



Published in final edited form as:

Circ Heart Fail. 2012 July 1; 5(4): 462–466. doi:10.1161/CIRCHEARTFAILURE.111.966200.

Circulating Matrix Metalloproteinases in Adolescents with Hypertrophic Cardiomyopathy and Ventricular Arrhythmia

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Abstract

Background—Myocardial fibrosis is a hallmark of hypertrophic cardiomyopathy (HCM) and a risk factor for ventricular arrhythmia. Fibrosis can be reflected in circulating matrix remodeling protein concentrations. We explored differences in circulating markers of extracellular matrix turnover between young HCM patients with versus without history of serious arrhythmia.

Methods and Results—Using multiplexed and single ELISA, MMP 1,2,3,9; tissue inhibitor of metalloproteinase (TIMP) 1,2,4; and collagen I carboxyterminal peptide (CICP) were measured in plasma from 45 young HCM patients (80% male, median age 17 years [IQR 15-20]). Participants were grouped into serious ventricular arrhythmia history (VA) versus no ventricular arrhythmia history (NoVA). Differences in MMPs between groups were examined nonparametrically. Relationships between MMPs and ventricular arrhythmia were assessed with linear regression, adjusted for interventricular septal thickness, family history of sudden death, abnormal exercise blood pressure, and implantable cardioverter defibrillator (ICD). In post-hoc sensitivity analysis, age was substituted for ICD. The 14 VA patients were older than 31 NoVA patients (Median 19 vs. 17 years, $p=0.03$). All 14 VA and 12 NoVA patients had ICD. MMP3 concentration was significantly higher in the VA group (VA median 12.9 [IQR 5.7-16.7] mcg/mL vs. NoVA 5.8 [IQR 3.7-10.0] mcg/mL; $p=0.01$). On multivariable analysis, VA was independently associated with increasing MMP3 (standardized b 0.37, $p=0.01$). Post hoc adjustment for age attenuated this association.

Conclusions—Circulating MMP3 may be a marker of ventricular arrhythmia in adolescent patients with HCM. Due to our role as pediatric providers, we cannot exclude age related confounding.

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Journal Subject Code: [5] Arrhythmias; [16] Myocardial cardiomyopathy disease; [115] Remodeling; [41] Pediatric and congenital heart disease; [134] Pathophysiology; [104] Structure

Disclosures

Dr. Walsh has received modest honoraria from St. Jude Medical Inc. (Minneapolis, MN). Dr. Alexander has received modest honoraria from Up-To-Date Inc. (Waltham, MA) and Best Doctors Inc. (Boston, MA).

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Keywords

hypertrophic cardiomyopathy; fibrosis; arrhythmia; matrix; metalloproteinases; pediatric

Ventricular tachyarrhythmia is a prominent cause of sudden death in patients with hypertrophic cardiomyopathy (HCM). Previously reported risk factors for sudden death in pediatric HCM cohorts such as personal history of syncope, ventricular arrhythmia, interventricular septal thickness greater than 3 cm, abnormal blood pressure (BP) response to exercise, and family history of sudden death do not completely stratify risk, leaving room for additional markers.¹ Histologic findings of interstitial fibrosis and myofibrillar disarray are characteristic features of myocardial specimens from patients with HCM.^{2,3} Recent studies have identified fibrosis and scar in HCM patients, both by gadolinium-enhanced cardiac magnetic resonance imaging (MRI) and by circulating markers of extracellular matrix (ECM) remodeling.^{4,5} Delayed enhancement is associated with circulating markers of ECM turnover.⁶ As cardiac MRI is contraindicated in patients with implantable cardioverter defibrillators (ICDs), investigation of the link between higher-grade ventricular arrhythmias and myocardial scar with imaging is challenging. In this study of primarily adolescent patients with HCM, we examined the ability of circulating markers of ECM remodeling to distinguish adolescents with versus without a history of serious ventricular arrhythmia.

Methods

This study enrolled consecutive patients with clinical diagnosis of HCM who were greater than 13 years of age from January 2009 to March 2010. Patients were diagnosed as HCM by findings on echocardiography of either interventricular septum or left ventricular wall thickness greater than 15mm or greater than 3 z-scores relative to body surface area for smaller patients.⁷ We focused on patients older than 13 years of age because no surviving HCM patients younger than this age had a history of serious ventricular arrhythmia (VA). We defined VA as a) history of a cardiac arrest; b) documented sustained ventricular tachycardia, ventricular fibrillation, appropriate defibrillator discharge; or c) syncope deemed by the primary cardiologist not to be related to outflow tract obstruction, neurocardiogenic syncope, neurological syncope/seizure, or other documented syncope cause and that contributed to the decision for an ICD.⁸ There were 4 patients with syncope and 1 with exertional syncope. One of the 4 patients with nonexertional syncope had a coexistent accessory pathway and documented atrial fibrillation/flutter that was likely the cause of his syncope. This patient was not included in the VA group. The other 4 syncope patients had ICDs implanted shortly after the syncope event and so were included as VA. Exclusion criteria included anatomic left ventricular outflow tract obstruction; age below 13 years; confounding conditions such as cancer, myocardial infarction, syncope, surgery or other invasive procedure within the previous 6 months, inflammatory conditions, acute or recent convalescence from infectious illness, musculoskeletal injuries or conditions; and genetic syndromes associated with HCM including LEOPARD syndrome, Friedrich ataxia, Noonan syndrome, and Costello syndrome. Samples were not obtained during active menstruation to limit confounding. At the time of clinical visit, informed consent was obtained and venous blood sample was drawn. Salient clinical history, anthropometrics, demographics, clinical echocardiogram data from within the last year, exercise data, and clinical cardiac MRI data from within the last 3 years were collected. We do not perform cardiac MRI on patients with ICD. Of 58 eligible patients, 45 participated, 4 declined, 4 were missed. Five were excluded for illness, inflammatory bowel disease, menses, LEOPARD syndrome, and recent mitral valve surgery. This study was approved by the

Departmental Scientific Review committee and institutional Committee on Clinical Investigation.

Laboratory assays

Venous blood samples were drawn into sodium heparin collection tubes on ice. These samples were immediately centrifuged under refrigeration to obtain platelet poor plasma and stored at -20 degrees Celsius until assays were performed. Plasma concentrations of matrix metalloproteinase 1, 2, 3, 9; tissue inhibitor of metalloproteinase 1,2,4; and collagen I carboxy terminal propeptide (CICP) were measured in triplicate, utilizing a multimarker approach for MMPs and TIMPs due to their overlapping substrate specificities and functions. Plasma MMPs and TIMPs were analyzed using Fluorokine MultiAnalyte Profiling F-MAP kits (R&D Systems, Minneapolis, MN) as previously described.⁹ F-MAP kits use microspheres with highly specific antibodies and unique fluorescent intensity to bind plasma MMPs/TIMPs while unbound microspheres are washed away. The addition of biotinylated MMP antibodies followed by phycoerythrin-labeled streptavidin labels microsphere-MMP-antibody complexes. Plasma-MMP-microsphere complex fluorescence was quantitated on a Luminex 100 Bioanalyzer (Luminex Corp., Austin, TX). CICP was measured using MicroVue ELISA (Quidel Corp, Santa Clara CA). The C-terminal propeptide is cleaved from collagen during deposition and therefore indexes collagen deposition. Intrassay coefficient of variation (CV) were MMP1 13%, MMP2 6%, MMP3 5%, MMP9 4%, TIMP1 5%, TIMP2 8%, TIMP4 8%, and CICP 3%.

Statistical Analysis

Participants were divided into those with VA and those without history of ventricular arrhythmia (noVA). The primary analysis examined univariate differences in MMPs, TIMPs, and CICP between VA and noVA patients using nonparametric Mann-Whitney rank sum testing. In secondary analysis backward elimination multivariable adjusted linear regression was utilized to examine the association between MMPs identified in the primary analysis as the response variable and VA versus noVA status as the predictor. Due to skewed biomarker distributions, MMPs were natural logarithm transformed. Adjustment binary covariates including family history of sudden death, interventricular septal thickness greater than 30 millimeters, and exercise blood pressure augmentation less than 20mmHg were considered in the stepwise approach with removal criteria of <0.1 and retention for <0.05 . In a pre-specified sensitivity analysis the presence of ICD was substituted for exercise blood pressure. After examining baseline differences, post-hoc sensitivity analysis replaced ICD with age in years in the multivariable model. In a second sensitivity analysis, those patients with syncope as their VA event were reclassified as noVA. All preceding univariate and multivariable regression analyses were repeated and were substantially similar in result. Therefore the former analyses with syncope included as VA are presented. Analyses were performed with PASW 17.0 (SPSS Inc; Chicago, IL). Nominal significance level was 0.05. All authors have reviewed and accepted the manuscript.

Results

Baseline Characteristics

The VA (n=14) patients were older and had less hypertrophy, as indexed by thickest ventricular segment, compared to noVA (n=31) [Table 1]. Of VA patients, 5 had ventricular tachycardia or fibrillation with arrest, 5 had documented ventricular tachycardia without arrest, 3 had syncope without alternate cause, and 1 had exertional syncope. These clinical scenarios contributed to ICD placement in each of these 14 patients. Twelve noVA patients had primary prevention ICD, 1 had a pacemaker placed as part of a separate study in the

remote past, and 2 had history of atrial fibrillation in the presence of Wolff-Parkinson-White syndrome.

None of the 29 patients undergoing exercise testing had hypotension with exercise. Exercise blood pressure augmentation of less than 20 millimeters mercury was seen in 7 of 9 VA patients and 12 of 20 no VA patients. There were no significant differences with respect to sex distribution, dyspnea, or left ventricular outflow tract obstruction. Gadolinium enhanced cardiac MRI identified scar in 15/19 (79%) noVA subjects. No VA patients had cardiac MRI within the preceding 3 years. Twenty participants had genetic testing (1 VA and 19 noVA), with 9 (45%) having beta myosin heavy chain 7 mutation, 7 (35%) having myosin binding protein C3 mutations, 1 having both myosin binding protein C3 and troponin I mutation, and 1 with myosin light chain 2 mutation.

Outcomes: Univariate comparison and Multivariable adjusted associations

As detailed in Table 2, VA patients had significantly higher MMP3 levels than noVA patients (median 13 [IQR 6, 17] vs. 6 [4,10] mcg/mL, $p=0.01$). No other significant differences were found between groups, although CACP trended for a higher value in the noVA group.

In multivariable adjusted regression models, higher \ln MMP3 level was associated with history of VA (regression coefficient 0.67 [95% CI 0.17, 1.17], $p=0.01$) with consistent results using either abnormal exercise BP or ICD in the model. Replacement of ICD with age in the stepwise model attenuated this association (0.47 [0.03, 0.97], $p=0.06$) and left age as the only predictor.

Discussion

In this investigation of predominantly adolescent patients with HCM, MMP3 is elevated in those with remote history of serious ventricular arrhythmia or unexplained syncope. This finding was robust in the face of adjustment for conventionally accepted risk factors including family history of sudden death and septal thickness greater than 3 centimeters, as well as the presence of an ICD. However, as pediatric providers, the young adult patients in our practice tend to be those following secondary prevention ICD placement. Therefore, we cannot exclude age-related confounding in this association. Our results must be considered hypothesis generating.

Excessive collagen content is a recognized histological feature of HCM.¹⁰ The regional deposition pattern of collagen in HCM patients is reflected in the pattern of late gadolinium enhancement, which commonly affects non-contiguous myocardial segments.^{11;12} The presence, but not extent, of enhancement appears associated with low-grade ventricular arrhythmias and possibly higher grade as well.^{5;13-15} The propensity for arrhythmia in HCM patients with delayed enhancement is broadly consistent with the well established link between collagenous scar and arrhythmia foci and circuits.⁶ In the myocardium, this transition from ECM dominated by collagen III to collagen I is recognized in physiologic and pathologic processes including aging, post-myocardial infarction repair, and nonischemic cardiomyopathy.¹⁶ However, data are conflicting on the respective roles of collagen I and III in the fibrosis of HCM. Previous work described a remodeling milieu favoring collagen III deposition and collagen I degradation in established HCM, but more recent work suggests collagen I deposition is an early feature of HCM prior to phenotypic presentation.^{17;18} There is a role for more sensitive markers of the link between ECM remodeling and arrhythmia as ventricular thickness does not correlate with fibrosis and the extent of delayed enhancement also does not correlate with arrhythmia.^{4;5;17} Thus relevant

circulating biomarkers, once validated, may have advantages in sensitivity and reassessment over time.

MMP3 sits at the nexus of multiple ECM remodeling pathways with varied substrates including collagen 3, basement membrane components, proteoglycans, fibronectin, as well as a role activating other MMPs.¹⁹⁻²¹ MMP3 responds to upstream clinical events and other stimuli by cleaving and activating these ECM elements. In this linker function, MMP3 has been implicated in the progression of multiple myocardial pathologies. MMP3 is elevated in tissue and serum of patients with idiopathic dilated cardiomyopathy.²² After myocardial infarction, MMP3 prospectively predicts LV dysfunction, remodeling, and mortality.²³ MMP3 genotypic variants are associated with risk of myocardial infarction and hemorrhagic stroke.^{24;25} Our results suggest MMP3 may be a marker of enhanced myocardial ECM turnover in HCM patients with substrate for serious arrhythmia.

While MMP3's role in myocardial ECM turnover is the most straightforward explanation, it is also possible that the MMP3 is acting upon vasculature. MMP3 is involved in the regulation of angiogenesis including facilitating vascular sprout invasion into tissue by ECM lysis as well as regulation of angiogenesis proteins including vascular endothelial growth factor (VEGF) and antiangiogenic endostatin.^{19;26;27} Tumors expressing MMP3-processed VEGF have large blood vessels with poor sprouting and low vascular density, also called vascular paucity.²⁶ Microvascular paucity is a known feature of pathologic hypertrophy in general and HCM in specific.²⁸ Also, MMP3 is implicated in the health of existing blood vessels, including epicardial artery disease and in venous disease.^{25;29} Indeed myocardial ischemia is seen in HCM, although none of our patients had suggestion of cardiac events within the preceding 6 months.³⁰ We are unable to discern if myocardial or microvascular sources are responsible for the observed elevation in MMP3. We could not confirm previous links between MMP9 and delayed enhancement or between other MMPs previously implicated in HCM including CICP, MMPs 2, 1 and TIMP1.^{5;18} This may be due to the comparison in this study between 2 groups of HCM patients rather than comparing HCM to unaffected persons. We are also unable to determine whether there are differences in tissue level activation of these other MMPs, including from MMP3 activation, as the assay we used quantifies active and inactive forms together.

Limitations

First, as noted, we are unable to eliminate age-related confounding. Future work will need to enroll VA and noVA patients at both ends of the age spectrum. Second, we cannot exclude reverse causation in this cross-sectional study. While we are unaware of any reports detailing MMP elevation months to years after acute cardiac events without some underlying ongoing process like ischemic cardiomyopathy, it is possible that MMP3 is elevated due to the VA event. Third, these results may have selection bias since we only examined survivors. Future work would ideally be prospective. Finally, while all comparisons were specified a priori, we did not perform correction for multiple comparisons. Therefore these results must be considered hypothesis generating.

Conclusions

MMP3 may be a circulating biomarker for ventricular arrhythmia in persons with hypertrophic cardiomyopathy, reflecting its role in ECM turnover and microvascular biology. Additional studies with broader cohorts and prospective design are warranted.

Acknowledgments

Sources of Funding

This work was supported by National Institutes of Health/ National Heart Lung and Blood Institute T32 HL007572 (JPZ) and UL1 RR 025758 (Harvard Catalyst/The Harvard Clinical and Translational Science Center).

This work was presented as an abstract at the American College of Cardiology Scientific Sessions 2011.

Abbreviations

HCM	hypertrophic cardiomyopathy
MMP	matrix metalloproteinase
TIMP	tissue inhibitor of metalloproteinase
CICP	collagen I carboxyterminal peptide
VA	ventricular arrhythmia
noVA	no ventricular arrhythmia
ICD	implantable cardioverter defibrillator
ECM	extracellular matrix

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CLINICAL SUMMARY

Previous investigators have demonstrated the importance of fibrosis as a cardinal feature of HCM through post-mortem pathology and contemporary imaging techniques. Serum biomarkers of collagen metabolism may also be a useful reflection of the fibrotic process within the myocardium, which may create a milieu for ventricular arrhythmias. Therefore, in consecutive adolescent patients with HCM, we compared circulating plasma concentrations of fibrosis biomarkers between those with remote history of serious ventricular arrhythmia versus those without ventricular arrhythmia history. We found that levels of matrix metalloproteinase 3 were significantly higher in patients with arrhythmias, and this difference persisted after adjustment for accepted sudden death risk factors in HCM. The association was attenuated after adjustment for age, as the older patients in our cohort tend to be those followed after ICD placement for serious arrhythmia. These data suggest that that arrhythmia propensity may be reflected in circulating biomarkers of extracellular matrix turnover. Future studies may determine whether these biomarkers may be able to predict serious arrhythmia in HCM patients and the precise mechanisms by which the processes reflected by these biomarkers may alter arrhythmia risk.

Table 1
Baseline Characteristics

	VA	noVA	p value
Age (years)	19 (17, 22)	17 (15, 19)	0.03
Female (%)	21%	19%	0.8
ICD (%)	100%	40%	<0.0001
Maximum Ventricular Thickness (mm)	23 (12, 27)	29 (20, 34)	0.03
Maximum Septal Thickness (mm)	23 (12, 27)	28 (20, 40)	0.03
Maximum LV free wall Thickness (mm)	11 (10, 12)	10 (9, 12)	0.4
Maximum thickness >30mm (%)	8%	47%	0.01
LVOT MIG (mmHg)	26 (0-49)	14 (0-38)	1.0
LVOT obstruction moderate or severe (%)	21%	10%	0.3
Dyspnea at rest or on exertion (%)	7%	10%	0.6
Family History of HCM (%)	43%	55%	0.3
Family History of Sudden Death (%)	29%	23%	0.5

All data reported as proportion or median with 25%,75% range

VA- history of serious ventricular arrhythmia, noVA- no history of serious ventricular arrhythmia, ICD- implantable cardiac defibrillator, LVOT- left ventricular outflow tract, MIG- echo derived maximum instantaneous gradient

Table 2**Primary Outcome**

	VA	NoVA	p value
MMP1 (ng/mL)	156 (111, 254)	184 (97, 337)	0.7
MMP2 (mcg/mL)	178 (168, 213)	191 (182, 212)	0.3
MMP3 (mcg/mL)	13 (6, 17)	6 (4, 10)	0.01
MMP9 (mcg/mL)	33 (24, 41)	35 (28, 46)	0.3
TIMP1 (mcg/mL)	74 (69, 81)	68 (58, 77)	0.1
TIMP2 (mcg/mL)	115 (101, 128)	107 (91, 118)	0.3
TIMP4 (ng/mL)	1339 (1132, 1642)	1434 (1069, 1625)	0.8
CICP (ng/mL)	10 (8, 16)	14 (10, 21)	0.06

Data reported as median with 25%, 75% range. Significance tested using Mann Whitney U test.

VA- history of serious ventricular arrhythmia, noVA- no history of serious ventricular arrhythmia, MMP- matrix metalloproteinase, TIMP- tissue inhibitor of metalloproteinase, CICP- collagen I carboxyterminal peptide