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RESEARCH PAPER

Imbalanced synthesis of cyclooxygenase-derived thromboxane A_2 and prostacyclin compromises vasomotor function of the thoracic aorta in Marfan syndrome

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Background and purpose: Thoracic aortic dissection is a life-threatening complication of Marfan syndrome, a connective tissue disorder caused by mutations in the gene encoding fibrillin-1. We have demonstrated that nitric oxide-mediated endothelial-dependent relaxation is impaired in the thoracic aorta in Marfan syndrome. In the present study, we determined whether the cyclooxygenase (COX)-pathway is involved in the compromised aortic vasomotor function.
Experimental approach: Thoracic aortae from mice at 3, 6 and 9 months of age, heterozygous for the *Fbn1* allele encoding a cysteine substitution (*Fbn1*$^{C1039G/+}$, 'Marfan', $n=35$), were compared with those from age-matched controls ($n=35$).
Key results: Isometric force measurement revealed that preincubation with indomethacin, a non-specific COX inhibitor, but not valeryl salicylate, a specific COX-1 inhibitor, improved the phenylephrine-induced contractions (at 6 months, EC_{50} and E_{max} were increased 4.5-fold and by 45%, respectively) in Marfan aortae. Sensitivity to acetylcholine-induced relaxation was improved 10-fold. Blockade of the thromboxane-endoperoxide receptor by SQ-29548 did not affect phenylephrine-mediated contractions in Marfan aortae, although they did respond to the thromboxane analogue, U46619. From 6 months on, phenylephrine-induced secretion of prostacyclin and thromboxane A_2 in Marfan aortae was 200% and 40%, respectively, of those in controls. Reduced COX-1 expression was detected in Marfan aortae at 3 and 9 months, whilst COX-2 expression was increased from 3 months on.
Conclusions and implications: The compromised vasomotor function in Marfan thoracic aortae is associated with an imbalanced synthesis of thromboxane A_2 and prostacyclin resulting from the differential protein expression of COX-1 and COX-2.
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Keywords: Marfan syndrome; cyclooxygenase; vasoconstriction; vasorelaxation; endothelial function; thoracic aorta; age-dependent disease progression; thromboxane A_2; prostacyclin; acetylcholine

Abbreviations: ACh, acetylcholine; COX, cyclooxygenase; eNOS, endothelial nitric oxide synthase; Fbn, fibrillin; KCl, potassium chloride; NO, nitric oxide; PGI_2, prostacyclin; TXA_2, thromboxane A_2

Introduction

Marfan syndrome is a genetic disorder of connective tissue caused by mutations in the gene encoding fibrillin-1 (*Fbn1*; Dietz *et al*., 1991). The most life-threatening complication is progressive aortic aneurysm, leading to aortic dissection and rupture (Pyeritz, 2000). Fbn1-rich microfibrils are essential in the formation of elastic fibres in large artery and the connective tissue intermediately subjacent to endothelial cells (Kielty *et al*., 1996). Nevertheless, smooth muscle attachment to neighbouring aortic elastic laminae is dependent on the connecting filaments, which are composed of Fbn1 (Bunton *et al*., 2001). For these reasons, it is likely that the functions of both endothelial and smooth muscle cells and, as a consequence the net vasomotor response are modified in Marfan syndrome.

Vascular endothelium regulates vasoconstriction and vasorelaxation through the synthesis of vasoactive mediators (Furchgott and Vanhoutte, 1989). Nitric oxide (NO) constitutively synthesized from the endothelial nitric oxide

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synthase (eNOS) causes relaxation of the underlying smooth muscle cells (Cohen and Adachi, 2006). Thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) are functionally antagonistic prostanoids that are generated from the metabolism of arachidonic acid through the rate-limiting enzyme, cyclooxygenase (COX; Furchgott and Vanhoutte, 1989; Smith *et al.*, 2000). Under normal physiological conditions, TXA_2 induces vasoconstriction, smooth muscle proliferation and migration, leukocyte activation, as well as platelet aggregation, while PGI_2 opposes these biological actions. Two isoforms of COX have been identified in the vascular endothelium and smooth muscle cells (DeWitt *et al.*, 1983). COX-1 is constitutively expressed and activated, and is the main source of basal TXA_2 (Ge *et al.*, 1995; Heymes *et al.*, 2000). COX-2 is subject to rapid induction by a number of cardiovascular risk factors, such as cytokines, cholesterol, hypoxia or after vascular injury (Smith *et al.*, 2000), and COX-2-derived PGI_2 is the most abundant prostanoid (Bunting *et al.*, 1977; Moncada *et al.*, 1977; Qi *et al.*, 2006) in the vasculature.

In vascular pathologies such as diabetes, hypertension or ageing, endothelial dysfunction is recognized and characterized by a reduced release of endothelium-derived relaxing factors and a concomitant increase in release of endothelium-derived contracting factor (Matz *et al.*, 2000; Hermann, 2006). During the progression of Marfan syndrome, we have previously demonstrated that endogenous endothelial NO production and eNOS downstream signalling are significantly impaired in the thoracic aorta (Chung *et al.*, 2007); nevertheless, vasoconstriction is greatly suppressed regardless of the means of stimulation. It is therefore important to delineate the mechanisms involved in the reduction in relaxation and impairment of force generation of the aorta in Marfan syndrome.

Although COX-derived prostanoids are important in the regulation of vasoconstriction and vasorelaxation, the role of COX in the development of Marfan syndrome has never been studied (no single reference related to Marfan syndrome and COX pathways could be found). Following up from our previous study, and from the present results, we concluded that the COX pathway contributes to the compromised aortic vasomotor function in Marfan syndrome by causing an imbalance in the release of endothelial relaxant (for example, PGI_2) and constricting (for example, TXA_2) prostanoids.

Methods

Experimental animals and tissue preparation

Heterozygous (*Fbn1*$^{C1039G/+}$) mice were mated to C57BL/6 mice to produce equal numbers of *Fbn1*$^{C1039G/+}$ 'Marfan' subjects ($n=35$) and wild-type 'control' ($n=35$) as described previously (Chung *et al.*, 2007). Both strains were housed in the institutional animal facility (Child and Family Research Institute, University of British Columbia), and all animal procedures were approved by the Institute's Animal Ethics Board. Mice at the ages of 3 ($n=25$), 6 ($n=25$) and 9 ($n=20$) months were killed by an overdose of a mixture of the anaesthetic ketamine hydrochloride (80 mg kg^{-1}) and xylazine hydrochloride (12 mg kg^{-1}, i.p.).

Measurement of isometric force

Thoracic aortic segments (2 mm) were mounted isometrically in a small vessel myograph (A/S Danish Myotechnology, Aarhus N, Denmark) for measuring generated force (Chung *et al.*, 2007). Optimal tension was determined in the preliminary experiments. Briefly, aortic segments were subjected to different resting tensions (from 0.5 to 10 mN) then stimulated with 80 mM KCl. At all the resting tensions applied, the control thoracic aorta generated significantly more force than the Marfan aorta. The optimal tension for both control and Marfan groups was set as 6.0 mN, at which the maximal contraction was achieved in both strains.

Aortic segments were stretched to the optimal tension for 20 min. Thereafter, the vessels were challenged twice with 80 mM KCl before the experiments were continued. To investigate the possible participation of prostanoids in the reduction of contraction and acetylcholine (ACh) relaxation in the Marfan aorta, aortic segments were incubated with the COX-1/COX-2 inhibitor indomethacin (10 μM) for 30 min before the addition of phenylephrine (1 nM–3 μM). When the force generated reached a plateau, ACh was added in a cumulative manner (10^{-10}–10^{-6} M). Since the phenylephrine-induced precontraction was different in the two strains, the ACh relaxation response was expressed as percentage of relaxation compared to the initial phenylephrine-induced contraction (Chung *et al.*, 2007). Concentration–response curves of phenylephrine-induced contraction and ACh-induced relaxation were constructed. The negative logarithm (pD_2) of the concentration of phenylephrine or ACh giving half-maximum response (EC_{50}) was assessed by linear interpolation on the semilogarithm concentration–response curve [$pD_2 = -\log(EC_{50})$].

To clarify whether COX-1 or COX-2 was involved in vasomotor function, aortic segments were incubated with the specific COX-2 inhibitor NS-398 (1 μM) or specific COX-1 inhibitor VAS (3 mM). Aortic segments were also incubated with SQ-29548 (TXA_2/PGH_2 receptor antagonist; 1 μM) to evaluate the possible participation of vasoconstricting prostanoids.

Production of TXA_2 and PGI_2

These experiments were performed independently of the isometric tension experiments. To measure the release of TXA_2 and PGI_2, aortic rings were placed in 1 ml Krebs solution at 37°C in a 95% O_2 and 5% CO_2 incubator. After 15 min equilibration, phenylephrine (1 μM) was added for 10 min. Then the incubation medium was collected and the concentrations of TXB_2 and 6-keto-$PGF_{1\alpha}$, the stable metabolites of TXA_2 and PGI_2, respectively, were measured by using enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI, USA), according to the manufacturer's instructions. The aortic segment was placed in a dry bath for 10 min and weighed. The amount of prostanoid released was expressed as pg ml^{-1} mg^{-1} dry weight of aorta. In some experiments, aortic segments were preincubated with indomethacin (10 μM), NS-398 (1 μM) or VAS (3 mM) for 30 min before the addition of phenylephrine.

Western immunoblotting

The procedures used for protein homogenization and western immunoblotting were as described previously (Chung *et al*., 2007). In brief, 40 μg of protein sample were separated on 9% sodium dodecyl sulphate–polyacrylamide gel electrophoresis, and then transferred to polyvinyldifluoride membranes (Biorad, Hercules, CA, USA). Membranes were first incubated with primary antibodies: rabbit polyclonal anti-COX-1 or anti-COX-2 (dilution 1:200) antibodies, then with IgG peroxidase-conjugated secondary antibodies (dilution 1:2500). Immunoreactive proteins were visualized by enhanced chemiluminescence kit (Amersham Life Sciences, Arlington Heights, IL, USA). To ensure equal loading, membranes were stripped and reprobed with antibodies against β-actin.

Statistics

Data are presented as means$\pm$s.e.mean. from at least three independent experiments. Statistical analysis and construction of concentration–response curves were performed using GraphPad Prism software (San Diego, CA, USA). Differences between control and Marfan groups were determined by Student's two-tailed *t*-test. Differences between concentration–response curves were analysed by two-way analysis of variance. Statistical significance was defined as P-values<0.05.

Drugs

Ketamine hydrochloride and xylazine hydrochloride (Research Biochemicals International, Natick, MA, USA); TXA_2 and/or prostaglandin H_2 receptor antagonist SQ-29548, valeryl salicylate (VAS), rabbit polyclonal anti-COX-1 and anti-COX-2 antibodies, enzyme immunoassay kits for measuring TXB_2 and 6-keto-$PGF_{1\alpha}$ (Cayman Chemical); NS-398 (Calbiochem, San Diego, CA, USA); phenylephrine, ACh, KCl, L-NAME, 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$ (U-46619, TXA_2 receptor agonist), indomethacin, IgG peroxidase-conjugated secondary antibody and chemicals for preparing Krebs solution (Sigma-Aldrich, Oakville, Ontario, Canada).

Results

Effects of COX blockade on vasoconstriction

To study the contribution of COX in the regulation of vasoconstriction, we preincubated the aorta in indomethacin (10 μM), a nonspecific COX-inhibitor, before the addition of phenylephrine. Indomethacin potentiated the phenylephrine-induced contraction only in the Marfan aortae, but the effect was age-dependent. At 6 months, indomethacin significantly augmented phenylephrine E_{max} value from 5.39 to 7.69 mN (Figure 1a). The sensitivity to phenylephrine was improved as the concentration–response curve was shifted leftward and the EC_{50} value was increased by 4.5-fold (Figure 1a). At 9 months, the E_{max} value was elevated from 3.72 to 6.43 mN (Table 1).

In contrast, indomethacin had no effect on the phenylephrine-induced contraction in the control aorta in any age group (Figure 1b, Table 1). However, inhibition of COX-1 with a specific inhibitor, VAS (3 mM), suppressed the phenylephrine contraction by 60% in the control aorta at 6 months, but had no significant effect on the Marfan aorta.

In the vasculature, the major vasoconstricting prostanoid synthesized from COX-1 is TXA_2/PGH_2 (Ge *et al*., 1995; Heymes *et al*., 2000). While indomethacin blocks the production of both relaxant and contractile prostanoids, blockade of the thromboxane–endoperoxide (TXA_2/PGH_2) receptor by SQ-29548 antagonizes only the contractile effects. SQ-29548 had no effect on the phenylephrine contraction in Marfan aorta in all age groups (Figure 2a). In the control, SQ-29548 pretreatment reduced phenylephrine–pEC_{50} value from 7.26 to 6.68 and greatly suppressed contraction by 50% at 9 months (Figure 2b, Table 1).

To determine whether the TXA_2/PGH_2-mediated signalling is downregulated in the Marfan aorta, we directly stimulated this pathway by using the TXA_2 analogue, U46619. U46619-induced an increased contraction in the Marfan aortae from 3- and 9- month-old animals compared with the age-matched controls (Figure 3).

Release of TXA_2 and PGI_2

TXA_2 and PGI_2 are the major COX-derived vasoconstricting and relaxing prostanoids in mouse aorta (Qi *et al*., 2006), so we only measured the production of these prostanoids. In aorta of both control and Marfan mice, phenylephrine (1 μM) evoked the release of 6-keto-$PGF_{1\alpha}$ (stable metabolite of PGI_2) and TXB_2 (stable metabolite of TXA_2). However, from 6 months onward, the production of TXB_2 was significantly decreased in the Marfan aorta by 60%, whilst the level of 6-keto-$PGF_{1\alpha}$ was markedly enhanced by 200% compared with the controls (Figure 4). Indomethacin (10 μM), VAS (3 mM) and NS-398 (1 μM) reduced the release of both prostanoids in both strains. Similar results were observed at 9 months of age.

Effects of COX blockade on vasorelaxation

Indomethacin suppressed the ACh-induced relaxation of the phenylephrine-precontracted control aorta from 89 to 53% at 6 months of age (Figure 5b, Table 1). Although the maximum relaxation was not improved in the Marfan aorta, the sensitivity to the relaxant effects of ACh was increased 10-fold after the pretreatment with indomethacin (Figure 5a, Table 1). Similar results were observed after pretreatment with VAS (3 mM) and NS-398 (1 μM), a specific COX-2 inhibitor.

The ACh-induced relaxation response in the Marfan aorta was unaffected by SQ-29548 pretreatment. However, SQ-29548 reduced the E_{max} and pEC_{50} values of the response to ACh in control aortae from 6-month-old mice (Table 1).

Protein expression of COX-1 and COX-2

In Marfan aortae, the expression of COX-1 was decreased compared to the control at 3 and 9 months. The expression of COX-2 was greatly elevated in the Marfan aorta from 3 months onwards (Figure 6).

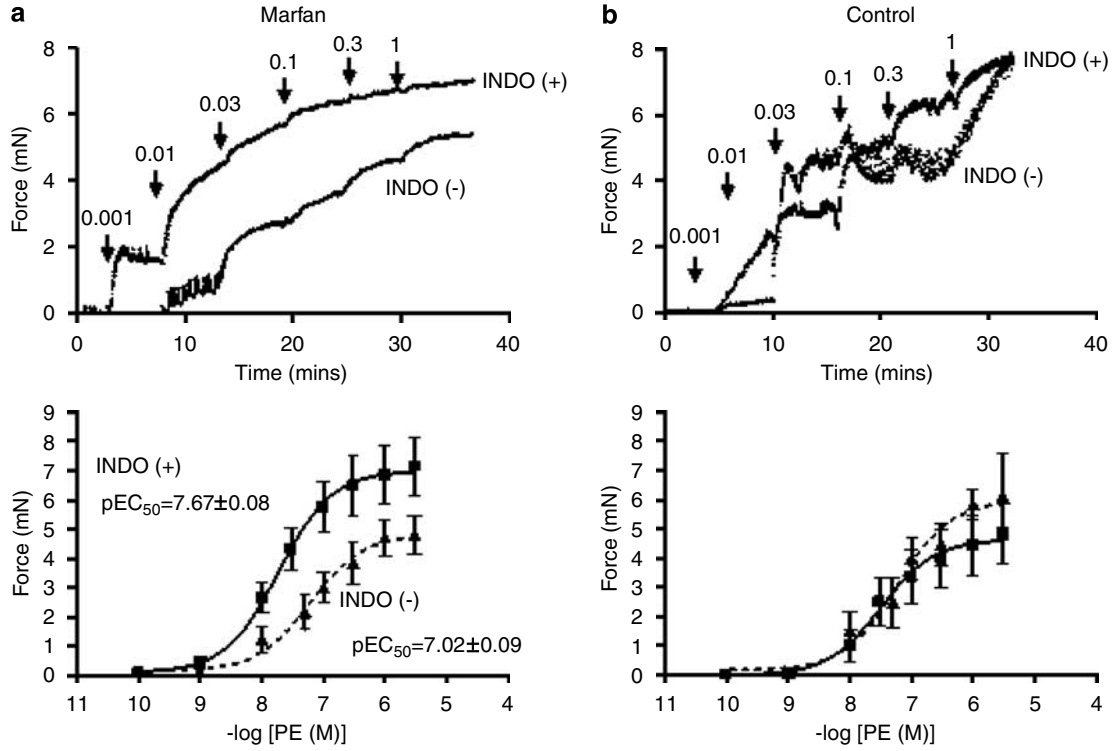

Figure 1 Effects of indomethacin on phenylephrine-induced contractions in thoracic aortae from (**a**) Marfan and (**b**) control mice at 6 months of age. Thoracic aortae were preincubated with 10 μM indomethacin for 30 min before phenylephrine was added at cumulative concentrations (arrows indicate the addition of phenylephrine and numbers represent the concentration in μM). Representative tracings from eight independent experiments demonstrate the contraction evoked by phenylephrine in the absence (INDO (−)) and presence (INDO (+)) of indomethacin. The lower panels are the corresponding concentration–response curves for phenylephrine-induced contractions.

Table 1 Summary of E_{max} and pEC_{50} values of phenylephrine-induced contraction (mN) and ACh-induced relaxation (% of maximal response) in the thoracic aorta from control and Marfan mice at different ages

Strain	*Control*			*Marfan*		
Age (in months)	*3*	*6*	*9*	*3*	*6*	*9*
E_{max} *(mN)*						
PE	4.32±0.43	6.28±0.56	6.47±0.88	2.93±0.47	5.39±0.49	3.72±0.63
PE + INDO	3.46±0.89	5.35±0.93	7.07±1.08	3.07±0.50	7.69±0.67*	6.43±0.69*
PE + SQ	3.97±0.51	6.90±0.82	3.17±0.26*	2.37±0.57	6.65±1.47	3.26±0.59
ACh	78.0±9.5	89.2±2.0	51.5±4.5	54.8±4.2	52.7±6.8	53.0±13.3
ACh + INDO	56.2±8.4*	53.4±7.5*	64.6±17.4	64.4±13.7	52.9±13.5	74.0±11.2
ACh + SQ	63.9±10.0	62.7±7.7*	53.0±9.4	68.2±19.2	34.5±8.7	77.5±9.3
pEC_{50}						
PE	7.13±0.10	7.19±0.09	7.26±0.07	7.55±0.28	7.02±0.09	6.76±0.07
PE + INDO	7.19±0.08	7.35±0.10	7.08±0.06	7.25±0.11	7.67±0.08*	6.98±0.11
PE + SQ	6.95±0.05*	7.03±0.04*	6.68±0.06*	6.91±0.19	7.20±0.26	6.70±0.08
ACh	7.06±0.31	7.29±0.18	7.80±0.46	6.50±0.53	5.85±0.22	7.14±0.35
ACh + INDO	6.94±0.28	6.86±0.26	7.12±0.55	6.71±0.37	6.85±0.44*	7.05±0.31
ACh + SQ	6.46±0.25	6.71±0.24*	6.87±0.32	6.70±0.40	6.32±0.38	6.89±0.23

Abbreviations: ACh, acetylcholine; PE, phenylephrine; INDO, indomethacin; SQ, SQ-29548.
*$P<0.05$, vs without inhibitors.

Discussion

Following on from our previous study of impaired smooth muscle and endothelial function in Marfan syndrome, we now show that the reduced aortic contractility and endothelial-dependent relaxation can be attributed, at least in part, to a shift in the balance of the basal production of COX-derived vasoconstrictors (for example, TXA_2) and vasodilators (for example, PGI_2). These changes in the production of prostanoids are probably associated with the differential expression of COX-1 and COX-2 in the aorta.

As we have demonstrated recently, Marfan thoracic aorta has an impaired contraction regardless of the means of stimulation (Chung *et al*., 2007). In the present study, we observed that the contractility of Marfan aorta at 6 and 9 months of age was significantly improved by pretreatment with the non-specific COX inhibitor indomethacin, but not the specific COX-1 inhibitor VAS (Figure 1). This could be due to an augmented synthesis of the COX-2-derived relaxant PGI_2 in the Marfan aorta (Figure 4). VAS suppressed the contraction in the control but not the Marfan aorta; this is probably related to the substantial release of the COX-1-derived vasoconstrictor TXA_2 in the control (Figure 4). In contrast, indomethacin did not affect contraction in the control thoracic aorta, implicating a balance between basal relaxant and constricting prostanoids (Figure 1).

An imbalance in the basal production of COX-derived vasoconstrictors and vasodilatators in the Marfan thoracic aorta was further revealed by blocking the TXA_2/PGH_2 receptors. SQ-29548 did not affect the contraction in Marfan aorta, but suppressed the force generated in the controls (Figure 2). It appears that the TXA_2/PGH_2-mediated pathway is not involved in the phenylephrine-induced vasoconstriction in the Marfan aortae. This may be due to the reduced basal production of the TXA_2/PGH_2 prostanoids or an impairment of the TXA_2/PGH_2 downstream signalling in these aortae. However, the latter possibility was ruled out since the contractile responses evoked by the TXA_2/PGH_2

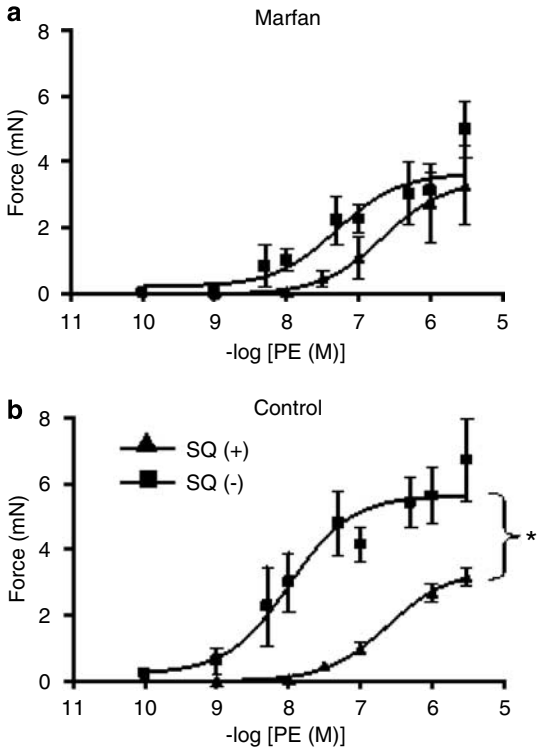

Figure 2 Effects of SQ-29548 on phenylephrine-induced contractions in thoracic aortae from (**a**) Marfan and (**b**) control mice at 9 months of age. Thoracic aortae were preincubated with 1 μM SQ-29548 [SQ (+)] for 30 min before phenylephrine was added at cumulative concentrations. Concentration–response curves were generated from six independent experiments, and compared with those without SQ-29548 pretreatment (SQ (−)). *$P<0.05$ vs SQ (−).

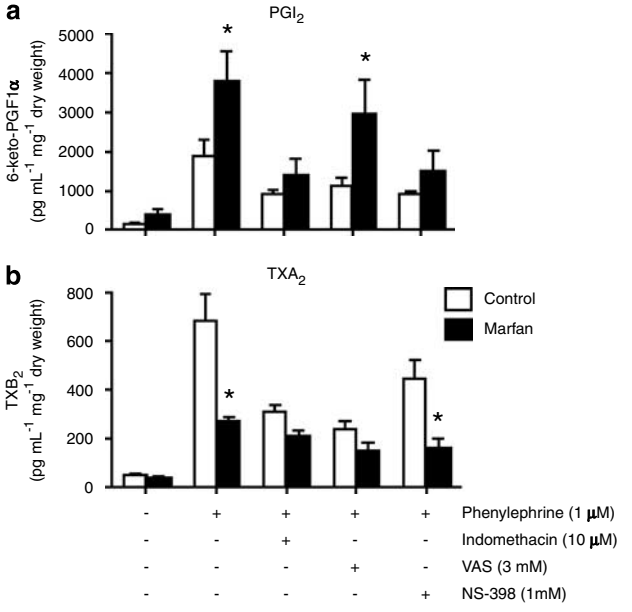

Figure 4 Release of PGI_2 and TXA_2. Aortic rings from control and Marfan strains of mice at 6 months of age were stimulated with phenylephrine (1 μM) with or without inhibitors for 10 min. The incubation medium was collected for the measurement of (**a**) PGI_2 and (**b**) TXA_2. $n=3$, *$P<0.05$ vs control.

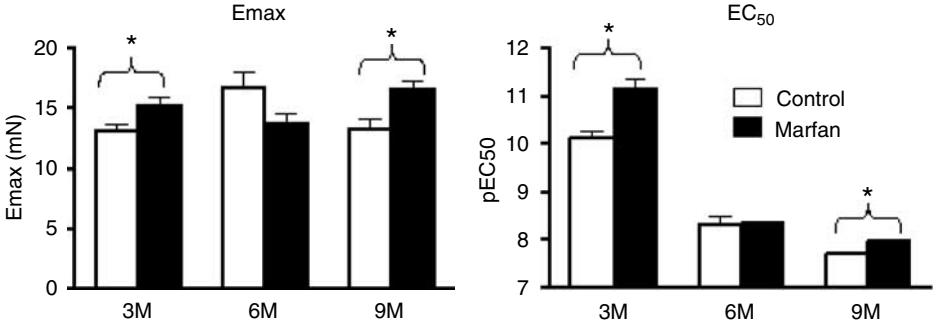

Figure 3 The values of E_{max} and pEC_{50} of U46616-induced contraction in the control and Marfan thoracic aorta at different ages. U46616 was added at cumulative concentrations (10^{-11}–10^{-8} M). Concentration–response curves were constructed as described in the Methods and E_{max} and pEC_{50} values were determined. $n=6$–10, *$P<0.05$ vs control.

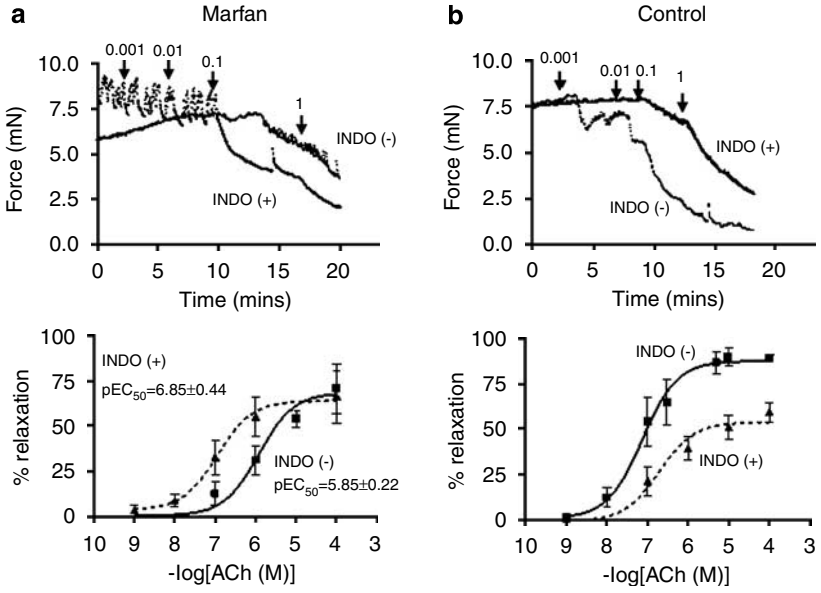

Figure 5 Effects of indomethacin on acetylcholine (ACh)-induced relaxation in thoracic aortae, from 6-month-old mice, precontracted with phenylephrine. (**a**) Marfan and (**b**) control aortae were preincubated with 10 μM indomethacin for 30 min and then contracted with phenylephrine (1 μM). ACh was added (as indicated by arrows) in cumulative concentrations (numbers represent the concentration in μM). The upper panels show representative traces from six independent experiments demonstrating the relaxation induced by ACh in the absence (INDO (−)) and presence (INDO (+)) of indomethacin. The lower panels are the corresponding concentration–response curves of ACh-induced relaxation.

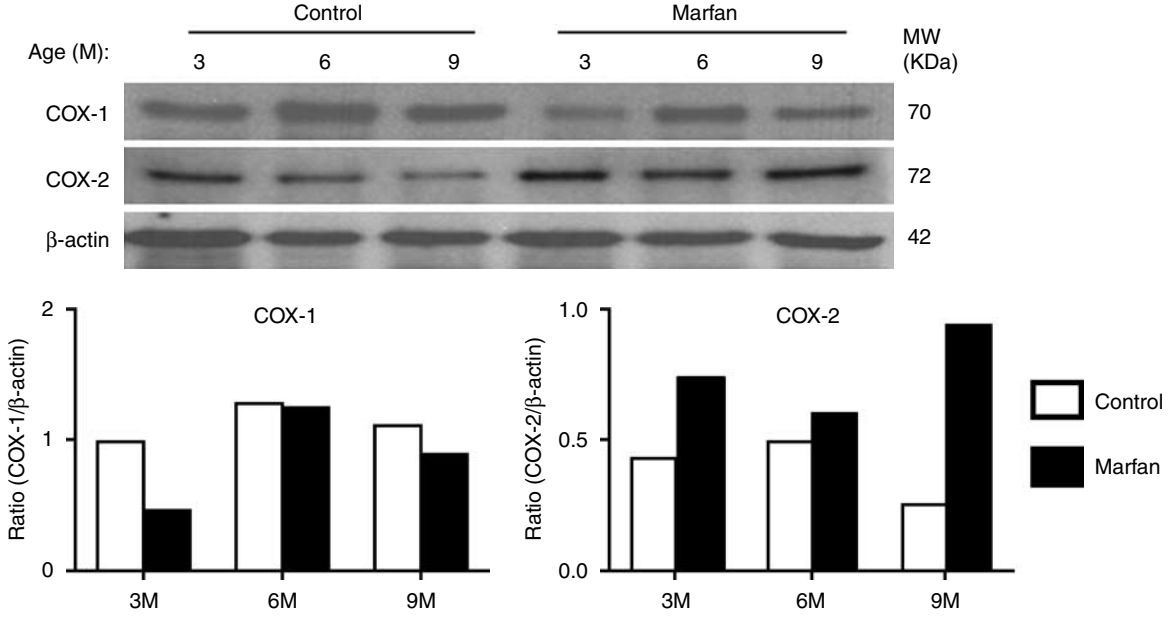

Figure 6 Differential expression of cyclooxgenase-1 (COX-1) and, cyclooxgenase-2 (COX-2) in Marfan aorta. Western immunoblotting showing the protein expression of COX-1 and COX-2 in the thoracic aortae from control and Marfan mice at different ages. Because of the limited aortic samples from each mouse, thoracic aortae were pooled from at least 15 mice from each group of animals at different ages. Expression of β-actin served as loading control. The histograms show the changing trend of the ratios of COX/β-actin expression.

receptor agonist U46619 in the Marfan aorta were similar to those in the control at 6 months of age. At 3 and 9 months, such contractile responses were indeed much greater in the Marfan aorta than those from the control, probably compensating for the reduced production of constricting prostanoids (Figure 3). From all these observations, we conclude that the diminished contraction in the Marfan thoracic aorta could be attributed to both reduced basal production of COX-1-derived TXA_2 and enhanced synthesis of COX-2-derived PGI_2.

We have demonstrated previously that the impairment of endothelial-dependent relaxation in Marfan thoracic aorta is

in part due to the downregulation of eNOS/Akt signalling (Chung *et al*., 2007). Several mechanisms have been implicated in the endothelial dysfunction associated with various pathological situations (for example, hypertension, diabetes, ageing and hypercholesterolaemia), including an increase in endothelium-derived vasoconstrictor production, for example, TXA_2 and PGH_2 (Matz *et al*., 2000; Tang *et al*., 2005; Hermann, 2006). However, the possibility that the ACh-induced response is due to TXA_2 release was ruled out in the Marfan aorta since SQ-29548 pretreatment did not affect the relaxation response to ACh in these aortae. In contrast, in the controls, especially at a young age, the endothelium-dependent relaxation to ACh was amplified after SQ-29548 treatment. It has been demonstrated that the endothelial-dependent relaxant response to ACh of rat aorta can be inhibited by the release of TXA_2/PGH_2 during stimulation with a high concentration (that is, 10–100 μM) of ACh (Koga *et al*., 1989; Kato *et al*., 1990; Tang *et al*., 2005). Furthermore, ACh induces a marked endothelial-dependent contraction in arteries from spontaneously hypertensive rats, and this is mediated by COX-derived TXA_2/PGH_2 (Kato *et al*., 1990; Ge *et al*., 1995). It is thus clear that COX-1 and COX-2 differentially regulate the cardiovascular function in both normal and pathological states by synthesizing vasoactive prostanoids.

In the Marfan aorta, we found that the expression of COX-1 was downregulated (Figure 6). In the vasculature, COX-1 is constitutively expressed and is the main source of the basal constricting prostanoid TXA_2 (Ge *et al*., 1995; Heymes *et al*., 2000). TXA_2 is primarily produced by platelets, but can also be synthesized from endothelial cells (DeWitt *et al*., 1983; Tazawa *et al*., 1996). In the aorta of spontaneously hypertensive rats, the expression of COX-1 has been shown to be augmented and the endothelium-dependent contraction is thought to require the activity of COX-1, rather than of COX-2, to produce endogenous agonists of the TXA_2/PGH_2 receptors (Ge *et al*., 1995; Heymes *et al*., 2000). Our present data indicate that the decreased TXA_2 secretion (Figure 4) in the Marfan aorta could be due to a significant reduction of COX-1 expression (Figure 6). The reason for this reduction of COX-1 and TXA_2 in the Marfan aorta is not known. However, TXA_2 is known to elevate blood pressure (Smith *et al*., 2000), promote inflammation (Thomas *et al*., 2003) and cause cardiac fibrosis (Francois *et al*., 2005). Specifically, COX-1-derived TXA_2 is believed to induce proliferation of vascular smooth muscle cells (Smith *et al*., 2000). Therefore, downregulated TXA_2 synthesis could attenuate its inflammatory effects on endothelial and smooth muscle cells during aneurysm formation.

Although COX-2 is much less abundant than COX-1, its expression was upregulated in the Marfan aorta (Figure 6). In the vascular endothelium and smooth muscle cells, COX-2-derived PGI_2 is the most abundant prostanoid (Bunting *et al*., 1977; Moncada *et al*., 1977; Qi *et al*., 2006). We believe that the increased release of PGI_2 is associated with an elevated expression of COX-2 in the Marfan aorta (Figures 4 and 6). The reason for the upregulation of COX-2 in Marfan aorta is also not known. However, it is possible that the loss of vessel elasticity and increase in pulse wave velocity in the Marfan aorta (Vitarelli *et al*., 2006) could induce COX-2 expression (Topper *et al*., 1996; Rudic *et al*., 2005). In mice deficient in eNOS, it has been shown that COX-2 is upregulated (Zhou *et al*., 2006), and COX-dependent vasodilatation can compensate for the loss of flow-mediated vasodilatation (Chataigneau *et al*., 1999). Furthermore, if COX-2 is inhibited in the vasculature with limited NO bioavailability, as observed in Marfan syndrome (Chung *et al*., 2007), thrombosis would be stimulated (Anning *et al*., 2006). Inhibition of COX-2 and deletion of PGI_2 receptors elevate blood pressure, increase injury-induced restenosis, thrombotic events, atherosclerosis, as well as vascular hyperplasia and remodelling (Cheng *et al*., 2002; Egan *et al*., 2005; Francois *et al*., 2005; Rudic *et al*., 2005). Nevertheless, the PGI_2 analogue iloprost induces differentiation of vascular smooth muscle cells from the synthetic, proliferative phenotype to a quiescent, contractile phenotype (Fetalvero *et al*., 2006). This may help maintain the contractile function and prevent the detrimental effects of dedifferentiation, such as reduction of lumen size due to smooth muscle migration, proliferation and matrix deposition. However, COX-2 expression induces metalloproteinases (Tsujii *et al*., 1997), the activation of which and resultant extracellular matrix degradation is essential for the formation of aortic aneurysm and vascular remodelling (Gitlin *et al*., 2007). Therefore, at present, it is not known whether the elevation of COX-2 and the consequent increase in PGI_2 production would have a beneficial effect in the aorta of patients with Marfan syndrome.

To elucidate the role of COX in the progression of Marfan syndrome, the age-matched control littermates were used for comparison. COXs are abundantly expressed in both vascular endothelial and smooth muscle cells, and they probably contribute to the abnormalities of the vascular wall with respect to functional changes during ageing (Heymes *et al*., 2000). It has been shown that indomethacin has a beneficial effect on endothelium-dependent relaxation in animal models of ageing and in the vasodilator response to ACh in elderly patients (Kato *et al*., 1990; Taddei *et al*., 1995). This suggests that the release of COX-derived vasoactive factors may contribute to the endothelial dysfunction that occurs in ageing. However, we did not observe either a potentiating effect of indomethacin on ACh-induced relaxation in the control aorta or an upregulation of COX-2 expression with age in our experiments (Figure 6). Therefore, these mechanisms may only be relevant after 1 year of age (Heymes *et al*., 2000; Matz *et al*., 2000).

In conclusion, we demonstrated that the impairment of contraction in Marfan thoracic aorta can be attributed to an elevated basal production of PGI_2, and this results from upregulation of COX-2 expression. The reduction in COX-1 could account for the decreased production of TXA_2 in the Marfan aorta. The imbalance between COX-derived vasoconstrictor and relaxant prostanoids could contribute to the compromised vasomotor function in Marfan syndrome.

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Conflict of interest

The authors state no conflict of interest.

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