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ORIGINAL ARTICLE
Adult congenital heart disease

Exaggerated elastin turnover in young individuals with Marfan syndrome: new insights from the AIMS trial

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Aims

The fragmentation and loss of elastic fibre in the tunica media of the aorta are pathological hallmarks of Marfan syndrome (MFS) but the dynamics of elastin degradation and its relationship to aortic size and physiological growth remain poorly understood.

Methods and results

In this *post hoc* analysis of the AIMS randomized controlled trial, the association of plasma desmosine (pDES)—a specific biomarker of mature elastin degradation—with age and aortic size was analysed in 113 patients with MFS and compared to 109 healthy controls. There was a strong association between age and pDES in both groups, with higher pDES levels in the lower age groups compared to adults. During childhood, pDES increased and peaked during early adolescence, and thereafter decreased to lower adult levels. This trend was exaggerated in young individuals with MFS but in those above 25 years of age, pDES levels were comparable to controls despite the presence of aortic root dilation. In MFS children, increased aortic diameter relative to controls was seen at an early age and although the increase in diameter was less after adolescence, aortic root size continued to increase steadily with age. In MFS participants, there was an indication of a positive association between baseline pDES levels and aortic root dilatation during up to 5 years of follow-up.

Conclusion

This study has shown that developmental age has a significant effect on levels of elastin turnover as measured by pDES in MFS individuals as well as healthy controls. This effect is exaggerated in those with MFS with increased levels seen during the period of physiologic development that plateaus towards adulthood. This suggests an early onset of pathophysiology that may present an important opportunity for disease-modifying intervention.

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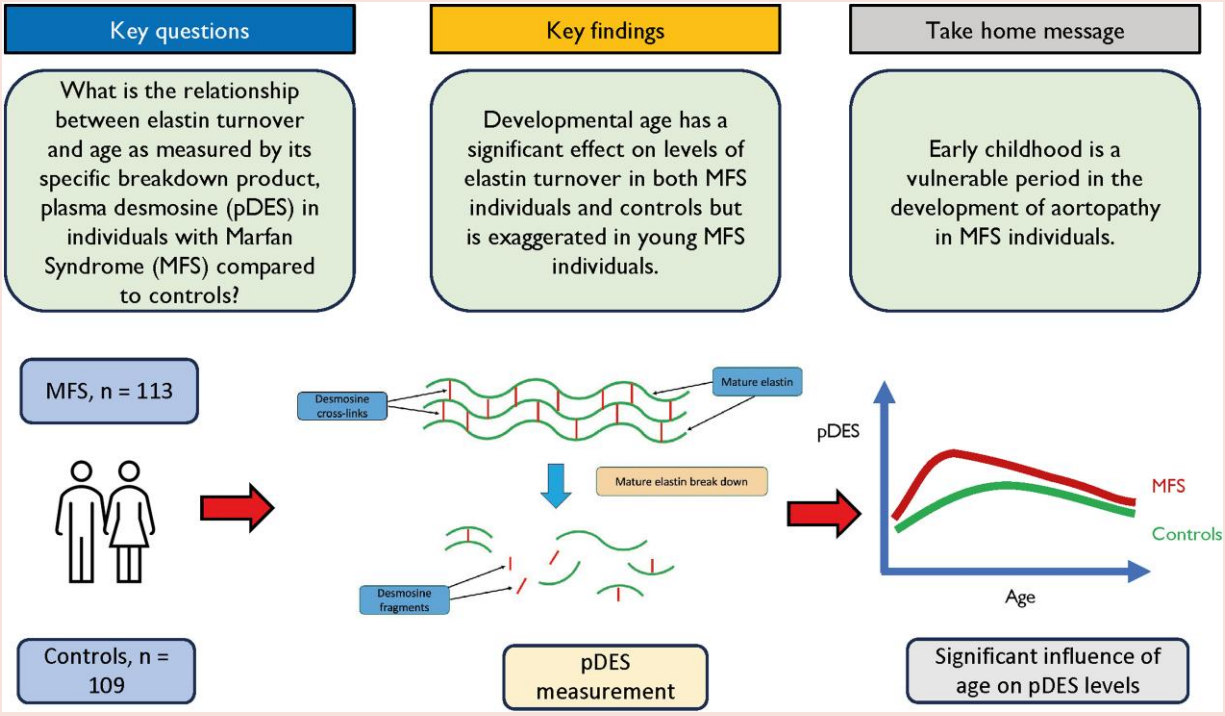
Graphical Abstract



Keywords Marfan syndrome • Biomarkers • Aortopathy • Desmosine • Elastin

Introduction

Patients with Marfan syndrome (MFS) are at increased risk of morbidity and mortality, largely from the development of aortic root dilatation and resulting hazard of aortic dissection that is inevitably fatal if untreated. Despite extensive research and discovery of pathogenic variants in the fibrillin-1 (*FBN1*) gene associated with MFS, there remains residual uncertainty surrounding the pathophysiology of aortopathy in MFS.[1] Excessive transforming growth factor beta (TGF-β) signalling has been hypothesized as playing a key role, and its modulation with angiotensin receptor blockers (ARB) has been the basis of multiple randomized controlled trials to date.[2–7] Fibrillin-1 is a glycoprotein that forms a major structural component of elastic fibres and plays a regulatory role in extracellular matrix (ECM) homeostasis; therefore, pathogenic variants of the *FBN1* gene can result in weaker and reduced elastin fibre formation contributing to the pathogenesis of aortic dilatation and rupture. Elastin homeostasis has been little studied in patients with MFS. Fragmentation and reduced elastin content with defective elastin cross-linking have been reported in aorta from adult MFS, however, it is not clear if the elastin abnormalities seen are due to abnormal elastin fibre formation during aortic development in childhood to adulthood or accelerated degradation throughout life.[8–11] Therefore, the aim of our study was to examine elastin turnover in relation to age in a cohort of MFS participants from the Aortic Irbesartan Marfan Study (AIMS) trial compared to healthy controls.[12] To assess elastin turnover, plasma desmosine (pDES)—the lysine cross-link in mature elastin that is released exclusively from breakdown of mature elastin—was measured by liquid chromatography-tandem mass spectrometry.[13] We have previously shown that pDES is a pathologically relevant biomarker of disease activity in atherosclerotic abdominal aneurysm and therefore may also be a useful tool to assess elastin turnover in relation to age in MFS compared to healthy controls.[14] Although the source of pDES is not exclusively derived from elastin in the cardiovascular tissues, the arterial tissues have greater relative size compared to other elastin containing tissues, and have, in particular the aorta, the highest elastin content by dry weight.[13,15,16] Therefore, the contribution from the cardiovascular system to circulating desmosine is considered greater than the lungs.[16] Importantly, previous work on observational data relating to elastin breakdown has been shown to potentially be a predictor of adverse cardiovascular outcomes across multiple different diseases.[14,17,18] Therefore, to understand its role in patients with MFS—a condition with elastin degradation—who are at high risk of cardiovascular morbidity and mortality, we sought to explore how elastin breakdown relates to various stages of growth in MFS and controls, as well as its relationship with aortic size.

We hypothesized that firstly, elastin turnover is increased in MFS compared to controls and that early years of growth may be associated with higher levels of pDES reflecting highly dynamic elastin turnover and secondly, pDES levels are associated with aortic root growth in MFS.

Methods

Design

We conducted a *post hoc* analysis of pDES levels in available consented baseline plasma samples from participants in the AIMS trial (ISRCTN90011794, EuDraCT No.2010-019302-16). Full details of the study have been published previously.[19] In brief, the AIMS trial was a double-blind, placebo-controlled randomized controlled trial studying the effects of irbesartan on the rate of aortic dilatation in children and adults with MFS. Participants were given open label

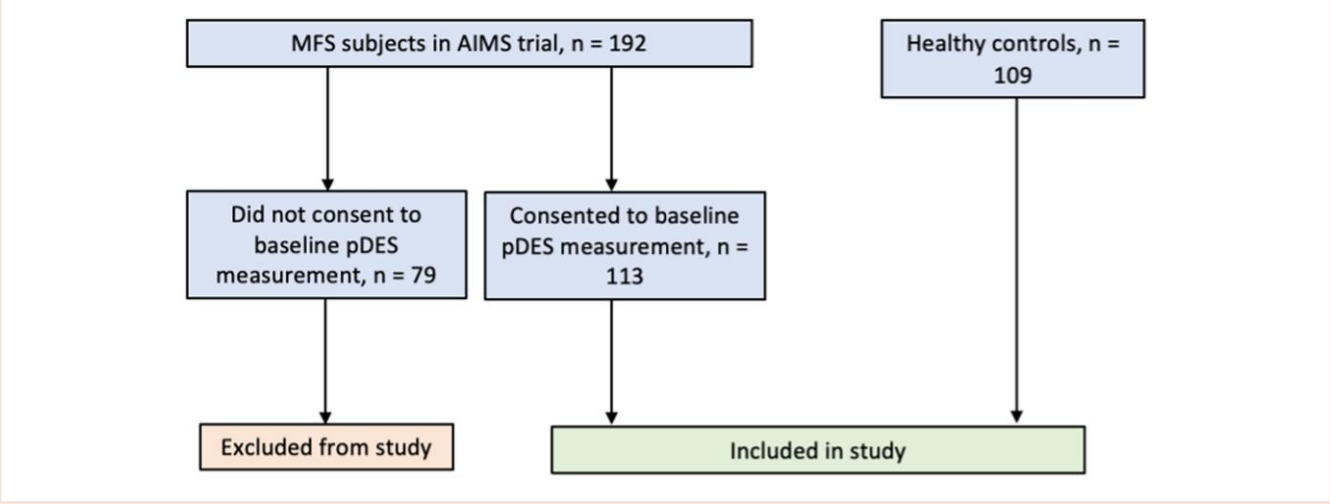


Figure 1 Study flow chart. MFS, Marfan syndrome; pDES, plasma desmosine.

irbesartan 75 mg once daily for 4 weeks and then randomly assigned to 150 mg of irbesartan (increased to 300 mg as tolerated) or matching placebo once daily for a maximum follow-up period of 5 years. Aortic diameter was measured by echocardiography at baseline and then annually. From the 192 participants recruited to the main AIMS trial, 113 provided consented plasma samples at baseline before randomization to placebo or irbesartan (*Figure 1*), and all were used in this study (NHS REC Ref: 10/H0720/28). Out of these participants, 63 of them provided a second sample at the 1-year follow-up. To study the interaction of age on elastin turnover, pDES levels were analysed according to three age ranges that reflect periods of development while retaining sufficient numbers within each category as follows: childhood to pubescent growth (≥6 to ≤15 years), post-pubescence to early adulthood (>15 to ≤25 years), and adulthood (>25 to 40 years).

Participants

Individuals were eligible for recruitment to the AIMS trial if they were aged between 6 and 40 years with a clinically confirmed diagnosis of MFS based on the revised Ghent diagnostic criteria and an aortic Z-score of more than zero at baseline echocardiography.[19,20] Baseline plasma samples were available in 113 participants. Controls were obtained from existing databases of healthy volunteers participating in three unrelated observational studies. Most of the adult controls (63%) were volunteers in the Molecular and Imaging Studies of Cardiovascular Health and Disease (CHD) biobank programme at the National Heart Centre Singapore (age range 20 to 70 years old) (https://ichgcp.net/clinical-trials-registry/NCT02804269). The CHD biobank is a programme that collects biological samples, health information, and imaging data from consented patients to study the molecular, imaging, and outcomes of cardiovascular health and disease. The remaining adult controls (37%) were from the Desmosine in Hereditary Aortopathy Study (DESMA), University of Dundee (NHSREC ref:17/EM/0450, IRAS ID:238727). Paediatric controls were children aged 6–18 attending for electrophysiologic study at the Bristol Royal Hospital for Children and Bristol Heart Institute with no history of lung disease or structural cardiovascular disease, who were enrolled in the Outcome Monitoring After Cardiac Surgery (OMACS) Trial, University of Bristol (ISRCTN: 17650644, NHS REC ref: 19/SW/0113, IRAS: 261397). The OMACS study is an ongoing cohort study of cardiac surgery patients at University Hospitals Bristol and Weston NHS Foundation Trust. Antihypertensive medications were prescribed in only two adult controls. All control samples were collected with ethical approval and patient consent.

Aortic root measurements

Aortic root measurements were obtained by transthoracic echocardiograms in the AIMS trial and the DESMA and OMAC studies, and by cardiac MRI in the CHD biobank.[21] Aortic root diameter was measured in the parasternal long axis view using inner-edge to inner-edge technique during peak systole at the level of the Sinus of Valsalva with the tip of open cusps at 90° to the direction of flow and at end-diastole. Aortic *Z*-score was calculated based on aortic sinus diameter and body surface area.[22]

Laboratory analyses

Desmosine analysis

The levels of pDES, which include free and peptidyl desmosine and isodesmosine, were quantified using a validated liquid chromatography-tandem mass spectrometry that has been previously described.[23] Lower limit of quantification is 0.1 ng/mL.

Statistical analysis

Baseline characteristics of participants in the MFS and control cohorts were summarized using either means and standard deviations (SDs) or medians and interquartile ranges (IQR) for continuous variables and counts and percentages for categorical variables. A linear regression model containing an indicator variable for cohort (MFS vs. controls), a categorical variable for age, and an interaction between these two variables was used to assess differences in mean pDES between the MFS and control cohorts. Age categories of >6 to ≤15 years, >15 to ≤25 years, and >25 to ≤40 years were used in order to categorize age into approximate tertiles and also to reflect periods of physiologic growth. The association between age and pDES was assessed using a piecewise linear regression model. This model allowed the slope for the association between age and pDES to differ within strata of cohort (MFS and controls) and age (≥6 to ≤15 years, >15 to ≤25 years, and >25 to ≤40 years). Finally, a linear mixed effects model for repeated measures was used to examine the association between pDES and aortic dilatation in the MFS cohort using aortic root measurements collected annually up to a maximum of 5 years in the AIMS trial. This model contained linear effects of pDES and time as predictors, along with a pDES–time interaction, and adjustment for randomized treatment group. An unstructured variance–covariance matrix was used to allow for the anticipated correlation between repeated aortic root measurements. For all of the models described, estimated effects and corresponding 95% confidence intervals and *P*-values were calculated. Subsequent models included adjustments for age, sex, ethnicity, height, and where appropriate, aortic root diameter and systolic blood pressure. Scatter plots showing the associations between age and pDES, age and aortic diameter, and aortic diameter and pDES in the two cohorts were produced including Lowess smoothing curves. In order to assess the impact of age on the association between aortic diameter and pDES, scatter plots of aortic diameter against pDES are shown by age category. In addition, since MFS participants had increased pDES and aortic diameter at baseline, separate regression analyses adjusted for age

Table 1 Baseline characteristics of those with Marfan syndrome vs. controls ($n = 222$)

	MFS ($n = 113$)	Controls ($n = 109$)	*P*-value
Plasma desmosine, pDES (ng/mL)	0.51 (0.29) $n = 113$	0.30 (0.13) $n = 109$	<0.0001
Age (years)	18.2 (9.4)	26.1 (9.5)	<0.0001
≥6 to ≤15	49 (43.4)	22 (20.2)	
>15 to ≤25	36 (31.9)	26 (23.9)	
>25 to ≤40	28 (24.8)	61 (56.0)	
Sex			0.35
Male	54 (47.8)	59 (54.1)	
Female	59 (52.2)	50 (45.9)	
Ethnicity			<0.0001
Asian	6 (5.3)	69 (63.3)	
Black African or Caribbean	1 (0.9)	0	
Caucasian	102 (90.3)	37 (33.9)	
Mixed	2 (1.8)	0	
Other	2 (1.8)	0	
Unknown	0	3 (2.8)	
Height (cm)	170 (21) $n = 113$	166 (13) $n = 109$	0.003
Weight (kg)	58.3 (24.9) $n = 113$	63.7 (17.0) $n = 109$	0.020
Body mass index, BMI (kg/m^2)	19.5 (6.1) $n = 113$	22.8 (4.5) $n = 109$	<0.0001
Body surface area, BSA (m^2)	1.66 (0.41) $n = 113$	1.70 (0.28) $n = 109$	0.68
Systolic blood pressure (mmHg)	109 (16) $n = 112$	126 (16) $n = 80$	<0.0001
Diastolic blood pressure (mmHg)	64 (12) $n = 112$	77 (10) $n = 80$	<0.0001
Aortic root diameter (mm)	33.8 (5.4) $n = 113$	27.8 (3.8) $n = 109$	<0.0001
Baseline aortic root *Z*-score	3.4 (2.1)	−0.1 (1.6)	<0.0001

P-values calculated using either Fisher's exact tests or Mann–Whitney tests. Data presented as mean (SD) or *n* (%).

Table 2 Mean plasma desmosine (ng/mL) in those with Marfan syndrome vs. controls, by age category ($n = 222$)

Age (years)	MFS ($n = 113$)	Controls ($n = 109$)	Unadjusted difference in mean pDES (ng/mL) (95% CI)	*P*-value*	Adjusted difference in mean pDES (ng/mL) (95% CI)[a]	*P*-value**
≥6– ≤ 15	0.64 (0.32)	0.46 (0.17)	0.19 (0.09 to 0.29)	0.0002	0.25 (0.15 to 0.35)	<0.0001
>15– ≤ 25	0.49 (0.20)	0.30 (0.07)	0.19 (0.09 to 0.29)	0.0001	0.16 (0.05 to 0.28)	0.006
>25– ≤ 40	0.30 (0.15)	0.25 (0.07)	0.05 (−0.04 to 0.14)	0.27	0.05 (−0.09 to 0.18)	0.51

Data presented as mean (SD).
[a]Adjusted for sex, ethnicity, height, and aortic root diameter.
*Interaction *P*-value 0.043.
**Interaction *P*-value 0.031.

within each age category were conducted for MFS participants and controls to assess the association between pDES and aortic diameter. All analyses were conducted using Stata IC version 16.0.

Results

Plasma desmosine was measured in 113 participants with MFS and 109 healthy controls (*Table 1*) at baseline. In our cohorts, participants with MFS were younger with a mean age of 18.2 years (SD 9.4) and 48% were male, compared to healthy controls with a mean age of 26.1 years (SD 9.5) and 54% male.

There was a strong association between pDES and age seen in both MFS participants and controls, where pDES was higher in the lower age groups compared to adults (*Table 2* and *Figure 2*). During childhood and into adolescence, pDES appeared to increase and peak during early adolescence, corresponding to the phase of aortic growth, and thereafter decreased to lower adult levels and remained relatively unchanged (*Table 3* and *Figure 3*). This trend was exaggerated in young individuals with MFS. In contrast, in MFS participants aged >25 years old, levels of pDES were comparable to controls. Formal interaction tests supported this finding (*Table 2*). In MFS and controls, pDES levels were higher in males than females, with and without adjustment for age

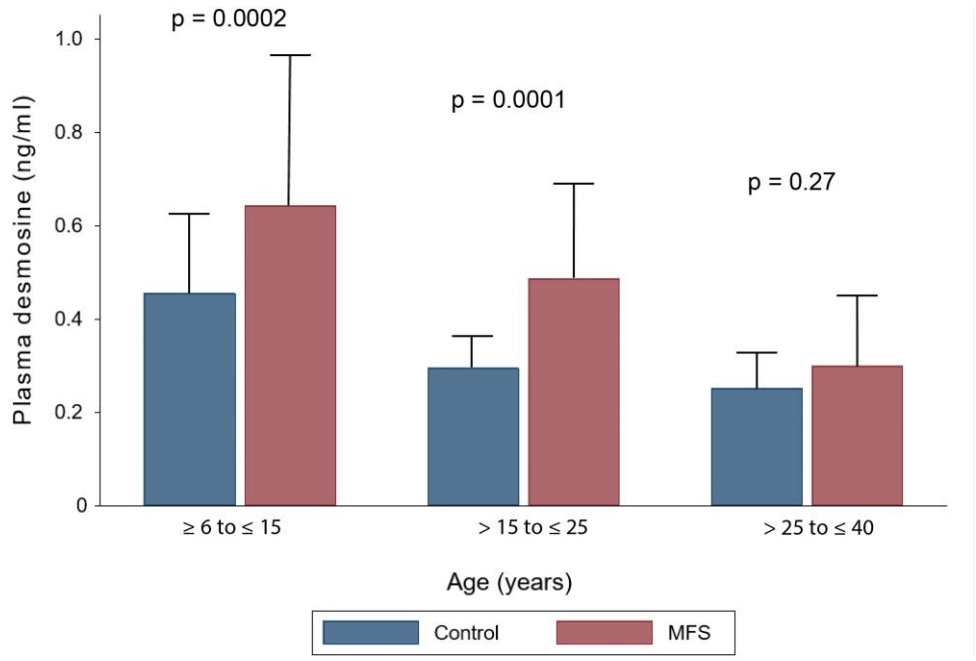


Figure 2 Mean (standard deviation) plasma desmosine (ng/mL) in those with MFS vs. controls, by age category ($n = 222$). MFS, Marfan syndrome.

Table 3 Association between age and plasma desmosine (ng/mL) in those with Marfan syndrome vs. controls, by age category ($n = 222$)

Age (years)	Unadjusted effect				Adjusted effect[b]			
	MFS ($n = 113$)		Controls ($n = 109$)		MFS ($n = 113$)		Controls ($n = 109$)	
	Effect on pDES[a] (ng/mL) (95% CI)	*P*-value	Effect on pDES[a] (ng/mL) (95% CI)	*P*-value	Effect on pDES[a] (ng/mL) (95% CI)	*P*-value	Effect on pDES[a] (ng/mL) (95% CI)	*P*-value
≥6– ≤ 15	0.45 (0.09 to 0.80)	0.014	0.24 (0.05 to 0.44)	0.013	0.39 (−0.11 to 0.89)	0.13	0.14 (−0.14 to 0.42)	0.32
>15– ≤ 25	−0.43 (−0.68 to −0.19)	<0.001	−0.05 (−0.14 to 0.04)	0.28	−0.35 (−0.63 to −0.08)	0.013	0.06 (−0.12 to 0.25)	0.50
>25– ≤ 40	0.04 (−0.09 to 0.16)	0.56	−0.02 (−0.08 to 0.03)	0.41	0.03 (−0.09 to 0.14)	0.66	−0.01 (−0.07 to 0.04)	0.66

[a]Per 10-year increase in age.
[b]Adjusted for sex, ethnicity, height, and aortic root diameter; 95% CIs estimated using robust standard errors.

(see Supplementary material online, *Table S1*). Adjusting for sex, ethnicity, height, and aortic root diameter resulted in similar effect estimates, though confidence intervals were generally wider.

Aortic root diameter increased with age in MFS children and controls (*Figure 3B*). In controls, aortic root growth diminished in early adolescence and aortic root size was relatively unchanging with age thereafter. In contrast, in MFS children, increased aortic diameter relative to controls was seen at an early age and although the increase in diameter was less after adolescence, aortic root size continued to increase steadily with age. There appeared to be an association between pDES and aortic diameter at baseline particularly before 15 years of age, which diminished between 15 and 25 years, and then disappeared after 25 suggesting the importance of the adolescent period (*Figure 4*). In addition, in MFS participants, there was an indication of a positive association between baseline pDES levels and aortic root dilatation during up to 5 years of follow-up, though this did not reach statistical significance. The effect of baseline desmosine (per 0.5 ng/mL increase) on rate of aortic dilatation (mm/year) adjusted for treatment group, age, sex, ethnicity, height, and systolic blood pressure was 0.18 (95% CI −0.03 to 0.39; $P = 0.10$) using data on 112 AIMS participants and the unadjusted effect was 0.19 (95% CI −0.01 to 0.40; $P = 0.062$) (*Table 4*). The unadjusted effect of baseline desmosine on aortic root *Z*-score was 0.01 (95% CI −0.01 to 0.04; $P = 0.38$) (see Supplementary material online, *Table S1*). In 63 AIMS participants who had paired samples at baseline and 1-year follow-up, irbesartan had little effect on levels of pDES at 1 year (*Table 5*).

Discussion

In this study using circulating pDES as a specific biomarker of mature elastin breakdown, we have demonstrated important novel findings

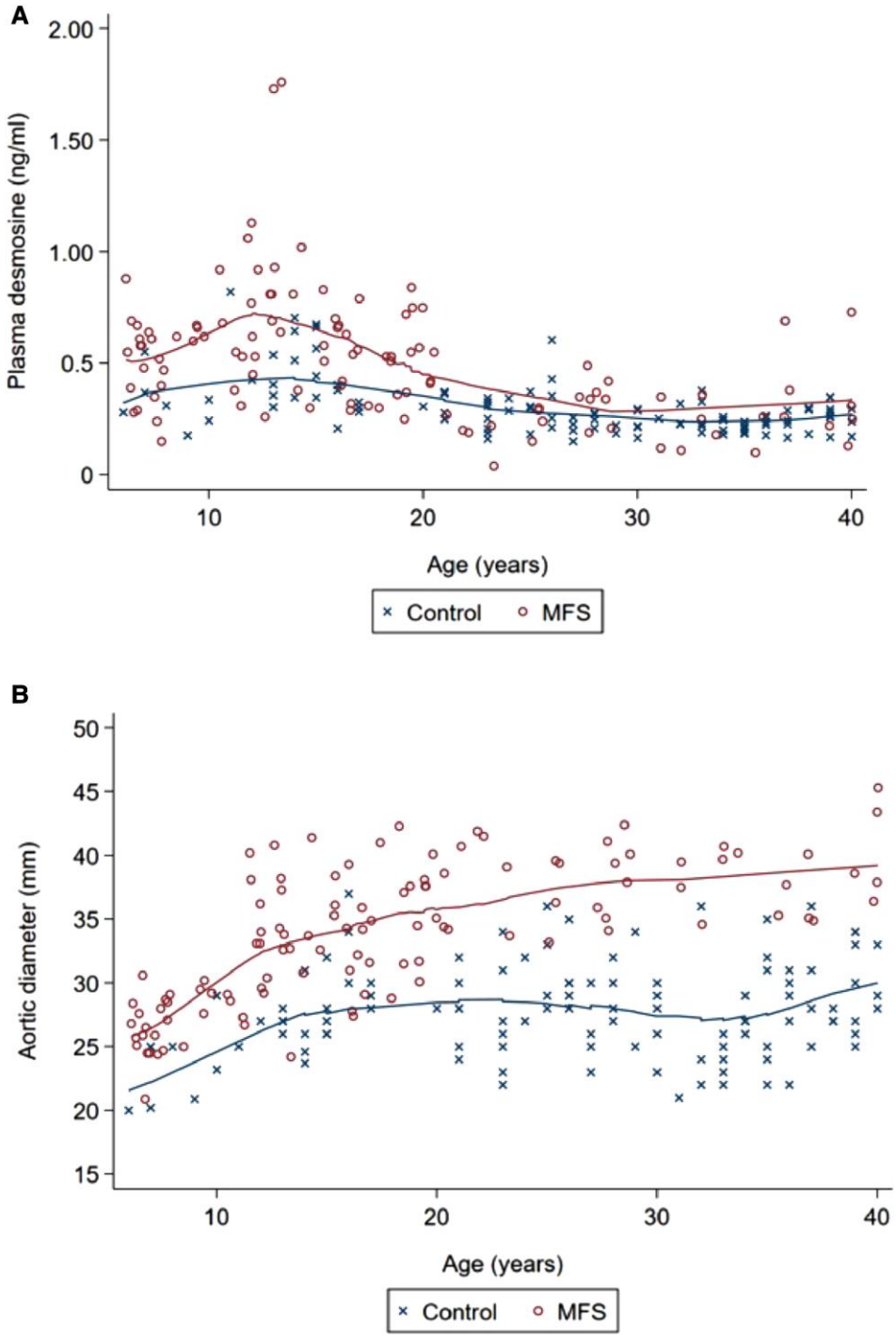


Figure 3 (*A*) Association between age and plasma desmosine in those with MFS vs. controls ($n = 222$). (*B*) Association between age and aortic diameter in those with MFS vs. controls ($n = 222$). MFS, Marfan syndrome.

of elastin turnover in relation to age in health and in MFS. We have shown for the first time that developmental age has a significant effect on levels of elastin turnover as measured by pDES in both MFS individuals as well as healthy controls, with increased levels seen during the period of physiologic development that coincides with aortic growth.[24,25] These findings indicate firstly that increased elastin turnover occurs during physiologic growth in childhood but diminishes in later adolescence. Secondly, elastin turnover appears to be exaggerated in young MFS individuals with aortopathy. Thirdly, in MFS adults, pDES was comparable to controls despite the presence of aortic root dilation, suggesting that progressive aortic root dilatation in adults may not be significantly attributable to ongoing elastin degradation and

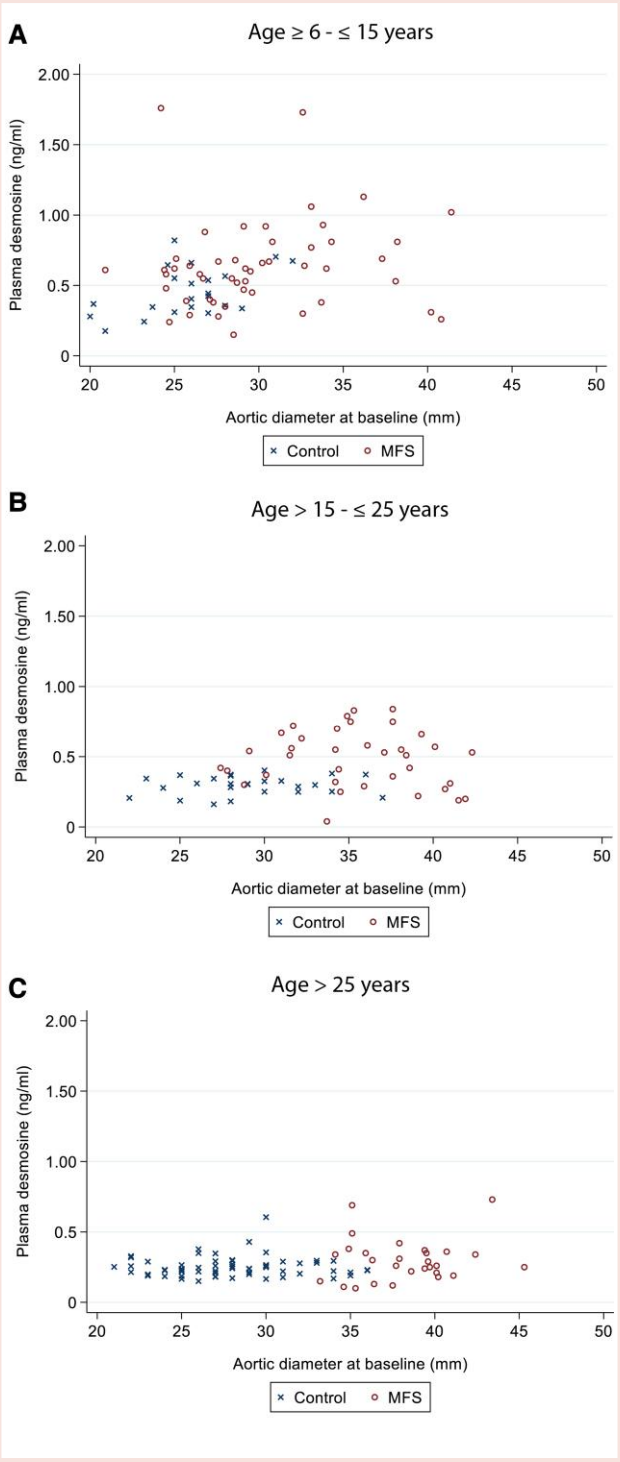


Figure 4 Associations between plasma desmosine levels and aortic root diameter in different age groups. MFS, Marfan syndrome.

Table 4 Effect of baseline plasma desmosine on the annual rate of aortic dilatation ($n = 112$)

	Rate of aortic dilatation (mm/year) (95% CI)	*P*-value
Unadjusted effect of baseline pDES[a] (per 0.5 ng/mL increase)	0.19 (−0.01 to 0.40)	0.062
Adjusted effect of baseline pDES[b] (per 0.5 ng/mL increase)	0.18 (−0.03 to 0.39)	0.10

CI, confidence interval.
[a]Adjusted for treatment group only.
[b]Adjusted for treatment group, age, sex, ethnicity, height, and systolic blood pressure.

Table 5 Effect of treatment group (irbesartan vs. placebo) on plasma desmosine (ng/mL) at 1 year ($n = 63$)

	Difference in mean pDES at 1 year (ng/mL) (95% CI)	*P*-value
Unadjusted effect of treatment group[a]	0.05 (−0.04 to 0.15)	0.27
Adjusted effect of treatment group[b]	0.02 (−0.07 to 0.12)	0.63

CI, confidence interval.
[a]Adjusted for baseline desmosine only.
[b]Adjusted for baseline desmosine, age, sex, ethnicity, height, aortic root diameter, and systolic blood pressure.

instead may be due to other factors, including accelerated vascular ageing.[26] Finally, there was a suggestive trend of association between levels of pDES and the growth of aortic root diameter in MFS.

Elastin is a major component of elastic fibres that form the elastic laminae that are sheet-like structures that form concentric layers around the circumference of the aorta and large elastic arteries. The number of layers of elastic laminae is established early in development, and the aortic medial thickness is due to accumulation of elastin fibres.[27,28] While it is known that elastin gene expression and protein synthesis begin in late embryonic development and terminate in adolescence, corresponding to physiological growth, the mechanism of circumferential aortic growth during development has not been fully elucidated.[11] It has been suggested that new elastin is deposited around breaks in the elastin laminae to allow circumferential expansion. This has been shown to occur around fenestrations in the elastic laminae in young mice but there may be additional mechanisms in larger mammals. In bovine pulmonary artery and developing sheep aorta, cell nests containing fragmented elastin laminae have been demonstrated in the outer media.[29–31] These cell nests were negative for the elastin precursor tropomyosin, while the adjacent surrounding cells showed strong positive signal for tropoelastin expression.[31] In addition, these cell nests contained abundant microfibrils that are essential for elastin assembly, and it has been demonstrated that elastolysis in the arterial wall results in large accumulations of microfibrils.[32] As a mechanism for elastic laminae growth, it has been postulated that the cells within these cell nests may degrade the elastin while the surrounding cells contribute to new elastin to fill the breaks, thereby allowing enlargement of the elastic laminae.[33] Our finding of increased levels of pDES indicating increased elastolysis of mature elastin, corresponding to periods of aortic development, supports this hypothesis of elastin turnover being integral to circumferential aortic growth, as opposed to growth arising solely from new elastin deposition.

The exaggerated levels of pDES in young MFS individuals compared to controls correspond to the early aortic root dilation in this age group as reported in a longitudinal study of aortic growth in MFS.[25] However, the elevated levels of pDES remain unexplained. Abnormal fibrillin

resulting from *FBN1* pathogenic variants may result in abnormal elastin fibres that predispose to increased elastolysis, as well as altered ECM regulation.[34] Our finding of early exaggerated elastin turnover in MFS children is concordant with the clinical observation that aortopathy occurs early in MFS. Indeed, in an observational study in young MFS patients, 35% of patients developed aortic root dilatation by the age of 5 years and 77% before the age of 19 years.[35] Our study findings provide indirect evidence that the pathology underlying aortic dilatation that occurs very early in childhood development is related to abnormal elastin turnover. Although there have been many studies in animal models of MFS, the pathophysiology of childhood aortopathy in human MFS is unknown.[36,37] Fibrillin-1 sequesters the large latent complex of TGF-β-binding protein and regulates its bioavailability for activation. Dysregulation of TGF-β activation is an important pathogenetic factor contributing to the genesis of MFS.[38] Angiotensin activity can influence TGF-β signalling by stimulating TGF-β messenger RNA (mRNA) and protein expression. In an early clinical study in 18 paediatric MFS patients with aggressive aortic disease despite standard treatment with betablockers, angiotensin-1 (AT-1) receptor blockade with losartan resulted in dramatic stabilization of the aortic root diameters.[39] However, since then, a meta-analysis of five randomized controlled trials investigating losartan in MFS has shown equipoise with regard to the benefit of losartan alone or in combination with a betablocker to reduce aortic growth and complications.[4,40–44] To date, there have been few studies specifically in paediatric and adolescent MFS patients. The largest study, a randomized, controlled single-blinded study, in 608 young MFS (mean age 11 years) did not demonstrate a significant difference between losartan compared to atenolol in the rate of aortic root dilatation between the two treatment groups over a 3-year period.[4] However, in the subgroup analysis, treatment at younger age with atenolol or losartan was associated with larger reduction in aortic growth rate, compared to adults.[4] A smaller, open-labelled randomized controlled study in children of similar age showed that when added to betablocker, losartan significantly reduced aortic dilatation.[5] In a prospective study of losartan in 20 unselected paediatric and adolescent MFS participants, significant reduction in normalized aortic dimensions was seen with losartan, and notably, a better response to therapy was seen when started at an earlier age.[45] Compared to losartan, irbesartan has greater bioavailability, longer duration of action, and greater blood pressure-lowering effect. The AIMS trial (Aortic Irbesartan in Marfan Study) investigated irbesartan in a slightly older age group (mean age 18).[12] In this study of 192 children and young adults randomized to irbesartan or placebo, irbesartan was associated with a significant reduction in the rate of aortic dilatation. This effect in terms of the difference in rate of aortic root diameter change (mm/year) appeared greater in younger participants, defined as <18 years (−0.34; −0.61 to −0.07) compared to those >18 years (0.05; −0.32 to 0.21), however, the study was underpowered to show this conclusively. This consistent trend seen in these studies showing the increased efficacy of ARB treatment when started at an early age indicates that the pathophysiology underlying aortic dilatation starts very early in MFS. These observations together with our findings provide indirect evidence that early childhood represents a vulnerable and criterial period in the development of MFS aortopathy, and that early initiation of treatment may be critical for disease modification.

Our incidental finding that in early adulthood, pDES levels in MFS participants fall to levels comparable to healthy controls despite the presence of increasing aortic diameter, was surprising as elastin fragmentation is a hallmark of aortopathy in MFS, however, this observation offers interesting insights. Firstly, putative regulatory dysfunction of the ECM and TGF-β activation resulting from the fibrillin pathogenic variants does not appear to be associated with persistent increased elastin degradation in adulthood. Our findings are similar to transgenic *FBN1* mouse experiments showing that significant fragmentation of aortic elastin occurs in early in life and thereafter stablizes.[26] Likewise, an observational study in MFS showed that loss of aortic distensibility occurs at an early age but stabilizes in adulthood.[46] Secondly, continued aortic expansion is not associated with increased elastin degradation, suggesting other factors such as abnormal aortic distensibility and the replacement of elastin with collagen may be of increased importance at this stage of life.[12,47] Collagen's higher tensile strength and increased deposition in early adulthood secondary to elastin loss may be a mode of healing for the aorta similar to developing scar tissue, and initially slow down the rate of aortic growth, until collagen breakdown associated with vascular ageing occurs.[16] We found higher levels of pDES in males compared to females, in keeping with the recent reports in MFS of greater aortic root dilation and lesser aortic distensibility in males compared to females, providing indirect evidence linking abnormal elastin turnover with aortic pathophysiology.[25,46] Our study only included patients up to 40 years of age, and it is plausible that in later life, premature degradation of extracellular matrix related to pathogenic vascular aging can occur in pre-existing weakened aortic media, representing a different pathophysiology of aortic root dilatation and escalation of risk of rupture.[14,26]

There was a borderline association between the levels of baseline pDES in MFS participants and aortic root diameter growth over the three years of follow-up. These observations are in keeping with our previous study in atherosclerotic abdominal aortic aneurysm where pDES levels predicted disease activity and clinical outcomes.[14] However, further studies are required to investigate a similar prognostic role in MS.

Limitations

There are several limitations to our study. Firstly, the age, sex, and ethnicity distribution between the controls and MFS participants were unbalanced due to the nature of retrospective analysis. However, all analyses conducted were stratified by age and models adjusted for age, sex, and ethnicity. Furthermore, although there are small differences in normal aortic diameter across ethnicities, these differences are small relative to measurement error and reproducibility and therefore are unlikely to be clinically relevant. Secondly, we did not study subjects with prior cardiac surgery as this was an exclusion criterion in the original design of the AIMS trial, and thus would inherently lead to the exclusion of severe cases. This could explain in part why higher pDES levels in the older age group were not observed. In addition, we were not able to measure serial pDES to study the association with aortic growth at an individual level as serial plasma sampling was not part of the AIMS protocol. Thirdly, we were unable to stratify our analysis according to type of genetic variants due to limited number of participants who gave consent to share their mutation report. This would be of interest as dominant negative variants potentially have a worse outcome in children compared to haploinsufficiency variants.[25] Fourthly, imaging modalities used to measure the aortic root size in the study were different for the majority of the adult controls, however, a recent large population-based study has reported that differences between the two modalities are negligible and supports an interchangeable application of transthoracic echocardiogram and standard cardiovascular magnetic resonance imaging for screening of aortic root diseases.[48] Lastly, there were only six cases of elective surgery in the AIMS trial, and no significant aortic events (death, rupture, emergency surgery), so we were not able to evaluate the association of baseline pDES with these events.

Conclusion

In conclusion, we have shown that developmental age and normal aortic growth are associated with increased elastin turnover and in MFS, this is exaggerated from an early age, suggesting an early onset of pathophysiology. In contrast, adulthood pDES levels were not significantly

elevated, indicating that the aortic root growth in adulthood is not associated with increased elastin breakdown, but may be mediated by other factors such as altered tensile strength and pathological vascular aging. Our study suggests that early childhood may be a vulnerable period in the development of aortopathy and presents an important opportunity for disease-modifying intervention.

Lead author biography

Dr Zaid Iskandar is currently a consultant interventional cardiologist working in Raigmore Hospital, Inverness, Scotland. He completed his medical degree at the University of Manchester and subsequent cardiology training including a higher research degree at the University of Dundee studying biomarkers in aortopathy. He is also a previous complex PCI and CTO fellow at the renowned Bristol Heart Institute.

Data availability

The data underlying this article will be shared on a reasonable request to the corresponding author.

Supplementary material

Supplementary material is available at *European Heart Journal Open* online.

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