], Usha Narayana Pillai[1], N. Madhvan Unny[1], K.D. John Martin[3], T.V. Aravindakshan[4]

10.18805/IJAR.B-4347

ABSTRACT

Background: Dilated cardiomyopathy is the important myocardial disease and one of the most common cause of death in the medium to large size dog breeds worldwide. The disease is characterized by dilatation of cardiac chambers and thinning of walls leads to systolic failure. Mutations in some sarcomere genes leads to cardiomyopathy in humans. Sarcomere is an important multifunctional protein network involved in the signal reception and transduction. Mutations in *β-MYH7, MYBPC3* and *TCAP* genes produce alterations in the morphology of heart (hypertrophy or dilatation).

Methods: In this study twenty apparently healthy and twenty five dogs with dilated cardiomyopathy (DCM) were selected from patients reported or referred to University Veterinary Hospital and Teaching Veterinary Clinical Complex, Mannuthy (2015-2017) based on the clinical examination, radiographic, electrocardiographic, haematobiochemical and echocardiographic studies cardiac disorders (Dilated cardiomyopathy and hypertrophic cardiomyopathy) were confirmed.

Result: In the present study we investigated genetic alterations of exon 12 of *β-MYH7*, 16 of *MYBPC3* and 2 of *TCAP* gene in dogs by polymerase chain reaction -single stranded confirmation of polymorphism (PCR-SSCP). Polymerase chain reactions were analysed using acrylamide gel and samples with different pattern of bands were sequenced. Polymerase chain reaction-SSCP showed different migration of band pattern in the intron 1 of *TCAP* gene in one sample.

Key words: Dilated cardiomyopathy, Mutations, Sarcomere, SSCP.

INTRODUCTION

Dilated cardiomyopathy is a myocardial disease of heart in dogs characterized by dilated cardiac chambers with systolic dysfunction. It can also be seen in all age groups from juvenile to adult. Mutations in *β-MYH7, MYBPC3* and *TCAP* sarcomeric genes produce alterations in the morphology of heart (hypertrophy or dilatation). In human cardiomyopathies, polymorphisms in sarcomere genes and their pattern of inheritance reported. Sarcomeres are important multifunctional ordered protein network involved in the signal reception and transduction. Major cardiac specific sarcomere proteins are coded through *β-MYH7* and *MYBPC3* genes. These genes provide instructions for making protein that are involved in sarcomere organisation and rigidity and binds with thick filaments. Beta myosin heavy chain 7 (*β-MYH7*) and myosin binding protein (*MYBPC3*) are cardiac isoforms of protein extensively expressed in heart muscle. Titin is giant cardiac protein that extends half the length of a sarcomere with kinase activity. Telethonin (Titin-cap or *TCAP*) gene produces titin cap protein and it acts as a scaffold to which myofibrils and other related proteins attach and suggests an important role of *TCAP* in the assembly of sarcomere.

The present study includes the screening of polymorphisms in the above-mentioned genes to find out

[1]Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy-680 651, Kerala, India.
[2]University Veterinary Hospital and TVCC, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy-680 651, Kerala, India.
[3]Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy-680 651, Kerala, India.
[4]Department of Animal Breeding and Genetics, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy-680 651, Kerala, India.

Corresponding Author: R.B. Vishnurahav, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy-680 651, Kerala, India.
Email: vishnu.vet6@gmail.com

How to cite this article: Vishnurahav, R.B., Ajithkumar, S., Pillai, U.N., Unny, N.M., Martin, K.D.J. and Aravindakshan, T.V. (2023). Investigation of Mutations in Exon 12 Of *β-MYH7*, 16 Of *MYBPC3* and 2 Of *TCAP* Gene in Dogs with Dilated Cardiomyopathy using PCR-SSCP Technique. Indian Journal of Animal Research. 57(6): 692-697. doi: 10.18805/IJAR.B-4347.

Submitted: 28-10-2020 **Accepted:** 05-03-2021 **Online:** 15-04-2021

genetic variations and underlying pathophysiology of dilatation or hypertrophy. These variations might be helpful in the early diagnosis of cardiomyopathies. Single strand confirmation polymorphism (SSCP) is an ideal technique used to detect polymorphisms.

The genes (*i.e.* cardiac troponin T, α-actin and β-myosin heavy chain) suspected for the pathogenesis and familial transmission of DCM in dogs were studied in animal models like mice, rats and hamsters (Chorro *et al.*, 2009). Common genetic variations in of *β-MYH7* gene (exons 7, 12, 19 and 20) were reported in human cardiomyopathies. All these genotypes were homozygous in DCM and three genotypes were heterozygous in hypertrophic cardiomyopathy (Tanjore *et al.*, 2010). Artur and Anna (2012) discovered a protein that converted chemical energy into mechanical force and it was controlled by a myosin heavy chain encoded by *β-MYH7* gene.

Myosin binding protein C (MyBP-C) was a thick filament that constituted two to four per cent of the myofibril and was involved in the regulation of muscle contraction. It regulated sarcomere organisation and rigidity. MyBP-C bound to myosin at two binding sites, namely C-terminal and N-terminal where the N-terminal site consisted of two immunoglobulin domains (C1 and C2). These domains connected by a flexible linker, which interacted with the S2 segment of myosin in a manner of regulated phosphorylation (Ababou *et al.*, 2008). Tanjore *et al.* (2008) reported mutations in the exon regions of 16 and 24 of *MYBPC3* gene in human patients with hypertrophic cardiomyopathy in India[9]. Mutations in MyBP-C gene were the second most common cause of hypertrophic cardiomyopathy (HCM). Mice with homozygous cardiac myosin binding protein C-deficient (Mybpct/t) developed intense cardiac dilation immediately after birth (almost two fold increase in the heart size). Majority of these cardiac MYBPC3 mutations encoded truncated proteins which lacked the portion of either titin binding domain or the carboxyl myosin. These mutations were thought to cause cardiac hypertrophy by inducing myocyte hypertrophy (increased cell size). Heterozygous cardiac myosin binding protein (MYBPC3+/-) mice developed intense myocyte hypertrophy (increase in cell size). Homozygous cardiac myosin binding protein (Mybpc3-/-) mice primarily developed myocyte hyperplasia (Jianga *et al.*, 2015). Moss *et al.* (2015) reported that the rate and force of myocardial contraction through interactions with either actin or myosin or both were regulated by cardiac myosin binding protein C gene (MYBPC3). The binding capability of this protein was dependant on its phosphorylation status.

A 19-kDa protein named telethonin (TCAP) was responsible for the modulation of the elastic properties of giant protein titin. It also involved in coupling action with Z-disc during stretching and contraction of sarcomere in cardiac myocytes (McNally *et al.*, 2013). Telethonin (titin-cap or t-cap) was found to be expressed almost exclusively in cardiac and skeletal muscle, with a single isoform encoded by the TCAP gene and high sequence homology across species. The mutations of TCAP gene were associated with cardiac and some other skeletal myopathies. Cardiac telethonin was noticed to act as an interaction partner for the protein kinase. Several missense TCAP mutations associated with human cardiomyopathies (DCM and HCM) was known to introduce single residue substitutions within the C-terminal 36 amino acids of TCAP protein, which might interfere with the phosphorylation status of telethonin (Candasamy *et al.*, 2014).

MATERIALS AND METHODS

Samples

In this study twenty apparently healthy and twenty five dogs with dilated cardiomyopathy (DCM) were selected from patients reported or referred to University Veterinary Hospital and Teaching Veterinary Clinical Complex, Mannuthy based on the clinical examination, radiographic, electrocardiographic, haematobiochemical and echocardiographic studies cardiac disorders were confirmed. The inclusion criteria for DCM were Electrocardiographic evidence of arrhythmias, which included atrial fibrillation, ventricular premature complexes and ventricular tachycardia. Radiographic evidence of chamber enlargement either, left atrium or ventricle and right atrium or ventricle or whole heart. Echocardiographic evidence of chamber enlargement included dilated left ventricle and atrium or whole heart, increased EPSS, poor ejection fraction (<40%), fractional shortening (<20-25%) in absence of other acquired or congenital or stenotic cardiac lesions (McEwan *et al.*, 2003; Borgarelli *et al.*, 2006 and Jeyaraja *et al.,* 2015).

After confirmation of DCM required blood samples (2 ml) were collected into BD Vacutainers with spray coated 5.4 mg ethylene diamine tetra acetic acid (EDTA) as anticoagulant under aseptic conditions. The samples were brought to the laboratory at 4°C, temperature being maintained with the aid of ice packs and then transferred to -20°C until used for DNA extraction. The DNA of samples collected were extracted. DNeasy Blood and Tissue Kit was used for DNA extraction (QIAGEN Cat No. 69504).

The concentration, purity and quality of DNA were checked by NanodropTM 1000 spectrophotometer (Thermo Scientific, USA). The purity of the DNA was verified by measuring absorbance at 260 nm and 280 nm. A 260/280 ratio of approximately 1.8 is generally accepted as “pure” for DNA. The DNA samples with value between 1.7-1.8 were used for further downstream processing.

Primers

In humans, common genetic variations in the exon 12 of *β-MYH7*, 16th exon of *MYBPC3* and second exon of *TCAP* gene were observed in both the HCM and DCM. The primers for amplification of exon sequences were designed based on the 12th exon of canine *β-MYH7* (GenBank Accession No: DQ 227285.1), 16th exon of *MYBPC3* (GenBank Accession No: NM001048106.1) and second exon of *TCAP* gene (GenBank Accession No: NC_006591.3) obtained from

NCBI using the tool Primer 3 (V.0.4.0). Primers were custom synthesized commercially (Sigma-Aldrich) and obtained in lyophilized form.

Primers (5′-3′) designed to amplify exon-12 (303 bp fragment) of *β-MYH7* gene, exon-16 (438 bp fragment) of *MYBPC3* and exon-2 of *TCAP* gene were given below:

Exon-12 of *β-MYH7* gene
FP: ATTGGCCTC TCCC TG AGT,
RP: TCCTGATACTGCCC CTGAAC,
Exon-16 of *MYBPC3* gene
FP: AGTGTGAGGTGTCCAGG AG,
RP: AAAAGTGAGGCTCGGTGTGT
Exon-2 of *TCAP* gene
FP: ACCCCTTCTGTATCCCAGGT,
RP: CCTCTCAGCCTCTCTGTGCT

The primers containing vials were centrifuged in a micro centrifuge for about 30 seconds to prevent the loss of contents. They were reconstituted in sterile de-ionized double distilled water to a concentration of 100 pM/µl. A particular amount of nuclease free water as specified by the manufacturer was added to obtain a stock concentration of 100 pM/µl. Stock solutions were incubated at room temperature for one hour and working solution was prepared in sterile 1.5 ml micro centrifuge tube with a concentration of 50 pM/µl and stored at -20°C.

Polymerase chain reaction

The PCR was used to amplify the exon 12 of *β-MYH7*, exon 16 of *MYBPC3* and exon 2 of *TCAP* gene fragments. The PCR master mix (2X) (Catalog number: K0171) was used for amplification. The optimization of PCR conditions was achieved through gradient PCR (Bio-Rad thermal cycler). For these, modifications in different time-temperature combinations of annealing and extension steps were used. The temperature gradient, which provided the best results for amplification was selected for all downstream use. The reaction was carried out in 0.2 ml PCR tubes and details of PCR reaction mix are provided below. The master mix prepared was spun briefly. Polymerase chain reaction was performed in a Bio-Rad thermal cycler (USA). Electrophoresis was carried out at 4V/cm until the bromophenol blue dye migrated more than two by third length of the gel and was photographed in a Gel Doc System (Bio-Rad, USA).

PCR was performed under standard conditions in a 50 µL reaction mixture containing 5 µL of template cDNA, 5 µL of 50 pM/µL of each primer, 25 µL of PCR master mix (2X) (Thermo scientific) and 18 µL of nuclease free water.

The reaction mixture was initially denatured for 5 min at 95°C and incubated for 34 cycles (denaturing for 30 s at 95°C, annealing for 30 s at 60°C and extending for 30 s at 72°C). Final extension was continued for 5 min at 72°C for the amplification of exon-12 of *β-MYH7* gene (303 bp fragment).

The reaction mixture was initially denatured for 5 min at 95°C and incubated for 34 cycles (denaturing for 30 s at 95°C, annealing for 30 s at 61°C and extending for 30 s at 72°C). Final extension was continued for 5 min at 72°C for the amplification of exon-16 of *MYBPC3* gene (438 bp fragment).

The reaction mixture was initially denatured for 5 min at 95°C and incubated for 34 cycles (denaturing for 30 s at 95°C, annealing for 30 s at 58.5°C and extending for 30 s at 72°C). Final extension was continued for 5 min at 72°C for the amplification of exon-2 of *TCAP* gene (508 bp fragment).

PCR-SSCP analysis of mutations

PCR-SSCP was performed to genotype all the samples. This technique involves denaturation of the double-stranded PCR product with heat and formamide and resolution of single-stranded DNA fragments on polyacrylamide gel. During electrophoresis, single stranded DNA fragments fold into complex three dimensional structures as a result of intra-strand base pairing. Even a single nucleotide difference can change the folding pattern of the strand and therefore, single strands of equal length but different sequence form separate bands.

Single strand confirmation polymorphism was performed using PCR products of exon-12 of *β-MYH7,* 16 of *MYBPC3* and 2 of *TCAP* genes. Aliquots of five microlitre PCR products were mixed with a 10 ml denaturing solution (9.5 ml formamide deionized, 0.4 ml of 0.5*M* EDTA, 2.5 mg xylene-cyanole and 2.5 mg bromophenol blue), heated for 10 minutes at 95°C and snap chilled immediately in ice for 10 minutes. Denatured PCR products were subjected to polyacrylamide gel electrophoresis (PAGE).

For Polyacrylamide gel electrophoresis, the glass plates, spacers and combs were washed with soap water and air dried in a dust free chamber. Glass plates were assembled vertically on the casting tray with the spacer placed between the glass plates.

Polyacrylamide gel solution was prepared by mixing acrylamide and bis acrylamide in the ratio of 29:1 and gel solution excluding 10X TBE, ammonium persulphate (APS) and N,N,N',N', Tetra Methyl Ethylene Diamine (TEMED) was prepared and stored under refrigeration in amber coloured bottle. The composition of 12 per cent polyacrylamide gel was used for this study. For preparing of gel solution, reagents were taken in a beaker, mixed well and the mixture was poured between the glass plates avoiding air bubbles. The 15 wells comb was inserted on top carefully. One hour was allowed to set the gel and plates were assembled in the sequencer. The comb was removed and wells were cleaned with buffer. The upper and lower buffer tanks were filled with 1X TBE buffer. Now, the denatured ssDNA samples were loaded into the wells. The gel was run at constant voltage for a fixed time at 12°C, in a vertical electrophoresis apparatus (Hoefer, USA). The voltage and running time for each fragment were standardized for each fragment. The SSCP pattern were visualized by silver staining and photographed and analysed. Silver staining was carried out according to the procedure described by Byun *et al.* (2009).

Electrophoresis (PAGE) conditions for each fragment of *β-MYH7*, *MYBPC3* and *TCAP* gene were given below: Exon 12 of *β-MYH7* gene (303 bp fragment): 9.3V/cm with the running time of 19 hours, exon 16 of *MYBPC3* gene (438 bp fragment): 15V/cm with the running time of 25 hours and exon 2 of *TCAP* gene (508 bp fragment): 10.6V/cm with the running time of 25 hours.

Representative PCR products showing different SSCP band patterns were selected and sequenced using respective forward and reverse primers to detect the polymorphisms, if any, at nucleotide level. Sequencing was performed by automated sequencer (ABI prism) using Sanger's dideoxy chain termination method at SciGenom Labs Pvt. Ltd., Cochin. The obtained sequences were aligned with other sequences in GenBank using BLASTn and EMBOSS merger.

RESULTS AND DISCUSSION

After amplification of DNA fragments isolated from blood samples of 25 patients, using PCR and SSCP procedures one sample with a change in the intron 1 of TCAP gene was observed. The PCR-SSCP analysis of the 508 bp fragment of *TCAP* gene showed two distinct band patterns representing two genotypes (AA and AB). Sequencing of representative products from each group revealed one novel SNP at position 36 (A→G transition) of the 508 bp amplicon (Position 262 of whole TCAP gene; chromosome 9). The polymorphism identified was in the region of intron one (position 149 of intron one) of *TCAP* gene fragment. According to the nucleotide combinations, two alleles A and B and two genotypes (AA and AB) were observed (Fig 1 and 2). Since the SNP was novel no published data is available on the frequency distribution. The genotype frequency observed for AA genotype was 0.04 and 0.96 for AB genotype in the whole population. The allele frequency of A and B were 0.52 and 0.48 respectively. No significant difference was noticed in genotype and allele frequencies. The 303 bp fragment of *β-MYH7* gene (exon-12) showed unique two band pattern and the animals studied were monomorphic (Fig 3 and 4). The 438 bp fragment of *MYBPC3* gene (exon-16) showed unique two band pattern and the animals studied were monomorphic (Fig 5 and 6). Both of these fragments indicating the highly conserved nature of this region.

Polymorphism studies

Polymorphism study of *β-MYH7, MYBPC3* and *TCAP* genes

Polymerase Chain Reaction-SSCP was performed to detect polymorphism in exon-12 of *β-MYH7,* exon-16 of *MYBPC3* and exon-2 *TCAP* gene

The 303 bp fragment of *β-MYH7* gene (exon-12) showed unique two band pattern and the animals studied were monomorphic indicating the highly conserved nature of this region. The 438 bp fragment of *MYBPC3* gene (exon-16) showed unique two band pattern and the animals studied were monomorphic indicating the highly conserved nature of this region.

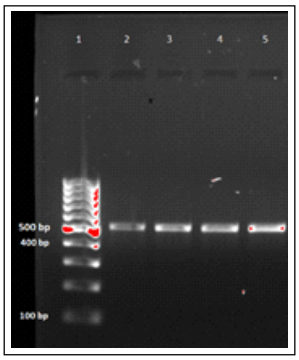

Fig 1: PCR amplification of 508 bp fragment of *TCAP* gene (exon 2). Lane 1: 100 bp DNA marker; Lane 2-5: 508 bp PCR product.

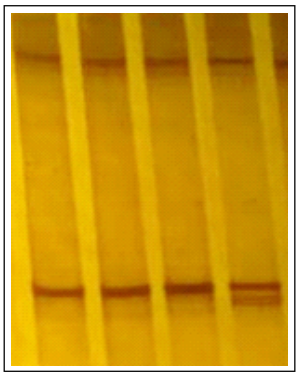

Fig 2: SSCP pattern of 508 bp fragment of *TCAP* gene (exon 2).

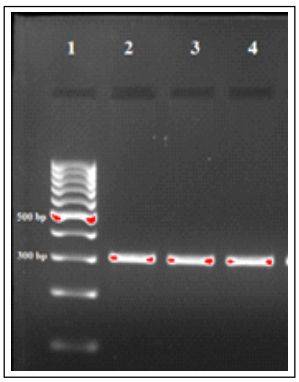

Fig 3: PCR amplification of 303 bp fragment of *β-MYH7* gene (exon 12). Lane 1: 100 bp DNA marker; Lane 2-5: 303 bp PCR product.



Fig 4: SSCP pattern of 303 bp fragment of *β-MYH7* gene (exon 12).

The PCR-SSCP analysis of the 508 bp fragment of *TCAP* gene showed two distinct band patterns representing two genotypes (AA and AB). Sequencing of representative products from each group revealed one novel SNP at position 36 (A→G transition) of the 508bp amplicon (Position 262 of whole TCAP gene; chromosome 9). The polymorphism identified was in the region of intron one (position 149 of intron one) of *TCAP* gene fragment. According to the nucleotide combinations, two alleles A and B and two genotypes (AA and AB) were observed (Fig 7 and 8). Since the SNP was novel no published data is available on the frequency distribution.

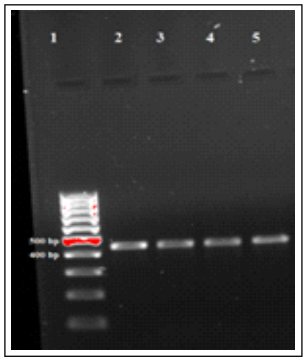

Fig 5: PCR amplification of 438 bp fragment of *MYBPC3* gene (exon 16).
Lane 1: 100 bp DNA marker; Lane 2-5: 438 bp PCR product.

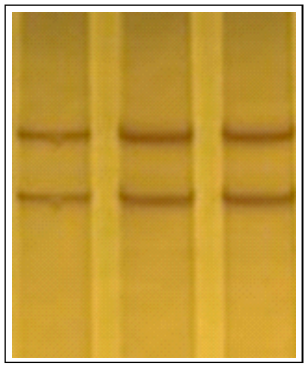

Fig 6: SSCP pattern of 438 bp fragment of *MYBPC3* gene (exon 16).

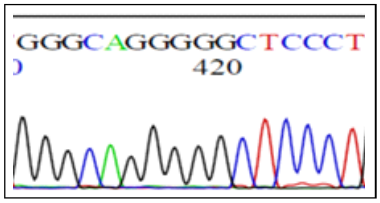

Fig 7: Sequence map of AB diplotype of *TCAP* gene (intron 1).

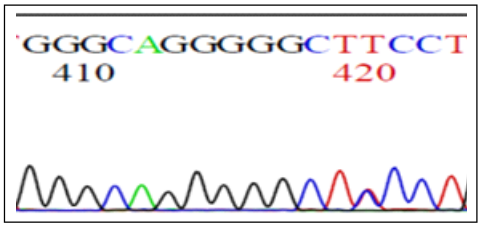

Fig 8: Sequence map of AA diplotype of *TCAP* gene (intron 1).

Sequence analysis

Nucleotide sequence homology

The exon 12 sequence of *β-MYH7* gene from dogs showed 100 per cent homology with *Canis lupus familiaris* slow myosin heavy chain beta coding sequence having 98 per cent with predicted cat and tiger sequence and 97 per cent with predicted leopard sequence. Exon 12 region of this gene was found to have higher per cent of identity with carnivores like cat, tiger and leopard. The exon 16 sequence of *MYBPC3* gene from dogs showed 100 per cent homology with *Canis lupus familiaris* was having 89 per cent identity with cattle, 98 per cent with predicted sheep sequence, 90 per cent with predicted buffalo sequence and 87 per cent with *Homo sapiens* sequence. Exon 16 region of this gene was found to have higher per cent of identity with herbivores and humans.

The exon 2 sequence of *TCAP* gene from dogs showed 100 per cent homology with *Canis lupus familiaris* was having 96 per cent identity with predicted Panthera pardus, Panthera tigiris and Felis catus and 87 per cent with *Homo sapiens* telethonin gene. Exon 2 region of this gene was found to have higher per cent of identity with dog, herbivores and humans. These exons of above mentioned genes are highly conserved in different animals.

CONCLUSION

Changes not found in the exon-12 of *β-MYH7,* exon-16 of *MYBPC3* and exon-2 *TCAP* gene but one novel SNP was observed in the intron 1 of TCAP gene in dogs with dilated cardiomyopathy.

ACKNOWLEDGEMENT

The authors are thankful to the Professor and Head, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Teaching Veterinary Clinical Complex and the Dean, College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University, India for providing necessary facilities and support for the study.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

Ababou, A., Rostkova, E., Mistry, S., Masurier, C.L., Gautel, M. and Pfuhl, M. (2008). Myosin binding protein C positioned to play a key role in regulation of muscle contraction: Structure and interactions of domain C1. J. Mol. Biol. 384: 615-630.

Artur, C. and Anna, J. (2012). Role of Genetic Factors in Dilated Cardiomyopathy. In: Cardiomyopathies- From Basic Research to Clinical Management, [Veselka, J. (ed.)], In Tech, Croatia, pp. 410-421.

Borgarelli, M., Santilli, R.A., Chiavegato, D., D'Agnolo, G., Zanatta, R., Mannelli, A. and Tarducci, A. (2006). Prognostic indicators for dogs with dilated cardiomyopathy. J. Vet. Intern. Med. 20: 104-110.

Byun, S.O., Fang, Q., Zhou, H. and Hickford, J.G.H. (2009). An effective method for silver-staining DNA in large numbers of polyacrylamide gels. Analytical Biochemistry. 385(1): 174-175.

Candasamy, A.J., Haworth, R.S., Cuello, F., Ibrahim, M., Aravamudhan, S., Krüger, M., Holt, M.R., Terraccian, O.C.M.N., Mayr, M., Gautel, M. and Avkiran, M. (2014). Phosphoregulation of the titin-cap protein telethonin in cardiac myocytes. J. Biological Chem. 289(3): 1282-1293.

Chorro, F.J., Such-Belenguer, L. and Lopez-Merino, V. (2009). Animal models of cardiovascular disease. Rev. Esp. Cardiol. 62: 69-84.

Jeyaraja, K., Arun, A., Yamini, H.S., Sesh, P.S.L. and Nambi, A.P. (2015). Diagnostic evaluation of dilated cardiomyopathy in Labrador Retrievers. Int. J. Advanced Res. 3(12): 628-655.

Jianga, J. Burgona, P.G., Wakimotoa, H., Onouea, K., Gorhama, J.M., O'Mearaf, C.C., Fomovskyf, G., McConnella, B.K., Leef, R.T., Seidmana, J.C. and Seidmana, C.S. (2015). Cardiac myosin binding protein C regulates postnatal myocyte cytokinesis. PNAS. 112(29): 9046-9051.

McEwan, J.D. Borgarelli, M., Tidholm, A., Vollmar, A.C. and Häggström, J. (2003). Proposed guidelines for the diagnosis of canine idiopathic dilated cardiomyopathy. J. Vet. Cardiol. 5: 7-19.

McNally, E.M., Golbus, J.R. and Puckelwartz, M.J. (2013). Genetic mutations and mechanisms in dilated cardiomyopathy. J. Clin. Invest. 123(1): 19-26.

Moss, R.L., Fitzsimons, D.P. and Ralphe, J.C. (2015). Cardiac MyBP-C regulates the rate and force of contraction in mammalian myocardium. Circulation Res. 116: 183-192.

Tanjore, R., Rangaraju, A., Kerkar, P.G., Calambur, N. and Nallari, P. (2008). MYBPC3 gene variations in hypertrophic cardiomyopathy patients in India. Can. J. Cardiol. 24(2): 127-130.

Tanjore, R., Rangaraju, A., Vadapalli, S., Remersu, S., Narsimhan, C. and Nallari, P. (2010). Genetic variations of *β-MYH7* in hypertrophic cardiomyopathy and dilated cardiomyopathy. Indian J. Hum. Genet. 16: 67-71.