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Mutation in Collagen Gene Induces Cardiomyopathy in Transgenic Mice

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Abstract In many remodeling tissues, such as the heart, collagen degradation to provide new integrin-binding sites is required for survival. However, complete loss of integrin signaling due to disconnection from extracellular matrix (ECM) leads to apoptosis and dilatation. To test the hypothesis that a mutation in type I collagen gene induces cardiomyopathy, we employed a metalloproteinase-resistant collagen mutant homozygous transgenic male (B6,129-Colla-1) and compared with age-sex matched wildtype C57BL/J6 control mice. At the age of 38–42 weeks, aortic and left ventricle (LV) pressure were measured. The LV wall thickness and diameter were measured by a digital micrometer. The levels of matrix metalloproteinase-2 (MMP-2) activity and cardiospecific tissue inhibitor of metalloproteinase-4 (TIMP-4) were measured by zymography and Western blot analyses, respectively. The levels of collagenolysis were measured by Western blot using anti-collagen antibody. In transgenic and wildtype mice, end-diastolic pressure (EDP) was 8.3 ± 1.7 and 6.5 ± 1.1 mmHg; LV diameter was 3.43 ± 0.07 and 2.94 ± 0.05 mm; wall thickness was 1.18 ± 0.03 and 1.28 ± 0.04 mm; end-diastolic wall stress was 600 ± 158 and 347 ± 49 dynes/cm^2, respectively. The increase in LV wall stress was associated with increased MMP-2 activity, increased collagenolysis, and decreased levels of TIMP-4. This leads to reduced elastic compliance in collagen mutant transgenic mice. The occurrence of cardiomyopathy in adult Colla-1 mice may be a significant confounding factor as it may be indicative of increased basal levels of ECM disruption. This phenotype is what would be expected if collagen degradation normally supplies integrin ligands during cardiac muscle remodeling. J. Cell. Biochem. 85: 259–267, 2002.

Key words: gelatinase; disintegrin metalloproteinase; ECG; LVH; cardiac ring; gene expression; functional genomic; fibrosis; elastin; heart failure

The mechanism(s) of transition from compensatory remodeling to decompensatory heart failure in cardiomyopathy is unclear [Grossman et al., 1975; Tyagi et al., 1996a; Lorell, 1997]. The consequence of various types of cardiomyopathies is associated with a decrease in cardiac muscle contractile function [WHO-ISFC, 1980; Manolio et al., 1992; Hosenpud, 1994]. The extracellular matrix (ECM) surrounding the cardiac muscle plays an important role in regulating muscle contraction and relaxation [Watson, 1991; Tyagi, 1997]. Single gene mutation in one of the cytoskeletal and ECM components is associated with the pathophysiology of cardiomyopathy in various diseases [Thierfelder et al., 1994; Hunter et al., 1995; Burn et al., 1997; Marian et al., 1997; Niimura et al., 1998; Roig et al., 1998; Dalakas et al., 2000; Wu et al., 2000]. For example, the mutation in collagen III gene is associated with Ehlers-Danlos IV syndromes [O'Connor et al., 1985]. The mutation in elastin gene is associated with William's syndromes [Ewart et al., 1994]. The mutation in tissue inhibitor of metalloproteinase-3 (TIMP-3) gene is linked to Sorsby's fundus dystrophy [Weber et al., 1994]. The mutations in cytoskeletal desmin, dystrophin as well as myosin genes are associated with various types of

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cardiomyopathies [Boileau et al., 1993; Vikstrom et al., 1996; Stollberger et al., 1998]. However, the mechanisms by which mutations in ECM and cytoskeletal genes instigate cardiomyopathy are largely unknown. Previously, we have demonstrated upregulation of matrix metalloproteinase-2 (MMP-2) gene in dilated cardiomyopathy (DCM) [Tyagi et al., 1996b]. This upregulation was modulated, in part, by increased levels of a transcription factor, activator protein-1 (AP-1), in human heart end-stage failure secondary to DCM [Tyagi et al., 1996b]. In muscular dystrophy, the serum levels of MMP activity were positively correlated with the degree of cardiomyopathy [Sohar et al., 1988] and suggested the involvement of impairment of cardiac muscle function [Kherif et al., 1999]. We hypothesized that alteration in one of the members of ECM-cytoskeletal gene family leads to changes in the composition and concentrations of other ECM components and causes detrimental consequences such as overt heart failure. To test this hypothesis, we employed a transgenic mouse model in which collagen is mutated. The results suggest that the mutation in collagen gene leads to alterations in the composition and concentrations of other ECM components, eliciting abnormalities in the remodeling, structure, and function.

MATERIALS AND METHODS

Collagen Mutant Transgenic Mice

Four male and four female homozygous transgenic mice (B6,129-Colla-1) were obtained from Jackson Laboratories. The transgenics in these mice was created as described [Liu et al., 1995]. A linearized genomic DNA fragment that included about 17 kb of coding region and 3.7 kb of 5′ and 3 kb of 3′ flanking sequences of the Colla-1 gene carrying mutation IV was microinjected into the pronucleus of fertilized FVB eggs. The eggs were incubated overnight, and two-cell embryos were transplanted to the oviduct of pseudopregnant females. The J1 ES cells carrying the subtle mutations (mutation IV) at the collagenase cleavage site were injected into C57BL/J6 embryos as described [Liu et al., 1995]. Metalloproteinase active site in collagen gene is mutated to less susceptible for cleavage in transgenic mice [Liu et al., 1995]. The site gly-775-Ile-776 is cleaved by interstitial collagenase [Liu et al., 1995]. The substitution of a pro for Ile-776 or the stabilization of the helix by substitutions of pro for gln-774 and ala-777 at p2 and p2′ rendered native collagen produced resistant to collagenase digestion [Liu et al., 1995]. These mice were bread at breading facility of the University of Mississippi Medical Center. Because originally these mice were developed to study scleroderma, at ~38–42 weeks of age, homozygous male mice developed skin abnormalities consisting of hair loss and small ulcerations [Liu et al., 1995]. This criteria along with identification of isolated cardiac collagen resistant to degradation by purified interstitial collagenase were used to identify final phenotype [Liu et al., 1995]. Because only male homozygous developed severe skin abnormalities as compared to age matched homozygous female or heterozygous male and female, we used only the male homozygous. This may suggest that transgene itself is not cardiotoxic. Age-sex matched wildtype C57BL/J6 mice were used as control. All mice were given standard chow and water ad libitum. At the age of 38–42 weeks, male homozygous collagen mutant transgenic mice were studied. Prior to anesthetizing, 24-h urine was collected from each mice in metabolic cages. The urinary protein was measured by Bio-Rad dye binding assay. All studies conformed with the principles of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and the protocol was approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee.

Electrocardiogram (ECG)

Mice were anesthetized with inactin (100 g/kg IP). This anesthesia has minimal effect on cardiovascular function [Buelke et al., 1978]. The three arm ECG was recorded using a Micro-Med ECG system. The QRS duration was measured in milliseconds.

LV Parameters

A PE-10 catheter was inserted through the right common carotid artery into aorta. One side of the catheter was connected to a pressure transducer (Micro-Med, Corp) positioned at the level of the heart. Following a 10-min stabilization period, aortic pressure (AP) was measured. For measuring LV function, a catheter was inserted into the LV. The LV pressure, end-diastolic pressure (EDP), and derivative of fall in pressure ($-dP/dt$) were recorded.

Because $-dP/dt$ is afterload dependent, $-dP/dt$ was normalized with AP [Mujumdar and Tyagi, 1999].

Cardiac Reactivity

Norepinephrine (NE) response was measured by infusing NE via a catheter in the jugular vein. At each injection of NE, the LV pressure was measured. The concentration of NE infused in the blood was determined based on the assumption that mice contain ~5 ml of blood volume. Five minutes after each dose of NE infusion, developed LV pressure (LVP) was recorded. A NE dose-response curve with LVP was constructed. The half effective concentration (EC_{50}) was determined by non-linear least squares fit using: $LVP = (A/(1 + \exp^{B(Dose - C)})) + D$, where A and D are constants and B is EC_{50}.

After assessing LV function, the heart was arrested in diastole by injecting (i.p.) 0.2 ml/100 g body weight of a 20% solution of KCl, rapidly excised, and placed in cold freshly prepared physiological salt solution. To measure the ex vivo LV and RV function, cardiac rings were prepared as described [Tyagi et al., 1999]. LV and RV wall thickness and diameters were measured by a digital micrometer. The wall stress was calculated: wall stress = (p.r. r/w)/(2(r + w)); where p is LV pressure, r is LV radius; w is LV wall thickness.

LV and RV Length-Tension Relation

The "deli" shape LV and RV rings were mounted in a tissue myobath. To keep spherical shape of the ring, an esophagus pediatric balloon was placed in the ring. One of the two mounted wires was connected to a force transducer. A known amount of stretch was placed on the ring, and the tension was measured. The tension in grams was converted to atmospheric pressure, mmHg as follows: mmHg = (tension (g) × 0.735)/(area of ring in cm^2). The data were plotted as mmHg versus stretched length (mm). The validity of deli shape rings is described previously [Tyagi et al., 1999].

Cardiac Contractile Function

The ring was stretched and brought to resting tension at which 20 mM $CaCl_2$ was added. A dose-response curve of $CaCl_2$ and contraction was created. The data were plotted as mmHg versus $CaCl_2$ concentration, $-\log M$, or pCa.

Histological Analysis

For histology, after arresting the heart in diastole, a portion of heart was fixed with 10% zinc formalin. The tissue sections from wildtype and transgenic mice were stained with Masson's trichrome for collagenous matrix. Optical light microscopy was performed at 5 × and 40 × magnifications.

Tissue Homogenates and Zymography

LV tissue homogenates were prepared as described [Tyagi et al., 1996c]. A Bio-Rad dye-binding assay was applied to estimate total protein. To determine total MMP activity, gelatin-zymography was performed. SDS–PAGE containing 1% gelatin was used as an impregnated substrate for MMP. The gels were stained by Coomassie Blue and lytic bands were scanned by a Bio-Rad GS-700 densitometer.

TIMP-4 and Actin Western Blots

To determine the levels of cardiospecific TIMP-4, Western blot analysis was performed using anti-TIMP-4 antibody (Chemicon Corp). To determine whether total proteins loaded onto the gel were identical, β-actin Western blots were performed, using anti-actin antibody (Sigma Chem Co.). The alkaline phosphatase secondary antibody was used as detection system. The bands on the blots were scanned.

Collagen degradation was measured by Western blot analysis using anti-collagen I antibody (Sigma Chem Co). The immuno-reactive band below 100 kDa was assigned as collagen degradation peptides [Tyagi et al., 1996b,c]. The bands of these peptides were scanned. The specificity of collagen breakdown peptides was determined by immuno-precipitating collagen peptides with antibody conjugated sepharose beads prior to loading onto the gel.

Collagen and Elastin

Pro-contractile elastin and anti-contractile collagen contents in cardiac rings were measured biochemically [Hodgkin et al., 1992; Mujumdar and Tyagi, 1999]. The rings were autoclaved, separately, in double-distilled water twice at 108.9°C for 3 h. The supernatant was collected to remove soluble collagenous material. The rest of tissue was defatted by a chloroform/methanol solution (2/1) and dried to a constant weight. The collagen- and fat-free specimen were then dissolved in 0.1 N NaOH at 100°C for 30-min. This procedure removed

nonelastic proteins and gave maximum recovery of major elastin specific cross-link, desmosines. After rehydration in Tris-Cl (pH 7.5), samples were digested with 1 mg tissue/20 μg thermolysin. The digested solution was analyzed for desmosine absorption at 320 nm ($\varepsilon_{320\ nm}$ 7850 $M^{-1}cm^{-1}$). There are 7.5 desmosines per tropoelastin molecule. From this absorption, the concentration of elastin was measured. Another set of tissue was analyzed biochemically for collagen content. Samples were defatted as described above. Each sample was hydrolyzed in 0.5 ml of 6 N HCl in vacuum at 115°C for 24 h. After reconstitution in 4 ml of double-distilled water, the hydroxyproline content was determined by absorbance. The collagen content was calculated by multiplying hydroxyproline levels by a factor of 7.46, assuming that hydroxyproline constitutes 13.4% of the collagen molecule.

Statistical Analysis

Data were presented as mean ± SEM. Because the aim of this study was to compare homozygous male collagen transgenic mice with wildtype male mice, the data in Tables I, II, and figures were compared as: transgenic mice with wildtype mice, using Student's unpaired *t*-test.

TABLE I. Hemodynamic and LV Parameters of Wildtype and Transgenic Mice With Collagen Mutation

	Wildtype	Transgene
Body weight, g	32 ± 1	31 ± 1
Heart weight, g	0.19 ± 0.04	0.15 ± 0.06
HW/BW × 10^3	6.13 ± 0.04	4.67 ± 0.06
Kidney weight, g	0.18 ± 0.04	0.19 ± 0.02
KW/BW × 10^3	5.81 ± 0.01	5.94 ± 0.02
Proteinurea, μg/24 h	17 ± 3	28 ± 4*
LV diameter, mm	2.94 ± 0.05	3.43 ± 0.07*
LV wall thickness, mm	1.28 ± 0.04	1.18 ± 0.03*
RV diameter, mm	1.42 ± 0.28	2.06 ± 0.22*
RV wall thickness, mm	0.41 ± 0.11	0.43 ± 0.16
Hemodynamic parameters		
AP, mmHg	123 ± 28	85 ± 17*
SBP, mmHg	133 ± 23	94 ± 21*
DBP, mmHg	111 ± 32	79 ± 17*
HR, beats/min	496 ± 67	364 ± 135*
LV parameters		
LVP_{max}, mmHg	134 ± 19	94 ± 9*
EDP, mmHg	6.5 ± 1.1	8.3 ± 1.7*
−dP/dt, mmHg/sec^{-1}	2117 ± 210	486 ± 195*
(−dP/dt)/AP, sec^{-1}	17.2 ± 2.1	5.7 ± 2.3*
LV wall stress, $dynes/cm^2$	347 ± 49	600 ± 158*

The parameters were measured in anesthetized mice. RV, right ventricle; AP, aortic pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; LVP_{max}, LV pressure maximum; EDP, end-diastolic pressure. The wall thickness was measured from lumen to epicardium. The LV diameter was measured from lumen to lumen. There is a tendency to increase in RV Wall, however, this was not statistically significant.
*$P < 0.05$, when transgene was compared to wildtype, n = 4.

TABLE II. Levels of Collagen and Elastin in per mg of Tissue

	Wildtype	Transgene
Collagen, μg/mg		
LV	4.35 ± 0.56	6.97 ± 1.2*
RV	3.61 ± 1.01	4.36 ± 0.38
Elastine, μg/mg		
LV	0.20 ± 0.03	0.15 ± 0.02*
RV	0.24 ± 0.01	0.18 ± 0.05*

The collagen was measured by hydroxyproline estimation. The elastin was measured by desmosine/isodesmosine assays.
*$P < 0.05$.

RESULTS

MMP Activity and TIMP Expression in Transgene

Because MMP-2 is present in all mammalian and can be induced during pathogenesis, MMP-2 in the LV tissue homogenate was measured by gelatin zymography. The activity of MMP-2/actin was significantly increased in collagen mutant transgenic mice as compared to wildtype control (Fig. 1). The levels of TIMP-4/actin was decreased in transgenic mice when compared with same age and sex matched wildtype control mice (Fig. 1).

Degradation of Collagen

To determine whether increased MMP-2 activity is associated with collagen degradation, LV tissue homogenates were prepared and collagen peptides were analyzed by Western blot. In collagen mutant transgenic mice collagen degradation (3/4, 75 kDa; and 1/4, 25 kDa) peptides, normalized with actin, were elevated (Fig. 2).

Cardiac Fibrosis and Tissue Injury in Transgenic Mice

To determine whether collagen mutant transgenic mice develops cardiac fibrosis, LV tissue sections from collagen mutant transgenic and wildtype mice were stained with Masson's trichrome. The results suggested increased LV fibrosis in collagen mutant transgenic mice as compared to control wildtype (Fig. 3). The qualitative degree of fibrosis was quantitated by measuring hydroxyproline/collagen and elastin contents (Table II). The LV wall was thin in transgene than wildtype (Table I). The levels of proteinurea was increased in transgenic mice than wildtype (Table I).

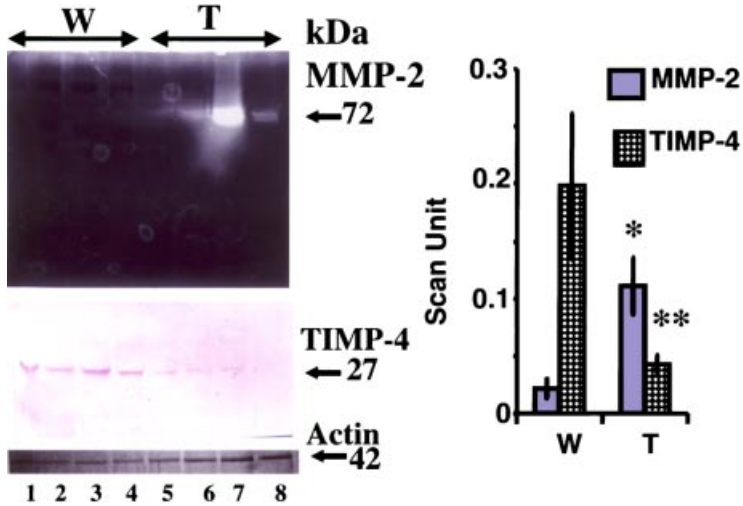

Fig. 1. MMP-2 (gelatinase A) activity and levels of TIMP-4 in Cola transgenic mice. The LV tissue extracts were prepared and analyzed by gelatin zymography for MMP-2, Westerns for TIMP-4, and actin (**left panel**). The levels of MMP-2 activity, and TIMP-4 were normalized with actin. **Lanes 1–4**, wildtype (W); **lanes 5–8**, transgene (T). The scanned lytic activity bands were normalized with corresponding levels of actin. The data are presented by histograms (**right panel**). The mean ± SEM from n = 4 in each group is reported. The $^{*}P < 0.001$; $^{**}P < 0.005$, when transgene is compared with wildtype mice. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Cardiac Contractility

To determine whether collagenolysis is associated with decreased cardiac contractility in transgenic mice, LV response to $CaCl_2$ was measured. The results suggested decreased kinetics of contraction in transgenic mice to $CaCl_2$ than wildtype mice (Fig. 4A). The magnitude of contraction was substantially decreased in transgene than wildtype control (Fig. 4B).

LV and RV Passive Diastolic Function

To determine whether collagenosis is associated with impaired cardiac compliance, LV and RV, pressure versus length relationships were performed. The results suggested that response was always lower in RV than LV and the response in both LV and RV of transgenic was significantly decreased as compared to wildtype (Fig. 5).

Cardiac Reactivity

Dose response curves suggested maximum LV pressure between 130–150 mmHg in wildtype; and 95–110 mmHg in transgenic mice (Fig. 6B). The EC_{50} of norepinephrine response in LVP was higher in transgene than wildtype

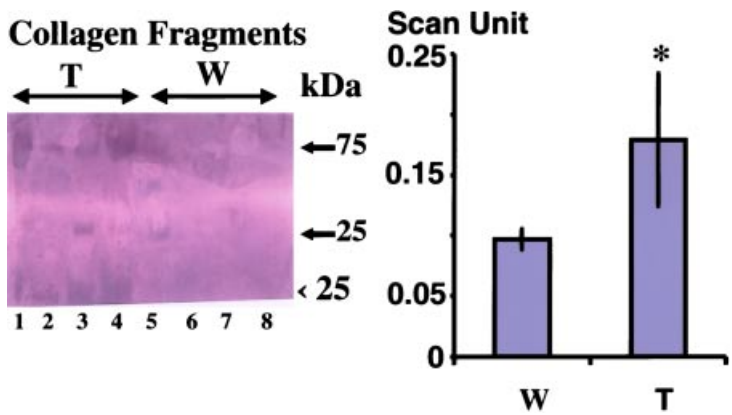

Fig. 2. Left panel: The levels of collagen degradation was estimated by measuring collagen fragments at 75, 25, and < 25 kDa by Western blot analysis. The LV tissue extracts were prepared and analyzed by anti-collagen antibody. The values of collagen fragments were normalized with actin. **Lanes 1–4** transgene (T); **lanes 5–8** wildtype (W). The bands below 100 kDa (intact α1 collagen chain) were scanned and normalized with actin. The mean ± SEM from n = 4 is reported by histograms (**right panel**). The $^{*}P < 0.01$, when transgene is compared with wildtype mice. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

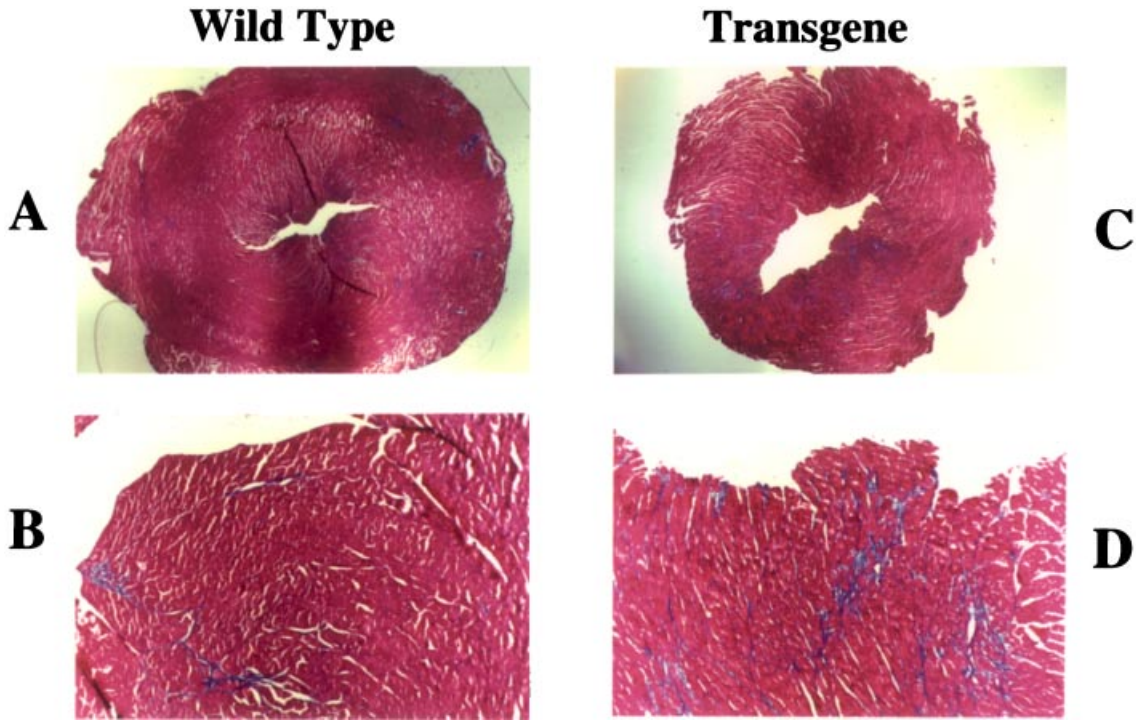

Fig. 3. LV fibrosis in transgene. Representative LV tissue sections from transgenic and wildtype mice were stained with Masson's trichrome for fibrotic collagen. **A** and **B**, wildtype; **C** and **D**, transgenic mice. A and C at 5 × magnifications; B and D at 40 × magnifications. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

control, suggesting decreased LV reactivity in transgenic mice than control mice (Fig. 6C).

LV Parameters

LV function, end-diastolic pressure (EDP), and (−dP/dt)/AP were measured. EDP was

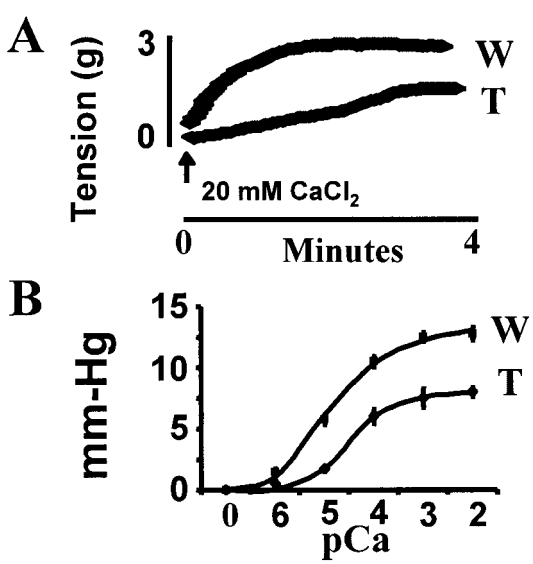

Fig. 4. LV contractile response. LV ring were prepared from transgene (T) and wildtype (W) mice. The response to $CaCl_2$ was measured in a tissue myobath. **A**: Time course of LV contraction to $CaCl_2$. The tension was recorded in grams. **B**: Dose-response curves of $CaCl_2$ contraction. Each data is a mean ± SEM of n = 4. The tension in grams was converted to dynes/cm^2 and to atmospheric pressure, mmHg.

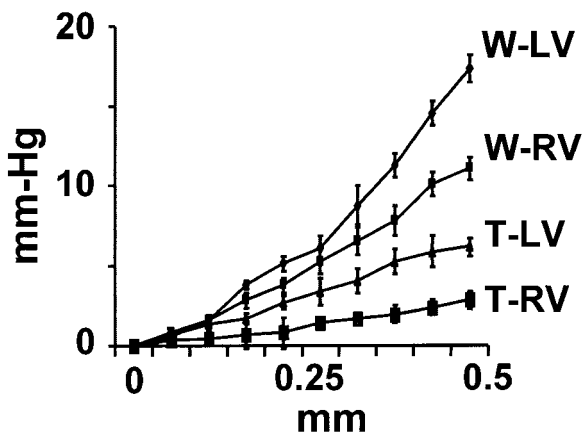

Fig. 5. LV passive diastolic function. The LV and RV length-tension relationship was measured by modification of Langendorff's preparation and by separating LV and RV, by creating ''deli'' shape rings from wildtype (W) and transgenic mice (T) in tissue myobath. The stretched length (mm) and tension (converted to atmospheric pressure mmHg) was recorded. Each data is a mean ± SEM of n = 4 rings from four animals.

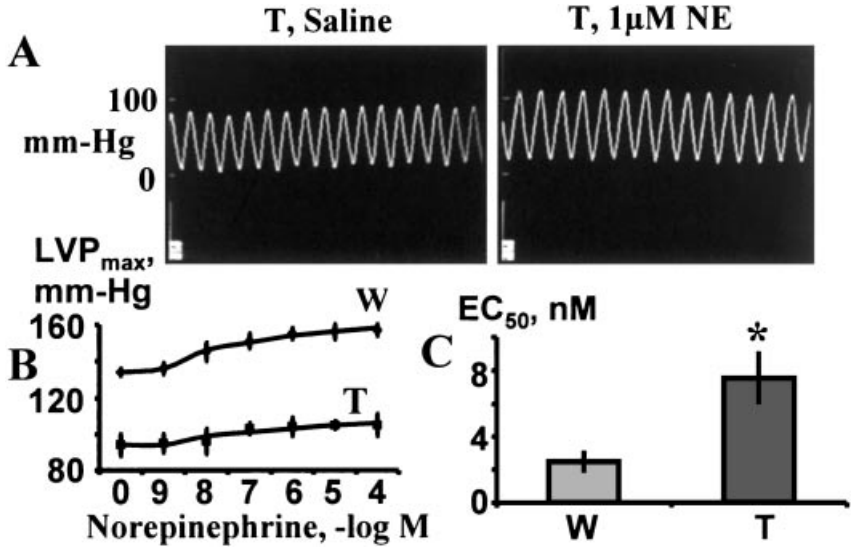

Fig. 6. LV reactivity. **A**: Tracing of wave of representing (transgene, T) in vivo LV blood pressure after saline or 1 μM norepinephrine (NE) infusion through jugular vein. **B**: Dose response curves of norepinephrine and LV pressure developed in wildtype (W) and transgenic (T) mice. **C**: Half maximal effective concentration (EC_{50}) calculated by a non-linear least squares fit to the data of norepinephrine dose and LV pressure developed. Each data was an average of at least four animals. Mean ± SEM was reported. *When T compared with W; $P < 0.001$.

increased and (−dP/dt)/AP was decreased in transgenic mice as compared to wildtype (Fig. 7).

Cardiac Conductance

To determine the degree of chaos in cardiac conduction, ECG was recorded. In transgenic mice, the R–R intervals were elongated (Fig. 8A). The duration of QRS complex (cardiac depolarization) was increased in transgenic mice as compared to wildtype control (Fig. 8B), suggesting reduction in the degree of chaos in transgenic mice.

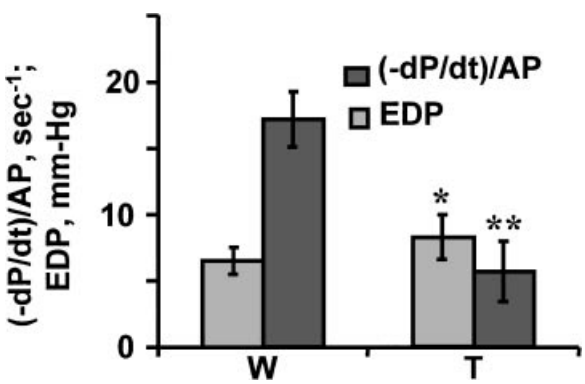

Fig. 7. LV active diastolic function. The EDP and (−dP/dt)/AP were measured in wildtype (W) and transgenic (T) mice. Each data was an average of at least four animals. Mean ± SEM was reported. *When T compared with W, $P < 0.03$; **When T compared with W, $P < 0.001$.

DISCUSSION

Results from this study suggest the two possibilities of why a specific collagen mutation causes generalized ECM disturbance; 1) the conformational changes in one member of the family lead to alteration in the composition and concentration in the others; 2) the mutation at the known collagenase site makes the collagen molecule more susceptible for degradation at

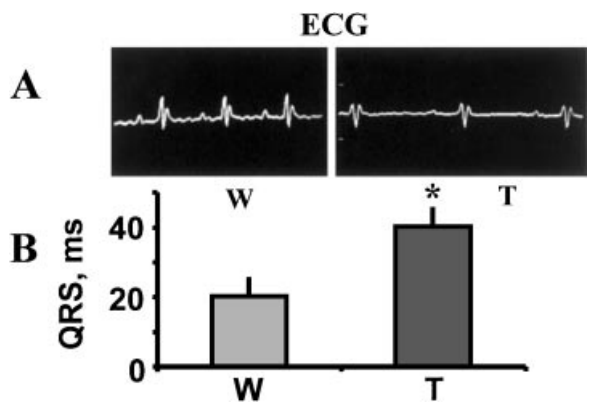

Fig. 8. Electrocardiograms of collagen mutant transgenic and wildtype mice. Mice were anesthetized by inactin (100 mg/kg, i.p.). The three electrode (left and right legs and right arm) ECG was recorded. The QRS duration (ms) was estimated by a computerized Micro-Med Software. **A**: ECG of wildtype (W) and transgenic (T) mice. **B**: Histographic representation of QRS duration. Each histographic data was an average of at least four animals. Mean ± SEM was reported. *When T compared with W, $P < 0.01$.

other sites. The levels of MMP-2 were increased and TIMP-4 decreased in collagen mutant transgenic mice, leading to collagenolysis. This was associated with decreased LV contractility.

In collagen, the Gly-Ile bond is the cleavage site of interstitial collagenase (MMP-1). The other less susceptible cleavage sites of other MMPs such as MMP-2 are hindered. However, in transgenic mice the Gly-Ile site is mutated to MMP-1-resistant. It is true that MMP-2 and TIMP-4 expressions are not directly linked to transgene expression. However, the results suggest that because MMP and TIMP are the members of ECM family, therefore, to compensate for the mutation in one member of the family, other members are affected. Because MMP-2 can degrade collagen [Aimes and Quigley, 1995], the collagen degradation products in the heart of these mice may be related to MMP-2 activation (Figs. 1 and 2). Trichrome staining of collagen shows increased fibrous collagen in the mutant mice as compared to wildtype control (Fig. 3). The levels of elastin are reversed i.e., decreased elastin content in transgenic mice as compared to wildtype (Table II). Although, collagen was resistant to collagenase digestion in transgenic mice (Fig. 3). It is known, however, that MMP-2 also degrades elastin [Senior et al., 1991; Aimes and Quigley, 1995]. Paradoxically, to reduce wall stress due to increase in workload, the metalloproteinase is activated to dilate the heart. However, metalloproteinase degrades elastin and ultrastructural collagen more efficiently than highly cross-linked collagen. The elastin turnover is slower than collagen, therefore, elastin is replaced by stiffer (highly cross-linked) collagen, and wall stress continues to increase.

To determine whether collagenolysis decreases cardiac contractility, $CaCl_2$-response to cardiac muscle in the ring was measured. The rate of cardiac contraction was decreased, and the magnitude of maximum contraction was lower in transgenic mice than wildtype control (Fig. 4). Myocardium compensates for workload by increasing its distensibility. The more there is elastin the more it is compliant (i.e., the slope of pressure-length curve stays left as compared to control). However, if there is less elastin and more collagen, the slope will shift to the right and compliance will be reduced. Because in RV there is higher elastin than LV, the curves always stay lower (Fig. 5). However, in transgenic mice the curves are below the wildtype control. These data may suggest that due to significant degradation of collagen and elastin in transgenic mice, the cardiac tensile strength is reduced. The dose response curve of NE demonstrated decreased cardiotonic receptor sensitivity in transgenic mice than wildtype control (Fig. 6). The arterial and LV pressure was significantly lower in transgenic mice than wildtype control (Table I). The force generated by cardiac muscle, i.e., $(-dP/dt)/AP$, is reduced in transgenic than wildtype. This leads to increased afterload and EDP (Fig. 7). It is possible that severe collagenolysis, bradycardia, proteinuria, and hypotension in transgenic mice lead to heart failure. The reduced degree of chaos in the spectrum of blood pressure has been suggested as a measure of degree of cardiac failure [Lombardi, 2000]. In transgenic mice at the age of 38–42 weeks, the degree of chaos was decreased, and QRS duration was increased. These results may elicit the discontinuity in cardiac conductance due to fibrosis in transgenic mice as compared to wildtype (Fig. 8). The degree of chaos is a protective mechanism of a vital organ, such as the noise in the heart waves, is a buffer to absorb any abnormality in the function. Similarly, sinus bradycardia with first degree AV block was present also in these mice. However, this is a secondary phenomenon and not a primary conduction abnormality given that the phenotype is complex. Although we did not measure physical properties of the vascular walls, it is presumed that collagen is mutated in all organs containing connective tissue. Because cardiac muscle is surrounded by collagen fibers, the cardiac muscle physiology is directly affected by the degree of fibrosis and dilatation.

Although, in adult ~12–16 week old transgenic mice, there was no significant collagenolysis, suggesting the compensatory response to mutation in the adulthood. However, at the age of 38–42 weeks, the collagen mutant transgenic mice elicit severe collagenolysis. It is important to compare the animal at different time points for collagen remodeling and hemodynamic function in these mice. Also, it is important to measure the reversibility of heart failure in these mice by inhibiting MMP activity by increasing TIMP. To this end, it is essential to administer exogenous cardiospecific TIMP-4 and measure the repair of cardiac function in collagen mutant transgenic mice. These studies are in progress.

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