Published in final edited form as:

Cardiovasc Pathol. 2009 ; 18(5): 253–261. doi:10.1016/j.carpath.2008.08.001.

Cardiac transgenic matrix metalloproteinase-2 expression induces myxomatous valve degeneration: a potential model of mitral valve prolapse disease

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Abstract

Introduction—Myxomatous mitral valve “degeneration” with prolapse (MVP) is the most frequent form of nonischemic mitral valve disease. In myxomatous valves, interstitial cells express extracellular matrix-degrading enzymes and it has been postulated that matrix metalloproteinases (MMPs) contribute to these changes.

Methods—We generated mice with cardiac-specific expression of constitutively active MMP-2 under the control of the α -myosin heavy chain promoter.

Results—These mice are normal at 4–6 months of age; at 12–14 months the mitral valves and chordae tendineae exhibit severe myxomatous change with echocardiographic MVP. Myxomatous change was also evident to a lesser extent in the aortic valves. Myxomatous changes were heterogeneous and limited to the left side of the heart with major disorganization of collagen bundles within the lamina fibrosa. Alcian blue/PAS-stained valves revealed massive accumulation of acidic glycosaminoglycans within the lamina spongiosa, consistent with valvular interstitial cell differentiation to a chondrocytic phenotype. Cells with the histologic features of hypertrophied chondrocytes were found within the chordae tendineae and the tips of the mitral papillary muscles.

Conclusion—This report demonstrates that increased activity of a single enzyme, MMP-2, within a transgenic context reproduces many of the features of the human MVP syndrome. The cardiac-specific MMP-2 transgenic mouse potentially provides a unique experimental platform for the evaluation of nonsurgical therapies based on the underlying pathophysiology of this disease. Published by Elsevier Inc.

Keywords

Mitral valve prolapse; Matrix metalloproteinase-2; Myxomatous degeneration; Chondrogenesis; Transgenic model

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1. Introduction

Mitral valve prolapse (MVP) caused by myxomatous degeneration has an estimated prevalence of 2–3% and is the most common etiology for isolated mitral valve regurgitation in the United States [1,2]. Nonsurgical treatment for MVP has been greatly hindered by a lack of understanding of the molecular pathogenesis of this disorder and the absence of a relevant animal model with which to test possible therapies. While inherited connective tissue disorders, such as Marfan syndrome, have provided potential mechanistic insights into the pathogenesis of this disorder, the majority of MVP cases are sporadic. Furthermore, the murine fibrillin-1 knockout model of Marfan syndrome bears only a partial resemblance to human MVP [3,4].

Myxomatous degeneration refers to the accumulation of primarily acid glycosaminoglycans within the spongiosa layer of the valve, with attendant disruption of the fibrous skeleton and loss of mechanical integrity. Familial inheritance with highly variable age- and sex-dependent penetrance is characteristic of most cases of idiopathic or nonsyndromic MVP [1,2]. Several chromosomal loci have been mapped, but the underlying genetic defects remain undefined [2]. In myxomatous valves, interstitial cells express extracellular matrix-degrading enzymes and it has been postulated that matrix metalloproteinases (MMPs) contribute to these changes [5,6]. Although less common, myxomatous degeneration of the aortic valve is well described, particularly in cases of isolated or idiopathic aortic regurgitation [7,8].

Myxomatous degeneration is characterized by the transition of the normally quiescent valvular interstitial cell to a proliferative, myofibroblastic phenotype [5,6], a process we have shown in renal tissues to be directly driven by the extracellular matrix-degrading enzyme, matrix metalloproteinase-2 (MMP-2 [9,10]). We recently reported on mice with cardiac-specific transgenic (TG) expression of active MMP-2 [11]. These mice exhibit a complex phenotype typified by age-dependent ventricular remodeling, systolic dysfunction, cardiomyocyte apoptosis and structural and functional mitochondrial defects [11]. While echocardiographic and histologic examination of younger mice revealed normal valvular structure and function, evaluation of older mice demonstrated mitral valve thickening and prolapse with myxomatous degeneration, the characterization of which is detailed in this report.

2. Materials and methods

2.1. Cardiac-specific MMP-2 TG mice

Cardiac-specific MMP-2 TG mice were generated as described in detail [11]. The transgene consisted of the full-length murine MMP-2 cDNA encoding a constitutively active MMP-2 protein due to an introduced V¹⁰⁷→G¹⁰⁷ mutation in the prodomain sequence. Cardiac-specific expression was driven by the α -myosin heavy chain promoter. Transgenic animals were maintained as heterozygotes within a CD-1 background.

Age-matched wild-type (WT) littermates were used as controls. All experiments and animal husbandry were carried out following the NIH Guide for the Care and Use of Laboratory Animals and local IACUC approval.

2.2. Histology and quantitative morphologic analyses

Hearts from 12.5- to 14-month-old transgenics and age-matched littermate controls ($n=8$ for each study group) were arrested in diastole with KCl at 20-mmHg pressure and fixed in buffered 4% paraformaldehyde. Multiple serial sections (5 μ m) through the levels of the mitral and aortic valves were prepared. The mitral valve apparatus, including the papillary

muscles, and the aortic root were dissected out, and paraffin-embedded sections were prepared using standard methodology. Masson trichrome, Picrosirius red (PSR) and PAS/Alcian blue stains were performed using standard methodology.

Mitral valve length (posterior leaflet) was determined by examining serial sections extending from the valve origin to the valve tip using a 10- μ m calibration standard in each image. Quantitation of the number of mitral valve interstitial cells was performed by counting cells within a 25 $\times$ 25- μ m grid placed halfway between the valvular origin and valvular tip of the posterior leaflet. Serial sections ($n=4$) were assessed for each valve preparation and data from four WT and four MMP-2 TG mice analyzed.

The severity of myxomatous degeneration was graded on PAS/Alcian blue-stained serial sections as mild (Stage I), moderate (Stage II) to severe (Stage III) according to the amounts of acidic glycosaminoglycans in the zona spongiosa (Stage I: <25%; Stage II: 25–50%; Stage III: >50%) according to the classification reported by Akiyama et al. [8].

Collagen volume fractions (CVF) of the posterior mitral valve leaflet ($n=4$ WT and 4 TG mice) were determined from digitized images of PSR-stained serial sections. The CVFs were quantified using ImageJ as detailed [11].

For c-myc epitope tag immunohistochemistry, paraformaldehyde-fixed isolated mitral valves were incubated with murine monoclonal anti-c-myc (9E11, Abcam, Cambridge, MA, USA), followed by the M.O.M. kit (Vector, Burlingame, CA, USA) and development with VIP purple substrate (Vector). Noncounterstained sections were examined using Nomarski interference contrast microscopy with a Zeiss Axiophot microscope.

2.3. Echocardiography

Transthoracic echocardiography was performed with a commercially available system (Acuson Sequoia c256, Acuson, Siemens) using a 15-MHz linear array transducer. After the anterior chest was shaved, mice were inserted into a plastic cone and maneuvered into a prone position. The cone was fixed with adhesive tape, and warm ultrasound transmission gel was used to fill the gap between the chest and the cone, so that two-dimensional imaging could be performed through the cone. Care was taken to avoid excessive pressure on the thorax, which can induce bradycardia. Two-dimensional long-axis images of the left ventricle were obtained at the plane of the aortic and mitral valves where the LV cavity is largest and a short-axis image was recorded at the level of the papillary muscles. M-mode echocardiograms were recorded through the anterior and posterior walls at a sweep speed of 200 mm/s using two-dimensional guidance. Images were acquired digitally and stored on a magneto-optical disk. All measurements were made from digital images captured on cine loops at the time of study with the use of a specialized software package (Acuson Sequoia). The LV end-diastolic dimension (LVEDD) and end-systolic dimension (LVESD) were determined as the largest and smallest dimensions of the LV, respectively, on M-mode images, and fractional shortening (FS) was derived from the following equation: $FS = (LVEDD - LVESD) / LVEDD$. The LV end-diastolic volume (LVEDV) was calculated using the following two-dimensional area-length method: $LVEDV = (5/6)A \cdot L$, where A is the endocardial parasternal short-axis area at end-diastole and L is the parasternal long-axis length.

2.4. Mitral valve RT-PCR

RNA was prepared from excised mitral valves ($n=3$ for each group) at 8 months of age. RNA integrity and quantity were determined with the Agilent 2100 Bioanalyzer. cDNA templates were generated by oligo-dT priming (Transcriptor, Roche, Alameda, CA, USA). PCR (Agilent 9800) for cartilage oligomeric matrix protein (COMP), Sox9 (SOX9),

matrillin 1 (MATN1) and normalizing GAPDH was performed with the following primer pairs:

COMP: ($T_m=62^\circ\text{C}$) 5'-GGAAACAGATGGAGCAGACG-3'/5'-CCGGGACCTGTAGAGGACTT-3'

SOX9: ($T_m=62^\circ\text{C}$) 5'-CAGCAAGACTCTGGGCAAG-3'/5'-TCCACGAAGGGTCTCTTCTC-3'

MATN1: ($T_m=58^\circ\text{C}$) 5'-TGCAGAAACAAATCTGTGTGG-3'/5'-TGGCCTCAAATTCAGTATGG-3'

GAPDH: ($T_m=60^\circ\text{C}$) 5'-TGACATCAAGAAGGTGGTGAAGCAGGCAT-3'/5'-CACCTGTTGCTGTAGCCGTATTCATTGTCAT-3'

Results are normalized to fold change in transgenics compared to wild type.

2.5. Statistics

All hemodynamic results are expressed as mean $\pm$ S.D.; *P* values were calculated using a two-tailed *t*-test. The data were analyzed using PVAN software (version 3.2; Millar Instruments) and statistical comparisons were performed with JMP (version 5.1; SAS Institute).

3. Results

The echocardiographic data from seven WT compared with eight MMP-2 TG mice at an average age of 4 months (range 3.5–4.5 months) are summarized in Table 1. All studies were done in conscious animals. There was no evidence for mitral valve thickening or prolapse in mice at this age and most cardiac functional parameters were the same in both the WT and MMP-2 TG mice. There was a modest, but significant, decrease in left ventricular fractional shortening in the TG mice at this time.

The echocardiographic data from seven WT mice compared with five MMP-2 TG mice at 14 $\pm$ 0.5 months are summarized in Table 1. There was no difference in heart rate between the two groups. As reported [11], the MMP-2 TG mice exhibited marked impairment of LV function with significant declines in LV ejection fraction and fractional LV shortening. In the transgenics, LV end-systolic volumes were more than twice the WT values. LV mass was also significantly elevated, as were long axis dimensions in both diastole and systole. Of note, aortic root dilatation was also present in the transgenics (Table 1 and Fig. 3). The MMP-2 TG mice consistently displayed echocardiographic evidence of mitral valve thickening and prolapse (Fig. 1I).

Fig. 1II (Panel A) shows a normal mitral valve apparatus stained with PSR for interstitial collagen from a 14-month-old WT mouse. The anterior and posterior valve leaflets are of normal dimensions and contain a well-defined, brightly stained lamina fibrosa composed of organized collagen. Immediately adjacent there is a small amount of ground substance (glycosaminoglycans) which characterizes the normal lamina spongiosa. In contrast, the mitral valve apparatus from the MMP-2 TG hearts is grossly distorted. Both the anterior and posterior leaflets were elongated and redundant with focal areas of massive spongiosa expansion. We measured the lengths of the mitral valve posterior leaflets from the WT and MMP-2 TG mice. The posterior leaflets from the WT mice averaged 135 $\pm$ 25 μm in length, while the posterior leaflets from the MMP-2 transgenics averaged 210 $\pm$ 13 μm in length ($n=4$ for each group; $P<.05$). Valve thickness was not determined as there was extensive variation along the length of any individual leaflet due to zones of either rarefaction or massive acidic glycosaminoglycan accumulation in the zona spongiosa that precluded accurate quantitation.

Collagen volume fractions of the posterior mitral valve leaflets were determined on PSR-stained sections. The total amounts of interstitial collagen in the WT and MMP-2 TG posterior mitral valve leaflets were not significantly different (WT: $29.7 \pm 4.8\%$; TG: $31.5 \pm 4.2\%$, $P > .05$, $n=4$ for each group). There was a major loss of collagen bundle organization within the lamina fibrosa in the MMP-2 TG mice (Fig. 1II, cf. Panels A and B). The collagen bundles comprising the lamina fibrosa are tightly organized in the WT mitral valves, with clear delineation from the surrounding ground substance. In sharp contrast, the collagen bundles in the TG valves are loosely associated and expanded within areas of intervening ground substance. As shown in the inset in Fig. 2II (Panel B), there are nodules of highly disorganized collagen in the TG valves.

Similar collagen organizational changes, although less dramatic, were seen in the aortic valve cusps of the MMP-2 transgenics (Fig. 1III, cf. Panels A and B). The aortic valve lamina fibrosa was disorganized and there was moderate expansion of the spongiosa. Valvular myxomatous degeneration was limited to the left side of the heart, as the tricuspid and pulmonary valves in the MMP-2 transgenics were structurally intact (data not shown).

Combined PAS and Alcian blue staining was performed on isolated mitral valves to determine whether neutral (PAS) or acidic (Alcian blue) glycosaminoglycans accumulated in the lamina spongiosa of the MMP-2 transgenics. Representative sections of WT (Panels A and B) and MMP-2 transgenics (Panels C and D) are shown in Fig. 2. The normal mitral valves contain modest amounts of neutral (pink) and acidic (blue) glycosaminoglycans within the lamina spongiosa. In contrast, there is massive accumulation of acidic glycosaminoglycans (chondroitin-6-sulfate, hyaluronate) within the lamina spongiosa of the MMP-2 transgenics. Expansion of the spongiosa in the MMP-2 transgenics was not uniform and was distributed in a nodular or irregular pattern. Expansion of the spongiosa in the MMP-2 transgenics was accompanied by a modest, but significant, increase in the number of interstitial valvular cells (WT: 23.5 ± 3.8 cells/ $625 \mu\text{m}^2$; TG: $34.7 \pm 3.9/625 \mu\text{m}^2$; $P = .02$). The valvular interstitial cells did acquire an elongated morphology characteristic of a myofibroblast phenotype (Fig. 2, Panel D insert). Similar to findings in human disease, the chordae tendineae from the MMP-2 transgenics demonstrated extensive myxomatous degeneration, with focal accumulation of acidic glycosaminoglycans (Fig. 2, Panel E). There was also evidence for fibrin deposition and hemorrhage within the chordae tendineae. Cells with the morphologic features of hypertrophic chondrocytes were detected within the mitral valve and the tips of the papillary muscles in the MMP-2 transgenics (Fig. 2, Panel F).

Analysis of serial sections of the proximal aortas confirmed the finding of aortic root dilatation in the MMP-2 transgenics obtained by ultrasound studies. Representative sections are shown in Fig. 3. The aortic valves were irregularly thickened along the length of the cusps primarily due to substantial expansion of the spongiosa. In addition, the TG valves demonstrated the hypercellularity of the interstitial cells along with occasional foci of inflammatory cells (Fig. 3, Panel B, blue arrow).

Aortic dilatation in the MMP-2 transgenics was confined to the level of the coronary sinus and in the example shown there was a greater than 1.5-fold increase in diameter at this level. Aortic diameters more distally were not enlarged in the MMP-2 transgenics and we did not detect histologic evidence for medial cystic degeneration or dissection. The limited extent of the aortic dilation may be the consequence of limited diffusion of the TG MMP-2 protein from the surrounding cardiomyocytes.

A key question relates to how cardiomyocyte expression of active MMP-2 under the control of the α -MHC promoter contributes to valvular myxomatous degeneration. Possible explanations include the effects of systolic dysfunction and ventricular remodeling induced

by MMP-2 on mitral and aortic valve functional dynamics, or possible diffusion of active MMP-2 from the cardiomyocytes into the valvular structures, per se. Alternatively, it was possible that MMP-2 transgene expression was induced in the setting of progressive ventricular failure due to activation of the α -myosin heavy chain promoter. To gain insight into this issue, we performed immunohistochemical staining of isolated mitral valves from 14.5-month-old mice for the c-myc epitope encoded at the C-terminal of the TG MMP-2 expression cassette. In our initial efforts, we detected little c-myc epitope staining in the mitral valves of the MMP-2 transgenics when the sections were conventionally processed with a methyl green counterstain and examined under standard microscopic conditions. However, examination of noncounterstained sections using Nomarski interference contrast microscopy revealed detectable Vector Purple reaction product within the mitral valve leaflets of the MMP-2 transgenics with a pattern consistent with valvular interstitial cell localization (Fig. 4). No c-myc epitope immunoreactive product was detected in isolated mitral valves from 4- to 6-month-old TG mice, a time which precedes the development of myxomatous degeneration (data not shown). These results are consistent with delayed expression of the MMP-2 transgene by valvular interstitial cells, presumably due to recruitment of α -myosin heavy chain promoter activity.

The cellular morphology of the myxomatous valves, coupled with the massive expansion of acidic glycosaminoglycans, is consistent with differentiation of the interstitial valve cells to a chondrocytic or chondroblastic phenotype. To substantiate this impression, we performed RT-PCR on excised mitral valve tissues and assessed the expression of three discrete genes. SOX9 is a transcription factor expressed during osteoblast and chondroblast differentiation and during gonadal differentiation [12]. Matrilin-1 is a cartilage-specific noncollagenous protein expressed during cartilage development [13]. Cartilage oligomeric matrix protein is an acidic oligomeric protein detected only in differentiated cartilage [14,15]. RT-PCR of extracted mitral valve RNA from 8-month-old mice did not reveal any detectable SOX9 or matrilin-1 transcripts in either the WT or TG mitral valves (data not shown). In contrast, low levels of COMP transcript were detected in the wild types and there was a significant, 2.7-fold increase in COMP transcript abundance in the TG mitral valves (Fig. 5). These results are consistent with a partial differentiation of the MMP-2 TG interstitial valve cells to a chondrocytic phenotype and confirm the impressions obtained with cellular morphology and expression of acidic glycosaminoglycans.

4. Discussion

Prior studies have provided evidence consistent with a role for MMP-2 in the pathogenesis of MVP. Rabkin et al. [5] reported that interstitial myofibroblasts were activated in human myxomatous valves and strongly expressed several MMPs, among them MMP-2, which was increased twofold compared with normal valves. In human specimens, Togashi et al. [16] provided gelatin zymographic evidence of major increases in MMP-2 expression in eight of nine cases of “floppy” valves, and the ratio of active/total MMP-2 was increased in these valves compared with normal specimens. Immunohistochemical studies demonstrated MMP-2 protein expression within elongated valvular interstitial cells in areas of myxomatous degeneration [16].

Immunohistochemical studies have confirmed abnormalities in the architecture and distribution of fibrillin, elastin and collagens I and II within the myxomatous leaflet tissue [17–19], and MMP-2 is capable of degrading all of these molecules, including fibrillin [20]. Furthermore, the MMP-2 TG valves had massive increases in acidic glycosaminoglycans, which have been shown to parallel the severity of valvular mechanical alterations [21].

Our data demonstrate that cardiac TG expression of a discrete matrix metalloproteinase, MMP-2, leads to pathological findings that resemble in many aspects those described in humans with the mitral valve prolapse syndrome. These features include development of the phenotype in middle age (CD-1 mice normally live 24–30 months), localization to the left side of the heart, greater mitral than aortic involvement, heterogeneous expansion of the lamina spongiosa and involvement of the chordae tendineae. In our model, the echocardiographic features of progressive atrial and ventricular enlargement and elongated chordae resemble those of advancing MVP in patients [2].

Caira et al. [22] recently noted that human myxomatous mitral valve disease is associated with the acquisition of a chondrocytic phenotype by valvular cells. Consistent with these observations, cells with the morphologic features of hypertrophic chondrocytes were detected within the myxomatous valves of the MMP-2 transgenics. The large increases in lamina spongiosa glycosaminoglycans were composed of acidic forms normally found in cartilage, and RT-PCR analysis of mitral valve RNA templates demonstrated a significant increase in expression of the differentiated chondrocyte marker, COMP. Furthermore, MMP-2 is required for limb bud mesenchymal cell differentiation to chondrogenic competent cells [23]. The murine MMP-2 knockout model displays multiple defects in skeletal development with extensive loss of cartilage [24], suggesting that MMP-2 drives the differentiation of valvular interstitial cells to a chondrocytic phenotype.

Mechanistically, MMP-2 involvement in the pathogenesis of MVP disease in the MMP-2 TG model very likely represents the accumulation of multiple abnormalities. This includes induction of MMP-2 transgene expression in the valvular tissues with chondrocytic differentiation in the setting of advancing MMP-2-mediated ventricular remodeling and systolic dysfunction. Mechanical forces including pressure and shear stress in cardiac valves as a consequence of ventricular failure also augment MMP-2 activity [25]. Cardiac-specific transcription of the MMP-2 gene is highly regulated, including activation by discrete AP-1 complexes following redox stress [26,27], both in vitro and in vivo. A further level of complexity may be due to the presence of functional haplotypes in the MMP-2 promoter [28], which could conceivably contribute to the variable penetrance so characteristic of this disorder [1,2].

Recently, considerable interest has focused on the possible role of TGF- β signaling in the pathogenesis of mitral valve prolapse as it relates to the Marfan syndrome [3]. In the mouse model studied in this report, enhanced TGF- β -mediated signals were a consequence of fibrillin-1 deficiency. As pointed out by Weyman and Scherrer-Crosbie [4], such abnormalities have not been demonstrated in sporadic MVP patients or families with the disorder. Other discrepancies of this model with the clinical presentation of MVP include adult onset compared with onset in infancy in Marfan syndrome, and homogeneous valve involvement with extensive valvular interstitial cell proliferation in the latter [3].

Recognition of a mediating role for MMP-2 in the pathogenesis of MVP may have practical implications. While nonselective MMP inhibitors, including the tetra-cyclines, doxycycline and minocycline have been evaluated with variable success in animal models of cardiovascular disease, the recent development of potent, highly selective MMP-2 inhibitors [29] provides valuable reagents to assess the efficacy of specific interventions in the MMP-2 TG model. Future studies will examine the timing and duration of MMP-2 inhibitor treatment on the development of valvular myxomatous degeneration in this model, thereby hopefully providing further insights into the pathogenesis and possible treatment of MVP.

Acknowledgments

This work was supported by National Heart, Lung, and Blood Institute Grant PO1-HL-68738 (J.S. Karliner and D.H. Lovett).

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Fig. 1.

(I) Representative echocardiograms on 14.5-month-old conscious WT (Panel A) and MMP-2 TG (Panel B) mice. Studies were performed as detailed in Materials and Methods. The WT mice demonstrate normal mitral structure and motion. The anterior leaflet of the mitral valve is thickened (upper arrow) in the MMP-2 TG hearts and there is prominent prolapse of the posterior mitral valve leaflet (lower arrow). (II) Picrosirius red staining of isolated mitral valves. (Panel A) WT mitral valve with well-organized lamina fibrosa (black arrow) in both the anterior (AL) and posterior (PL) leaflets of the mitral valve. There is a normal amount of ground substance in the lamina spongiosa (blue arrow). (Panel B) MMP-2 TG mitral valve demonstrates gross disruptions in structure with nodular expansion of the lamina spongiosa (blue arrow). Both leaflets are lengthened and thickened in a heterogeneous pattern. There is an increase in PSR-stained interstitial collagen (black arrow), but this is highly disorganized (insert, black arrow). (Magnification: A $\times 100$; B $\times 60$; insert $\times 200$.) (III) Picrosirius red staining of aortic valves. (Panel A) The noncoronary aortic cusp of the WT valve has a well-organized lamina fibrosa (black arrow) with a small amount of ground substance in the spongiosa. (Panel B) Aortic cusps from the MMP-2 TG valve are heterogeneously thickened, the lamina fibrosa is poorly organized (black arrow) and there is a moderate increase in the spongiosa. (Magnification: A and B $\times 60$.)

Fig. 2. MMP-2 TG valves and chordae tendineae exhibit myxomatous degeneration and chondrocyte transformation. Isolated mitral valve/papillary muscle preparations were sequentially stained with PAS and Alcian blue to define neutral (PAS) and acidic (Alcian blue) glycosaminoglycans. (Panels A and B) Normal mitral valve posterior leaflet from WT mice. Note the normal lamina fibrosa (black arrow) and Alcian blue-positive spongiosa (white arrow). (Panel C) The posterior mitral valve leaflet is grossly abnormal in the MMP-2 transgenics with irregular massive accumulations of Alcian blue-positive acidic mucopolysaccharides. (Panel D) Note the major expansion of the spongiosa with minimal increases in valvular interstitial cell number (insert). (Panel E) Chordae tendineae from a MMP-2 TG mouse display substantial myxomatous degeneration with large accumulations of Alcian blue-positive material. There are fibrin deposition and hemorrhage at a site of chordae rupture. (Panel F) Cells with histologic features of hypertrophic chondrocytes (arrow) are present in the tips of the papillary muscles/chordae tendineae in the MMP-2 transgenics. (Magnification: A $\times 150$; B $\times 300$; C $\times 150$; D $\times 300$; E $\times 300$; F $\times 450$.)

Fig. 3. Transgenic MMP-2 induces aortic root dilatation. (Panel A) Masson trichrome stain through the midsection of the aortic root from WT mice with normal sinuses of Valsalva. (Panel B) The aortic root diameter at the level of the sinuses of Valsalva is increased by nearly 50% in this specimen, and myxomatous thickening of the aortic cusps is readily apparent. A focus of inflammatory cells is present in the sinus (blue arrow).

Fig. 4.

Expression of c-myc-tagged MMP-2 transgene in posterior leaflet mitral valve at 14.5 months. (Panel A) Wild-type control leaflet stained for the c-myc epitope tag without counterstaining and photographed using Nomarski optics. Minimal background staining is evident. (Panel B) MMP-2 TG mitral valve leaflet with valvular interstitial cell staining for the c-myc epitope (purplish granules). (Magnification: A and B $\times 100$.)

Fig. 5. RT-PCR analysis of COMP expression. RT-PCR was performed on RNA templates isolated from 8-month-old WT and MMP-2 TG mitral valves. Relative transcript abundance of GAPDH-normalized PCR products is given, in which the control, WT abundance of COMP is assigned a value of 1. (Data expressed as mean $\pm$ 1 S.D.; $n=3$ for each group; * $P<.05$.)

Table 1

Echocardiographic assessment of function in control and MMP-2 transgenic mice

	WT (Control) (n=7)	TG (3.5–4.5 months) (n=8)	TG (14.5 months) (n=5)
LVEF (%)	82.7±1.0	77.6±1.6	66.1±4.0 *
LVFS (%)	55.7±1.4	49.0±1.7 *	38.9±3.5 *
HR (bpm)	609±7	623±4	600±6
EDV (μl)	37.1±4.2	36.7±2.0	49.8±4.0 *
ESV (μl)	6.6±1.0	8.2±0.6	17.3±3.3 *
LV mass (mg)	67.8±4.3	64.7±2.7	81.9±2.9 *
LV-Ld (mm)	5.5±0.07	5.9±0.06 *	6.1±0.06 *
LV-Ls (mm)	4.8±0.06	5.0±0.08	5.5±0.11 *
LV Epicardial area (mm ²)	0.33±0.02	0.33±0.01	0.41±0.03 *
LV Endocardial area (mm ²)	0.09±0.01	0.10±0.04	0.14±0.02 *
Aortic root diameter (mm)	1.43±0.03	1.6±0.04	1.8±0.05 *

LVEF=left ventricular ejection fraction; LVFS=left ventricular fractional shortening; HR=heart rate (beats/min); EDV=end-diastolic volume; LV-Ld=LV long-axis length; LV-Ls=LV short-axis length.

* $P<.05$ TG vs. control by ANOVA and regression equation.