Aortic microcalcification is associated with aortopathy in Marfan Syndrome; potential marker for imaging.

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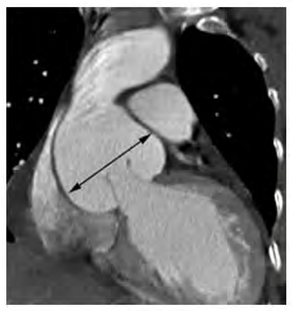
Introduction

Marfan syndrome (MFS) is a genetic connective tissue disorder (FBN1 gene), in which aortic rupture is the major cause of death. MFS patients with an aortic diameter below the advised limit for prophylactic surgery (<5cm) may unexpectedly experience an aortic dissection or rupture, despite yearly monitoring. Hence, there is a clear need for improved prognostic markers to predict such aortic events. We hypothesize that elastin fragments play a causal role in aortic calcification in MFS and that microcalcification serves as a novel marker for aortic disease severity. To address this hypothesis, we analyzed MFS patient and mouse aortas.



Aim

To establish if elastin fragments play a role in aortic microcalcification in Marfan syndrome

Elastin peptides involved in aortic calcification

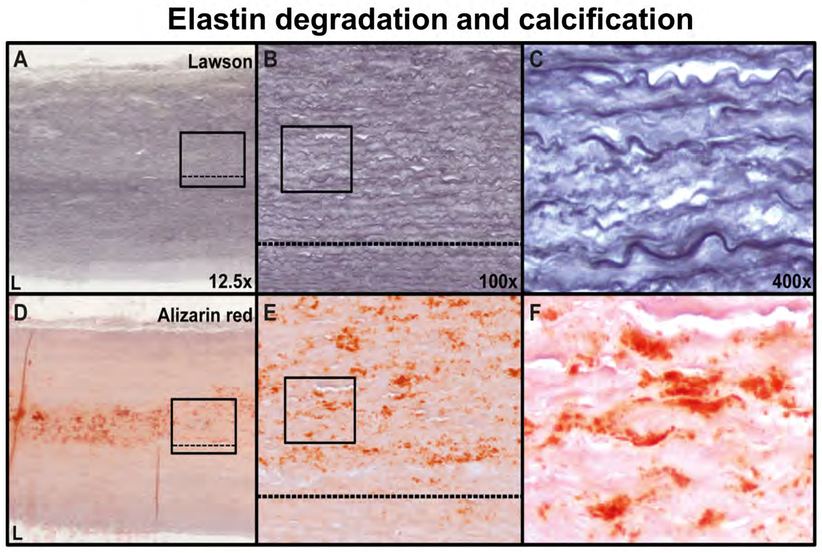


Figure 1. In human Marfan aortic sections, the area of progressive elastin degradation (Lawson staining, upper panels) co-localizes with the area of vascular calcification (alizarin red staining, lower panels).

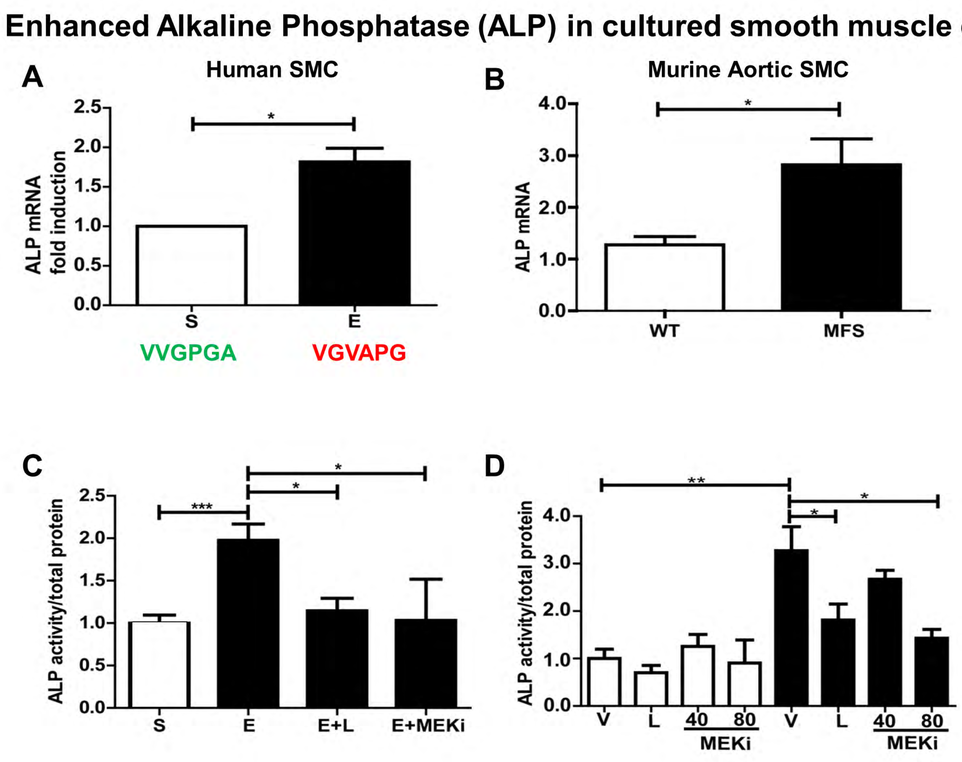


Figure 2. Vascular calcification is controlled by balancing extracellular levels of the mineral ions calcium and inorganic phosphate (Pi) versus mineralization inhibitors and inorganic pyrophosphate (PPi). Alkaline phosphatase (ALP) decreases extracellular PPi levels and thereby enhances vascular calcification.

Elastin peptides (E= **VGVAPG**) enhance ALP mRNA (**A**) and activity (**C**) in cultured human umbilical cord artery SMC as compared to incubation with control scrambled peptides (S= **VVGPGA**). Basal ALP is already enhanced in murine Marfan (MFS) aortic smooth muscle cell (*Fbn1*$^{C1039G/+}$ mice) cultures (**B,D**) as compared to wild type (WT littermates) aortic SMC cultures. The increase in ALP is probably mediated via the elastin receptor complex, which can be blocked by incubation with lactose or blockade of the ERK1/2 signalling cascade. Indeed, simultaneous treatment with lactose (L), or the MEK1/2 inhibitor (MEKi), attenuates the effect of elastin peptides on ALP activity (**C**) and in the MFS aortic SMC (**D**).

*= P<0.05, **= P≤0.01, ***= P<0.001.

Macrocalcification in aortic root of Marfan mice

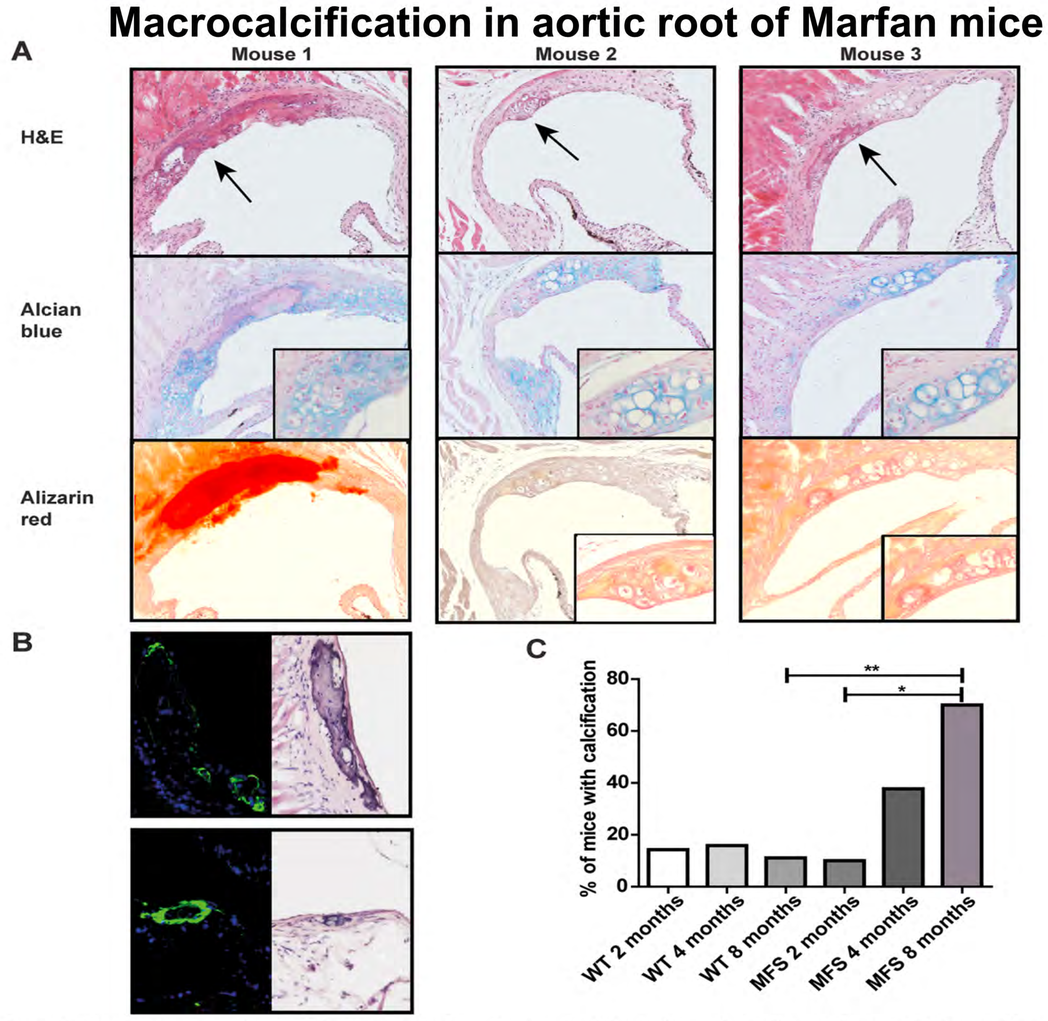


Figure 3. Aortic root sections of three MFS mice show osteoblast-like cells in the aortic medial layer (**A**; arrows, upper panels) of the MFS mice. Glucosaminoglycans (alcian blue; middle panels) and calcium deposits (alizarin red; lower panels) surround these bone-like cells. Inlays show osteoblast-like cells in more detail. Upon Injection with the near infrared fluorescent OsteoSense-800 calcification probe (PerkinElmer), incorporation of the probe over 2 days could be observed in the calcified areas in aortic root (**B**). In MFS mice, the percentage of mice with macrocalcifications increases with age, while this is not observed in WT mice (**C**).

Microcalcification in the ascending aorta

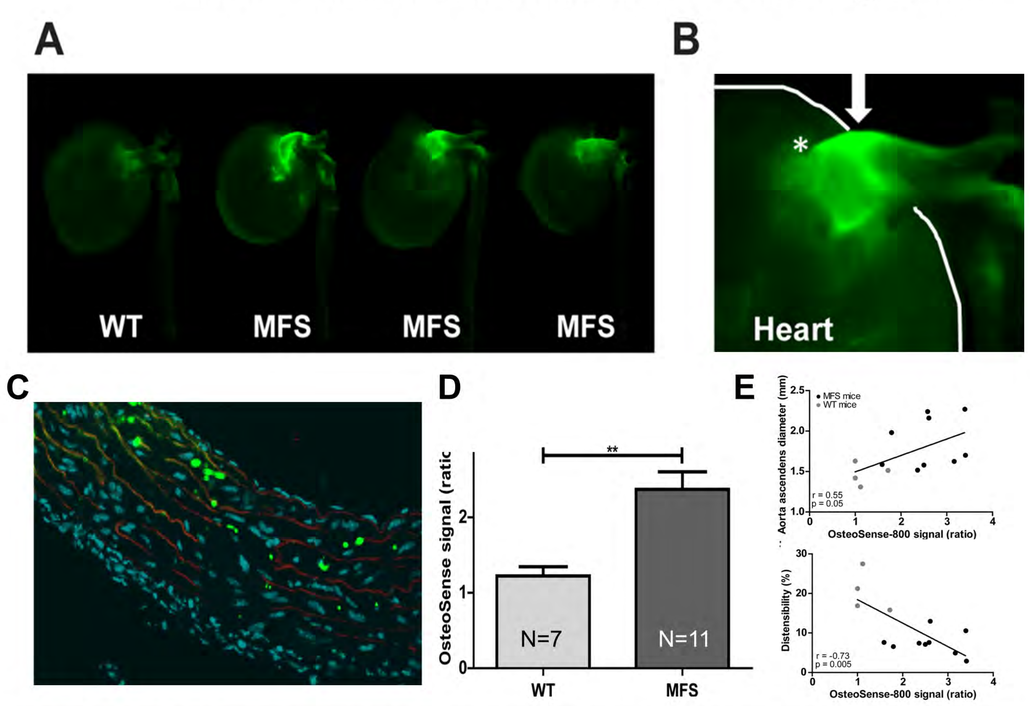


Figure 4. The biphosphonate conjugated fluorescent probe OsteoSense-800 was injected i.p. in 8-month old WT and MFS *Fbn1*$^{C1039G/+}$ mice. Especially the ascending aorta was highly fluorescent in MFS mice after 2 days incorporation of the OsteoSense-800 probe as visualized (green) ex vivo using an Odyssey scanner (**A-D**, arrow). The amount of OsteoSense-800 accumulation correlates with ascending aorta diameter and reduced aortic distensibility (**E**). Gray dots= WT mice, black dots= MFS), L= lumen, A= adventitia

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Conclusion

We have shown that elastin fragments promote SMC microcalcification, which is enhanced in Marfan SMC, and in particular in the dilated ascending aorta in Marfan mice and patients, where most of the elastin fragmentation was observed. We propose that elastin fragment-mediated microcalcification may contribute to aortic disease in Marfan and that it may serve as a novel imaging marker to detect aortic damage.