ESC European Society of Cardiology

Cardiovascular Research (2020) **116**, 457–465
doi:10.1093/cvr/cvz095

Celiprolol but not losartan improves the biomechanical integrity of the aorta in a mouse model of vascular Ehlers–Danlos syndrome

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Received 25 January 2019; revised 8 March 2019; editorial decision 3 April 2019; accepted 4 April 2019; online publish-ahead-of-print 8 April 2019

Time for primary review: 41 days

Aims Antihypertensive drugs are included in the medical therapy of vascular Ehlers–Danlos syndrome (vEDS). The β-blocker celiprolol has been suggested to prevent arterial damage in vEDS, but the underlying mechanism remains unclear. It is also unknown whether the widely used angiotensin II receptor type 1 antagonist losartan has a therapeutic effect in vEDS. Here, we evaluated the impact of celiprolol and losartan on the biomechanical integrity of the vEDS thoracic aorta.

Methods and results We established a new approach to measure the maximum tensile force at rupture of uniaxially stretched murine thoracic aortic rings. In a vEDS model, which we (re-)characterized here at molecular level, heterozygous mice showed a significant reduction in the rupture force compared to wild-type mice, reflecting the increased mortality due to aortic rupture. For the assessment of treatment effects, heterozygous mice at 4 weeks of age underwent a 4-week treatment with celiprolol, losartan, and, as a proof-of-concept drug, the matrix metalloproteinase inhibitor doxycycline. Compared to age- and sex-matched untreated heterozygous mice, treatment with doxycycline or celiprolol resulted in a significant increase of rupture force, whereas no significant change was detected upon losartan treatment.

Conclusions In a vEDS model, celiprolol or doxycycline, but not losartan, can improve the biomechanical integrity of the aortic wall, thereby potentially reducing the risk of dissection and rupture. As doxycycline is a broad-spectrum antibiotic with considerable side effects, celiprolol may be more suitable for a long-term therapy and thus rather indicated for the medication of patients with vEDS.

Keywords Aortic dissections • Aneurysms • Medical therapy • *COL3A1* • Collagen

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1. Introduction

Vascular Ehlers–Danlos syndrome (vEDS; OMIM #130050) is a rare autosomal-dominant connective tissue disorder caused by heterozygous mutations in the *COL3A1* gene (OMIM *120180) with an estimated prevalence of ∼2 in 100 000 individuals.[1] Typical vEDS is characterized by thin, translucent skin, easy bruising, characteristic facial features, and fragility of connective tissues, affecting hollow organ walls (e.g. uterus, intestine) including medium- and large-sized arteries.[2] Some *COL3A1* mutations, however, may not cause typical clinical signs of vEDS other than vascular events. The increased risk of arterial dissection or rupture may lead to the life-threatening complications of vEDS, even at normal diameter and younger age.

For vEDS, no targeted therapy is available and there is no consensus on clinical management.[3,4] Current management includes antihypertensive drugs as well as medical and surgical treatment of symptoms and complications. In 2010, a clinical trial showed that the cardioselective β-blocker celiprolol with β2-agonist vasodilatory properties[5,6] reduces the risk of arterial dissection and rupture, regardless of the underlying *COL3A1* mutation.[7] However, it remains unclear whether celiprolol exerts its beneficial effect by the β-blocker-typical prevention of excessive and short-term rises in blood pressure and heart rate or by improving the biomechanical integrity of the aortic wall. Another potential therapeutic approach involves the broad-spectrum antibiotic and matrix metalloproteinase (MMP) inhibitor doxycycline. In a mouse vEDS model (*Col3a1*^{tm1Jae}), doxycycline has been proven to normalize aortic tissue MMP activity, thereby attenuating the degradation of collagen and ameliorating the susceptibility to stress-induced aortic lesions.[8,9]

In contrast, the medical treatment of aortic aneurysms in Marfan syndrome (MFS; OMIM #154700), a connective tissue disorder often in differential diagnosis with vEDS, has been extensively studied in mice and humans. Losartan, an angiotensin II receptor type 1 (AGTR1) antagonist and indirect inhibitor of transforming growth factor beta (TGFβ)-signalling, prevented aortic aneurysm formation in a mouse model of MFS[10] and reduced the rate of aortic root dilation in paediatric[11] and adult MFS cases.[12] Controversially, a meta-analysis of six randomized, but pharmacogenetically non-stratified, trials revealed no clinical outcome benefits in MFS patients treated with losartan.[13] Nevertheless, losartan is often used to protect MFS patients from unexpected and/or emergency aortic complications.[14] Even though phenotypic overlap between MFS and vEDS exists, the success of medical therapies can profoundly differ and the therapeutic role of losartan in vEDS remains elusive.

The first described mouse vEDS model (*Col3a1*^{tm1Jae}) harbours a null mutation. Homozygous mice are embryonic lethal, whereas heterozygous mice do not show any obvious phenotype and have no increased mortality.[15] More than a decade after initial description, thorough histological examination of the aorta of heterozygous mice revealed the presence of a spectrum of lesions in the aortic walls similar to those observed in human patients.[16] The second mouse vEDS model (*Col3a1*^{m1Lsmi}) was generated spontaneously during unrelated experiments and was described as truly haploinsufficient for *Col3a1* due to a deletion comprising exon 1. Similar to the first described mouse vEDS model, homozygous mice are embryonic lethal. However, ∼28% of heterozygous mice die due to aortic dissection and/or rupture reflecting the key characteristic of vEDS and thus we used this model for our study.[17] Recently, an additional mouse model (*Col3a1*$^{Tg\text{-}G182S}$) was reported, overexpressing a mutant transcript with a glycine substitution and showing reduced longitudinal tensile strength of the thoraco-abdominal aorta as well as severe skin lesions in males[18] (Supplementary material online, *Table S1*).

No previous studies have attempted to confirm or assess the therapeutic effect of celiprolol or losartan in vEDS. Here, we use a novel read-out system with doxycycline as a proof-of-concept drug to assess whether or not treatment with celiprolol or losartan strengthens the weakened aortic wall in the *Col3a1*^{m1Lsmi} mouse vEDS model and thus could be the therapy of choice in vEDS.

2. Methods

2.1 Animals and treatment

All animal experiments were approved by the local animal ethics committee (Kantonales Veterinaeramt Zurich, approval reference numbers: ZH051/12 and ZH096/15) and performed according to local guidelines (Swiss Animal Protection Ordinance, TschV, and Swiss Animal Protection Law, TschG), conforming to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

The vEDS mouse model *Col3a1*^{m1Lsmi} obtained from MRC Harwell Laboratories (Oxfordshire, UK)[17] was bred in-house, genotyped by PCR with DNA isolated from ear biopsies, and further characterized using whole-genome sequencing, long-range PCR followed by long-read sequencing using MinION (Oxford Nanopore, Oxford Science Park, UK), and subsequent transcript analysis as described in the Supplementary material online. Due to a mixed genetic background (C57BL/6J and 129P/OlaHsd), mice were backcrossed to the C57BL/6J strain (Charles River Laboratories, Wilmington, MA, USA) prior to the rupture force measurements. Hereafter, wild-type mice are referred to as *Col3a1*$^{+/+}$ and heterozygous mice as *Col3a1*$^{m1Lsmi/+}$.

According to previous animal studies, 4-week-old *Col3a1*$^{m1Lsmi/+}$ mice underwent treatment with doxycycline ($n = 16$, ∼100 mg/kg/day),[8] celiprolol ($n = 10$; ∼200 mg/kg/day),[19] or losartan ($n = 10$; ∼180 mg/kg/day).[10] Mice were weighed at the beginning and end of each treatment. Littermates were used for untreated control groups of *Col3a1*$^{+/+}$ and *Col3a1*$^{m1Lsmi/+}$ mice, receiving normal drinking water or the corresponding control diet. All treatments were conducted for 4 weeks and in the case of losartan ($n = 10$; ∼180 mg/kg/day) for 8 weeks as well (i.e. from ages 4 to 12 weeks). Prior to the measurements, genotype and treatment were blinded to the investigator. Treatment details are available in the Supplementary material online.

2.2 Microscopy

Samples from the thoracic aorta and skin were prepared as reported[20] and examined under a FEI CM100 transmission electron microscope (FEI, Eindhoven, The Netherlands). Using multiphoton microscopy, the collagen microstructure of aortic tissue under stretching was investigated with a dedicated *in situ* experimental setup.[21,22] 3D stacks were taken at an unstretched configuration and at three stretching steps (from 0 to 1.9, 2.6, and 3.2 mm). Microstructural parameters were quantified from microscopy images containing only a second harmonic generation signal using custom scripts in Matlab (The MathWorks Inc., Natick, MA, USA). Details are available in the Supplementary material online.

2.3 Rupture-force measurement

Immediately following sacrifice using CO_2, the thoracic aorta was isolated, excised, and carefully cleaned of adherent connective tissue and fat in 3-(*N*-morpholino)-propanesulfonic acid (MOPS) buffer. Subsequently, 1.5-mm-long aortic ring sections (S1–S3) were cut from the ascending (S1) and descending aorta (S2 and S3) by a custom-made device

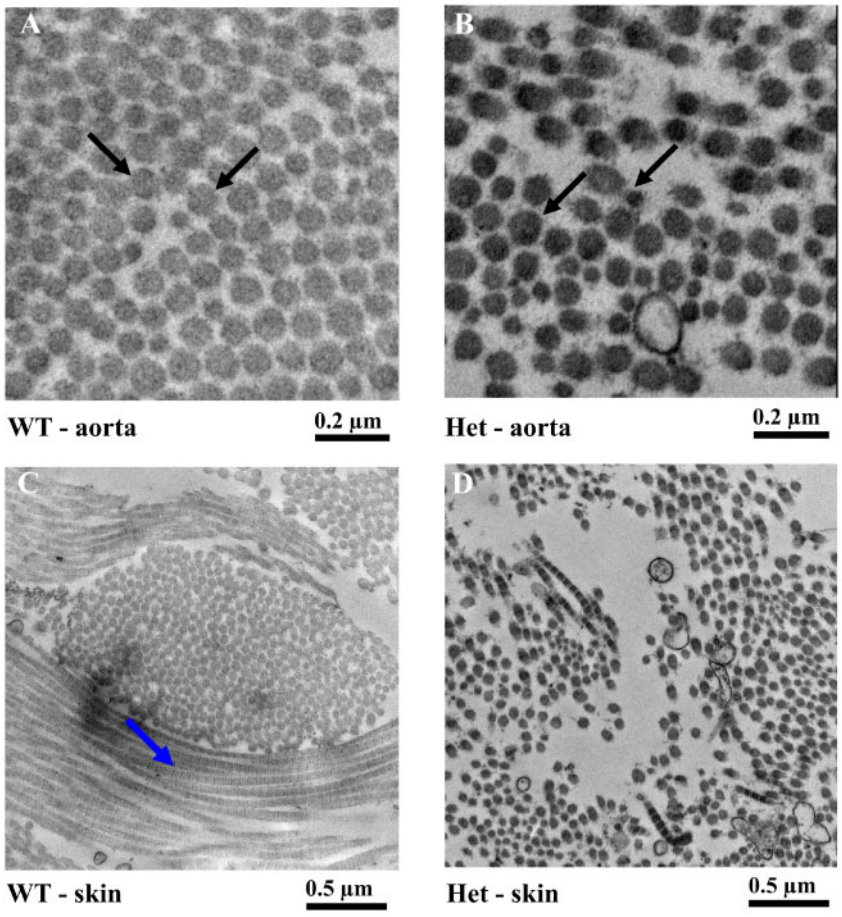

Figure 1 Transmission electron microscopy images. (*A* and *B*) Cross sections of collagen fibrils in the adventitia of the thoracic aorta from (*A*) *Col3a1*$^{+/+}$ (WT) and (*B*) *Col3a1*$^{m1Lsmi/+}$ (Het) mice. Black arrows point to individual fibrils. (*C* and *D*) Skin sections of (C) WT and (*D*) Het mice are shown. Blue arrow indicates longitudinal aligned bundles of collagen fibrils, which are only displayed in the tissue from WT animals. Magnification: 24 500×. For collagen fibril diameter distribution, see Supplementary material online, *Figure S4*.

(Supplementary material online, *Figure S1A*) and mounted on two 200-µm-diameter stainless steel wires placed in MOPS buffer tempered to 37°C to mimic physiological conditions. The mounted aortic rings were stretched at a constant speed of 5 µm/s until tissue rupture using a uniaxial tension device (Tissue Puller 560TP, Danish Myo Technology, Aarhus, Denmark), thereby recording the maximum stretching/tensile force in mN (Supplementary material online, *Figure* S1B, *C*). Details on the custom-made cutting and the uniaxial tension device as well as the calculations of relative forces used for the comparison of data from different experiments are available in the Supplementary material online.

2.4 Statistical analysis

For arithmetic means, lower and upper confidence limits [95% confidence intervals (CIs)] were calculated using critical values of paired *t*-test distribution (vassarstats.net/conf_mean.html). The appropriate test to calculate numeric *P*-values for statistical significance between groups was determined as described in the Supplementary material online, *Figure S2*. Using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA) differences between two groups were assessed by two-sample *t*-test (normal distribution, equal sample variance), Welch's *t*-test (normal distribution, unequal sample variance), or Mann–Whitney *U* test (non-normal distribution), while *P*-values for statistical significance among three groups were calculated by one-way ANOVA (normal distribution) with Tukey's correction or non-parametric Kruskal–Wallis test (non-normal distribution) with Dunn's correction. Statistical tests were performed for planned comparisons only and exact numeric *P*-values were reported for $P \geq 0.0001$ and $P \leq 0.9999$.

3. Results

3.1 Mouse model characterization

Whole-genome sequencing and subsequent transcript analysis revealed that the deletion in *Col3a1*$^{m1Lsmi/+}$ mice (Chr1:g.45 338 538_45 340 967delins2061) does not lead to a *Col3a1* null allele (true haploinsufficiency) as initially reported,[17] but to a shortened transcript with an in-frame deletion of exons 33-39 [NM_009930.2:c.2281_2820del, NP_034060.2:p.(Pro762_Gly941del)] potentially affecting normal collagen processing and/or assembly (Supplementary material online, *Figure S3*). Indeed, imaging showed the effect of this in-frame deletion on protein level. Transmission electron microscopy (TEM) revealed a

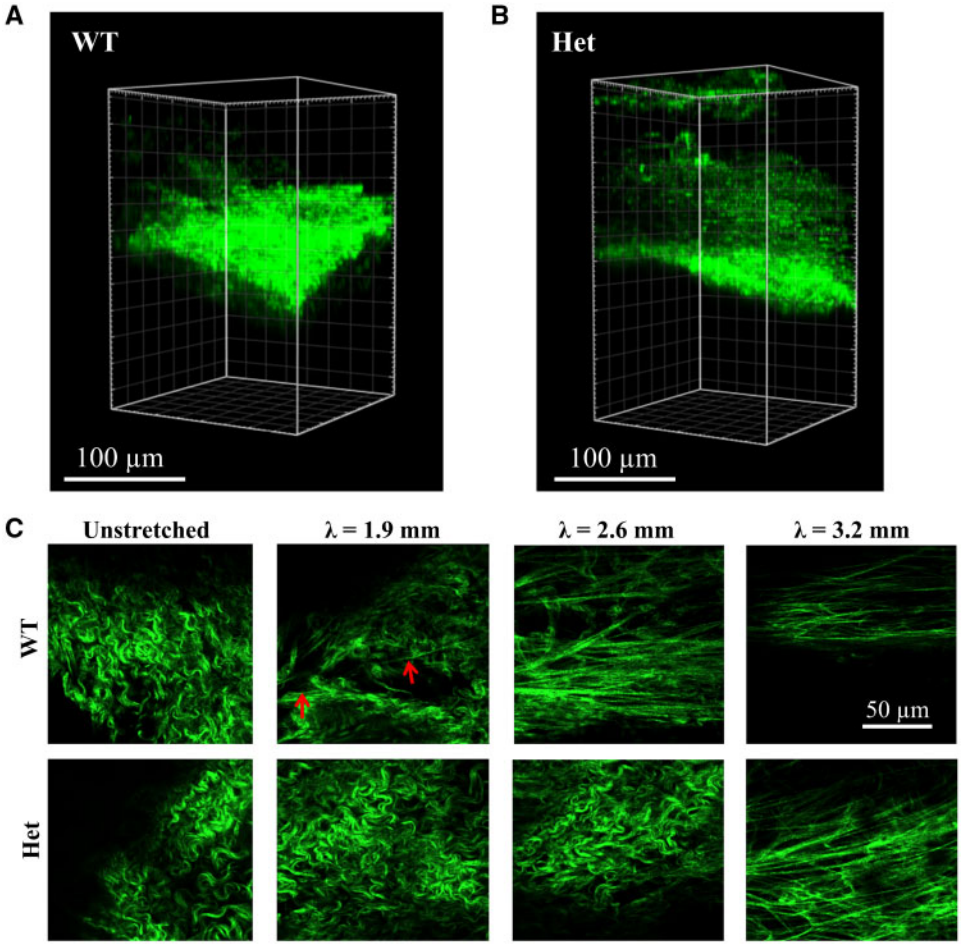

Figure 2 Collagen distribution in the aortic wall imaged by multiphoton microscopy. (*A–C*) Second harmonic generation signal emitted by collagen in the aortic wall of 11-week-old mice is shown in green. (*A* and *B*) 3D images of a cross-section of the aortic wall at unstretched configuration of (*A*) *Col3a1*$^{+/+}$ (WT) and (*B*) *Col3a1*$^{m1Lsmil/+}$ (Het) mice. (*C*) 2D images made at relaxed configuration and displacements (λ) of 1.9, 2.6, and 3.2 mm. Red arrows indicate stretched collagen structures in the tissue from a WT mouse.

reduced number as well as more variable and oversized diameters of collagen fibrils in the adventitia of the thoracic aorta and in the skin of *Col3a1*$^{m1Lsmil/+}$ mice compared to *Col3a1*$^{+/+}$ animals (*Figure 1*; Supplementary material online, *Figure S4*). Accordingly, multiphoton microscopy (MPM) images of aortic wall samples revealed considerably less collagen amount [981 570 (95% CI 889 968–1 073 173) µm^3 vs. 1 443 702 (95% CI 966 868–1 920 535) µm^3] and density [0.070 (95% CI 0.052–0.087) vs. 0.132 (95% CI 0.061–0.203)] in a relaxed configuration as well as delayed and reduced reorienting and aligning upon stretching in *Col3a1*$^{m1Lsmil/+}$ mice in comparison to *Col3a1*$^{+/+}$ mice (*Figure 2*; Supplementary material online, *Table S2*).

In order to assess whether or not there is a measurable difference in the biomechanical integrity of the thoracic aorta between *Col3a1*$^{m1Lsmil/+}$ and *Col3a1*$^{+/+}$ mice, the maximum tensile force of 1.5-mm-long aortic rings was measured for three aortic sections (S1–S3) (*Figure 3*A). In *Col3a1*$^{+/+}$ mice, the maximum tensile force decreases with increasing distance from the heart regardless of age (*Figure 3B*; Supplementary material online, *Table S3*). This tendency was also present in aortic sections of *Col3a1*$^{m1Lsmil/+}$ mice, although less pronounced (*Figure 3C*; Supplementary material online, *Table S3*). More importantly, maximum tensile forces of aortic rings from *Col3a1*$^{m1Lsmil/+}$ mice were significantly lower compared to *Col3a1*$^{+/+}$ mice in all three aortic sections at ages from 6 weeks [e.g. S1 in 6- to 7-week-old males: 347.0 (95% CI 279.3–414.7) mN vs. 638.2 (95% CI 573.2–703.2) mN] (*Figure 3C*; Supplementary material online, *Table S3*). No considerable differences in rupture forces were measured between male and female mice (Supplementary material online, *Figure S5* and *Table S3*).

3.2 Doxycycline, celiprolol, and losartan treatments

Premature deaths of *Col3a1*$^{m1Lsmil/+}$ mice due to aortic ruptures were observed during the early phase of treatments (doxycycline: 7/16; losartan 4 weeks and 8 weeks: each 1/10; celiprolol: 0/10) and are in relation to the respective untreated heterozygous control groups (doxycycline: 4/16; losartan and celiprolol 4 weeks: 0/10; losartan 8 weeks: 2/10) (Supplementary material online, *Tables S4* and *S5*). Measurements in the remaining mice revealed that treatment with doxycycline increased the maximum tensile force of the thoracic aortic wall in *Col3a1*$^{m1Lsmil/+}$ mice [S1 470.3 (95% CI 443.0–497.5) mN, S2 404.8 (95% CI 381.7–427.8) mN, S3 281.9 (95% CI 248.6–315.2) mN] to levels comparable to *Col3a1*$^{+/+}$ mice [S1 523.1 (95% CI 498.4–547.9) mN, S2 405.9 (95% CI

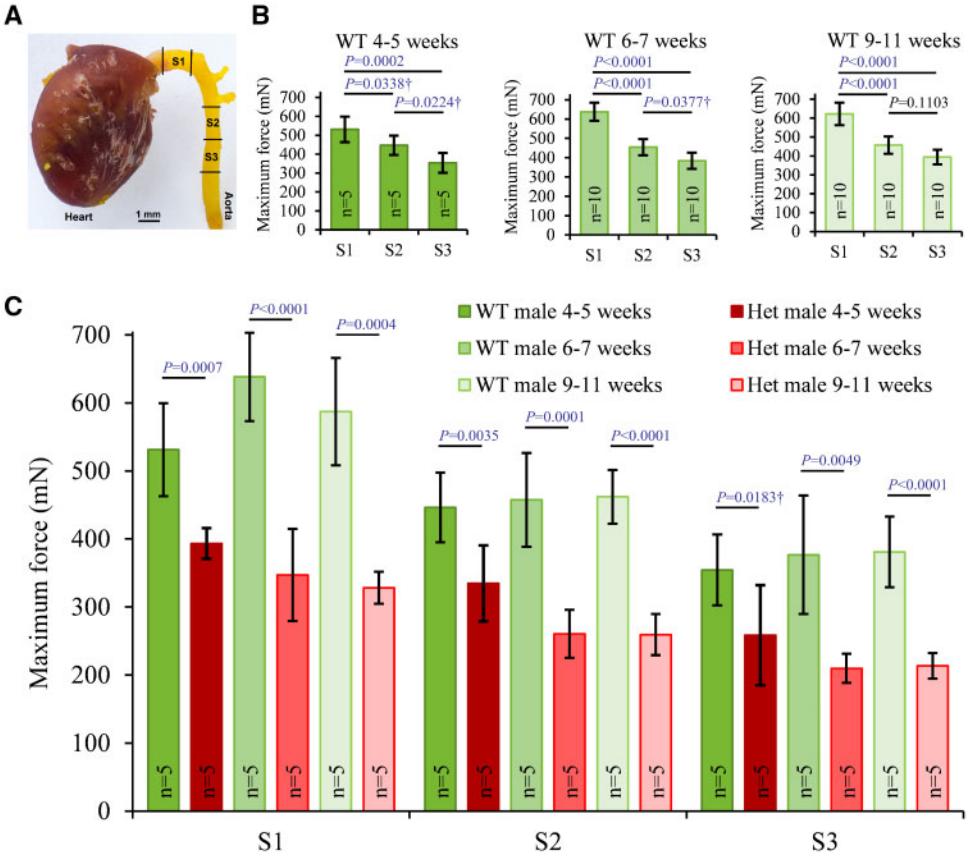

Figure 3 Tensile force at rupture of the murine thoracic aorta. (*A*) Location of the three 1.5-mm-long ring sections in the ascending (section S1) and descending (sections S2 and S3) aorta. Aorta and heart from a *Col3a1*$^{+/+}$ mouse were stained with yellow latex as described in the Supplementary material online. (*B*) Maximum tensile forces (mN) of the three aortic sections from *Col3a1*$^{+/+}$ mice and (*C*) comparison between *Col3a1*$^{+/+}$ (WT) and *Col3a1*$^{m1Lsmi/+}$ (Het) male mice at ages of 4–5, 6–7, and 9–11 weeks. For comparison to data of females see Supplementary material online, *Figure S5*. Sample size (*n*) is displayed and individual values are listed in Supplementary material online, *Table S3*. Data are means with error bars indicating 95% CIs. Significant differences ($P < 0.05$) are shown in blue. One-way ANOVA analysis with Tukey's correction (*B*) and unpaired two-sample *t*-test or Welch's *t*-test was performed where appropriate (*C*). †denotes statistically significant difference between groups (means) of measurements with overlapping CIs.

371.2–440.7) mN, S3 333.7 (95% CI 305.8–361.6) mN] (*Figure 4A*; Supplementary material online, *Table S6*). This is in line with previous findings in a different mouse model of vEDS [*Col3a1*^{tm1Jae}mice][8,9] and thus acts as a proof-of-concept for our novel approach and read-out system. Similarly, 4-week treatment with the β-blocker celiprolol [S2 395.2 (95% CI 363.5–426.9) mN, S3 303.3 (95% CI 281.6–325.1) mN] led to a significantly higher rupture force in the descending aorta (S2 and S3) when compared to untreated *Col3a1*$^{m1Lsmi/+}$ mice [S2 296.7 (95% CI 261.9–331.5) mN, S3 245.5 (95% CI 220.7–270.3) mN]. A similar trend, although not significant, was observed for the segment derived from the ascending aorta [S1 483.9 (95% CI 448.8–519.0) mN vs. 400.3 (95% CI 347.3–453.3) mN] (*Figure 4B*; Supplementary material online, *Table S6*). In contrast, no significant increase was detected in the rupture force upon 4-week losartan treatment [S1 358.5 (95% CI 332.7–384.3) mN, S2 290.9 (95% CI 246.4–335.4) mN, S3 239.4 (95% CI 200.0–278.8) mN] (*Figure 4C*; Supplementary material online, *Table S6*). Comparison among the normalized data of treated mice showed that celiprolol treatment was not significantly different from doxycycline treatment, while both treatments resulted in significantly higher rupture forces than the treatment with losartan (*Figure 4D*). To assess whether losartan requires a longer time span to elicit its actual potential, *Col3a1*$^{m1Lsmi/+}$ mice underwent an 8-week losartan treatment as well. However, even after 8 weeks, losartan did not significantly increase the rupture force of the thoracic aorta (Supplementary material online, *Figure S6* and *Table S7*).

4. Discussion

Using a novel read-out system, we confirmed the beneficial effect of doxycycline in mice modelling vEDS and showed that celiprolol considerably increases the biomechanical integrity of the thoracic aorta, whereas we did not observe any improvement upon losartan treatment (*Figures 4* and *5*). To date, studies testing novel medical therapeutic approaches for aortic diseases have mostly been based on morbidity (e.g. aneurysm progression, dissection), mortality, or histological analyses to assess treatment efficacy. However, while these methods provide qualitative insights, they cannot objectively reveal how the treatment influences the strength (i.e. biomechanical integrity) of the aortic wall which ultimately determines whether or not a drug is effective to prevent aortic dissection and rupture. Thus, we developed a novel approach

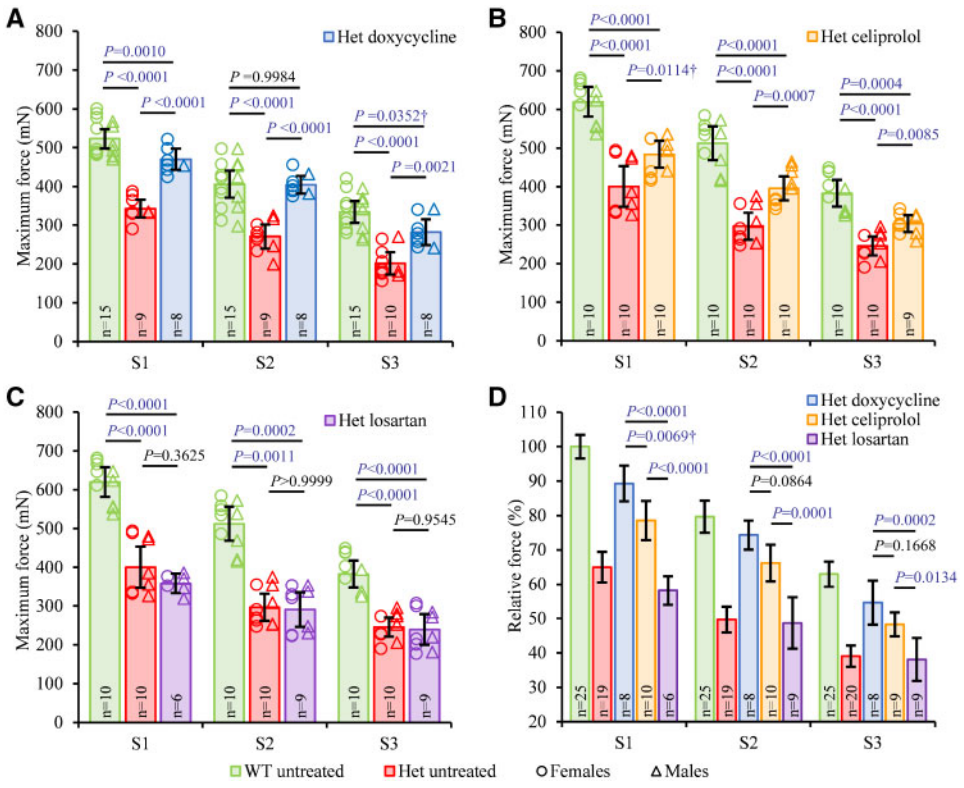

Figure 4 Treatment with doxycycline or celiprolol but not losartan increases the biomechanical integrity in *Col3a1*$^{m1Lsmi/+}$ mice. (*A–C*) *Col3a1*$^{m1Lsmi/+}$ (Het) mice underwent a 4-week treatment with (*A*) doxycycline, (*B*) celiprolol, or (*C*) losartan. Maximum tensile force (mN) was measured for three thoracic aortic sections (S1–S3) (*Figure 3A*) and compared to untreated *Col3a1*$^{+/+}$ (WT) and Het controls. (*D*) Normalized comparison of the effects of the different treatments on maximum tensile force (significant differences to untreated controls are not marked). Note that the same control groups were used for the celiprolol and losartan treatments. Sample size (*n*) is displayed. Aortas of mice that died prematurely or that were damaged during preparation were excluded (Supplementary material online, *Table S4*). Data are means with error bars indicating 95% CIs. Significant differences ($P < 0.05$) are shown in blue. One-way ANOVA analysis with Tukey's correction or Kruskal–Wallis test with Dunn's correction was performed where appropriate. †denotes statistically significant difference between groups (means) of measurements with overlapping CIs.

to measure the tensile force at rupture of uniaxially stretched murine aortic rings as an objective read-out of the biomechanical integrity of the aortic wall.

A first indication of the validity of our novel read-out system is the, despite potential variability in the aortic segment length of 1.5 mm, significantly lower maximum tensile force measured in *Col3a1*$^{m1Lsmi/+}$ compared to *Col3a1*$^{+/+}$ mice, reflecting the increased mortality due to aortic rupture.[17] Second, these results were reproduced in three different measured sections (S1–S3) (*Figure 3A*). Third, we provided proof of concept for the suitability of the used maximum tensile force measurements and *Col3a1*$^{m1Lsmi/+}$ mouse model by confirming the positive effect of MMP inhibition by doxycycline reported for the true-haploinsufficient vEDS mouse model *Col3a1*^{tm1Jae}.[8,9] Consequently, our read-out system is suitable to test candidate substances for their potential to reduce the risk for aortic ruptures.

Significant differences between *Col3a1*$^{+/+}$ and *Col3a1*$^{m1Lsmi/+}$ mice from an early age are likely explained by differences in intramural collagen content. At a molecular level, we showed that the spontaneously derived mutation in *Col3a1*$^{m1Lsmi/+}$ mice results in a shortened transcript (in-frame deletion) but not in true haploinsufficiency as initially suggested.[17] This finding may explain the more severe aortic phenotype compared to the true haploinsufficiency model *Col3a1*^{tm1Jae} (Supplementary material online, *Table S1*). In *Col3a1*$^{m1Lsmi/+}$ mice, TEM and MPM revealed a reduction in total collagen content, higher variability in collagen fibril diameter, and delayed alignment of collagen structures upon tensile stress. These data suggest reduced type III collagen secretion as shown in vEDS patients with in-frame transcript deletions.[23,24] As most vEDS-causing mutations, including the most frequently identified glycine substitutions, lead to the impaired secretion of type III collagen and a reduced total collagen content,[18] our results obtained with the *Col3a1*$^{m1Lsmi/+}$ mice are expected to be transferable to most cases of vEDS. Moreover, the mutation-independent effect of celiprolol is supported by the clinical study in 2010, in which the risk of arterial dissection and rupture was reduced regardless of the underlying *COL3A1* mutation.[7] Since neither the applied TEM nor MPM techniques allow discrimination between different types of collagens, however, it remains to be determined whether the observed differences in rupture force and collagen characteristics solely reflect a reduced amount of normal type III collagen or whether other types of collagens are affected as well. Indeed, copolymers between type I and type III collagens can form[25,26] and disturbed fibrillogenesis of heterotypic type III:I collagen fibril assembly has recently been reported in mice overexpressing *Col3a1* harbouring a glycine substitution (Supplementary material online, *Table S1*).[18]

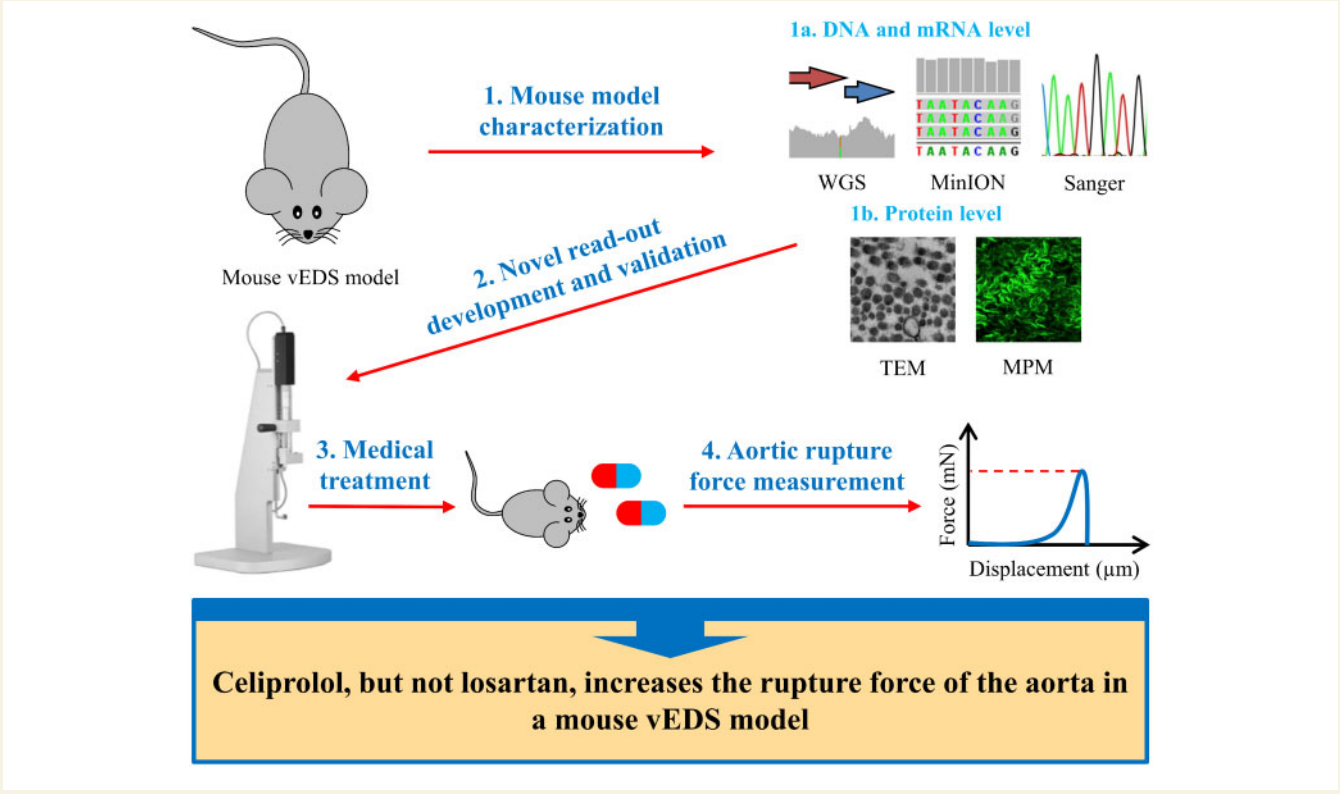

Figure 5 Schematic representation of the workflow in this study. (1) The mouse vEDS model *Col3a1*^{m1Lsmi} was characterized at DNA, mRNA, and protein levels. (2) A novel read-out using the Tissue Puller 560TP (DMT) to measure the aortic rupture force was developed and subsequently validated using doxycycline as a proof-of-concept drug. (3) Mice modelling vEDS were treated with celiprolol or losartan. (4) The aortic rupture force was measured to compare the treatment efficacy, showing that celiprolol but not losartan increases the rupture force of the aorta in this experimental vEDS model. MPM, multiphoton microscopy; TEM, transmission electron microscopy; vEDS, vascular Ehlers–Danlos syndrome; WGS, whole-genome sequencing.

Our results demonstrate that doxycycline significantly improves the biomechanical integrity of the thoracic aorta in *Col3a1*$^{m1Lsmi/+}$ mice (*Figure 4A*). This finding confirms and extends previous histological evidence that doxycycline ameliorates aortic lesions in the true-haploinsufficient vEDS mouse model *Col3a1*^{tm1Jae} by MMP inhibition.[8,9] However, considering that doxycycline is a broad-spectrum antibiotic and has considerable side effects including severe photosensitivity[27] it becomes an unattractive choice for life-long therapy. In contrast, antihypertensive drugs such as the β-blocker celiprolol or the AGTR1-antagonist losartan have milder side effects and thus are more suitable for a long-term therapy. For example, celiprolol, which was designated orphan drug status by the FDA in 2015 for the treatment of vEDS,[28] may lead to fatigue and an upset stomach,[29] whereas the most common side effects of losartan are dizziness, acute nasopharyngeal infections, and musculoskeletal pain.[30]

This is the first study demonstrating that celiprolol improves the biomechanical integrity of the thoracic aorta in an experimental model of vEDS (*Figure 4B*). In humans, celiprolol was previously shown to reduce heart rate as well as mean and pulsatile pressures in essential hypertension by blocking β1-adrenoceptors, thereby moderating the positive chronotropic effects of sympathetic arousal and highly likely decreasing the continuous and pulsatile mechanical stress on collagen fibres within the arterial wall.[31,32] The clinical trial with celiprolol, however, revealed no reduction in brachial systolic and diastolic blood pressure but an increased stiffness of the common carotid artery, regardless of the underlying *COL3A1* mutation.[7] In consideration of the findings of our study, the increased arterial stiffness after long-term treatment of vEDS patients with celiprolol[7] might reflect an improved biomechanical integrity of the aortic wall. Indeed, besides the β-blocker typical action, celiprolol stimulates NO production and thus may reduce vascular oxidative stress,[19,33,34] which could beneficially impact the extracellular matrix, ensuring a more intact aortic wall. However, the understanding of the underlying effect of celiprolol causing a strengthening of the aortic wall remains to be elucidated.

Considering that losartan has previously been shown to reduce aortic aneurysm progression in mice modelling MFS,[10] another clinically highly relevant finding of our study is that losartan does not improve the biomechanical integrity of the thoracic aorta neither after 4 nor after 8 weeks of treatment in a mouse vEDS model (*Figure 4C*; Supplementary material online, *Figure S6*). Although it is believed that the blockade of AGTR1 and thus indirect inhibition of TGFβ-signalling confers the positive effect of preventing aortic root dilation in MFS, recent studies are challenging the pathogenic driver role of TGFβ and its signalling[35,36] as well as the mode of action of losartan.[37] Nevertheless, evidence that losartan does not improve the biomechanical integrity of the aorta is essential, even derived from an experimental model, as losartan is not only widely used for disease management of MFS but is also considered for related aortic diseases including vEDS.

We administered losartan and celiprolol in dosages comparable to previous animal studies.[10,19] For the clinical translation, the human equivalent dose can be estimated by applying a species conversion factor of 0.081 based on body surface and weight.[38] The resulting human

equivalent dosages for a 75-kg patient (~1200 mg celiprolol or ~1100 mg losartan per day) are substantially higher than the routinely administered safety approved doses (up to 400 mg celiprolol or 150 mg losartan per day). The approved lower dosage of celiprolol (100–400 mg per day), which has been shown to be efficient in vEDS after long-term treatment,[7] is likely sufficient and more adequate for vEDS patients.

Moreover, human dosages may need to be further adjusted according to individual pharmacogenetic predisposition. For instance, losartan requires CYP2C9-mediated activation into its more potent metabolite E-3174,[39] of which ~20–30% of Caucasians are possible poor metabolizers[40] and thus may require higher dosages. The mouse genome contains 2 homologues and 10 orthologues of the *CYP2C9* gene,[41] likely leading to increased losartan activation and thus hampering the mouse-to-human dosage translation. In contrast, celiprolol is only metabolized to a minor extent,[31,42] representing an additional advantage over losartan. To enable optimal treatment through precision medicine, further appropriately stratified studies and thoughtful dosage regimens are required.

There are some limitations to our study: (i) the maximum tensile forces determined *ex vivo* by our read-out system might not entirely reflect rupture forces in physiological *in vivo* conditions with long-term pulsatile pressure in arteries, which could lead to rupture at lower loads. Moreover, in our set up, using aortic rings, we apply only circumferential stress but not axial stress, the influence of which is difficult to predict but expected to be low or neglectable since according to the Laplace law, the circumferential component of the stress generated by blood pressure is higher than the axial component. (ii) The role of blood pressure on the mortality as well as the influence on treatment efficacy remains open and needs to be assessed in future studies.

Taken together, we demonstrated in a mouse vEDS model that celiprolol rather than losartan beneficially impacts the biomechanical integrity of the thoracic aorta, providing evidence for celiprolol as medical therapy of choice in vEDS. This result supports previous data suggesting that the delay or prevention of arterial events is not solely achieved by celiprolol's blood pressure modulating capacity but also by a direct impact on the arterial wall.[7] Moreover, a blood pressure-independent mode of action is supported by the finding that losartan, another tested antihypertensive drug, did not improve the biomechanical integrity of the aorta in the same vEDS model. However, losartan, effective in MFS patients, is increasingly considered or even prescribed for the non-evidence-based treatment of patients with MFS-related aortic conditions. Thus, there is a need to rethink the current paradigm that a medical therapy may be of benefit in phenotypically similar diseases but rather to consider the disease-underlying molecular mechanisms.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

Acknowledgements

The authors thank Dr Arabella Mauri and Prof. Dr Edoardo Mazza for help in biomechanics, multiphoton microscopy, and custom-made device development (manufacturing work by Jean-Claude Tomasina) as well as for comments on the manuscript. They also thank Carmen Kaiser and Gery Barmettler for help with non-linear laser scanning and electron microscopy; Prof. Dr Max Gassmann for helpful discussions; Prof. Dr Edouard Battegay, Dr Indranil Bhattacharya, Julien Courseau, Judith de Vos, Dr Cecilia Giunta, Dr Stephan Gysi, Dr Elvira Haas, and Dr Raimund Jaeger for support.

Conflict of interest: none declared.

Funding

This work was supported by funding from the Gebauer Stiftung; the Isaac Dreyfus-Bernheim Stiftung; and the Wolfermann-Nägeli-Stiftung.

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Translational perspective

Aortic dissection and rupture belong to the major complications of vascular Ehlers–Danlos syndrome (vEDS). Medical management includes antihypertensive drugs but there are no comparative data for drug selection. This study shows that the β-blocker celiprolol significantly improves the biomechanical integrity of the thoracic aorta in an experimental model of vEDS, explaining the treatment effect in a clinical trial. In contrast, the AGTR1-antagonist losartan, widely used to prevent or stabilize aortic dilation in the phenotypically overlapping Marfan syndrome, did not have a beneficial effect. These findings provide further evidence that celiprolol rather than losartan may be the medical therapy of choice in vEDS.