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Increased Circulating Matrix Metalloproteinase-2 in Patients With Hypertrophic Cardiomyopathy With Systolic Dysfunction

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Background Some patients with hypertrophic cardiomyopathy (HCM) develop left ventricular (LV) wall thinning associated with LV dilatation and systolic dysfunction. Recently, matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) were reported to be involved in ventricular remodeling, however, little is known about MMPs and TIMPs in patients with HCM.

Methods and Results Enzyme-linked immunoassays were used to measure the plasma concentrations of MMP-2, MMP-3, MMP-9, TIMP-1, and TIMP-2 in 11 patients with HCM accompanied by systolic dysfunction (fractional shortening (FS) <25%, group A), 17 patients with HCM who had preserved systolic function (FS ≥25%, group B), and 50 age-matched clinically healthy control subjects (mean age: 57 years). The concentration of MMP-2 in group A was significantly higher than in group B and the control subjects (1,124±84, 792±49, 809±26 ng/ml, respectively), whereas there was no significant difference between group B and the control subjects. MMP-2 concentrations significantly increased as the New York Heart Association functional class increased in patients with HCM. TIMP-2 was also significantly higher in group A patients than in group B and the control subjects (45.3±4.7, 34.6±2.2, 33.7±1.8 ng/ml, respectively), but there was no difference between group B and control subjects. TIMP-1 was significantly higher in HCM patients than in control subjects. MMP-3 and MMP-9 concentrations did not differ among the 3 groups. Both MMP-2 and TIMP-2 correlated significantly with FS and LV dimension, negatively and positively, respectively.

Conclusions These results suggest that changes in the release and activity of MMP-2 and TIMP-2 may be associated with the mechanisms responsible for cardiac remodeling in patients with HCM. (*Circ J* 2004; **68:** 355 – 360)

Key Words: Cardiomyopathy; Matrix metalloproteinase; Remodeling; Systolic dysfunction

Hypertrophic cardiomyopathy (HCM) is characterized by disproportionate left ventricular (LV) hypertrophy and LV diastolic dysfunction. However, some patients with HCM develop LV wall thinning associated with LV dilatation and systolic dysfunction[1–7] The pathological features reveal that such patients have myocardium with marked fibrosis and abnormal collagen matrices[8] Evidence suggests that LV myocardial collagen matrix disorganization and changes in the fibrillar collagen network contribute to the progression of LV dilatation and dysfunction[9,10] Matrix disorganization in LV dilatation has, in turn, been attributed to enhanced collagen degradation through changes in the concentrations of matrix metalloproteinases (MMPs) or their tissue inhibitors (TIMPs)[11–13]

It has been suspected that the MMPs play an important role in tissue remodeling in both normal and pathological conditions[14–22] Enhanced activity of MMPs could lead to increased collagen turnover and has been reported in idiopathic dilated cardiomyopathy (DCM)[19] tachycardia-induced heart failure[12,20] and pressure-overload hypertrophy[13] Elevated concentrations of the serum markers of collagen turnover have also been reported in patients with idiopathic DCM[21] Soejima et al showed a significant negative correlation between the concentration of serum MMP-1 and LV ejection fraction (LVEF)[22] Collectively, these findings suggest that changes in collagen metabolism may be associated with LV remodeling and the progression of LV systolic dysfunction in patients with HCM. However, the exact details and sequence of clinicopathological events remain uncertain.

The present study was conducted in order to test the hypothesis that circulating MMPs and TIMPs may be altered in patients with HCM with systolic dysfunction and may be associated with LV remodeling in HCM.

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Table 1 Clinical Characteristics

	Control	Group A	Group B	p value
No. of cases	*50*	*11*	*17*	
Age (years)	*56.5±1.1*	*59.8±3.0*	*55.3±3.1*	*NS*
Male	*25 (50%)*	*3 (27%)*	*11 (64.7%)*	*NS*
Family history of HCM	*–*	*11 (100%)*	*11 (64.7%)*	*NS*
Family history of SCD	*–*	*6 (54.5%)*	*8 (47.1%)*	*NS*
History of chest pain	*–*	*9 (81.8%)*	*6 (35.3%)*	*0.0238*
History of syncope	*–*	*5 (45.5%)*	*0 (0%)*	*0.0047*
VT or VF	*–*	*6 (54.5%)*	*1 (5.9%)*	*0.0069*
NYHA functional class				*<0.0001*
1	*50 (100%)*	*1 (9.1%)*	*10 (58.8%)*	
2	*0 (0%)*	*1 (9.1%)*	*7 (41.2%)*	
3	*0 (0%)*	*8 (72.7%)*	*0 (0%)*	
4	*0 (0%)*	*1 (9.1%)*	*0 (0%)*	
Echocardiography				
IVST (mm)	*–*	*10.3±1.3*	*16.5±1.0*	*0.001*
PWT (mm)	*–*	*10.2±3.1*	*12.4±3.0*	*NS*
IVST/PWT	*–*	*1.12±0.17*	*1.39±0.11*	*NS*
LAD (mm)	*–*	*46.5±1.8*	*41.1±1.7*	*0.041*
LVDd (mm)	*–*	*57.3±2.1*	*46.2±1.2*	*<0.0001*
LVDs (mm)	*–*	*47.5±2.7*	*27.8±0.9*	*<0.0001*
FS (%)	*–*	*17.7±1.7*	*39.7±1.1*	*<0.0001*
Medical treatment				
β-blockers	*–*	*7 (63.6%)*	*3 (17.6%)*	*0.0204*
Ca antagonists	*–*	*3 (27.3%)*	*9 (52.9%)*	*NS*
ACE inhibitors	*–*	*5 (45.5%)*	*1 (5.9%)*	*0.0221*
Antiarrhythmic agents	*–*	*6 (54.5%)*	*4 (23.5%)*	*NS*

SCD, sudden cardiac death; VT, ventricular tachycardia; VF, ventricular fibrillation; NYHA, New York Heart Association; IVST, interventricular septal thickness; PWT, left ventricular posterior wall thickness; LAD, left atrial diameter; LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; FS, fractional shortening; ACE, angiotensin-converting enzyme.

Methods

Study Subjects

The study population consisted of 28 patients with HCM (14 men, 14 women; mean age, 57 years; range, 27–81). The diagnosis of HCM was based upon the echocardiographic demonstration of a maximum LV wall thickness ≥13 mm and the absence of any causes of ventricular hypertrophy specified by the criteria of Maron et al[23] Echocardiographic examination revealed that 23 of the 28 patients had a LV wall hypertrophy ≥13 mm. Although each of the other 5 patients had a LV wall thickness <13 mm, all of them had systolic dysfunction and a family history of HCM, and all were found to have the same gene mutation as the proband with HCM. In addition, 2 of these patients previously had demonstrated LV wall hypertrophy ≥13 mm. Therefore, these 5 patients were also enrolled in the present study as cases of 'burned out' HCM. Of the 28 HCM patients, 19 had a mutation in the cardiac troponin I (Lys183 deletion) or cardiac troponin T (Arg92Trp or Lys273Glu) gene. The patients were classified as follows: 11 patients, New York Heart Association (NYHA) functional class I; 8 patients, class II; 8 patients, class III; and 1 patient, class IV. The 28 patients with HCM were divided into 2 groups according to their fractional shortening (FS) as determined by echocardiography: 11 patients with systolic dysfunction (FS <25%) were assigned to group A, and 17 with preserved systolic function (FS ≥25%) were assigned to group B. The study included 50 age-matched clinically healthy control subjects (25 men, 25 women; mean age: 57 years; range: 41–77). Patients with chronic inflammatory disorders, liver disease, renal disease, diabetes mellitus or malignant tumors were excluded from this study, because they have high concentrations of circulating MMPs or TIMPs[14–19] The age and gender ratios of the control subjects were similar to those of the 28 patients with HCM. Informed consent was obtained from all subjects or their guardians in accordance with the guidelines of the Bioethical Committee on Medical Researches, School of Medicine, Kanazawa University.

Measurements of MMPs and TIMPs

Blood samples were taken from peripheral veins after an overnight fast and collected in ice-cold vacuum glass tubes (Venoject®, Terumo, Tokyo, Japan) containing ethylenediamine tetraacetic acid (EDTA). Next, the plasma was separated by centrifugation at 1,000 G for 10 min at 4°C. These samples were immediately frozen and stored at –80°C. Plasma concentrations of MMP-2, MMP-3, MMP-9, TIMP-1 and TIMP-2 were determined by a one-step sandwich enzyme immunoassay (EIA) method using commercially available kits with monoclonal antibodies against each substance (Daiichi Fine Chemical Co, Ltd, Toyama, Japan)[24–28] The one-step EIA system for MMP-2 recognizes both the free form of pro-MMP-2 and its complex with TIMP-2, but does not detect the active form of MMP-2. The sensitivity of the enzyme-linked immunosorbent assay (ELISA) for MMP-2 was 0.24 ng/ml[24] The EIA system for MMP-3 is capable of measuring both pro-MMP-3 and the active forms of MMP-3 as well as the forms of MMP-3 complexed with TIMPs, but MMP-3 existed only as the precursor form in human blood. The sensitivity of the ELISA for MMP-3 was 9.2 ng/ml[25] The EIA system for MMP-9 detects pro-MMP-9 (the 83 kDa intermediate species) and the complex form with TIMP-1. The sensitivity of the ELISA for MMP-9 was 0.24 ng/ml[26] The EIA system for TIMP-1 can detect both free TIMP-1 and that complexed with active forms of MMP-2, MMP-3, MMP-9

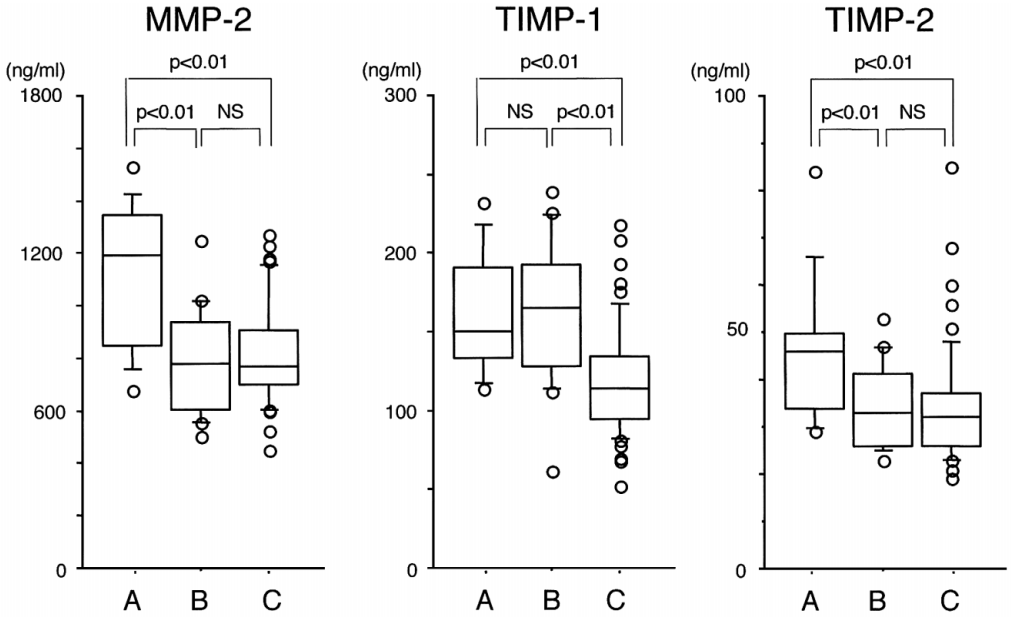

Fig 1. Plasma concentrations of matrix metalloproteinases and their tissue inhibitors. (A) Group A; (B) group B; (C) control subjects.

and pro-MMP-9. The sensitivity of the ELISA for TIMP-1 was 0.15 ng/ml.[27] The EIA system for TIMP-2 detects a free form of TIMP-2 and that complexed with active forms of MMPs, including MMP-2, MMP-3, MMP-9, but not TIMP-2 complexed with pro-MMP-2. The sensitivity of the ELISA for TIMP-2 was 1.6 ng/ml.[28]

Electrocardiographic and Echocardiographic Evaluations

Standard 12-lead electrocardiograms and 24-h Holter ECG recordings were obtained in all patients. Ventricular tachycardia (VT) was defined as >3 consecutive ventricular premature beats (wide-complex tachycardias, QRS complex >120 ms) at >120 beats/min if there was no evidence for supraventricular tachycardia with aberrant conduction.

All subjects underwent standard M-mode and 2-dimensional echocardiography to identify and quantify the morphologic features of the left ventricle. LV dimensions and the thickness of the septum and posterior LV wall were measured at the level of the mitral valve leaflet tips. When the endocardial echo was unclear, the interventricular septal thickness (IVST) and posterior wall thickness (PWT) were measured at the most distinct portion between the mitral valve and the papillary muscle level using 2-dimensional echocardiograms. The fractional shortning (FS) was calculated as the difference in end-diastolic and end-systolic diameters divided by the end-diastolic diameter.

Statistical Analysis

All values are expressed as means ± SEM. Fisher's exact test or the unpaired Student's t-test were used to compare the clinical characteristics of the study patients and control subjects. The differences between 2 independent groups regarding the concentrations of MMPs and TIMPs were tested using a non-parametric method (Mann-Whitney U-test). Among 3 groups, the Kruskal-Wallis H-test was used for analyses followed by the multiple comparison test. Linear regression analysis was employed to determine the relationship between continuous variables. All statistical analyses were performed with the Stat View 5.0 system (Abacus Concepts, Berkeley, CA, USA). A p-value <0.05 was considered statistically significant.

Results

Patient Characteristics

Table 1 summarizes the baseline characteristics of each group. The frequency of chest pain (by history), syncope (by history), and malignant ventricular arrhythmias (VT or ventricular fibrillation) was significantly higher in group A than in group B. Echocardiography revealed that the IVST was significantly smaller in group A than in group B (p=0.001). The left atrial diameters and the LV end-diastolic and end-systolic diameters were significantly larger in group A than in group B (p=0.041, p<0.0001, p<0.0001, respectively). In group A, the FS was significantly smaller than in group B.

Plasma MMPs and TIMPs Concentrations in HCM

The plasma concentrations of MMP-2 in group A were significantly higher than those in group B and the control subjects (1,124±84, 792±49, 809±26 ng/ml, respectively), as shown in Fig 1, but there was no significant difference in these concentrations between group B and the control subjects. TIMP-2 was also significantly higher in group A than in group B or the control subjects (45.3±4.7, 34.6±2.2, 33.7±1.8 ng/ml, respectively), but there was no difference between group B and control subjects in terms of TIMP-2. TIMP-1 was significantly higher in both HCM groups (groups A and B) than in the control subjects, but there was no significant difference between TIMP-1 concentrations for groups A and B (Fig 1). MMP-3 and MMP-9 concentrations did not differ among the 3 groups.

Noticeably, MMP-2 concentrations significantly increased as the NYHA functional class increased in patients with HCM (Fig 2).

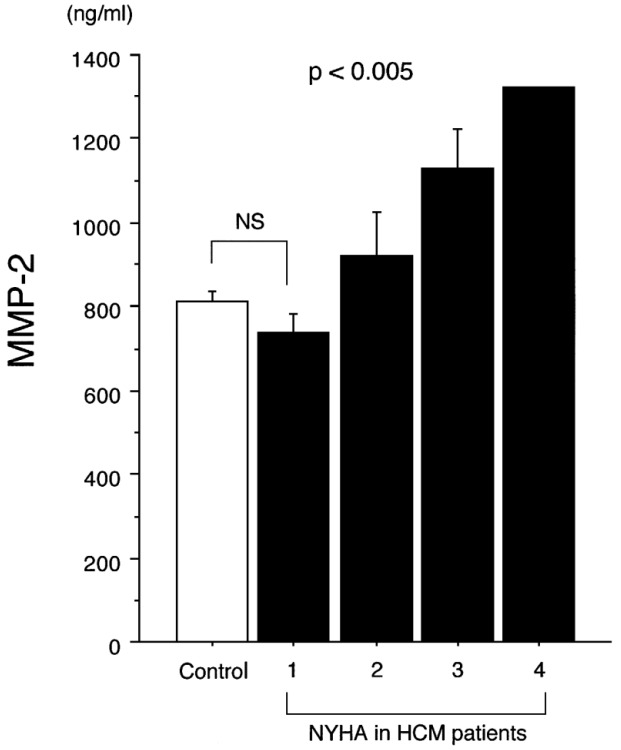

Fig 2. Plasma concentrations of matrix metalloproteinases (MMP-2) in patients with hypertrophic cardiomyopathy (HCM) according to New York Heart Association (NYHA) functional class.

Correlations Between Plasma Concentrations of MMPs and TIMPs and the Echocardiographic Parameters in Patients With HCM

There were significant negative correlations between plasma MMP-2 concentrations and FS (r=–0.61, p<0.001) and between plasma TIMP-2 concentrations and FS (r=–0.52, p<0.005), as shown in Fig 3. Plasma MMP-2 concentrations correlated positively with LV end-diastolic and end-systolic diameters (r=0.52, p<0.01, r=0.61, p<0.001, respectively), as shown in Fig 4. Plasma TIMP-2 concentrations also correlated positively with these diameters (r=0.44, p<0.05, r=0.57, p<0.005, respectively). Plasma concentrations of MMP-3, MMP-9 and TIMP-1 did not show any significant correlations with these parameters.

Discussion

Cardiac Remodeling and Progression to Dilated Phase in HCM

In some patients with HCM, LV wall thinning is associated with the development of systolic dysfunction and congestive heart failure (ie, the 'burned out' phase),[29–33] and usually develops during middle age in a relatively small but important subset of patients estimated to be approximately 10–15% of the referral-based HCM population.[1,2] In the present study, 19 of the 28 HCM patients (68%) had a mutation in the cardiac troponin I gene (Lys183 deletion) or 1 of 2 mutations in the cardiac troponin T (Arg92Trp or Lys273Glu) gene. Our previous results indicate that these 3 mutations show a high degree of penetrance and early pro-

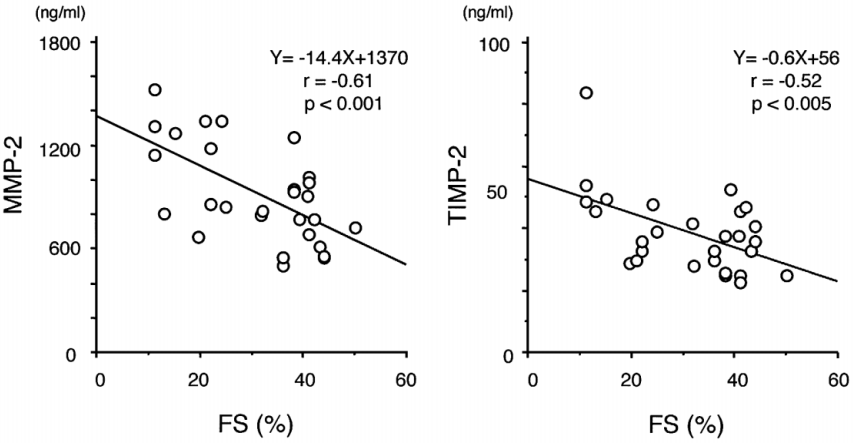

Fig 3. Correlation between plasma MMP-2 concentration, TIMP-2 concentration and fractional shortening (FS) in patients with hypertrophic cardiomyopathy (HCM).

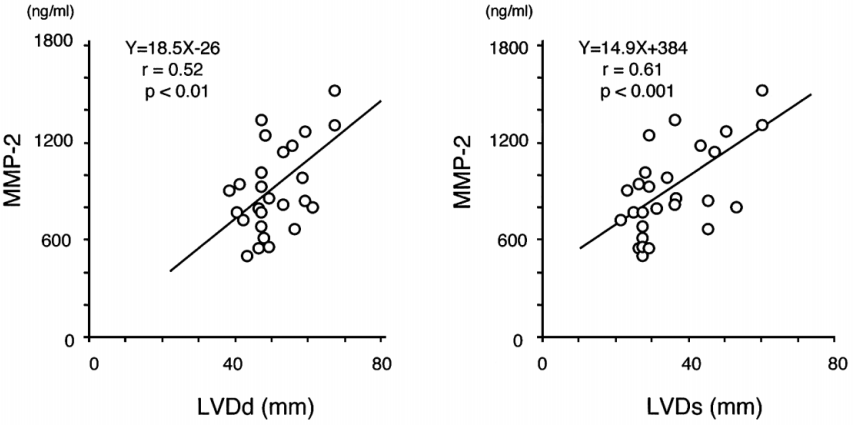

Fig 4. Correlation between plasma MMP-2 concentration and left ventricular dimensions in patients with hypertrophic cardiomyopathy (HCM). LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter.

gression to dilated cardiomyopathy-like features?[3–5]

MMPs and TIMPs in HCM

Recently, it has been suggested that MMPs become activated within the failing myocardium![11–13,20–22,33] Progressive activation of MMPs might be expected to lead to progressive degradation of the extracellular matrix, which would then lead to mural realignment of myocyte bundles and/or individual myocytes within the LV wall, and thus account for the LV wall thinning and the dilatation that occurs in heart failure patients?[34,35] MMPs are produced as a zymogen (pro-MMPs) that needs proteolytic activation by eliminating the N-terminal propeptide for the enzymes to function. To maintain tight control, MMPs are regulated at 3 main levels: transcription, activation of latent proenzymes (pro-MMPs) by several factors, and inhibition of activity by TIMPs![15] TIMPs are capable of regulating the activation of MMPs by binding to and preventing these enzymes from degrading the collagen matrix of the heart?[35,36] The presence of TIMPs is an important control point of MMP activity. Four different TIMP species have been identified and bind to activated MMPs in a 1:1 stoichiometric ratio. TIMP-1 is highly inducible by cytokines and hormones, whereas TIMP-2 expression is largely constitutive, following the pattern of expression of MMP-2, with which it interacts specifically![14,15,37] Yokoseki et al demonstrated that LVEF significantly correlated with the expressions of both MMP-2 and TIMP-2 in right ventricular endomyocardial biopsy samples from patients with idiopathic dilated cardiomyopathy?[38] Because both MMP-2 and TIMP-2 were increased in the present group A, one may hypothesize that there was no net increase in MMP-2 activity. However, there are several facts suggesting that the plasma concentrations of TIMP-2 do not directly reflect the actual values MMP inhibition. First, certain TIMPs bind to pro-MMPs and thereby form MMP–TIMP complexes. The functional significance of these pro-MMP–TIMP complexes remains incompletely understood, but they may actually facilitate MMP activation. For example, it has been demonstrated that TIMP-2 forms a complex with membrane-type MMPs and that this complex enhances the activation of pro-MMP-2. TIMP-2 promotes activation of pro-MMP-2 when membrane-type (MT)-MMP is present at low concentrations and inhibits it at higher concentrations?[39] In addition to binding to MMPs, TIMPs appear to influence cell growth and metabolism in vitro![40,41] Second, TIMP-2 measured with the kit used in the present study does not reflect the concentrations of pro- and active-MMP-2-TIMP complexes. Accordingly, increased circulating TIMP-2 concentrations might not reflect the MMP-2 inhibition simply or directly, and might be a consequence of the elevated expression of MMP-2 in HCM hearts with systolic dysfunction. In contrast to TIMP-2, the TIMP-1 concentration reflects all of the pro-MMP, active-MMP, pro-MMP–TIMP complex, and active-MMP–TIMP complex. Therefore, when the ratios of plasma MMP-2 to TIMP-1 (MMP-2/TIMP-1) were compared between groups A and B, they were found to be significantly higher in group A than in group B (7.04±0.47 vs 5.54±0.76, respectively, p=0.026). These findings suggest that an increase in MMP-2 in group A may reflect an increase in collagen degradation.

Study Limitations

One of the limitations of our study is that it included a relatively small number of patients because HCM with systolic dysfunction is not common. In addition, HCM patients having cardiac troponin I or T gene mutations were predominantly selected because they are likely to develop LV remodeling. The patients with chronic inflammatory disorders, liver disease, renal disease, diabetes mellitus or malignant tumors were excluded because they have high concentrations of circulating MMPs or TIMPs. For these reasons, the number of study patients was small. Further investigations including larger samples are necessary to confirm and clarify our results.

We could not compare the MMP-1 concentrations, which is also reported to be important in LV remodeling?[22] among study participants.

Nor did we evaluate the tissue concentrations of MMPs; previous studies have demonstrated that the myocardium is an important source for changes in the plasma concentrations of MMPs?[42,43] On that basis, our current findings led us to consider that the concentrations of circulating MMPs and TIMPs may reflect the histopathological events occurring in the failing heart of HCM patients.

All patients received medication for the treatment of heart failure and these medications may have had some influence. For medical and ethical reasons, treatments were not interrupted.

Conclusion

We report herein and for the first time that the plasma concentrations of MMP-2 and TIMP-2 increase with the severity of LV systolic dysfunction in HCM patients. In addition, they correlate negatively with the FS and positively with the LV dimensions in those with HCM. These results suggest that changes in the release and activity of MMP-2 and TIMP-2 may be associated with the mechanisms responsible for cardiac remodeling in patients with HCM.

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