Cardiac remodeling in the mouse model of Marfan syndrome develops into two distinctive phenotypes


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Marfan syndrome (MFS) is a systemic disorder of connective tissue caused by mutations in fibrillin-1. Cardiac dysfunction in MFS has not been characterized halting the development of therapies of cardiac complication in Marfan syndrome. We aimed to study the age-dependent cardiac remodeling in the mouse model of MFS Fbn1039G+/- mouse (Marfan HT mouse) and its association with valvular regurgitation. Marfan HT mice of 2-4 months demonstrated a mild hypertrophic cardiac remodeling with predominant decline of diastolic function and increased TGF-β canonical (p SMAD2/3) and non-canonical (p ERK 1/2 and p p38 MAPK) signaling and up regulation of hypertrophic markers natriuretic peptides atrium natriuretic peptide (ANP) and brain natriuretic peptide (BNP). Among older HT mice (6-14 months) cardiac remodeling was associated with two distinct phenotypes manifesting either dilated or constricted LV chamber. Dilatation of LV chamber was accompanied by biochemical evidence of greater mechanical stress, including elevated ERK1/2 and pMAPK38 phosphorylation and higher BNP expression. The aortic valve regurgitation was registered in 20% of constricted group and 60% of dilated, while mitral insufficiency was observed in 40% of constricted group and 100% of dilated group. Cardiac dysfunction was not associated with the increase of interstitial fibrosis and non-myocyte proliferation. In the mouse model fibrillin-1 haploinsufficiency results in the early onset of non-fibrotic hypertrophic cardiac remodeling and dysfunction independently from valvular abnormalities. MFS heart is vulnerable to stress-induced cardiac dilatation in the face of valvular regurgitation and stress-activated MAPK signals represent a potential target for cardiac management in MFS.

**Key words:** fibrillin 1, Marfan syndrome, myocardial hypertrophy, cardiac function
1. Introduction

Marfan syndrome (MFS) is an autosomal-dominant systemic connective-tissue disorder affecting approximately 1 in 5,000 to 1 in 10,000 people caused by heterozygous mutations in the gene encoding fibrillin-1 (26). Fibrillin-1 (FBN-1) is 350-kDa calcium binding matrix glycoprotein that assembles to form 10-12 ηm myofibrils in the extracellular matrix. This protein has two key physiologic functions: participating in the assembly of specialized matrices that define structural properties of connective tissue, and providing extracellular control for TGF-β and bone morphogenic growth factors signaling (22). There are a number of skeletal and ocular symptoms characteristic for Marfan syndrome ranging from joint supermobility to abnormally flat cornea to scoliosis to bone overgrowth (4). The most important cardiovascular manifestation consists of aortic root dilatation and subsequent predisposition for aortic root dissection and/or rupture; the later being a leading cause of morbidity and mortality in patients with MFS. Other cardiovascular manifestations of MFS include mitral valve prolapse, calcification of the mitral valve annulus, dilatation of the main pulmonary artery and dilatation or dissection of the descending aorta (3, 21, 25). Vascular manifestations of Marfan syndrome stem from overall abnormality in the homeostasis of the extracellular matrix, in which reduced or mutated forms of fibrillin-1 lead to alterations in the mechanical properties of tissues, cell-matrix interactions and impaired hemodynamic load sensing (24, 28, 34, 38). Affected tissues have a signature of increased TGF-β signaling with increased phosphorylation and nuclear translocation of the TGF-β receptor-activated SMAD proteins (SMAD2 and SMAD3), increased expression of TGF-β-responsive gene products such as collagen, connective tissue growth factor and plasminogen activator inhibitor-1 and/or increased activation of non-canonical TGF-β signaling cascades including mitogen-activated protein kinase 42/44 (ERK1/2) (2, 5, 10, 18, 23).
The abnormality of the extracellular connective tissue matrix and matrix-cardiomyocyte interaction associated with fibrillin-1 mutation may affect the cardiac muscle. Fibrillin-1 fibers are oriented in the longitudinal axis of cardiomyocytes and play an important role in transmitting contractile forces from myocytes to the extracellular connective tissue framework (3). An impaired force transmission and consequent decreased tensile strength of the myocardium associated with fibrillin-1 mutation may lead to compensatory neuro-humoral activation to support cardiac output and, eventually, to a hypertrophic remodeling. Left ventricular enlargement observed by echocardiography had been reported in a mouse model of Marfan syndrome (30). However, cardiac manifestations were described in context of abnormal mitral valve morphology, significant mitral regurgitation and/or aortic valve insufficiency resulting in hemodynamic overload imposed on left ventricle. Whether cardiac remodeling, adaptive or maladaptive, could develop due to fibrillin-1 mutation independently from hemodynamic overload remains unclear. We examined the age-dependent pathophysiologic consequences of FBN-1 deficiency on left ventricular remodeling and cardiac function in a mouse model of Marfan syndrome. Analysis of systolic and diastolic left ventricular functions, as well as assessment of cardiac remodeling were performed in Fbn1039G+/- mouse (Marfan HT mouse) harboring a cbEGF domain cysteine substitution (C1039G) in an endogenous allele (16, 29). The Fbn1039G+/- mouse model manifests the effects of heterozygous missense mutations, analogous to the clinical manifestations in affected humans.

2. Materials and Methods

2.1 Transgenic mice
A male pair of Marfan mice, provided by Dr. Dietz, Johns Hopkins School of Medicine, had C57 background. Wild type mice and mice heterozygous for the C1039G mutation (Fbn1039G+/−, Marfan HT mice) were studied at two age groups 2-4 months and 6-14 months of age. Both male and female mice in 50%/50% proportion were used. Experiments were conducted in accordance with the National Institutes of Health guidelines and with the NIA Animal Care and Use Committee approval.

2.2 Echocardiography

Marfan HT mice and age-matched wild type littermates of different age groups were assessed in vivo by transthoracic echocardiography using a probe with a center frequency of 30 MHz (Visualsonics, Toronto). The mice were placed under anesthesia using isoflurane (2.5 % in oxygen) and maintained in the supine position on the heated pad. Measurements of aortic root diameter were made at systole in parasternal long-axis view at sinus level. The heart was imaged in the two-dimensional mode (M-mode) in the parasternal long axis view at the plane of the aorta and mitral valve with visualization of the left ventricular (LV) apex. The M-mode tracings of the LV were obtained and analyzed from the parasternal long-axis view of the left ventricle. The intra-ventricular septal wall (IVS) thickness, left ventricular posterior wall thickness (LWPW), and left ventricular internal diameters (LVID) were measured in end-systole and end-diastole.

Left ventricular fractional shortening and LV mass were calculated using software provided by manufacturer as previously described (36).

The presence of aortic and mitral valve insufficiencies resulting in regurgitation flow was assessed by Doppler interrogation. Doppler recordings were performed on semilunar valve outflow in the parasternal long axis view and on the atrio-ventricular valve (mitral valve) inflow in the apical four-chamber view. All measurements were obtained using a 45° angle of
interrogation. Aortic regurgitation was assessed as reversal of outflow in diastole. Mitral valve regurgitation (reversal of inflow in systole) (11), was defined as valve incompetence if regurgitation exceeded one standard deviation of the average regurgitation for WT mouse of the same age.

2.3 Pressure-volume measurement

In vivo left ventricular function was assessed by pressure-volume technique as previously described (39, 40). Mice were anesthetized with isoflurane (2.5 % in O₂), intubated and ventilated at 120 breaths/min and a tidal volume of 200 μL and maintained on a heated pad. A standard 1.4 French pressure-conductance catheter (SPR 839; Millar Instruments, Houston, TX) was volume calibrated for the conversion of relative volume units (RVU) to absolute volume. The left ventricular apex was exposed and pressure-volume catheter was advanced through apex. The catheter was positioned along the cardiac longitudinal axis with the distal electrode in the aortic root and the proximal electrode in the LV apex. Pressure-volume data were obtained at steady state and during transient reduction in venous return by occluding the inferior vena cava (IVC) (39). All steady-state and caval occlusion pressure-volume loops were acquired with the PVAN software (Conductance Technologies, San Antonio TX and Millar Houston TX) acquisition system while the ventilation was momentarily turned off. Pressure-volume (P-V) loops were analyzed with the PVAN 3.5 software package (Millar Instruments). The major load-dependent and load-independent parameters of contractility and stiffness of the left ventricle included end-systolic volume (ESV) and end-diastolic volume (EDV), arterial elastance (Ea), stroke work (SV), preload recruitable stroke work (PRSW), arterio-ventricular coupling (Ea/Ees), the slope of end-systolic (ESPVR, Ees) and end-diastolic pressure-volume
relationship (EDPVR, Eed), maximal rate of pressure raise (+dP/dt) and decline (-dP/dt), relaxation time constant calculated by Weiss (tau w) (40).

2.4 Histological Assessment

For histological assessment mice were anesthetized with pentobarbital sodium (100mg/kg of body weight) intraperitoneally. The hearts were removed and weighed (wet weight), fixed with 10% formalin overnight, and embedded in paraffin. The hearts were further cut into two pieces through the long axis, measured, sectioned at 5 μm slices, and subjected to hematoxylin-eosin staining. Digital images of stained sections were obtained from light microscopy and analyzed using a digital imaging analysis system (MCID, InterFocus Imaging Ltd, Cambridge, UK). Myocyte diameter was measured as the shortest distance across the nucleus in transverse cell sections. Diameters of 100 myocytes from 5 randomly selected microscope fields (×200 magnification) from the LV posterior wall were averaged to represent the myocyte diameter. Myocyte density was calculated from the same area in the same fashion. Myocardial tissue fibrosis was measured in Masson's trichrome-stained sections and was expressed as a fraction of a microscopic field (×100 magnification) of the LV posterior wall. An average of 5 randomly selected fields represented results of a given specimen.

2.5 Western blot analysis

Protein analysis was performed using standard techniques. Left ventricular tissues were homogenized with RIPA buffer with phosphatase inhibitors cocktails I and II (Calbiochem) and protease inhibitor cocktail (Cell Signaling). Samples were clarified by centrifugation at 14,000 rpm, the proteins fractionated on 8-20% SDS-polyacrylamide gels. Immunoblotting was carried out with antibodies (from Cell Signaling) using the following titers: SMAD 2/3 (1:500),
phosphor-SMAD2/3 (1:250), ERK1/2MAPK (1:1000), phosphor-ERK1/2 MAPK (1:500),
p38MARK (1:1000), phosphor- p38MARK (1:500), ANP (1:250, Santa Cruz), BNP (1:250,
Abcam), alpha-SMA (1:1000, Sigma), GAPDH (Sigma, 1:1000). Detection was by enhanced
chemiluminiscence (ECL Select Western Blotting Detection). Band intensity was quantified by
Kodak image software. The ratio for protein examined was normalized against GADPH.

2.6 Statistical analysis

All data are reported as means± SE. Differences were analyzed by one- and two-way ANOVA
with Bonferroni post-test correction. Statistical significance was accepted at P<0.05.

3. Results

3.1 Cardiac remodeling and its association with valvular function in MFS mice

A scattered diagram (Figure 1A) presents results of sonographic measurements of LV internal
end-diastolic diameter (LVIDd) in young and older adult WT and HT mice. Average LV end-
diastolic diameter in young WT mice was 3.43±0.35 mm. Variability of this index among young
HT was slightly higher than in young WT mice (SD±0.39 mm), however only in 2 HT mice it
was outside of one standard deviation of mean for WT (in one mouse it was higher than the
average for WT, in another it was lower). In contrast, among older animals the distribution of the
LV internal diameters in HT mice was much wider than in WT (SD±0.24 in WT vs. ±0.84 in
HT); LVIDd of only one older HT mouse was within one standard deviation of mean for the
older WT. Distribution of LVIDd in older HT mice clearly indicated two different phenotypes of
remodeling with either dilated or reduced (constricted) LV chamber. Such a distribution strongly
suggests that separation into 2 phenotypes, dilated and non-dilated (constricted), was not age-dependent.

Representative echocardiographic images of young and older adult WT and HT mice in M-mode are presented in Figure 2A, and numerical results of echocardiographic assessment in the Table 1. Based on 2 phenotypes shown in Figure 1, Table 1 presents 2 groups of older adult HT mice - mice with either dilated or constricted LV chamber. At 2-4 months of age HT mice have exhibited some characteristics of LV remodeling: thickness of LV posterior wall and intraventricular septum were noticeably higher in HT than in WT (p<0.05). At the same time diameter of LV chamber, both in systole and diastole, in young adult mice did not differ between WT and HT, and FS was not affected by genotype (Table 1).

On the contrary, among older groups noticeable thickening of septum and LV posterior wall in HT vs WT (p<0.05) was accompanied by significant changes in LV chamber dimension – the LV diameters (both systolic and diastolic) were significantly reduced in constricted HT group and increased in dilated HT (p<0.05 vs WT). Consequently, LV diameters in constricted and dilated HT groups were significantly different from each other (p<0.05). Thickness of septum and posterior wall were also higher in HT groups vs WT (p<0.05), but only in posterior wall the thickness was significantly less expressed in HT dilated group than in constricted. LV remodeling in older HT dilated group was also accompanied by a functional decline – FS in dilated HT was significantly smaller than in older WT (p<0.05). Small increase of FS in constricted HT group did not reach statistical significance in comparison with WT.

Comparing with WT, aortic root in HT mice was dilated in both age groups (Table 1); however, in the young group the increase of aortic diameter did not reach statistical significance. At the older age (6-14 months age group), the aortic diameter was markedly increased in HT
mice exceeding that of WT by 29% for constricted group (p<0.05) and by 61% in dilated group (p<0.05); the increase of diameter of aortic root in the dilated HT group significantly exceeded that of constricted group (p<0.05).

Representative Doppler sonograms reflecting aortic and mitral valve function are shown on Figure 3. No aortic insufficiency was recorded among young WT or HT groups or among older adult WT mice (Table 1). Among older HT mice, the aortic valve regurgitation was observed in 20% of constricted group and 60% of dilated. Mitral valvular insufficiency was defined as velocity of backflow exceeding the average backflow velocity of WT of corresponding age group by more than 1SD. Mitral valve insufficiency was not observed in young or older WT mice. It was recorded, however in 13% of young HT mice. Statistical analysis of echo parameters presented in the Table 1 was performed for young HT excluding animal with mitral regurgitation. Resultant statistics demonstrated statistically significant changes in the thickness of left ventricular posterior wall and ventricular septum indicating mild cardiac hypertrophy. Among older HT mice, mitral valve insufficiency was observed in 40% of concentric group and in all (100%) of dilated (Table 1). On average, the increase of severity of mitral valve insufficiency (average velocity of regurgitation) among older mice in HT concentric group was not statistically different from WT (Figure 3C), while average outflow velocity was significantly higher in dilated HT group vs WT.

Myocardial hypertrophy in young and older adult HT mice revealed by echo measurements was confirmed by higher heart weight/body weight ratio in HT mice compared with WT counterparts measured postmortem (Figure 2B) and by higher cardiomyocyte thickness measured histologically (Figure 4 A, C-E), however in young HT mice the increase of cardiomyocyte short diameter drawn through a nucleus did not reach statistical significance. Traditional
hypothesis. It is possible that relatively modest changes in cardiomyocyte size in young HT mice were not detected with HE staining.

Note that myocardial hypertrophy in HT mice was not accompanied by the increase in collagen content (Fig. 4B).

3.2 MFS mice demonstrate complex systolic and diastolic dysfunction

Representative P-V loops for each studied group are shown on Figure 5. Results of measurements are presented in the Table 2. The LV chamber volumes were compatible with LV dimensions obtained by Echo measurements: in young HT mice LV chamber did not differ from WT either in diastole or in systole; among older groups in dilated HT both end-diastolic and end-systolic volumes were significantly larger (p<0.05) than in WT mice, but in constricted HT group the end-diastolic LV chamber was significantly smaller than in WT.

In the younger group P-V loop analyses revealed significant diastolic dysfunction in HT shown by load-dependent indices (reduction of -dP/dt and increase of tau, p<0.05). Diastolic dysfunction in young HT was confirmed by significant increase of load independent index, Eed (p<0.05 vs WT). Arterio-ventricular coupling (Ea/Ees) among young HT mice was maintained at WT level because small decline of the index of intrinsic contractility (Ees) was balanced by small elevation of arterial elastance (Ea). Nevertheless, systolic function was also affected as indicated by a significant reduction of +dP/dt and by significant reduction of the index of systolic reserve, PRSW (p<0.05).
Among older mice, the diastolic dysfunction in HT was clearly presented in both dilated and constricted groups: while tau was significantly elevated in constricted HT only, \(-\frac{dP}{dt}\) was significantly reduced in both HT groups. Moreover, the load independent diastolic index (Eed) was doubled in both HT groups comparing to WT. Decline of systolic function in older HT groups was obvious not only through a significant reduction of preload recruitable stroke work (PRSW), but also through substantial reduction of Ees, an index of intrinsic contractility: Ees in constricted HT group was less than half of that in WT and even further reduced in dilated HT (p<0.05). Massive loss of intrinsic myocardial contractility in HT mice resulted in a significant arterio-ventricular uncoupling indicated by an elevation of Ea/Ees, which was only slightly less expressed in dilated HT group due to reduction of Ea.

**3.3 ANP and BNP hypertrophic markers and TGF-β dependent signaling in Marfan HT mice**

Western blots analysis (Figure 6) of downstream targets of both canonical and non-canonical TGF-β signaling pathways showed a mild TGF-β activation in 2-4 months old HT mice as reflected in phosphorylation of SMAD2/3 transcriptional factor; in whole heart homogenates of HT mice p/t SMAD2/3 ratio was significantly higher than in WT. We observed an increase of p/t ratios of ERK1/2 and p38 MAPK in 2-4 months old HT mice. To address the role of TGF-β signaling in dilated and constricted hearts, p/t ratio of SMAD2/3, ERK1/2 and p38MAPK was compared between WT and HT mice with constricted and dilated hearts (Figure 7). Among mice of older age group the ratios of p/t ERK1/2 and p/t p38 MAPK were increased in HT mice over WT mice and in dilated group over constricted group. p/t SMAD2/3 levels were similar between WT and HT mice in constricted and dilated groups at the older age group.
Expression of cardiac hypertrophic markers - atrium natriuretic peptide (ANP) and brain natriuretic peptide (BNP) was increased in HT versus WT mice at 2-4 months age group. Both dilated and constricted hearts were characterized by increased expression of BNP compared to WT. The increase in BNP expression was more pronounced in mice with cardiac dilatation. Expression of ANP was increased only in the constricted group of older HT mice (Figure 7).

TGF-β dependent differentiation of fibroblast into myofibroblasts is identified by their expression of alpha-smooth muscle actin (alpha-SMA). Expression of myofibroblast marker alpha-smooth muscle actin was similar between WT and HT mice at both age groups (Figure 6, 7).

4. Discussion

Extensive characterization of cardiac function and remodeling in Fbn1C039G+/− mouse model of Marfan syndrome revealed factors contributing to the variances in clinical evaluation of cardiac dysfunction in MFS patients (7, 19, 20, 32, 33, 41). In our study, early remodeling was expressed as myocardial hypertrophy accompanied by up-regulation of biochemical indicators of hypertrophic growth such as natriuretic cardiac peptides ANP and BNP universally observed in young 2-4 months old HT mice. Histological examination with HE staining revealed a trend to increased diameter of cardiomyocytes in 2-4 months HT mice compared with WT mice. Technical limitation of HE staining for plasma membrane delineation may be a possible explanation of why histomorphometric measurements did not correlate strongly with hw/bw ratio and LV mass indexes of cardiac hypertrophy in young HT mice. Cardiomyocytes cross-sectional...
area evaluation using wheat germ agglutinin staining (WGA) provide better delineation of the
plasma membrane and detection of myocardial remodeling at the cellular level.

There were no indications for aortic valve insufficiency in this group; mitral valve
insufficiency was observed only in 13% of young HT mice, and arterial elastance was not
significantly elevated. Therefore, this early manifestation of cardiac remodeling was not
associated with hemodynamic overload, but likely was triggered by intrinsic mechanisms related
to the altered myocardial matrix causing persistent mechanical stress.

Diastolic dysfunction in the young HT mice was easily proven by a significant elevation
of Tau and by increased end-diastolic stiffness. Systolic dysfunction in young HT was revealed
only via pressure-volume analyses of LV performance, which demonstrated a significantly lower
PRSW – a load independent index of systolic function reflecting a reduction of systolic reserve.
These findings correspond to clinical observations: the Doppler-assisted measurements in
children with Marfan syndrome revealed a prolonged relaxation time, decreased deceleration
velocity, and decreased peak velocity ratio suggesting a selective impairment of LV relaxation.
This early diastolic dysfunction was not accompanied by any changes in fractional shortening
(33) i.e., systolic function appeared to be unaffected.

Stiffness of large arteries and associated elevation of pulse pressure is considered to be the
main determinant of aortic dilatation in MFS patients. There is also a prevailing view linking
hemodynamic afterload from increased pulse pressure to adverse cardiac remodeling and
outcome (8, 15, 16, 37). A significant increase in aortic elastance (Ea) was not detected in
younger group of HT mice, while they already demonstrated a hypertrophic remodeling and
tendency for aortic enlargement suggesting that intrinsic cardiac mechanisms were likely
involved in initiating a chamber remodeling process. In older mice, an increased aortic elastance
was observed in constricted phenotype where it could be a contributing hemodynamic factor in sustaining hypertrophic remodeling process.

One very important findings of the present study is that older MFS HT mice manifest distinct degrees in severity of cardiac remodeling that were not gender dependent. Deficient extracellular matrix (ECM) makes the heart vulnerable to severe cardiac dysfunction in the presence of valvular regurgitation or possibly other etiologies of hemodynamic overload. In addition to persistent mechanical stress from deficient extracellular matrix, dilated hearts are subjected to increased preload through deficient valves. In some older animals, severe vascular manifestations of Marfan syndrome (aortic dilatation and valvular insufficiencies) lead to increased hemodynamic stress and, combined with altered cardiac mechanobiology, resulted in cardiac dilatation. Our findings in the mouse model support clinical observations of cardiomyopathies in MFS patients, where cardiac dilatation are observed with various frequencies depending on age and gender of patients population, and different imaging techniques. Marfan patients often demonstrate rapid evolution of heart failure in the presence of hemodynamic overload well tolerated by the general population (7, 32, 41).

Myocyte-specific signaling mechanisms caused by deficient fibrillin microenvironments in MFS HT mice may be similar to vascular molecular signals leading to aortic aneurism initiation and progression, but may be different. Cardiac hypertrophy in Fbn1C1039G+/- mice may result from primary contractile dysfunction of the heart due to the underlying alterations in the mechanical features of the cardiac muscle. Both depressed contractility and relaxation may initiate signaling for cardiac remodeling reflecting complexity of calcium–dependent intracellular pro-growth pathways (9, 13).
Similarly to aortic growth and remodeling, the hypertrophic response in the heart with fibrillin-1 deficiency is likely to involve TGF-beta signaling network. Activation of TGF-β signaling, as a result of impaired sequestration of latent complexes in the extracellular matrix is likely to initiate an entire program of TGF-β disease-predisposing signals (12, 14, 27, 29, 31). This signaling includes non-canonical mitogen-activated protein kinase (MAPK) cascades such as extracellular signal activated kinase (ERK1/2), and network of kinases including p38 MAPK as well as ras-MEK, Rho GTPas, phosphoinositide-3- kinase. If canonical SMAD-dependent signaling pathway plays a major role in cardiac fibrosis, non-canonical myocyte-targeted TGF-β signaling have a central role in the maladaptive cardiac response to sustained pressure overload (21, 25, 35). In young Marfan HT mice, extracellular matrix abnormalities and phosphorylation of SMAD2/3 was not associated with the increase of interstitial fibrosis and therefore selectivity of TGF-β/SMAD2/3 axis for fibroblasts and resulting net fibrosis is not a detrimental contributor to aberrant cardiac function in early onset cardiac remodeling in Marfan HT mice. Transformation of fibroblast to myofibroblasts characterized by increased expression of alpha-smooth muscle actin (alpha-SMA) is associated with increased production of extracellular matrix components (35). The signaling axis of TGF-β dependent fibroblast trans-differentiation and up-regulation of myofibroblast marker SMA was not involved in the cardiac remodeling in our model of Marfan syndrome. In contrast to other MFS manifestations, profibrotic canonical TGF-β/SMAD2/3 signaling and non-myocyte proliferation does not play a prominent role in age-dependent cardiac remodeling in Marfan syndrome.

With age, a structurally deficient matrix component compromises the cardiac function as well as adaptation to increased workload resulting in the development of two distinct phenotypes of cardiac remodeling. These two distinct cardiac phenotypes have differences in cellular
signaling associated with severity of cardiac remodeling. Cellular pool of natriuretic peptides ANP and BNP was different in dilated and constricted group. The ANP expression was increased in constricted group only, suggesting that this important adaptive mechanism was inactivated in dilated group. Increased BNP expression in dilatation group may indicate severity of cardiac remodeling as BNP is the strongest biomarker in terms of its consistent activation with adverse cardiovascular disease outcome and heart failure (1).

Role of ERK1/2 and p38 MAP kinases are well established in cardiac pro-growth signaling network and may be a myocyte-targeted signaling pathway in Marfan syndrome related cardiac pathology. Cardiac dilatation was associated with biochemical evidence of greater hemodynamic stress associated with elevated ERK1/2 and p38MAPK activity and this hyperactivity paralleled the severity of cardiac remodeling in older HT mice. The degree of MAPK hyperactivity may depend on hemodynamic load and represent an informative biomarker of cardiac remodeling severity with the stress response ERK1/2 and p38 kinases serving as molecular readabouts of biomechanical stress. In hearts with fibrillin 1 deficiency, abnormal ERK1/2 signaling is likely to be independent from angiotensin II mediated TGF-β activation and may be activated through other upstream signaling pathways responsive to persistent mechanical stress leading to increased Angiotensin receptor 1 signaling and conformational changes in β-arrestin 2 pathway (6). Undoubtedly, mechanistic exploration and understanding of the specificity of TGF-β – dependent and independent pathways transmitting signals from extracellular matrix with deficient fibrillin-1 to cardiomyocytes will offer opportunities to clarify the mechanism of cardiac disease initiation and optimize treatment for cardiac rescue.

In the present study, we found that fibrillin-1 haploinsufficiency results in the display of early onset of cardiac hypertrophy and dysfunction. With age, cardiac remodeling progresses
into two distinct phenotypes characterized either by concentric hypertrophy or dilatation of the left ventricle. Dilatation of the LV manifests into progressive systolic and diastolic dysfunction in a face of valvular regurgitation.

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Disclosures

None

References


Figure Legend

**Figure 1.** Left ventricular internal diameter in end-diastole (LVIDd) in WT and HT mice of different ages. **A.** Scattered plot of LVIDd for WT and HT in young (left) and older (right) age groups. **B.** Scattered plot of LVIDd among older HT mice at different age. Solid horizontal line is the average LVIDd for a WT of corresponding age. Dotted horizontal lines reflect ±1 standard deviation of average LVIDd for WT of corresponding age.

**Figure 2.** Left ventricular chamber remodeling in Marfan HT mice. **A.** Representative sonographic (M-mode long-axis) images of the heart in Marfan HT mice and WT age-matched controls. Marfan HT mice display a mild cardiac hypertrophy at age 2-4 months and cardiac hypertrophy with constricted or dilated left ventricle at age 6-14 months. **B.** Quantification of ratio of heart weight to body weight n= 6 (wild type, 2-4 m), n=7 (Marfan HT, 2-4 m) n=13 (wild type 6-14 m), n=6 (Marfan HT, 6-14 m concentric hypertrophy), n=7 (Marfan HT, 6-14 m, dilatation). Values are as means±SE. * P<0.05

**Figure 3.** Representative Doppler profiles for aortic valve (AoV) outlow and mitral valve (MV) inflow velocity. At the 6-14 months age group Doppler interrogation demonstrates **A.** normal left ventricular outflow (AoV) tract without regurgitation **B.** mild aortic regurgitation and **C.**
severe aortic regurgitation  B. Mitral valve outflow a. normal mitral valve outflow b. significant
outflow (regurgitation) patterns are observed (arrows) in HT mice with chamber dilatation.  C.
Quantification of mitral valve outflow velocity (regurgitation). Outflow velocity was
significantly increased in 6-14 months old Marfan HT mice with cardiac dilatation. All values
are shown as means±SEM. * - P<0.05 wild type vs HT mice, n=5-8 animals per group.

Figure 4. Cardiomyocyte hypertrophy in Marfan HT mice of different age groups. A.
Quantification of ventricular myocytes transverse-section size in WT and Marfan HT mice. Data
represent the short diameter of cardiomyocyte drawn through a nucleus  B. Quantification of
interstitial fibrosis in WT and Marfan HT mice. * - P <0.05 wild type versus Marfan HT mice,
n=5-10 per group C-E. Representative histological sections of the myocardium from WT and
Marfan HT mice.

Figure 5. Representative pressure-volume loops during inferior vena cava occlusion in HT and
WT littermates at different ages. A. Representative pressure-volume loops at 2 months of age.
B. Representative pressure-volume loops at 6-14 months of age demonstrating two distinct
hypertrophic phenotypes (constricted and dilated) among HT.

Figure 6. A. Representative Western blots of natriuretic peptides ANP and BNP, alpha-SMA
and phosphorylated (p-) and total (t-) SMAD2/3, ERK1/2, p38 MAPK using cardiac tissue
lysates from wild type (WT) and Marfan HT mice at 2-4 months of age. B and C. Summary
data for immunoblots. * - P<0.05 wild type vs HT,  n=3 per group.

Figure 7. A. Representative Western blots of natriuretic peptides ANP and BNP, alpha –SMA
and phosphorylated (p-) and total (t-) SMAD2/3, ERK1/2, p38 MAPK from wild type (WT)
and dilated and constricted group Marfan HT mice at 6-14 months of age. **B and C. Summary**

data for immunoblots * P<0.05 wild types vs HT, # P < 0.05 Dil vs Cons, n=3
Table 1. Echocardiographic indices for WT and HT mice.

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<th>6-14 m, Older adult</th>
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<td>IVSd, mm</td>
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<td>1.19±0.1*</td>
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<td>frequency(%)</td>
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</tbody>
</table>

* P <0.05 and .01 WT vs HT in corresponding age groups #,## P<0.05 and .01 6-14m HT Constricted vs Dilated.

IVSd – intra-ventricular septum in diastole, LVIDd – left ventricular internal diameter in diastole, LVIDs-left ventricular internal diameter in systole, LVPWd- left ventricular posterior wall in diastole, FS –fractional shortening,* , ** P <0.05 and .01 WT vs HT in corresponding age groups #,## P<0.05 and .01 6-14m HT Constricted vs Dilated.
Table 2. Hemodynamic indices of WT and HT groups in young and older adult mice.

<table>
<thead>
<tr>
<th>Pressure-Volume Parameters</th>
<th>2-4 m, Young</th>
<th></th>
<th>6-14 m, Older adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT (n=7)</td>
<td>HT (n=6)</td>
<td>WT (n=12)</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>561±35</td>
<td>531±29</td>
<td>547±23</td>
</tr>
<tr>
<td>ESV (µL)</td>
<td>21.3±2.1</td>
<td>18.0±2.1</td>
<td>19.7±2.0</td>
</tr>
<tr>
<td>EDV(µL)</td>
<td>34.1±1.9</td>
<td>30.6±2.8</td>
<td>36.6±1.1</td>
</tr>
<tr>
<td>SV(µL)</td>
<td>14.4±1.7</td>
<td>11.6±2.3</td>
<td>13.9±1.4</td>
</tr>
<tr>
<td>Ea (mmHg/µL)</td>
<td>6.1±0.7</td>
<td>7.1±0.9</td>
<td>7.2±0.5</td>
</tr>
<tr>
<td>+P/dt (mmHg/sec)</td>
<td>8566±348</td>
<td>5122±313*</td>
<td>8507±849</td>
</tr>
<tr>
<td>-dP/dt- (mmHg/sec)</td>
<td>6713±654</td>
<td>4713±611*</td>
<td>6744±607</td>
</tr>
<tr>
<td>Tau W(ms)</td>
<td>7.9±0.2</td>
<td>13.2±1.1*</td>
<td>7.5±0.6</td>
</tr>
<tr>
<td>PRSW (mmHg*µL/µL)</td>
<td>69±6</td>
<td>45±4*</td>
<td>67±7</td>
</tr>
<tr>
<td>Ees (mmHg/µL)</td>
<td>8.9±1.2</td>
<td>6.9±0.7</td>
<td>8.5±1.1</td>
</tr>
<tr>
<td>Eed (10^{-3}*mmHg/µL)</td>
<td>0.22±0.02</td>
<td>0.39±0.03*</td>
<td>0.23±0.03</td>
</tr>
<tr>
<td>Ea/Ees</td>
<td>0.87±0.08</td>
<td>0.94±0.01</td>
<td>0.68±0.05</td>
</tr>
</tbody>
</table>

HR – heart rate, ESL-end-systolic volume, EDV-end-diastolic volume, SV-stroke volume, Ea – aortic elastance, +dP/dt-peak rate of pressure rise, -dP/dt-peak of pressure decline, tau –time rate of relaxation (Weiss), PRSW- preload recruitable stroke work. Ees- end-systolic elastance, Eed –end-diastolic elastance, Ea/Ees – arterio-ventricular coupling *, ** P<0.05 and 0.01. WT vs HT (in respective age groups); #, ## p<0.05 and .01 HT constricted vs. dilated (Bonferroni post hoc comparison)
Figure 2
Figure 3
Figure 4
Figure 5
Figure A shows the Western blot analysis of various proteins in WT and HT (2-4 m) groups. The proteins analyzed include alphaSMA, p-ERK1/2, t-ERK1/2, p-p38MAPK, t-p38MAPK, p-SMAD2/3, t-SMAD2/3, BNP, ANP, and GAPDH.

Figure B presents the quantification of p/p38MAPK and p/SMAD2/3 ratios, indicating significant changes in the HT 2-4 m group compared to the WT group.

Figure C illustrates the fold change in BNP, ANP, p/ERK1/2, and alphaSMA, with significant differences marked by asterisks (p<0.05).

The data suggests dysregulation in the HT 2-4 m group, particularly in the p38MAPK and SMAD2/3 pathways.
<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>HT Dil</th>
<th>HT Con</th>
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</thead>
<tbody>
<tr>
<td>alphaSMA</td>
<td></td>
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<tr>
<td>p- ERK1/2</td>
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<tr>
<td>t- ERK12</td>
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<tr>
<td>p-p38MAPK</td>
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<tr>
<td>t- p38MAPK</td>
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<tr>
<td>p-SMAD2/3</td>
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<tr>
<td>t- SMAD2/3</td>
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<tr>
<td>BNP</td>
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<td></td>
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<tr>
<td>ANP</td>
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<td></td>
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<tr>
<td>GAPDH</td>
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</table>

Figure 7

<table>
<thead>
<tr>
<th></th>
<th>WT 6-14 m, n=3</th>
<th>HT 6-14 m, Dil, n=3</th>
<th>HT 6-14 m, Con, n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/I ERK1/2</td>
<td>**</td>
<td>**##</td>
<td>**</td>
</tr>
<tr>
<td>P/I p38</td>
<td>**</td>
<td>**</td>
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