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Fibrillin-1 genetic deficiency leads to pathological aging of arteries in mice

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Abstract

Fibrillin-1, the major component of extracellular microfibrils that associate with insoluble elastin in elastic fibers, is mainly synthesized during development and postnatal growth and is believed to guide elastogenesis. Mutations in the fibrillin-1 gene cause Marfan syndrome, a multisystem disorder characterized by aortic aneurysms and dissections. The recent finding that early deficiency of elastin modifies vascular aging has raised the possibility that fibrillin-1 deficiency could also contribute to late-onset pathology of vascular remodeling. To address this question, we examined cardiovascular function in 3 week-old, 6 month-old, and 24 month-old mice that are heterozygous for a hypomorphic structural mutation of fibrillin-1 (*Fbn1*$^{+/mg\Delta}$ mice). Our results indicate that *Fbn1*$^{+/mg\Delta}$ mice, particularly those that are 24 month-old, are slightly more hypotensive than wild-type littermates. Additionally, aneurysm and aortic insufficiency were more frequently observed in aging *Fbn1*$^{+/mg\Delta}$ mice than in the wild-type counterparts. We also noted substantial fragmentation and decreased number of elastic lamellae in the aortic wall of *Fbn1*$^{+/mg\Delta}$ mice, which were correlated with an increase in aortic stiffness, a decrease in vasoreactivity,

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Authors declare no conflict of interest.

STATEMENT OF AUTHOR CONTRIBUTIONS

MB and FG conceived and carried out experiments, and analysed data. HP and RF conceived experiments. PM, EB, BS, AJP, SB, QD and JMP carried out experiments. All authors were involved in writing the paper and had final approval of the submitted and published versions.

altered expressions of elastic fiber-related genes, including fibrillin-1 and elastin, and a decrease in the relative ratio between tissue elastin and collagen. Collectively, our findings suggest that the heterozygous mgΔ mutation accelerates some aspects of vascular aging and eventually lead to aortic manifestations resembling those of Marfan syndrome. Importantly, our data also indicate that vascular abnormalities in *Fbn1*$^{+/mg\Delta}$ mice are opposite to those induced by elastin haploinsufficiency during aging that affect blood pressure, vascular dimensions and number of elastic lamellae.

Keywords

elastic lamellae; fibrillin-1; Marfan syndrome; arterial aging; aneurysm; transgenic mice

INTRODUCTION

Elasticity is a vital physiological property of large arteries. During systole, pressurized blood dilates large arteries while elastic components of the arterial wall store energy. During diastole, the arterial wall releases the accumulated energy by compressing the blood, therefore maintains blood pressure and flow into the arterial tree [1.]. Circumferentially-oriented elastic lamellae made of elastin and microfibrils fulfill this function in the closed circulatory system of higher vertebrates, whereas microfibrils devoid of elastin are the sole elastic components of invertebrate arteries owing to their limited stretching in response to mechanical force [2,3.]. Microfibrils are macromolecular structures that are mainly composed of fibrillin-1 and which also include microfibril-associated glycoproteins (MAGPs), latent TGF-β-binding proteins (LTBPs) and fibulins [4.]. Despite their relatively low abundance (~10% compared to elastin), microfibrils exert a significant mechanical function in the elastic fibers of large arteries as evidenced by the vascular manifestations of fibrillin-1 mutations in Marfan syndrome (MFS), which include increased arterial stiffness and abnormal aortic wall architecture that precipitate dissecting aneurysms [5–10.].

Elastic fibers undergo a slow age-dependent degradation due to the progressive imbalance between elastolytic enzymes and their inhibitors, leading to the release of elastin peptides with vasodilatory properties [11–13.]. In accordance with these findings, genetic elastin deficiency was recently shown to modify the physiological process of arterial aging in *Eln*$^{+/-}$ mice [14.]. As already alluded, several mouse strains with discrete *Fbn1* mutations have been created that replicate the clinical spectrum of MFS, including aneurysmal pathologies that manifest during neonatal or adult life [15–18.]. Amongst them is the so-called mgΔ mutation, a hypomorphic in-frame deletion of exons 19–24 of *Fbn1*, which in homozygosity causes dissecting aneurysm and death soon after birth but has no apparent effect on the viability and fitness of young heterozygous mutant mice [17.]. The present study employed *Fbn1*$^{+/mg\Delta}$ mice to investigate whether fibrillin-1 may also contribute to cardiovascular function during aging. The results of these analyses revealed that the *Fbn1* mutation promotes several negative changes in cardiovascular function as the mice ages, including an increased rate of aortic aneurysm and rupture.

METHODS

Animals

The present study employed mice heterozygous for a hypomorphic in-frame deletion in fibrillin-1 (*Fbn1*$^{+/mg\Delta}$), which were backcrossed for >5 generations into the C57Bl/6J background [17.]. The analyses were performed on male *Fbn1*$^{+/mg\Delta}$ mice and *WT* littermates (controls) of three age-groups: 3 week-old (young animals), 5–7 month-old (adult animals, called 6 month-old) and 23–27 month-old (old animals, called 24 month-old). No change in

neonatal mortality were observed in *Fbn1*$^{+/mg\Delta}$ mice compared to wild-type littermates. Housing and surgical procedures were in accordance with institutional guidelines.

Blood pressure and heart weight

Mice were anesthetized using isoflurane (1.5–2%), and placed on a heating table to maintain body temperature at 37°C. Immediately after completion of anesthesia, verified by absence of response to pinching of the mouse toes, a Millar probe SPR838 was inserted into the carotid artery then moved to the ascending aorta where the blood pressure was measured. In other animals, anesthetized with pentobarbital (60mg/kg, IP), hearts and left ventricles were dissected, washed, and weighed (wet weight).

Ultrasound

Transthoracic echocardiography was performed with a Toshiba Powervision 6000, SSA 370A device equipped with an 8- to 14-MHz linear transducer, as previously described [19,20.]. Procedures are detailed in Online Supporting Information 1.

Arterial desmosine and hydroxyproline content

Desmosine were determined by radioimmunoassay, as described [21.]. Total protein in the tissue hydrolysates was determined by a ninhydrin based method [22.]. Hydroxyproline levels were determined by amino-acid analysis using high-pressure ion-exchange chromatography on a Biochrom 30 amino acid analyzer. Norleucin was used as an internal standard. Results are expressed as amino-acid mass per vessel segment length and as amino-acid mass per vessel total protein content.

Aorta mechanics

Mechanical studies were performed on excised cannulated ascending aortae, as described [14,23.]. Procedures are detailed in Online Supporting Information 1.

Histological analyses

Aortae were classified as aneurysmal/symptomatic when obvious dilation of a vessel section was observed by gross anatomy/visual examination. Transverse sections of the paraffin-embedded ascending aorta (pathological or non-pathological) were stained with hematoxylin-eosin for cells, Weigert for elastic fibers, and picrosirius red for collagen. Some aortae were fixed by cardiac perfusion with 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.4), then embedded in Epon for ultrastructural analyses. Semi-thin sections were stained as described [24.], and stained with tannic acid before uranyl acetate.

RNA analyses

Total RNA was extracted from entire aortae and gene expression levels were evaluated by real-time PCR. Resulting values were normalized against the expression of the housekeeping gene Hypoxanthine guanine PhosphoRibosyl Transferase (HPRT) and total mRNA input. Amplification primers for the tested genes have been previously described [14.]. Procedures are detailed in Online Supporting Information 1.

Aorta reactivity

Variations of cannulated aorta diameters in response to 10μmol/L of the vasoconstrictor phenylephrine (PE), then 10 μmol/L PE + 10 μmol/L of the vasodilator acetylcholine (Ach) were assessed at 75 mmHg [14,23.]. Diameters of *Fbn1*$^{+/mg\Delta}$ mouse aortae were normalized against the mean diameter of *WT* aortae [11.].

Statistics

Two- or three-way ANOVA followed, when necessary, by Fisher's least significant difference test (LSD) or t-test for paired value comparisons, were used to evaluate the significance of most parameters. Nonparametric Mann-Whitney U test was used to assess vessel midwall strain, stress, incremental elasticity modulus and distensibility. The results are presented as mean values ± standard error of the mean (SEM). P values ≤ 0.05 were considered as statistically significant.

RESULTS

The mgΔ allele encodes a shortened fibrillin-1 molecule that is produced at ~10% of the normal level [17.]. Previous investigations showed that newborn *Fbn1*$^{mg\Delta/mg\Delta}$ mice succumb to cardiovascular and/or respiratory complications, but did not determine whether seemingly normal *Fbn1*$^{+/mg\Delta}$ mice display cardiovascular manifestations past their reproductive age [17,25.]. In the present study, we noted a statistically borderline loss of *Fbn1*$^{+/mg\Delta}$ viability only between 12 and 20 months of age (p=0.06; χ^2 test at 18 months) in the absence of substantial changes in body weight (Fig. 1A and Table I). We also found lower blood pressures and higher pulse pressure in *Fbn1*$^{+/mg\Delta}$ mice compared with *WT* littermates (three-way ANOVA, p≤0.05; Table I), conceivably as a result of aortic insufficiency in *Fbn1*$^{+/mg\Delta}$ mice (see below). As shown in a previous study in which blood pressure was found similar in awake or anesthetized mice, an effect of anesthesia explaining this inter-group difference could not be suspected [23.]. Hence, several critical parameters of cardiovascular physiology were compared between young (3 week-old), adult (6 month-old), and old (24 month-old) *Fbn1*$^{+/mg\Delta}$ and *WT* mice.

Cardiovascular function

Ultrasound measurements through the cardiac cycle of ascending aortae in adult *Fbn1*$^{+/mg\Delta}$ mice revealed increases of 38 % and 71 % in diastolic diameter and aortic surface, respectively (Table I and online supporting information 2A). The unusual presence of aortic aneurysm was also noted in 31% (4 out of 13) of adult *Fbn1*$^{+/mg\Delta}$ mice (vs. 7% in adult *Fbn1*$^{+/+}$ animals; 1 out of 15), which reached a significantly greater frequency than normal (67% or 8 out of 12 mice) in old *Fbn1*$^{+/mg\Delta}$ mice (vs. 17% in aged *Fbn1*$^{+/+}$ animals; 2 out of 12) (Fig. 1B). Additionally, aortic compliance (estimated from the ratio of the diameters and pressure gradients between systole and diastole) was reduced in some, not all, adult mutant animals, leading to a non-significant trend towards decreased compliance in this group (Table I). Retrograde flow (measured using color-Doppler mode) was also identified in some adult (2 out of 8) and some old (3 out of 5) *Fbn1*$^{+/mg\Delta}$ mice (Table I and online supporting information 2B,C). These aortic insufficiencies (AI) were either mild with no cardiac complications or severe with a consistent pattern of left ventricle (LV) dysfunction (as evidenced by signs of LV remodeling, such as dilation and wall thickening leading to hypertrophy) affecting both ventricular contraction and relaxation. On the contrary, in non-symptomatic (= non AI/non aneurysmal) *Fbn1*$^{+/mg\Delta}$ mice, cardiac dimensions and function were not modified. The variability within the *Fbn1*$^{+/mg\Delta}$ mice, i.e. presence of both clearly pathological and relatively unaffected animals, leads to a trend of structural and functional cardiac alterations in this group regarding some parameters, e.g. left ventricle end diastolic diameters and left ventricle weight to body weight ratios (Table I). Collectively, these in vivo data suggested that the mgΔ mutation accelerates some aspects of the physiological process of vascular aging and also leads to pathological vascular aging to different degrees in heterozygous mutant mice. Phenotypic heterogeneity of genetically identical *Fbn1*$^{+/mg\Delta}$ mice resembles the clinical variability of MFS.

Ascending aorta morphology

Histological examination of the ascending aortae documented significant disorganization of the medial layer in *Fbn1*$^{+/mg\Delta}$ mice of all ages, which is characterized by thinner and often disrupted elastic lamellae (Figs. 1C and 2, and Table II). Interestingly, morphometric analyses also revealed that the medial layer of fibrillin-1 haploinsufficient aortae contains one less elastic lamella than normal irrespective of the age of the mice (Table II). This observation is consistent with the concept that has emerged from the analysis of elastin haploinsufficient mice that the aortic wall undergoes adaptive changes in response to changes in hemodynamic stress [23,26.]. Additional findings in adult and old *Fbn1*$^{+/mg\Delta}$ aortae included more diffused collagen staining, as well as histological evidence of dilation and rupture (Fig. 1D and 2G,N,U). Dissecting aneurysm was most frequently observed in tissue samples from old mutant mice in association with a more dramatic disorganization of the medial layer and fragmentation of elastic lamellae (Fig. 1D and 2, and Table II). These in vitro data therefore corroborated the in vivo evidence that microfibril deficiency in *Fbn1*$^{+/mg\Delta}$ mice causes severe architectural changes that can predispose the aging aortic wall to structural collapse.

Ascending aorta biomechanics

In order to compare the changed structure to the function of *Fbn1*$^{+/mg\Delta}$ mouse arteries, biomechanical tests were performed on isolated aortic segments from young *Fbn1*$^{+/mg\Delta}$ mice (Fig. 3). Mutant tissues displayed increased diameter, strain and wall thickness without appreciable changes in wall stress and incremental elastic modulus (Einc). They also documented that aortae from young *Fbn1*$^{+/mg\Delta}$ mice are able of higher extension at imposed pressure below 100 mmHg and lower extension at imposed pressure above 125 mmHg, compared to aortae from *WT* mice (Fig. 3G). In adult and aged animals, the outer and inner diameters were higher and showed a greater age-related enlargement in *Fbn1*$^{+/mg\Delta}$ mice, which also showed an aortic wall thickness above normal in adults and abnormally thin in aged animals (Fig. 4A–C). Similar biphasic changes from controls were observed for aortic wall stress (σ) in that this parameter calculated at pressures above 125 mmHg is decreased in adult *Fbn1*$^{+/mg\Delta}$ mice and slightly increased in old mutant animals, compared to *WT* of matching ages (Fig. 4D). This is due to the opposite evolutions of σ, during aging, which decreased in WT because of wall thickening and increases in *Fbn1*$^{+/mg\Delta}$ mice because of wall thinning (Fig. 4C). Whereas no significant variances from control were noted in the strain value (ε) of mutant aortae in adults, vascular aging was associated with a relatively lower ε in *Fbn1*$^{+/mg\Delta}$ samples (Fig. 4E–F). Similarly, the results showed that the ability of mutant aortae to extend at imposed pressures above 75 mmHg was relatively lower than normal at all ages, and that the Einc value was substantially greater in old mutant animals at imposed pressure above 100 mmHg (Fig. 4G,H). Together, these results indicated that the mgΔ mutation substantially impairs the biomechanical properties of the aging aortic wall.

Ascending aorta biochemistry

Aortic function greatly depends on the proper extracellular deposition and assembly of collagenous and elastic macroaggregates [27.]. Desmosine and hydroxyproline contents were therefore measured in *Fbn1*$^{+/mg\Delta}$ aortae to quantify elastin and collagen contents, respectively [14.]. Increased hydroxyproline (collagen) levels and reduced desmosine (elastin) levels were found in mutant aortae, and both variances from normal increased with age relatively to unchanged amounts of total protein contents (Fig. 5A–E). These findings respectively corroborated the histological evidence of increased tissue remodeling and elastolysis in aging microfibril-deficient aortae. In addition, the decreased desmosine/hydroxyproline ratio (Fig. 5F) in *Fbn1*$^{+/mg\Delta}$ aortae is consistent with the decreased distensibility observed in the vessels of these adult and old animals.

Ascending aorta gene expression

Expression of key extracellular gene products, ratioed to the expression level of HPRT, was examined in the ascending aortae of young, adult, and old *Fbn1*$^{+/mg\Delta}$ mice to further support the above findings (Fig. 5G–K). As expected, *Fbn1* transcripts were significantly lower than normal (−48%) in mutant aortae of young animals and a slightly relative increase was noted in old tissues (Fig. 5G). We also observed an increase in *Fbn2* expression in *Fbn1*$^{+/mg\Delta}$ animals (Fig. 5H). These increases in *Fbn1* and *Fbn2* gene expression in aging *Fbn1*$^{+/mg\Delta}$ mice might be attributed to the unproductive tissue remodeling response that is mounted by smooth muscle cells in reaction to the formation of a structurally abnormal matrix. In accordance with this hypothesis, collagen I transcripts were also slightly elevated in the aortae of old *Fbn1*$^{+/mg\Delta}$ mice (decreased in youngs) (Fig. 5I). Furthermore, elastin (*Eln)* transcripts were significantly lower than normal in *Fbn1*$^{+/mg\Delta}$ aortae from young animals; a similar difference was noted for lysyl oxidase (*Lox)* mRNA (Fig. 5J,K). The same trends were confirmed when the expressions of these genes were ratioed to total mRNA (online supporting information 3). These results were consistent with the notion that *Fbn1* deficiency down-regulates the expression of other major contributors to collagen and elastic fiber assembly in early stages, while up-regulating the expression of most of these genes in late-life.

Ascending aorta reactivity

Lastly, we investigated how microfibril deficiency may affect aortic tissue ability to respond to vasoconstrictor (phenylephrine, PE) and vasodilator (acetylcholine, Ach) stimuli in adult and aged animals. Response to PE, significant in *Fbn1*$^{+/+}$ mice independently of age, was decreased and became non-significant in *Fbn1*$^{+/mg\Delta}$ aortae of all ages (Fig. 6A). When old tissues were separated into non-pathological and pathological (= aneurysmal) samples, we found that the mutant aortae of the former group reacted to PE treatment more strongly than the latter group (almost no response) and equally to WT samples (Fig. 6B). Non-pathological *Fbn1*$^{+/mg\Delta}$ vessels presented a lowered response to Ach (Fig. 6C), independently of age, while pathological vessels could not be tested for vasorelaxation induction by Ach due to the absence of pre-constriction in response to PE. These results indicate that fibrillin-1 insufficiency alters the capacity of the aortic wall to respond to physiological vasoactive agonists.

DISCUSSION

Elastic fiber components regulate cell proliferation, migration and arterial morphogenesis, in addition to imparting elastic properties to blood vessels [27–32.]. Interestingly, mutations in the major structural components of elastic fibers, elastin and fibrillin-1, have distinct vascular outcomes. On the one hand, elastin haploinsufficiency causes hyperproliferation of vascular smooth muscle cells and narrowing of large arteries in supravalvular aortic stenosis (SVAS) and Williams syndrome [33–38.]. Mutations in fibrillin-1, on the other hand, cause aortic aneurysms and dissections in MFS. The opposite effects of elastin and fibrillin-1 deficiencies on vascular morphogenesis and function have been replicated in genetically engineered mouse models of SVAS and MFS. Whereas the former mutant animals have provided important insights into elastin contribution to vascular aging, comparable information about fibrillin-1 is currently unavailable due to the fact that most studies have focused on homozygous mutant mice that die of dissecting aneurysm during the first 2 weeks of postnatal life or between 2 to 4 months of age. Here we elucidated the potential contribution of fibrillin-1 to vascular aging in *Fbn1*$^{+/mg\Delta}$ mice that live past sexual maturity and whose adult phenotype has not been characterized. There are two major findings of our analyses.

First, aging *Fbn1*$^{+/mg\Delta}$ mice are significantly more prone than wild-type littermates to develop dissecting aortic aneurysm. As shown in previous works, aneurysms appear earlier in *Fbn1*$^{mg\Delta/mg\Delta}$, which only produce about 10% of the normal amount of fibrillin-1 [17.], than in *Fbn1*$^{+/mg\Delta}$ animals, which contain about 60% of the normal content in fibrillin-1. Hence it is reasonable to argue that the presence of mutant and wild-type fibrillin-1 molecules in *Fbn1*$^{+/mg\Delta}$ mice could have a dominant-negative effect on microfibril assembly, leading to the observed vasculature abnormalities. Contrasting this hypothetical argument, however, is the factual evidence that mice under-expressing wild-type fibrillin-1 (*Fbn1*$^{mgR/mgR}$ mice) to nearly same extent as *Fbn1*$^{+/mg\Delta}$ mice develop aortic aneurysms as well but earlier in life (2–4 months of age) [18]. Taken together, these data strongly support the original hypothesis - that the mgΔ allele has little or no dominant-negative effect on the assembly of wild-type fibrillin-1 molecules [17]. It follows that the vascular phenotype of aging *Fbn1*$^{+/mg\Delta}$ mice probably reflects a gene-dosage effect that triggers aortic alterations later in life.

Aging *Fbn1*$^{+/mg\Delta}$ mice are slightly hypotensive and display arteries of abnormally larger diameters and with fragmented elastic lamellae, altered mechanical properties and reactivity, as well as increased signs of cardiac hypertrophy. These observations support the notion that microfibril deficiency modifies the process of vascular aging in a manner opposite to elastin haploinsufficiency [14,39.].

Impaired remodeling of the aortic wall in *Fbn1*$^{+/mg\Delta}$ mice, as evidenced by the progressive fragmentation of elastic lamellae, is likely to reflect the modified expression of at least *Fbn1*, *Fbn2*, *Eln*, *Lox* and *Col1* genes, as well as enhanced matrix degradation by matrix metalloproteases (MMPs). We rest the latter conclusion on published evidence of aortic elastic fiber degradation and disorganization as well as increased MMP activity in the aortae of MFS patients and other fibrillin-1 deficient mice, and on the ability of MMP inhibition to mitigate aneurysmal progression in mouse models of MFS [6,40–42.]. Expression of wild-type or mutant fibrillin-1 occurs predominantly throughout the arterial media and, to a much lower extent, in the adventitia [17.]. This supports the previously articulated alternative hypothesis that increased arterial diameter and aneurysms of *Fbn1*$^{+/mg\Delta}$ animals reflects in part a decreased tensile strength of the adventitia, in which fibrillin-1 microfibrils may be required to properly organize the collagenous extracellular matrix during development [17]. In this view, the mutant artery would be unable to accommodate the intraluminal blood pressure and abnormally inflate.

The fact that vascular disease in *Fbn1*$^{+/mg\Delta}$ mice, our model of MFS, progresses at a slower pace than in MFS patients probably reflects the lower mechanical constraints in the aortic wall of these mammalian species. In this regard, it is worth noting that a delay in the emergence of vascular abnormalities was also observed in *Eln*$^{+/-}$ mice in which, unlike SVAS and Williams patients, there is no appreciable arterial stenosis even though they display increased number of thinner lamellar units, cardiac hypertrophy, arteries of smaller diameter and decreased elastin content, hypertension and altered mechanics [14,23,26.].

The above findings clearly indicate that fibrillin-1 is an important structural and instructive determinant of arterial wall morphogenesis, mechanical compliance and tissue homeostasis. It is conceivable to argue that some of these functions are accounted for by the role of microfibrils in regulating cell performance through the interaction of fibrillin-1 with cells surface receptors, such as integrins and heparan sulfate proteoglycans, and latent TGFβ complexes. For example, fibrillin-1 triggers integrin-mediated signalling may regulate adhesion and spreading of VSMC that could be required for the proper organization of aortic tissue during development [43,44.]. Furthermore, improper activation of TGF signalling, as a result of impaired sequestration of latent complexes in the extracellular matrix, could also

promote changes in VSMC phenotype. Moreover, it has recently been shown that fibrillin-1 fragments and microfibrils, which can pass through the basement membrane and anchor the endothelial cells to the internal elastic lamina [45.], trigger integrin-mediated calcium signaling in endothelial cells [46.], whose dysfunction has clearly been involved in the pathogenesis of Marfan syndrome [41,47.]. A decrease in fibrillin-1 signaling, in mutant mice or human patients, could cause abnormal endothelial function and signaling to the medial VSMCs, and subsequent arterial wall dysorganisation. Such endothelial dysfunction, i.e. impaired response to acetylcholine, was evidenced in *Fbn1*$^{+/mg\Delta}$ mice in our experiments. Along the same lines, decreased number of elastic lamellae in adult *Fbn1*$^{+/mg\Delta}$ aortae could be viewed as a change in the remodeling capacity of *Fbn1*$^{+/mg\Delta}$ VSMC that in turn reflects changes in the biosynthetic properties of these cells due to impaired cell-matrix interactions and/or latent TGFβ sequestration.

The finding that aging *Fbn1*$^{+/mg\Delta}$ mice can display either seemingly normal arteries, except for some modified parameters (diameter enlargement, larger wall thickness, higher stiffness), or enhanced aneurysms, cardiac hypertrophy and aortic insufficiency suggests that, similarly to MFS patients, fibrillin-1 mutations have varied penetrance in this mouse model. In our genetically identical animals, these phenotypic variances likely involve epigenetic or environmental factors. More generally, our data imply that accumulation of age-related environmental insults that affect the aortic wall microenvironment may disrupt cell-matrix with the consequence of accelerating the physiological process of aortic tissue degradation. In this view, mutations (even sub-clinical mutations) that affect molecules involved in elastogenesis or elastic fiber interactions with structural, cellular or soluble molecules would be expected to predispose aortic tissues to late-onset pathologies and/or premature aging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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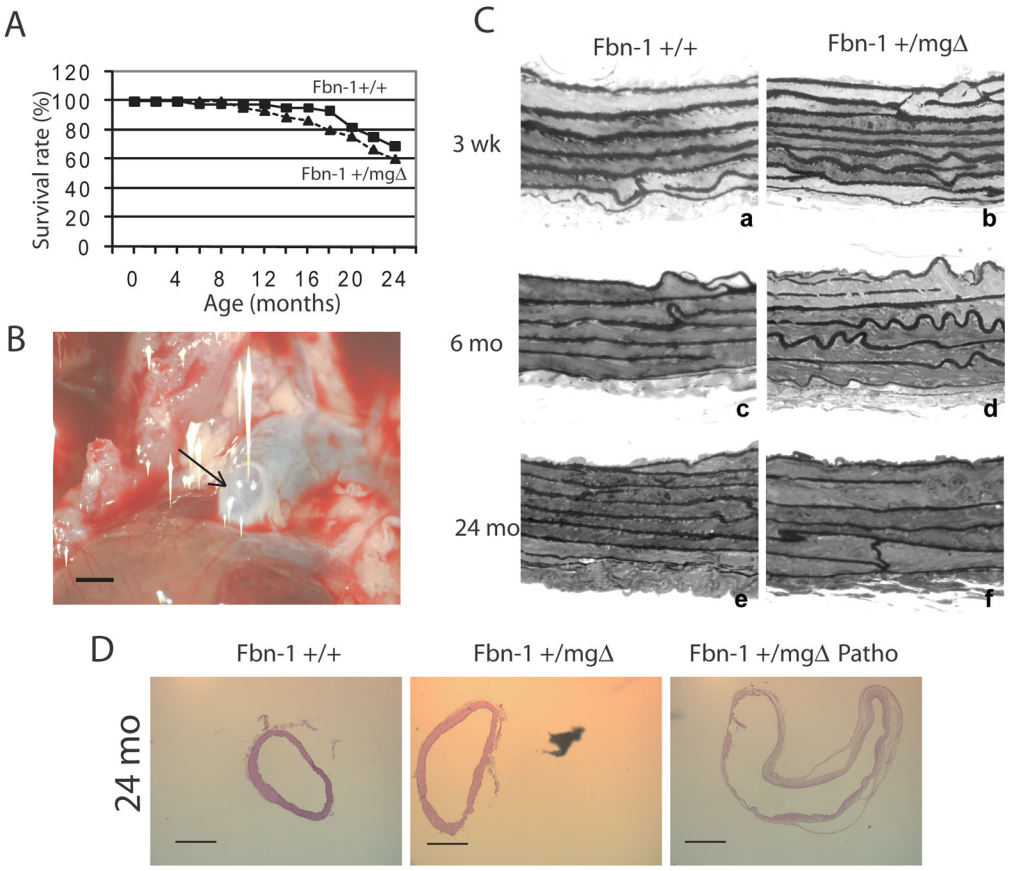

Figure 1.

Effect of aging and genotype on the survival rate (A), aneurismal ascending aorta of 24-month-old *Fbn1*$^{+/mg\Delta}$ mouse (B) (the arrow indicate the saccular aneurysm at the vessel root), and histological examination of semi-thin sections of ascending aorta of 3-week-old, 6- and 24-month-old mice (C). Eosin/Hematoxylin staining of cross-sections of ascending aorta of *Fbn1*$^{+/+}$, non-pathological *Fbn1*$^{+/mg\Delta}$, and pathological *Fbn1*$^{+/mg\Delta}$ (Patho) 24-month-old mice (D). n=45 for (A). Bar sizes: 1mm (B) and 500μm (D).

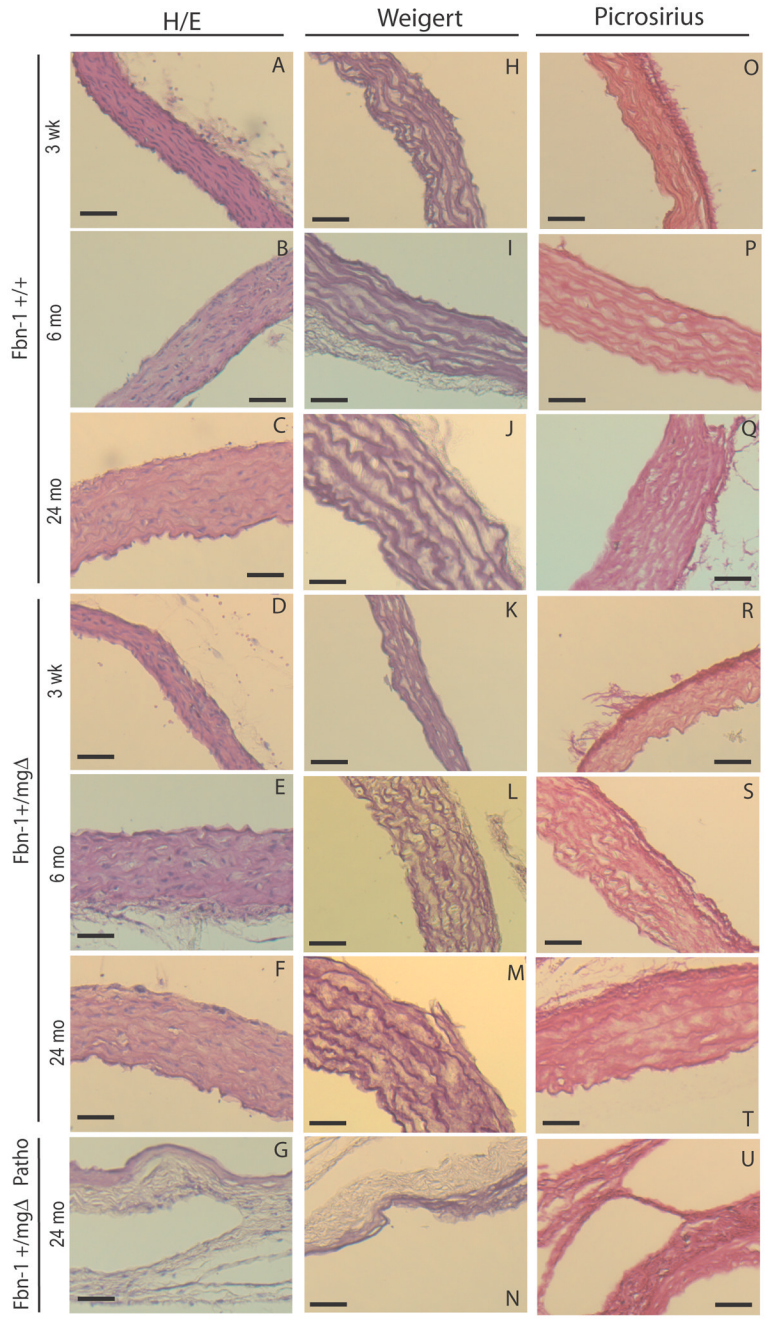

Figure 2.

Histological examinations of paraffin-embedded cross-sections of the ascending aorta of 3-week-old, 6- and 24-month-old *Fbn1*$^{+/+}$ and *Fbn1*$^{+/mg\Delta}$ mice, and 24-month-old pathological *Fbn1*$^{+/mg\Delta}$ mice (Patho). The elastic lamellae were thinner and disorganized in adult and aged *Fbn1*$^{+/mg\Delta}$ mice. H/E: Hematoxylin/Eosin staining. Bar size: 50 μm.

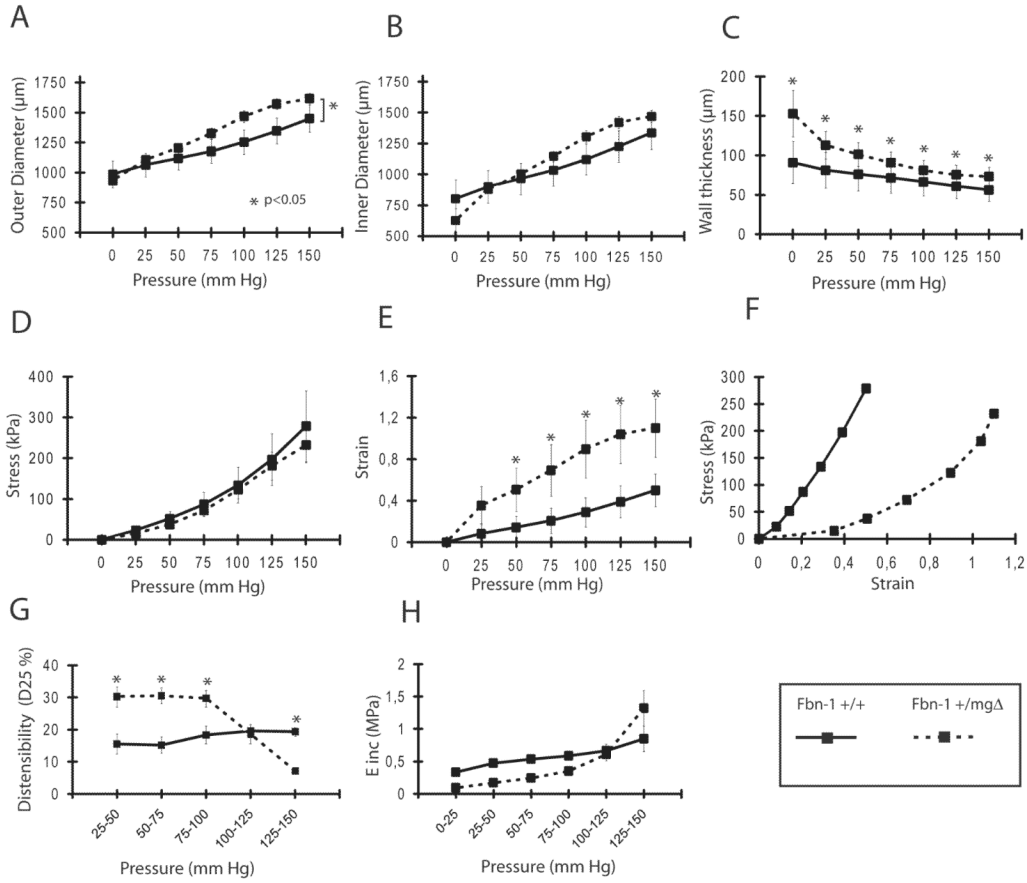

Figure 3.

Diameter-pressure curves and derived mechanical parameters of the ascending aorta of 3-week-old *Fbn1*$^{+/+}$ and *Fbn1*$^{+/mg\Delta}$ mice. *Significant difference between *Fbn1*$^{+/+}$ and *Fbn1*$^{+/mg\Delta}$ mice. n=4–7 per group.

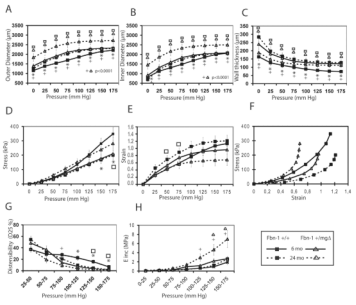

Figure 4.

Diameter-pressure curves and derived mechanical parameters of the ascending aorta of 6- and 24-month-old *Fbn1*$^{+/+}$ and *Fbn1*$^{+/mgΔ}$ mice. *,+Significant difference between 6-month-old *Fbn1*$^{+/+}$ and *Fbn1*$^{+/mgΔ}$ *mice* or between 24-month-old *Fbn1*$^{+/+}$ and *Fbn1*$^{+/mgΔ}$ mice, respectively. □,ΔSignificant difference between 6–month-old and 24-month-old values for *Fbn1*$^{+/+}$ and *Fbn1*$^{+/mgΔ}$, respectively. n=5–6 per group.

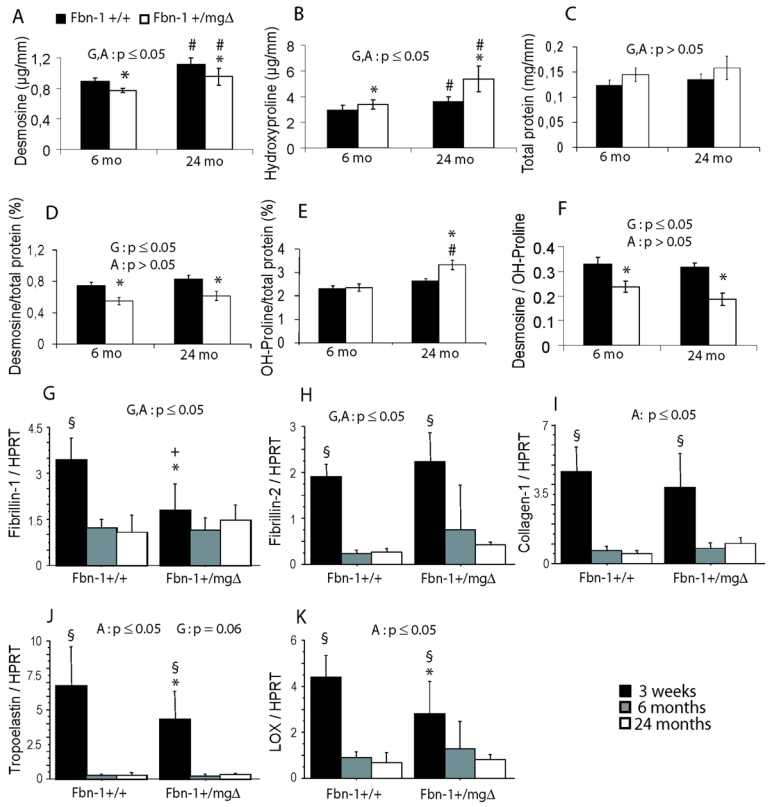

Figure 5.

Desmosine and hydroxyproline contents (A–F) and elastic fiber-related gene expression (G–K) of the ascending aorta of 3 weeks, 6 and 24-month-old *Fbn1*$^{+/+}$ and *Fbn1*$^{+/mg\Delta}$ mice. D,E present the desmosine and hydroxyproline ratios relative to total protein content. The general effect of genotype (G) and age (A) was assessed by two-way ANOVA separately in each condition (A to K), and followed by Fisher's LSD test for paired comparisons: *,#Significant difference between *Fbn1*$^{+/+}$ and *Fbn1*$^{+/mg\Delta}$ mice of the same age, and between 6- and 24-months-old mice of the same genotype, respectively. **§**Significant difference between young (3-week-old) on one hand and 6- and 24-month old animals of the same genotype on the other hand. +Significant difference between young (3-week-old) and 6-month old animals of the same genotype on the other hand. For desmosine and hydroxyproline dosages: n=5–7 (6-month-old mice) and n=3–5 (24-month-old mice) in each group. For mRNA expression: n=6 (3-week-old), n=8 (6-month-old) and n=5–7 (24-month-old) in each group.

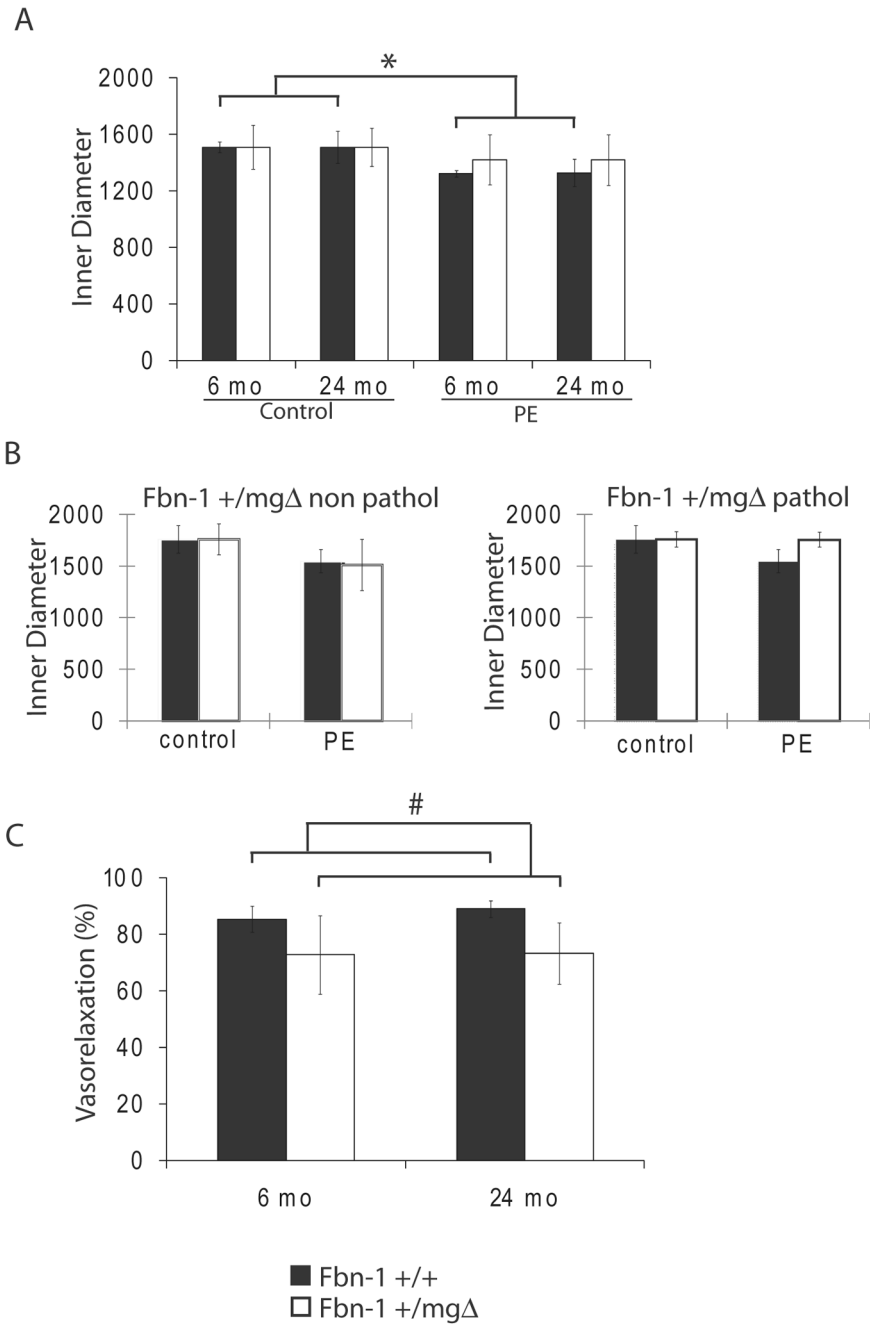

Figure 6.

Reactivity to vasoactive agents of the ascending aorta of 6- and 24-month-old *Fbn1*$^{+/+}$ and *Fbn1*$^{+/mgΔ}$. (A,B) 10μmol/L phenylephrine (PE). 24-month-old *Fbn1*$^{+/mgΔ}$ mice were pooled (A) or separated (B) in non-pathological and pathological group. Ach-induced vasorelaxation (10μmol/L phenylephrine + 10μmol/L acetylcholine) in non-pathological vessels is represented as the reversal of the PE-induced vasoconstriction, in percent (C). *Generally significant difference with the control value, independent of age. #Generally significant difference between *Fbn1*$^{+/+}$ and *Fbn1*$^{+/mgΔ}$ mice, independent of age. n=3–6 per group.

Table I

Hemodynamic and ultrasound parameters of ascending aorta and heart.

	5–7 month-old		23–27 month-old	
	Fbn1$^{+/+}$	**Fbn1$^{+/mg\Delta}$**	**Fbn1$^{+/+}$**	**Fbn1$^{+/mg\Delta}$**
Number of Animals	8	8	7	5
Body Weight (g)	29.5 ± 0.9	31.8 ± 0.7 *	33.2 ±1.3 †	31.6 ± 0.8
Entire Heart weight/BW (mg/g)	4.92 ± 0.13	5.99 ± 0.91	5.72 ± 0.35	6.25 ± 0.94
Systolic Arterial Pressure (mmHg)	105 ± 2	104 ± 3	110 ± 4	104 ± 5
Mean Arterial Pressure (mmHg)	85 ± 1	81 ± 2	84 ± 3	76 ± 6
Diastolic Arterial Pressure (mmHg)	75 ± 1	70 ± 2	72 ± 3	62 ± 6
Pulse pressure (mmHg)	30 ± 1	34 ± 3	38 ± 3	42 ± 2
Ultrasound study				
Ascending aorta:				
Systolic Diameter (mm)	1.70 ± 0.05	2.18 ± 0.17 *	2.11 ± 0.07 †	2.26 ± 0.19*
Diastolic Diameter (mm)	1.48 ± 0.04	2.04 ± 0.19 *	1.97 ± 0.08 †	2.05 ± 0.21*
Surface (mm^2)	2.28 ± 0.14	3.90 ± 0.66 *	3.53 ± 0.22 †	4.09 ± 0.66
Compliance (mm/mmHg)	0.720 ± 0.137	0.520 ± 0.112	0.383 ± 0.07 ‡	0.579 ± 0.173
Cardiac data:				
Heart Rate (bpm)	504 ± 11	485 ± 25	475 ± 16	456 ± 22
Left Atria Dimension (mm)	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.9 ± 0.4
Left Ventricle EDD	4.36 ± 0.18	5.00 ± 0.54	5.13 ± 0.15 †	5.11 ± 0.26
Left Ventricle EDD/BW (mm/g)	0.148 ± 0.005	0.158 ± 0.017	0.156 ± 0.008	0.162 ± 0.011
Fractional Shortening (%)	40 ± 2	31 ± 4	36 ± 3	35 ± 4
LV Weight/BW (mg/g)	3.58 ± 0.20	5.40 ± 1.56	4.32 ± 0.24 †	4.76 ± 0.72
Vcfc (circ/s)	2.9 ± 0.2	2.3 ± 0.3	2.5 ± 0.3	2.5 ± 0.2
Sa (cm/s)	2.93 ± 0.15	2.61 ± 0.21	2.84 ± 0.26	2.98 ± 0.50
Spw (cm/s)	2.94 ± 0.3	2.77 ± 0.26	3.22 ± 0.22	3.24 ± 0.22
Isovolumic Relaxation Time (ms)	17 ± 1	20 ± 2	19 ± 1	21 ± 1
Ea (cm/s)	4.53 ± 0.32	4.56 ± 0.37	4.74 ± 0.26	5.01 ± 0.77
Epw (cm/s)	4.34 ± 0.45	3.84 ± 0.4	4.97 ± 0.21	4.49 ± 0.49
E/Ea	20.6 ± 1.0	18.9 ± 2.5	21.6 ± 1.4	18.2 ± 2.4
Aortic Regurgitation Frequency	0/8	2/8	2/7	3/5

Values are mean ± sem.

† Significant difference with 5–7 month-old animals of the same genotype (†: P≤0.05; ‡: P=0.056). Compliance is a local estimation calculated from the Δdiameter/Δpressure between systolic and diastolic values. LVEDD: left ventricle end diastolic diameter; Vcfc: mean shortening velocity of circumferential fibers, corrected for time (=FS/rate-corrected ejection time); Sa, Spw: maximal systolic velocity of the mitral annulus and posterior wall, respectively. Ea, Epw: maximal diastolic velocity of the mitral annulus and posterior wall, respectively. E/Ea: maximal velocity of LV mitral inflow to Ea.

Table II

Histomorphometric parameters of ascending aorta wall.

Age	**3 weeks**		**6 month**		**24 months**	
Genotype	**Fbn1$^{+/+}$**	**Fbn1$^{+/mg\Delta}$**	**Fbn1$^{+/+}$**	**Fbn1$^{+/mg\Delta}$**	**Fbn1$^{+/+}$**	**Fbn1$^{+/mg\Delta}$**
Number of elastic lamellae (EL)	7.1±0.3	6.1±0.2*	7.7±0.5	7.1±0.4*	8.4±0.3	6.7±0.9 *
Distance between EL (μm)	0.85±0.34	0.70±0.27	0.92±0.24	1.18±0.37*	0.96±0.19	1.23±0.6*
Rupture of EL ($\times 10^{-4}$ per μm^2)	2±1	4±2.8*	2±1.4	6±2*	3.2±2.1	7.8±3.6*

Values are mean ± SEM.

*Significant difference between *Fbn1*$^{+/+}$ and *Fbn1*$^{+/mg\Delta}$ mice of matching age.