Searching for Nutrition Targets: Multi-Omics Study in Early-Stage Myxomatous Mitral Valve Disease in Dogs

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Abstract

Myxomatous mitral valve disease (MMVD), common in small breeds and older dogs, can progress to heart failure. It is of great importance to slow or prevent the progression of MMVD at its early stage to extend the longevity of the affected animals. Our goal was to identify and characterize cellular and molecular pathways that might contribute to the pathogenesis and progression of MMVD. Transcriptomics and metabolomics studies were performed using cardiac tissues and serum samples from both MMVD and control dogs. Cardiac tissues were collected from dogs humanely euthanized for reasons unrelated to our study. Our study documented numerous changes, including compromised energy metabolism, increased inflammation and oxidative stress, and altered extracellular matrix (ECM) homeostasis. Some of these changes may benefit from nutritional or medical management.

Glossary of Abbreviations
CHF: Congestive Heart Failure
DET: Differentially Expressed Transcript
ECM: Extracellular Matrix
LV: Left Ventricle
MMP: Matrix Metalloproteinase
MMVD: Myxomatous Mitral Valve Disease
MV: Mitral Valve

Introduction

MMVD, the common acquired cardiac disease in dogs, is characterized by progressive mitral degeneration, which can lead to congestive heart failure (CHF).[1,2] MMVD is common in small- to medium-sized dogs and increases with age.[3] The MMVD dogs typically experience a lengthy preclinical stage when dogs have structural heart disease but no clinical signs of CHF. Once advanced to CHF, the disease progresses much more rapidly.[4] Therefore, early intervention in the preclinical stage to slow or prevent the progression provides an opportunity to extend the life of the affected animals. A multiomics integrative study was conducted to identify molecular and metabolic pathways important for MMVD pathogenesis and progression and to generate testable hypotheses for nutritional or medical management.[5]

Metabolomics and Transcriptomics Analyses

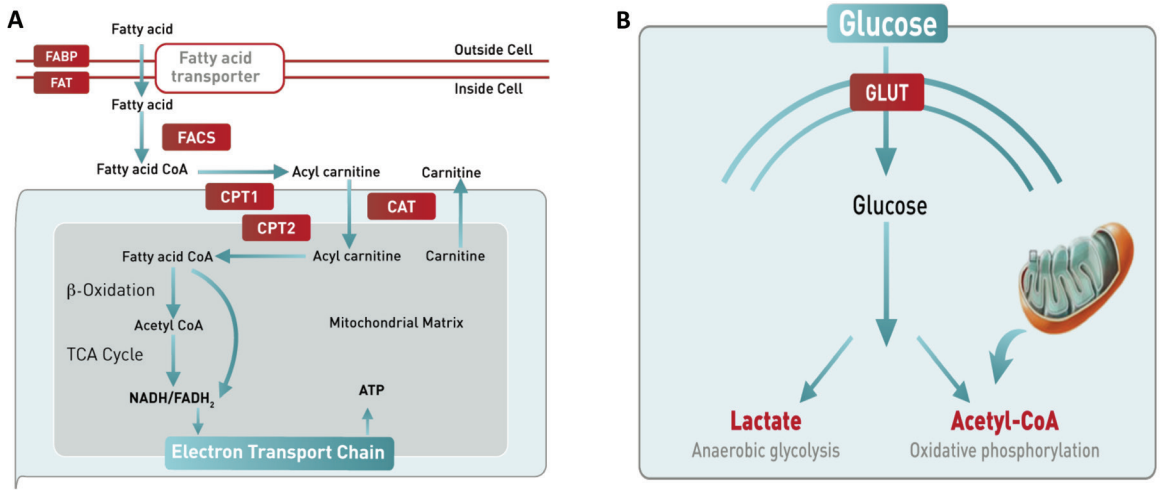
Serum samples from 18 preclinical MMVD and 11 age and sex-matched control dogs were submitted for metabolomics study. Cardiac tissues were collected from dogs humanely euthanized for reasons unrelated to this study. From those dogs, mitral valve (MV) tissues from three preclinical MMVD and three control dogs and free wall tissues of left ventricle (LV) from two preclinical MMVD and four control dogs were subject to RNA-seq transcriptomics study.

Fifty-four differentially expressed metabolites, 812 differentially expressed transcripts (DETs) from LV, and 263 DETs from MV were identified. There were 114 DETs common in LV and MV. Fifteen DETs from LV tissue were chosen for RT-qPCR validation, and 13 were confirmed.

Increases in Anaerobic Glycolysis and Decreases in Fatty-Acid Oxidation

Long-chain fatty acid (LCFA) oxidation provides more than 70% of energy for the normal adult mammalian heart.[6] Numerous changes in gene expressions related to energy metabolism pathways were observed in dogs with MMVD (Table 1).[5] The expression of three genes involved in LCFA transport to the cytoplasm, including fatty acid translocase (FAT), membrane-bound fatty acid binding protein (FABP4), and fatty acid transporter proteins (FATP6), were altered. Long-chain acyl-CoA synthetase (ACSL1 or LC-FACS), the enzyme responsible for activating LCFA to its CoA ester in the cell, was downregulated 2.6 and 3.0 folds in MV and LV of dogs with preclinical MMVD, respectively (Figure 1A). Serum concentration of deoxycarnitine, the immediate precursor of carnitine, was downregulated in MMVD dogs (Table 2), suggesting compromised fatty-acid oxidations. In addition, expression of 3-oxoacid CoA transferase 1, the rate-limiting enzyme for ketolysis and phytanoyl-CoA hydroxylase, which is important for branched-chain fatty acid oxidation, was downregulated in the mitral valve of MMVD dogs. Serum levels of three long-chain acyl carnitines also were lower in dogs with MMVD than control dogs, though the difference did not reach statistical significance. In a previous microarray gene expression study, Oyama and Chittur also observed a decreased expression in acyl-CoA synthetase in the MV of dogs with end-stage MMVD.[7]

Figure 1. Schematic representation of (A) long-chain fatty acid (LCFA) transport system in the cell membrane and mitochrondrial membrane. Fatty acid translocase (FAT), along with fatty acid-binding protein (FABP), bind LCFAs at the cell surface and transport them across the membrane. Some LCFAs also are transported by fatty acid transport proteins (FATPs). Once inside the plasma, LCFAs are activated by long-chain fatty acyl-CoA synthetase (LC-FACS or ACSL) to form acyl-CoA esters, which are converted to fatty acylcarnitine by carnitine palmitoyltransferase 1 (CPT1), also known as carnitine acyl transferase. Acylcarnitine is transported across mitochondrial membrane by carnitine/acylcarnitine transporter (CAT) for β-oxidation. (B) Glucose transport in the cell membrane. Glucose transport over the plasma membrane is facilitated by a group of membrane proteins called glucose transporters (GLUTs). Oxidation of glucose under aerobic conditions, oxidative phosphorylation, results in 32 ATP molecules per glucose molecule, while anaerobic glycolysis only generates two ATPs per glucose molecules.



In contrast, glucose uptake and anaerobic glycolysis were upregulated. Glucose transporter (GLUT) is a large family of membrane-bound proteins that facilitate the transport of glucose across the cell membrane (Figure 1B). Our gene expression study showed increased expression of GLUT3 in MV and LV and of GLUT6 in MV of MMVD dogs (Table 1). GLUT3 is a GLUT isomer with higher affinity and greater transport capacity for glucose than other isomers. In addition, serum metabolomics analysis showed lower concentration of glucose but higher lactate level in MMVD dogs compared to control dogs (Table 2). Our results suggested dogs with MMVD had compromised fatty acid oxidation and increased reliance on anaerobic glycolysis, where one glucose molecule produces only two ATPs or about 5% of its energy potential. Taken together, our study suggested energy insufficiency plays a role in the development and progression of MMVD in dogs.

Increases in Inflammation and Oxidative Stress

Glutathione S-transferases (GSTs) belong to a family of metabolic isozymes that catalyze the conjugation of reduced glutathione (GSH) to xenobiotic substrates or peroxidized lipids for the purpose of detoxification or reduction of oxidative stress. The expression of GSTP1, a GST isomer, was decreased in MMVD dogs (Table 1). Serum concentration of oxidized glutathione (GSSG) was significantly higher in MMVD dogs than in control dogs (Table 2). In addition, SIRT5, an NAD-dependent deacyclase that activates superoxide dismutase (SOD), was downregulated in MMVD dogs.[5] Previously Freeman, et al., reported that GSH:GSSG ratio and vitamin E concentration were significantly lower in dogs with CHF than in the controls.[8] Increased oxidative stress and reduced vitamin E concentrations also were reported in dogs with idiopathic dilated cardiomyopathy.[9]

In humans, impaired cardiac function was associated with elevated plasma levels of proinflammatory markers,[10,11] which decreased after treatment.[12] In dogs, increased concentration of C-reactive protein, a marker for inflammation, was associated with CHF.[13] Collectively, data from our study and others demonstrated increased inflammation and oxidative stress in dogs with MMVD.

Altered ECM Homeostasis

In the heart, dynamic homeostasis of ECM plays an important role in maintaining the structural integrity and function of normal MV.[14] The matrix metalloproteinases (MMPs) are the driving forces for ECM degradations, whereas their inhibitors, known as tissue inhibitors of MMPs (TIMPs), promote ECM synthesis.[15] Misregulation in these gene-expression programs has been implicated in the maladaptive ECM remodeling in canine MMVD.[14] In our current study, while no expression change in MMPs and TIMPs was observed in the MV tissue in MMVD dogs, greater than 100-fold increases in MMP8 and MMP9 and more than 3-fold decreases in MM11 and MMP15 were found in the LV of dogs with MMVD (Table 1). TIMP1 was upregulated by more than 50-fold in the LV, but no difference was found in MV. Interestingly, Oyama and Chittur previously documented a 4.5-fold increase in TIMP1 in the MV of end-stage MMVD dogs,[7] suggesting different regulatory programs in ECM homeostasis in dogs with early- and late-stage MMVD.

Table 1. Heat map of differentially expressed transcripts from the RNA-seq study on left ventricle and mitral valve tissues from dogs with myxomatous mitral valve disease (MMVD) and control dogs. All are significant (P<0.01) unless otherwise indicated.

Symbol	Functional Role	Mitral Valve	Left Ventricle	Description
		Fold change from control		
GLUT3	EM	7.49	16.51	Solute carrier family 2, facilitated glucose transporter member 3
GLUT6	EM	11.7	NS	Solute carrier family 2, facilitated glucose transporter member 6
ACOT6	EM	-3.33	NS	Acyl-CoA thioesterase 6
ACSL1	EM	-2.57	-2.98	Acyl-CoA synthetase long-chain family member 1
FABP4	EM	-2.91	NS	Homolog to human fatty acid binding protein 4, adipocyte
FATP6	EM	4.01	NS	Solute carrier family 27 (fatty acid transporter), member 6
PHYH	EM	-3.01	NS	Phytanoyl-CoA hydroxylase-like
OXCT1	EM	-2.5	NS	3-oxoacid CoA transferase 1
SIR5	OS	-2.31	NS	NAD-dependent protein deacylase sirtuin-5, mitochondrial
GSTP1	OS	-2.56	NS	Glutathione S-transferase pi 1
MMP8	EC	NS	162	Matrix metallopeptidase 8 (neutrophil collagenase)
MMP9	EC	NS	256	Matrix metalloproteinase-9
MMP11	EC	NS	-6.2	Matrix metallopeptidase 11 (stromelysin 3)
MMP15	EC	NS	-3.4	Matrix metallopeptidase 15 (membrane-inserted)
TIMP1	EC	NS	47.7	Tissue inhibitors of metalloproteinases -1
ADAMTS1	EC	NS	4.23	A disintegrin and metallopeptidase with thrombospondin repeats, 1
ADAMTS4	EC	7.45	13.4	A disintegrin and metallopeptidase with thrombospondin repeats, 4
ADAMTS7	EC	NS	-4.12	A disintegrin and metallopeptidase with thrombospondin repeats, 7
ADAMTS9	EC	NS	13.8	A disintegrin and metallopeptidase with thrombospondin repeats, 9

EM, extracellular matrix; OS, oxidative stress; EC, energy metabolism; NS, nonsignificance.
Red, green and gray colors indicate a significant increase, decrease and nonsignificance in gene expression, respectively. A positive number reflects increased expression in dogs with MMVD; a negative number reflects decreased expression in dogs with MMVD.
Adapted from Li, et al.[5] Permission to reproduce was obtained from Mary Ann Liebert Inc.

Table 2. Heat map of differentially expressed identifiable serum metabolites in dogs with myxomatous mitral valve disease (MMVD) and healthy controls.

RanF*	BIOCHEMICAL NAME	FC	PATHWAY	SUB PATHWAY
Y	Glutathione, oxidized	2.32‡	Amino acid	Glutathione metabolism
	Glucose	0.91§	Carbohydrate	Glycolysis, gluconeogenesis, pyruvate metabolism
	Lactate	1.32§	Carbohydrate	Glycolysis, gluconeogenesis, pyruvate metabolism
Y	Deoxycarnitine	0.85§	Lipid	Carnitine metabolism
Y	N-acetylneuraminate	1.88‡	Carbohydrate	Aminosugar metabolism
Y	N-glycolylneuraminate	2.51‡	Xenobiotic	Food/plant component

*RanF: Y = Random Forest Analysis identified this as important for separating samples between dogs with MMVD and controls
†Fold Change (FC) in concentration of metabolites in serum samples from dogs with MMVD and controls. Red and green indicate a significantly upregulated and downregulated metabolite, respectively. A number greater than 1 reflects a higher concentration in dogs with MMVD; less than 1 reflects a lower concentration in dogs with MMVD.
‡, § Statistical significance where ‡=P < 0.01 and §=P < 0.05.
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Numerous changes in yet another ECM metalloproteinase family, A Disintegrin and Metalloprotease with Thrombospondin Repeats (ADAMTS), were observed. ADAMTS1, ADAMTS4 and ADAMTS9 were upregulated, while ADAMTS7 was downregulated in the LV of MMVD dogs (Table 1). Such changes have been implicated in LV remodeling in MMVD.[16-18] ADAMTS4 also was increased in the MV of MMVD dogs.

Histological changes in MMVD include excessive deposition of proteoglycans and abnormal fibrillary ECM organization.[14] The sialic acid family includes a group of N- or O-linked derivatives of neuraminic acid of a 9-carbon backbone. The best-known member of sialic acid family is N-acetylneuraminate. The sialic acid linkage patterns were altered in the mitral valves of pigs affected with endocardiosis.[19] Increased concentrations in serum sialic acids have been associated with heart failure.[20] Increased sialic acid metabolites, N-acetylneuraminate and N-glycolylneuraminate, were found in the serum of dogs with MMVD (Table 2),

suggesting that changes in sialic acid metabolism also may contribute to the development of MMVD in dogs.

Conclusions

Our study demonstrated numerous molecular, cellular and metabolic changes in dogs with MMVD using an integrative transcriptomics and metabolomics analysis. Our results demonstrated increased reliance of anaerobic glycolysis in the context of reduced fatty acid oxidation in dogs with MMVD. Markers of oxidative stress and inflammation also increased. Other changes included alterations in ECM homeostasis. Many of these changes may benefit from nutritional or pharmaceutical management.

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