Clinical Investigation

The Role of Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases in Duchenne Muscular Dystrophy Cardiomyopathy

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ABSTRACT

Background: Cardiomyopathy is the leading cause of death in Duchenne muscular dystrophy (DMD). Standard cardiac biomarkers are poor indicators of DMD cardiovascular disease. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) regulate collagen turnover. Given the cardiac fibrosis seen in DMD, we hypothesized that MMPs and TIMPs correlate with severity of DMD cardiomyopathy.

Methods and Results: Prospectively enrolled DMD subjects (n = 42) underwent cardiac magnetic resonance imaging for function and late gadolinium enhancement (LGE), including LGE severity from 0 (no LGE) to 4 (severe). Serum from DMD and healthy male control subjects (n = 15) analyzed for MMPs 1, 2, 3, 7, 9, and 10 and TIMPs 1−4. MMP1, MMP7, and MMP10 were higher in DMD than in control (respectively, median 5080 pg/mL vs 2120 pg/mL [$P = .007$], 2170 pg/mL vs 1420 pg/mL [$P < .001$], and 216 pg/mL vs 140pg/mL [$P = .040$]); TIMP4 was lower in DMD (124 pg/mL vs 263 pg/mL; $P = .046$). Within DMD, MMP7 correlated inversely with left ventricular ejection fraction ($r = -0.40$; $P = .012$) and directly with strain ($r = 0.54$; $P = .001$) and LGE severity ($r = 0.47$; $P = .003$). MMP7 was higher in DMD patients with LGE compared with those without LGE and control subjects ($P < .001$).

Conclusions: Multiple MMPs are elevated in DMD compared with control subjects. MMP7 is related to DMD cardiac dysfunction and myocardial fibrosis, possibly through remodeling of the extracellular matrix. (*J Cardiac Fail 2019;25:259−267*)

Key Words: Duchenne muscular dystrophy, biomarkers, cardiomyopathy, fibrosis.

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Duchenne muscular dystrophy (DMD) is an X-linked disorder affecting 1 in 4700 male births.[1] Although perceived primarily as a skeletal myopathy, boys also develop insidious and progressive cardiomyopathy. In the current era, cardiomyopathy is the leading cause of mortality.[2] Because of skeletal muscle weakness, boys with cardiomyopathy are usually asymptomatic until they develop severe left ventricular (LV) dysfunction. Cardiac imaging is the primary modality for diagnosis of dysfunction. Unfortunately, standard heart failure biomarkers, such as B-type natriuretic peptide (BNP), are only increased at the end stage.[3,4]

Therapeutic options for DMD cardiomyopathy are limited. Standard heart failure medications, including angiotensin-converting enzyme inhibitors, beta-blockers, and aldosterone inhibitors, have demonstrated some level of efficacy.[5–8] However, therapeutic evaluation in DMD has been limited by small sample sizes and short duration of treatment, and these medications only serve to delay the inevitable decline in function.[9] Given the differences in pathogenesis, disease-specific therapeutics are necessary.[10]

DMD cardiomyopathy appears to begin with diffuse myocardial fibrosis, followed by larger areas of focal fibrosis and eventual overt myocardial dysfunction.[11,12] A better understanding of the molecular effectors leading to DMD fibrosis may help to identify novel biomarkers of disease progression or novel targets for drug therapy. These biomarkers could function as surrogate outcome measures or be used to monitor therapeutic response between cardiac magnetic resonance imaging (CMR) studies. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) regulate collagen turnover in the myocardial extracellular matrix and may play a role in DMD fibrosis.[13] We hypothesized that MMPs and TIMPs would be elevated in DMD compared with control subjects and would correlate with severity of DMD cardiomyopathy.

Methods

Enrollment

This prospective study was approved by the Vanderbilt Institutional Review Board, and the investigation conforms with the principles outlined in the Declaration of Helsinki. DMD subjects were enrolled from the neuromuscular cardiology clinic from 2012 to 2015. Informed consent was obtained from every subject (or their guardian) and appropriate assents were obtained. Inclusion criteria were (1) diagnosis of DMD with clinical phenotype and confirmation with either genetic testing or muscle biopsy, (2) blood obtained at time of CMR, and (3) able to tolerate CMR without sedation or anesthesia; given difficulties with breath-holds in younger children, the youngest age enrolled was 7 years. To enroll a population with a broad range of cardiovascular disease severity, no upper age limit was used for DMD patients. Exclusion criteria were (1) additional cardiac diagnoses that could confound biomarkers (1 patient who, in addition to a DMD mutation, also had 2 known disease-causing mutations for hypertrophic cardiomyopathy) and (2) inability to draw an adequate volume of blood for biomarker analysis. Pertinent clinical data were collected from patients and the electronic medical record. Enrolled DMD subjects underwent blood draw, CMR, and skeletal muscle strength assessment at a single time point.

Healthy male pediatric patients aged 8−18 years were enrolled as control subjects. These healthy children were recruited before treadmill testing for chest pain, syncope, palpitations, or tachycardia. Exclusion criteria were (1) abnormal treadmill test, (2) presence or concern for structural or functional cardiovascular disease (congenital heart disease, cardiomyopathy, or any secondary cardiovascular disease), (3) abnormal echocardiogram, and (4) arrhythmia or clinical concern for arrhythmia. All participants were determined by their primary cardiologist to be healthy after thorough evaluation as indicated by clinical presentation. All clinic notes and cardiac testing were reviewed by a study author (J.H.S.) to ensure that all subjects conformed with inclusion and exclusion criteria.

Biomarker Analysis

The Milliplex Map Human MMP Magnetic Bead Panels 1 and 2 and Human TIMP Magnetic Bead Panel 2 (EMD Millipore Corp, Billerica, Massachusetts; catalog nos. HMMP1MAG-55K, HMMP2MAG-55K, and HTMP2MAG-54K) were used to detect serum MMP1, MMP2, MMP3, MMP7, MMP9, MMP10, TIMP1, TIMP2, TIMP3, and TIMP4 according to the manufacturer's instructions. The Milliplex Map Human Cytokine/Chemokine Magnetic Bead Panel (EMD Millipore Corp; catalog no. HCYTOMAG-60K) was used to detect serum interleukin 1-beta (IL-1β) and tumor necrosis factor alpha (TNF-α). Seven working standards were generated by serial dilution (1:3) of the reconstituted standard provided in the kit. Two quality control samples were included in each plate run. Assay plates were read on a Luminex 200 with Xponent software using the parameters outlined in the assay kit instructions. The Milliplex Analyst 5.0 software was used for data analysis. The correlation efficiency (R) for the standard curve was >0.99 for each assay. All assays were run in duplicate, and the average percentage coefficient of variation (CV) was $<10\%$. Any individual samples with a CV $>25\%$ were repeated twice and if the subsequent CV was $>25\%$ the results were removed from analysis. The total DMD samples removed from analysis by biomarker were MMP1 (n = 9), MMP3 (n = 0), MMP7 (n = 3), MMP9 (n = 4), MMP10 (n = 2), TIMP1 (n = 4), TIMP2 (n = 5), TIMP3 (n = 0), TIMP4 (n = 0). MMP2 was not considered further owing to the large number of samples with CV $>25\%$. We also conducted a sensitivity analysis where, instead of removing samples with CV $>25\%$, we used the medians of all available values.

Cardiac Magnetic Resonance

CMR was performed for DMD subjects with the use of a 1.5 Tesla Siemens Avanto (Siemens Healthcare Sector, Erlangen, Germany). LV volume, mass, and function were

calculated as previously described.[12] A peripheral intravenous line was used to administer Gd-DTPA contrast (gadopentate dimeglumine; Magnevist, Bayer Healthcare Pharmaceuticals, Wayne, New Jersey) at a dose of 0.2 mmol/kg. Late gadolinium enhancement (LGE) was assessed by means of single-shot and segmented inversion recovery balanced steady-state free precession (bSSFP) with optimized inversion recovery to null the signal from the myocardium, and phase-sensitive inversion recovery bSSFP with an inversion time of 300 ms.

Myocardial tagging was performed in the short axis at the level of the papillary muscles with the use of a segmented k-space fast gradient echo sequence with electrocardiographic triggering. Grid tagging was performed with 9−13 phases. Typical imaging parameters included slice thickness 6−8 mm, field of view 340 mm × 340 mm, matrix size 256 × 192, and minimum echo time and repetition time. The sequences were breath-holds and parallel imaging with the use of GRAPPA (Siemens), and an acceleration factor of two was used. Analysis of myocardial tagged images was performed by means of harmonic phase methodology (Diagnosoft, Morrisville, North Carolina) as previously described.[14] One reader performed the analysis blinded to pertinent clinical data. A mesh was created by manually contouring the endocardial and epicardial borders at end-systole. The software then calculated the peak global and segmental circumferential strain (ε_{cc}) values; segmental values were calculated in the 6 segments at the middle portion of the LV by means of the standard 17-segment model.[15]

The presence or absence of LGE, as well as location according to the standard 17-segment model, was qualitatively assessed by 1 reader during initial CMR interpretation. LGE was confirmed by a second reader blinded to all clinical data. If the readers did not agree, the images were reviewed by both investigators and consensus was reached. Both readers assigned LGE severity with the use of a modification of the global severity score reported by Menon et al.[16] Each readers reviewed the complete short axis stack for all sequences performed (at least 1 single-shot inversion recovery short axis stack, 1 set of segmented inversion recovery images in the short-axis plane, and at least 1 phase-sensitive inversion recovery short axis stack). The readers cross-referenced areas of LGE with the 4-chamber, 3-chamber, and 2-chamber views to ensure that an accurate assessment of LGE severity was obtained. The score ranged from 0 (no LGE) to 4 (severe LGE) defined as the following: 0 = no LGE; 1 = LGE localized to basal or middle free wall; 2 = LGE involving free wall but less than one-third of free wall length, no or minimal septal involvement; 3 = LGE in multiple areas of LV free wall greater than one-third and mild septal involvement or more extensive LV free wall involvement (greater than two-thirds) without septal involvement; and 4 = extensive free wall and septal involvement. Percent LGE was calculated with the use of Medis software and the full-width half-maximum (FWHM) technique by 1 reader blinded to all clinical data. For consistency and quality (some subjects had difficulty with breath-holds for the segmented inversion recovery images), the single-shot phase sensitive inversion recovery short axis stack was used for these calculations. The epicardial and endocardial borders were manually contoured, avoiding the LV outflow tract in the basal slice and apical slices with significant through-plane motion. A reference region of interest was placed in myocardium without visible LGE. The percentage LGE was then calculated.

Quantitative Muscle Strength Testing

Quantitative muscle testing (QMT) is an objective reproducible method for upper and lower extremity strength evaluation in DMD.[17−19] QMT was performed on DMD subjects with the use of a handheld myometer by 1 investigator (W.B.B.) as previously described.[20] Arm QMT score was calculated by adding flexion and extension values for the right and left elbows, leg QMT score was calculated by adding flexion and extension values for the right and left knees, and total QMT score was calculated by adding the total arm score and the total leg score. The QMT in normal children increases with age but often plateaus or decreases with age in patients with DMD, so we partially corrected for this by indexing the QMT to age.

Statistical Analysis

Demographic variables were compared with the use of either a Wilcoxon rank-sum (continuous variables) or a chi-square or Fisher exact test (categoric variables). Correlations between continuous variables were evaluated with the use of Spearman rho. A Wilcoxon rank-sum test was used to evaluate the difference in continuous variables between 2 groups and a Kruskal-Wallis test to compare more than 2 groups.

LV dysfunction was defined as LV ejection fraction (LVEF) <55%. Because the analyses were nonparametric, biomarker levels that were below (or above) detection levels were assumed to be tied and lower (or higher) than all values in the detectable range.

Analyses were performed with the use of IBM SPSS statistics, version 24.0 (IBM Corp, Armonk, New York) and R studio 3.4.3 (available online at www.rstudio.com/). Study data were collected and managed with the use of the REDCap (Research Electronic Data Capture) electronic data capture tools hosted at Vanderbilt University.[21]

Results

Demographics

Forty-two DMD and 15 control subjects were enrolled. There was no significant difference in age between the DMD and control subjects (Table 1). As expected, DMD subjects were shorter and weighed less than control subjects.

DMD Cardiac Imaging

DMD subjects had a median (interquartile range [IQR]) LVEF of 57% (47%−59%) and a right ventricular EF of

Table 1. Demographics

Variable	Duchenne Muscular Dystrophy (n = 42)	Control (n = 15)	*P* Value
Age (y), median	12.8 (IQR 10.5−16.3; range 8−27)	14 (IQR 13−15) (range 8−17)	.663
Height (cm), median	147 (IQR 131−159)	168 (IQR 157−175)	.001
Weight (kg), median	51 (IQR 36−59)	63.5 (IQR 55−83)	.006
Male sex	100%	100%	
Race			.178*
White	88% (n = 37)	73% (n = 11)	
African American	5% (n = 2)	27% (n = 4)	
Asian	2% (n = 1)	0	
Mixed	5% (n = 2)	0	
Ethnicity			.927
Hispanic	14% (n = 6)	13% (n = 2)	
Current medications			
Glucocorticoids	57% (n = 24)		
ACEi	64% (n = 27)		
ARB	12% (n = 5)		
Beta-blocker	41% (n = 17)		
Aldosterone inhibitor	2% (n = 1)		
Ambulatory	16% (n = 9)		

IQR, interquartile range; ACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers.
*Statistical analysis of race categories performed as a comparison of white versus nonwhite.

57% (52%−61%). Eighteen subjects (43%) had abnormal function, defined as LVEF <55%. Of 41 patients administered gadolinium contrast, 28 (68%) had at least 1 segment with LGE. The median global severity score was 2 (0−3), with 5 subjects having a global severity score of 4 (most severe). Only 3 subjects had symptoms of heart failure, which is not unexpected given the decreased activity level of boys with DMD. Three subjects had a documented arrhythmia before enrollment in the study, 2 with supraventricular tachycardia and 1 with ventricular tachycardia.

DMD Medications

A total of 24 DMD subjects (57%) were taking corticosteroids at the time of the study (Table 1). Additional medications included: 27 (64%) taking angiotensin-converting enzyme inhibitors (ACEi), 5 (12%) taking angiotensin receptor blockers (ARB), 17 (41%) taking beta-blockers (BB), and 1 (2%) taking an aldosterone inhibitor.

Skeletal Muscle Strength Assessment

Only 9 patients were ambulatory at enrolment. The median (IQR) arm QMT was 22.3 (11.3−36.8) pounds, leg QMT 40.0 (24.4−66.4) pounds, and total QMT 64.5 (40.8−100.5) pounds. As expected, indexed arm, leg, and total QMT were higher in ambulatory than nonambulatory patients: 3.5 (2.6−4.2) versus 1.5 (0.5−2.1; $P = .002$), 7.3 (3.4−8.3) versus 2.5 (1.4−4.4; $P = .005$), and 11.4 (6.0−12.3) versus 4.0 (2.2−6.8; $P = .004$), respectively. Patients currently taking steroids had higher indexed arm, leg, and total QMT (2.4 [1.8−3.7] vs 0.8 [0.3−1.6], 5.0 [2.7−7.7] vs 2.2 [0.7−2.6], and 7.0 [4.9−11.7] vs 2.8 [1.0−4.3]; $P < .001$ for all), although this effect is likely related at least in part to patient age because patients not taking corticosteroids were significantly older: 11 years (10−12) versus 16 years (14−20); $P < .001$.

Biomarkers in DMD Versus Control

MMP1, MMP7, and MMP10 were elevated in DMD compared with control: median (IQR) 5080 pg/mL (2890−7900) versus 2120 pg/mL (1470−3380; $P = .007$), 2170 pg/mL (1630−4700) versus 1420 pg/mL (862−1630; $P < .001$), and 216 pg/mL [120−489] versus 140 pg/mL (55.2−170; $P = .040$; Table 2). TIMP4 was lower compared with control: 124 pg/mL (6.44−335) versus 263 pg/mL (87.2−426); $P = .046$.

Biomarkers and DMD Skeletal Muscle Strength

MMP7 correlated inversely with total indexed QMT (rho = −0.43; $P = .010$; Table 3). MMP1 and the MMP1/TIMP1 ratio also correlated inversely with total indexed QMT (rho = −0.44; $P = .016$; and rho = −0.55; $P = .002$) whereas TIMP2 correlated directly with total indexed QMT (rho = 0.44; $P = .009$). Results for indexed arm and leg QMT and absolute (nonindexed) QMT were similar except that MMP1 did not correlate with total absolute QMT. MMP7 was significantly higher in nonambulatory subjects

Table 2. Differences Between Matrix Metalloproteinase (MMP) and Tissue Inhibitors of Metalloproteinase (TIMP) Levels in Duchenne Muscular Dystrophy (DMD) and Control Subjects, Median (IQR)

Protein	DMD (n = 42)	Control (n = 15)	*P* Value
MMP1 (pg/mL)	5080 (2890−7900)	2120 (1470−3380)	.007
MMP3 (pg/mL)	8850 (2050−37400)	5090 (1930−7410)	.071
MMP7 (pg/mL)	2170 (1640−4700)	1420 (862−1630)	<.001
MMP9 (ng/mL)	78.2 (46.9−140)	79.0 (37.8−98.5)	.270
MMP10 (pg/mL)	216 (120−289)	140 (55.2−170)	.040
TIMP1 (pg/mL)	94.5 (76.4−131)	95.8 (68.8−126)	.752
TIMP2 (pg/mL)	45.5 (37.9−57.0)	45.2 (35.9−55.6)	.834
TIMP3 (pg/mL)	771 (293−1420)	1010 (263−2790)	.235
TIMP4 (pg/mL)	124 (6.44−335)	263 (87.2−426)	.046
MMP1/TIMP1	37.1 (22.9−95.6)	32.7 (18.8−37.7)	.155

Table 3. Correlations Between MMPs and TIMPs and Markers of Cardiovascular Disease and Skeletal Muscle Strength (rho; *P*)

Protein	LVEF	Global LGE	FWHM	ε_{cc}	QMT arm	QMT leg	Total QMT
MMP1 (pg/mL)	−0.3	0.36	0.34	0.13	−0.26	−0.35	−0.32
	.09	.043*	.060	.495	.168	.056	.09
MMP3 (pg/mL)	−0.07	−0.01	0.21	0.16	0.2	0.32	0.28
	.672	.944	.179	.329	.229	.048*	.089
MMP7 (pg/mL)	−0.4	0.47	0.44	0.54	−0.39	−0.36	−0.38
	.012*	.003*	.006*	.001*	.019*	.036*	.024*
MMP9 (ng/mL)	0.02	0.03	0.13	−0.08	−0.11	−0.07	−0.09
	.913	.856	.448	.626	.529	.705	.597
MMP10 (pg/mL)	0.17	−0.26	−0.20	0.03	−0.39	−0.35	−0.37
	.281	.114	.220	.845	.02	.034	.027
TIMP1 (pg/mL)	0.24	−0.07	0.12	0.13	0.05	0.16	0.13
	.146	.669	.49	.451	.78	.349	.425
TIMP2 (pg/mL)	0.06	−0.29	−0.32	−0.13	0.35	0.49	0.45
	.709	.078	.855	.447	.037*	.003*,*	.006*
TIMP3 (pg/mL)	−0.07	0.07	0.10	0.06	0.09	0.01	0.03
	.640	.644	.53	.724	.58	.951	.839
TIMP4 (pg/mL)	−0.12	0.35	0.28	0.26	−0.22	−0.3	−0.26
	.450	.026*	.076	.099	.177	.068	.11
MMP1/TIMP1	−0.39	0.33	0.21	0.039	−0.38	−0.48	−0.45
	.025*	.068	.244	.827	.038*	.007*	.012*

LVEF, left ventricular ejection fraction; LGE, late gadolinium enhancement; FWHM, percentage LGE measured using full-width half-maximum; ε_{cc}, circumferential myocardial strain; QMT, quantitative muscle testing; other abbreviations as in Table 2.
*$P < 0.05$.

(2420 pg/mL [1710−4910] vs 1410 pg/mL [860−1620]; $P = .007$) but there were no other significant differences. A subset analysis in the 9 subjects who were ambulatory revealed a correlation only between TIMP4 and arm QMT (rho = 0.73; $P = .039$), likely because of the small sample size.

Biomarkers and Current Medications

MMP1, TIMP4, and the MMP1/TIMP1 ratio were lower in patients on corticosteroids (3560 pg/mL [2210−7420] vs 7530 pg/mL [4190−12,800; $P = .017$]; 13.7 pg/mL [6.44−165] vs 285 pg/mL [107−667; $P = .004$]; 31.5 [18.0−55.1] vs 113 [36.2−162; $P = .007$]); there was a trend toward lower MMP7 in patients on corticosteroids ($P = .066$). MMP3 and TIMP2 were greater in patients on corticosteroids: 29,600 pg/mL (3630−57,900) versus 6200 pg/mL (1750−10,600; $P = .031$) and 51.0 pg/mL (45.3−62.1) versus 42.2 (35.0−49.5; $P = .022$). There were no significant differences in MMPs or TIMPs in patients taking ACEi, ARB, BB, or aldosterone inhibitors.

Biomarkers and DMD Cardiovascular Disease

A comparison between DMD without CM, DMD with CM, and control groups demonstrated a significant difference between them for MMP1 ($P = .003$), MMP7 ($P < .001$), TIMP4 ($P = .012$), and MMP/TIMP1 ($P = .026$; Table 4). When considering the relationship with cardiomyopathy, the most notable biomarker was MMP7. DMD subjects with abnormal function (defined as LVEF <55%) had elevated MMP7 compared with those with normal function and control subjects (Table 3; Fig. 1A). DMD subjects with LGE had higher MMP7 compared with those without LGE and

Table 4. Differences Between MMP and TIMP Levels in DMD Patients With and Without Left Ventricular (LV) Dysfunction and Control Subjects, Median (IQR)

Protein	A: Control (n = 15)	B: DMD Without LV Dysfunction (n = 24)	C: DMD With LV Dysfunction (n = 18)	*P* Value			
				Kruskal-Wallis	A vs B	A vs C	B vs C
MMP1 (pg/mL)	2120 (1470−3380)	3960 (2350−7420)	7530 (4430−12,400)	.003*	.076	.001	.041
MMP3 (pg/mL)	5090 (1930−7400)	14,790 (1840−57,900)	8850 (5070−13,000)	.194			
MMP7 (pg/mL)	1420 (860−1630)	1850 (1370−2420)	4240 (2000−6760)	<.001*	.004	<.001	.009
MMP9 (ng/mL)	79.0 (37.8−98.5)	73.2 (43.0−141)	100 (48.2−140)	.498			
MMP10 (pg/mL)	140 (55.2−170)	219 (149−289)	159 (111−319)	.071			
TIMP1 (pg/mL)	95.8 (68.8−126)	100 (74.7−131)	89.2 (74.7−145)	.855			
TIMP2 (pg/mL)	45.2 (35.9−55.6)	51.0 (41.9−57.4)	44.0 (35.0−54.8)	.373			
TIMP3 (pg/mL)	1010 (263−2790)	515 (179−1410)	870 (552−1470)	.348			
TIMP4 (pg/mL)	263 (87.2−426)	13.7 (6.44−247)	208 (29.8−667)	.012*	.004	.664	.034
MMP1/TIMP1	32.7 (18.8−37.7)	33.1 (17.9−66.2)	59.3 (35.0−162)	.026*	.741	.010	.030

Abbreviations as in Table 2.
*$P < 0.05$.

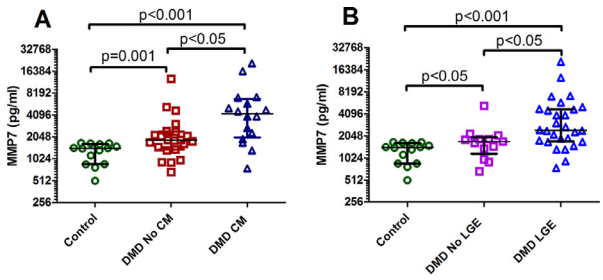

Fig. 1. (A) Comparison of matrix metalloproteinase (MMP) 7 levels in control subjects (n = 14), Duchenne muscular dystrophy (DMD) patients without cardiomyopathy (CM; n = 23), and DMD patients with CM (n = 16). (B) Comparison of MMP7 levels in control subjects (n = 14), DMD patients without late gadolinium enhancement (LGE; n = 13), and DMD patients with LGE (n = 25). Overall $P < .001$; P values for individual comparisons shown on figure. MMP7 plotted on a log2 scale. The horizontal lines represent the medians and interquartile ranges.

control subjects ($P < .001$ for Kruskal-Wallis; Fig. 1B demonstrates individual comparisons); DMD subjects without LGE also had higher MMP7 compared with control subjects (Fig. 1).

Within DMD, MMP7 levels correlated inversely with LVEF (Fig. 2A; rho = −0.40; $P = .012$) and directly with LGE global severity score and FWHM (rho = 0.47; $P = .003$; and rho = 0.44; $P = .006$; Table 3). MMP7 also correlated with E_{cc} in the mid-LV slice (rho = 0.54; $P = .001$; Fig. 2B). MMP7 correlated with indexed LV end systolic volumes (rho = 0.38; $P = .012$) and showed a trend for correlation with LV end-diastolic volumes (rho = 0.28; $P = .07$).

MMP1 and the MMP1/TIMP1 were greater in DMD patients with abnormal LVEF compared with those with normal function: 3960 pg/mL (2350−7420) versus 7530 pg/mL (4430−12,400; $P = .041$) and 33.1 (18.0−66.2) versus 59.3 (35.0−162; $P = .034$). TIMP4 was elevated in DMD subjects with abnormal function and with LGE compared with those with normal function and without LGE: 13.7 pg/mL (6.44−247) versus 208 pg/mL (29.8−667; $P = .034$) and 174 pg/mL (6.44−381) versus 6.44 pg/mL (6.44−184; $P = .047$). TIMP4 correlated with LGE global severity and FWHM (rho = 0.35; $P = .026$).

Inflammatory Markers

Given that the role of MMPs in regulating inflammation, TNF-α and IL-1β were also analyzed. Need to change these to fit with previous and subsequent changes were not increased significantly in DMD compared with control. Levels of TNF-α and IL-1β did not correlate with any of the MMPs or TIMPs analyzed. Moreover, TNF-α and IL-1β did not correlate with QMT or measures of ventricular function or myocardial fibrosis.

Sensitivity Analysis

When median values were substituted for levels that were removed for increased duplicate %CV, MMP1 and MMP1/TIMP1 were no longer significantly elevated in DMD patients with abnormal LVEF, although MMP1 remained elevated in DMD versus control. The remainder of the analyses were unchanged.

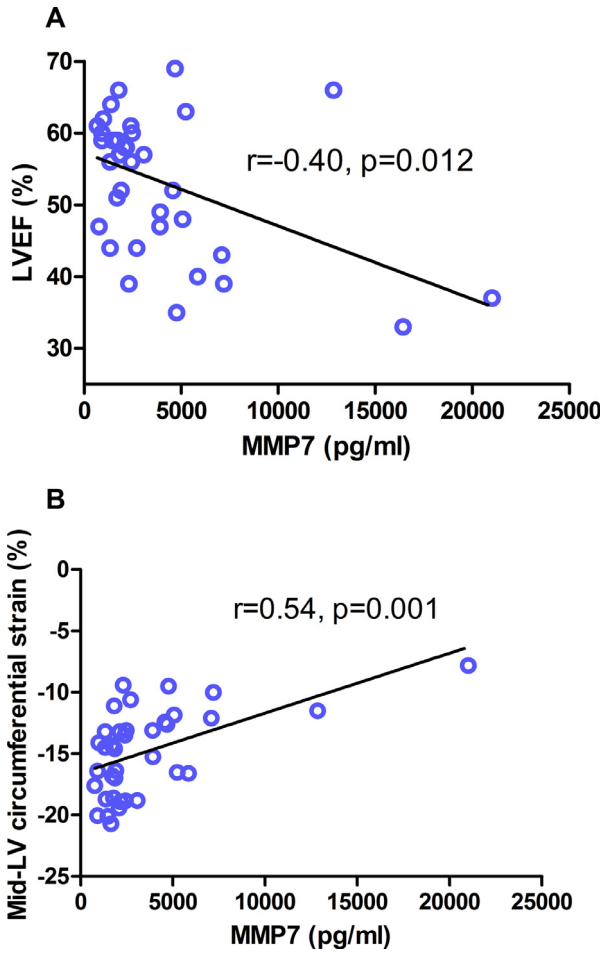

Fig. 2. (A) Correlation between matrix metalloproteinase (MMP) 7 and left ventricular ejection fraction (LVEF) (n = 39) and (B) circumferential strain at the level of the papillary muscles (n = 37).

Discussion

Our data demonstrate significant differences in MMPs and TIMPS between DMD and control subjects. In addition, multiple MMPs, MMP7 in particular, correlate with cardiovascular disease severity in DMD. These findings are important because they suggest abnormalities in extracellular matrix remodeling as an underlying cause of DMD cardiovascular disease progression. Given the dearth of DMD cardiovascular biomarkers, MMP7 has potential as a biomarker of cardiovascular disease severity.

Although standard biomarkers do not accurately reflect severity of DMD cardiovascular disease, recent investigations suggest the potential of interleukin-1 receptor−like 1 (ST2), brain-derived neurotrophic factor, and osteopontin to serve as DMD cardiovascular biomarkers.[22,23] Our data suggest that MMPs, particularly MMP7, could play a role in assessing severity of disease. Whether these laboratory values can predict disease progression or mortality will

require further study. It also must be noted that MMP7 correlates with QMT, suggesting that it is related to both cardiac and skeletal muscle fibrosis. This may limit cardiac specificity. In addition, it is likely that these correlations reflect the generalized inflammatory response known to occur in DMD, although this would not diminish the significance of the correlation with cardiovascular disease severity, and could provide a basis for assessment of therapeutics for both cardiac and skeletal muscle disease. Of note, the lack of correlation with TNF-α and IL-1β decreases the likelihood that MMPs are acting through inflammatory pathways but does not eliminate this possibility, because there are multiple other molecules through which MMPs could be signaling. A subset analysis in the 18 subjects not taking corticosteroids demonstrated a significant correlation only between MMP7 and LVEF (rho = −0.63; $P = .005$) but not measures of fibrosis. In patients taking corticosteroids, there were no significant correlations between MMP7 and LVEF or measures of fibrosis. Although these data could suggest the hypothesis that corticosteroids are acting through modulation of MMP pathways, this analysis was underpowered and further evaluation in a larger cohort is warranted before mechanistic studies are conducted.

MMPs and TIMPs regulate remodeling of the extracellular matrix and inflammatory response.[24–26] MMP7 is localized primarily in endothelial cells and vascular smooth muscle cells. There are limited reports evaluating MMP7 in DMD patients or in patients with cardiovascular disease. A previous study demonstrated elevated circulating MMP7 in DMD and in vitro studies demonstrate elevated MMP7 in DMD fibroblasts.[27,28] Nonhuman studies demonstrate improved survival after MI in mice with MMP7 gene deletion.[29] MMP7 levels also predict progression of lung disease in patients with idiopathic pulmonary fibrosis, suggesting the importance of MMP7 in multiple fibrotic disease processes.[30]

MMP1 levels are elevated in the setting of inflammation.[25] Given that inflammation is thought to be a major component of DMD progression, it is not surprising that we found elevated MMP1 compared with control. Interestingly, previous in vitro data suggest down-regulation of MMP1 in DMD fibroblasts.[27] We suspect this discrepancy is secondary to our measuring circulating MMP levels, not fibroblast expression. Indeed, other studies have suggested a down-regulation of MMP1 at the tissue level but an up-regulation in circulating MMP1.[24] Studies in adults with hypertension demonstrate elevated MMP1 associated with LV dilation and dysfunction.[31] Adults with dilated cardiomyopathy also have higher MMP1, as well as higher TIMP1 and MMP1/TIMP1, suggesting the important role of collagen homeostasis.[32]

Previous studies have demonstrated elevated MMP9 in DMD compared with control subjects and a progressive increase in MMP9 as DMD patients age.[22,33] It is notable that our data demonstrated nearly identical median values (Table 2) but a trend to elevated mean MMP9 in DMD that did not reach significance (104 ng/mL ± 70 vs 74 ng/mL ± 41; $P = .07$). The MMP9 discrepancy may be partially explained by differences in cohorts, including age and severity of skeletal muscle or cardiovascular disease, or differences in assays. Of note, ours is not the only study that has failed to detect a difference in MMP9 between DMD and control subjects.[34]

Determining factors involved in the pathogenesis of DMD cardiomyopathy may help to explain the mechanisms of current medications and help to develop novel therapeutics. Our data suggest that corticosteroids modulate MMPs and TIMPS. Corticosteroids are one of the few therapies to benefit the cardiac and skeletal myopathy in DMD, but their mechanism of action remains unclear.[35–37] It is possible that this modulation of MMPs and TIMPs is one of the reasons for corticosteroid efficacy. MMP inhibitors have also been studied for multiple disease processes, primarily oncologic. Batimistat inhibition of MMPs improved pathologic changes in skeletal muscle of mdx mice.[38] Nonspecific inhibition with the use of tetracyclines has also demonstrated promise in nonhuman animal models.[39] However, most human trials of MMP inhibitors to this point have been unsuccessful owing to poor efficacy and side-effects.[28,40] Newer and more specific medications hold promise for future studies.[41] Whether DMD patients would benefit from MMP inhibition, and which MMP should be inhibited, is unclear, but these data suggest that further study should be undertaken.

Study Limitations

Medication therapy may have modified the difference between DMD and control subjects. Unfortunately, given the early adoption of medication therapy by families of boys with DMD, it would be extremely difficult to enroll medication-naïve patients with a broad range of cardiovascular disease severity. Given the sample size limitations, we did not adjust for current or previous medications, though our data suggest that only corticosteroids led to a difference in levels. Except for MMP3 and TIMP2, most MMP and TIMP levels were lower in DMD patients on corticosteroids, suggesting that therapy would decrease the difference between DMD and control subjects. Aldosterone inhibitors are the most likely additional medication to modulate fibrosis, but only 5 patients were taking aldosterone inhibitors, making assessment of the effect of aldosterone inhibition on biomarker levels difficult. The effects of medications should be addressed in future multicenter studies.

This study evaluated the relationship of biomarkers to cardiac and skeletal muscle function at 1 point in time. Although these results are an important first step in identifying biomarkers for use in DMD, longitudinal analysis and evaluation of biomarkers in relation to future outcomes will be important before adopting these biomarkers in routine clinical practice. Owing to the smaller sample size, multivariable analysis could not be performed. Some samples were eliminated from analysis owing to suboptimal CV, despite multiple repeated assays. This suggests that, in

addition to research in a larger sample size, further optimization of the assay is necessary. Although there were multiple analyses performed in this study, all of the analyses were prespecified. However, some of the significant results may be due to a type I error. The conservative Bonferonni correction assumes that hypothesis tests are independent, but we suspect that our tests have varying degrees of correlation. Rather than make a formal adjustment for multiple comparisons, we included the results of all comparisons in Table 3.

Conclusion

MMPs and TIMPs, particularly MMP7, are related to DMD cardiac dysfunction and myocardial fibrosis, likely through their role in fibrosis and inflammation. MMP7 has potential as a biomarker of cardiovascular disease severity in DMD.

Disclosures

None.

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