Adamts1 in vascular homeostasis and remodelling

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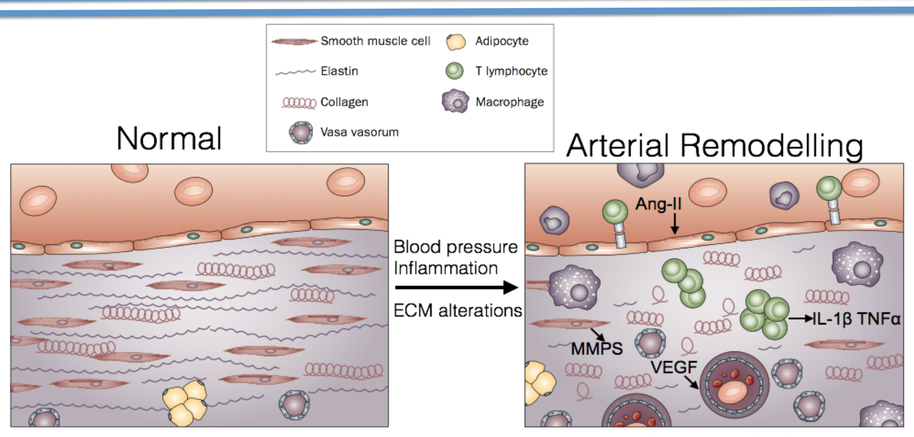
Introduction:

Aneurysms involving the aortic root and the ascending aorta leading to dissections are the major diseases affecting the aorta and a common cause of premature deaths ranking as high as the XXth cause of death in developed countries.

The major constituent of the vessel wall is the **extracellular matrix (ECM)**. It forms part of the basic structure of blood vessels and provides structural and mechanical support through elasticity, stiffness, and intercellular communication. Changes in ECM proteins expression, assembly, cross-linking, and degradation can trigger physiological pathological conditions in the vascular wall, including atherosclerosis, aneurysms, stenosis and hypertension (Hellenthal et al. 2009). Mutations in genes which encode ECM proteins which affects mechanical properties of tissues are present in some **inherited connective tissue disorders** such as Marfan syndrome (MS), Loeys-Dietz syndrome (LDS), vascular type of Ehlers-Danlos syndrome (EDS-IV), and familial forms of non-syndromic thoracic aneurysm and dissection (FTAAD) (Hoffjan 2012, Van Laer et al. 2014). **TGFβ signaling pathway** is over-activated in both syndromic and non-syndromic aortic diseases, TGFβ signaling pathway suggesting that it plays a pivotal role in these diseases.

The ADAMTS family of of extracellular metalloproteinases degrade proteoglycans and therefore have the potential to modify tissue architecture and function (Stanton 2011). Recently, different works have involved the families, ADAMTS and ADAMTSL (Adamts-like) in fibril microfiber formation thus suggesting a role of these genes in the regulation of TGFβ signalling (Hoffjan et al 2012). Different mutations in ADAMTS/ADAMTSL superfamily members has been described as causative of connective tissue disorders without aortic phenotype (Le Goff et al. 2011). **Adamts1** is widely expressed in aortic endothelial and VSMCs during development and in adulthood (Thai et al. 2002; Luque et. al. 2003) and under pathological vascular remodeling in (Jönsson-Rylanderand et al. 2005) and thoracic aneurysm (Pen et. al. 2013). However the role of this metalloproteinase in the vascular wall is poorly understood. Here, we show **the potential role of Adamts1 in vascular wall homeostasis** using two different approaches, a genetic model of **Adamts1 deficient mice** and a knocking-down model in aorta **using short-interference RNA (siRNA) expressing lentiviruses**. Both models, Adamts1 deficient mice and knocking-down present some vascular features that resembles aortic disorders, such as aortic ectasia, fibrosis, proteoglycan accumulation, elastin breaks, TGFb hyperactivation. These phenotype was exacerbated by AngII infusion. **These data supports that Adamts1 is essential for vascular integrity in homeostasis and remodeling**

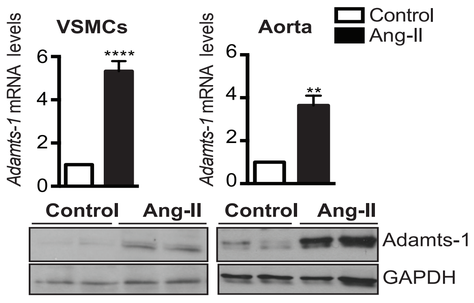


Modified from Hellenthal, F. A. *et al. Nat. Rev. Cardiol.* (2009)

Vascular Remodelling is mediated by changes in extracellular matrix
Vascular remodeling consists of alteration of structure and arrangement of blood vessels through changes in the different layers, which include cell migration and proliferation, cell death, and modifications in extracellular matrix (ECM) such as elastin degradation, collagen and proteoglycan deposition. The changes in ECM affects the mechanical properties of the vessel wall. This process is present in major vascular diseases such as hypertension, aneurysm, vascular stenosis, and atherosclerosis. Some vascular inherited disorders occurs with mutations in ECM codified genes produces

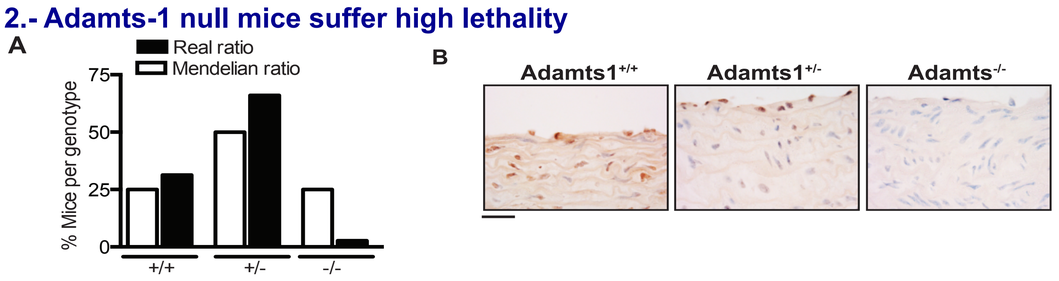
Results:

1.-Adamts-1 is basal expressed in vascular cells and vascular wall and up-regulated by pro-remodelling stimuli Ang-II



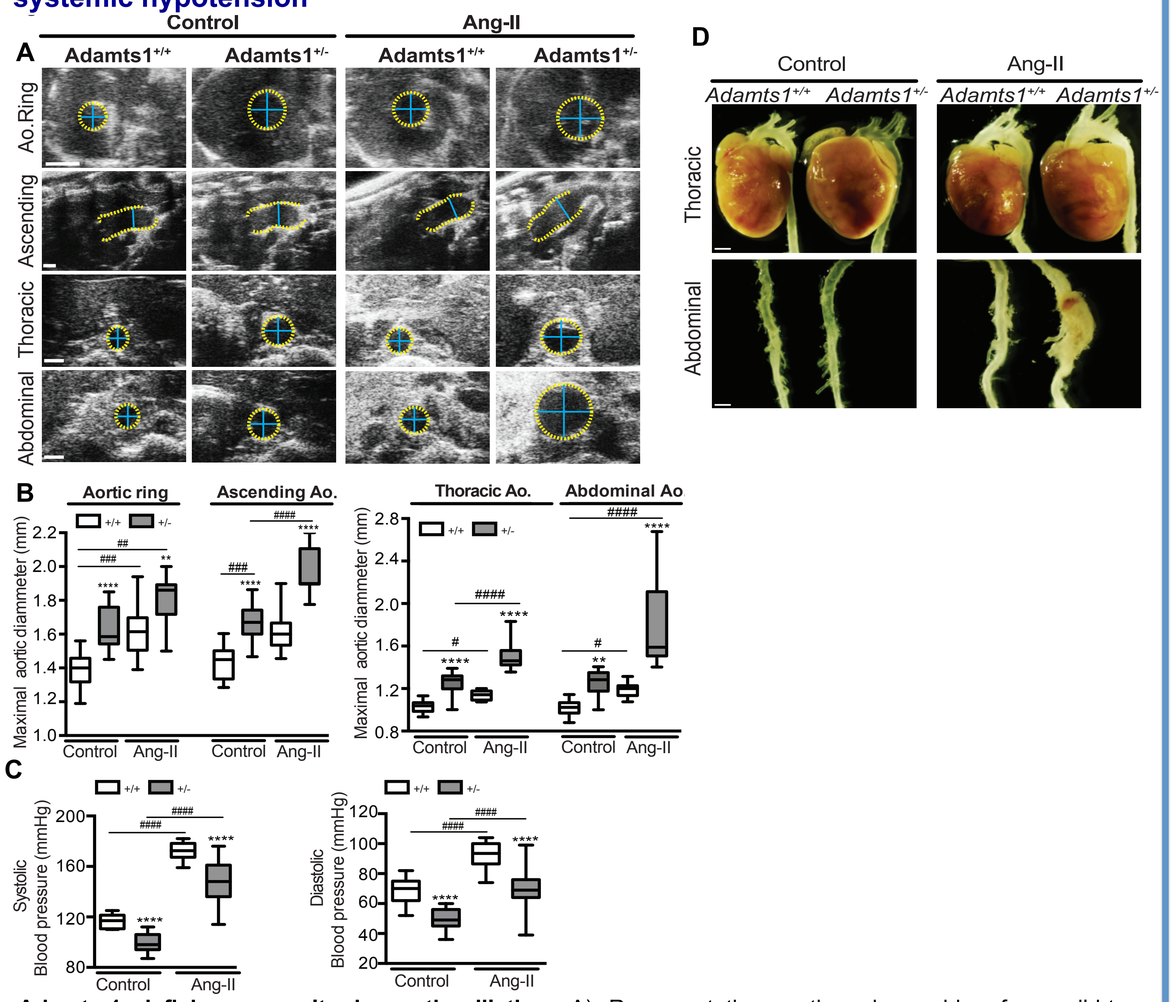
Vascular expression of Adamts-1 increases upon Angiotensin II (Ang II) stimulation. *Adamts-1* expression was analyzed in protein (western-blot) and RNA (qPCR) extracts from primary VSMCs and murine aortic tissue treated with Ang II for 6 hours or 3 days, respectively. In western blot assays, the expression of GAPDH was analyzed as loading control. Images are representative of three independent experiments. In qPCR experiments, levels of *Adamts-1* mRNA were normalized to *GAPDH* expression. Data are expressed as fold-increase relative to non-stimulated cells (Control) and shown as means ± SD of three independent experiments, $^{**}P < 0.01$ and $^{****}P < 0.0001$ versus control.

2.- Adamts-1 null mice suffer high lethality



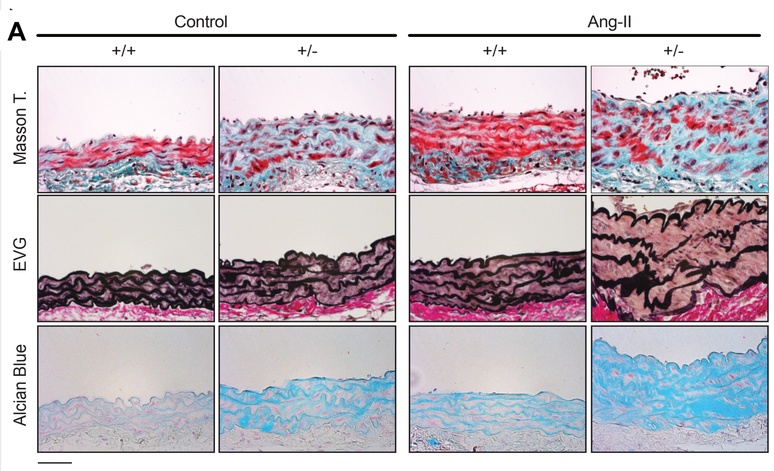
Adamts-1 deficiency results in high lethality A) Genotype of mice recovered at weaning in Adamts-1 heterozygous crosses. N= 151. +/+, wild-type; +/-, heterozygous; -/-, Adamts-1 null. B) Representative inmunostaining of Adamts-1 in aortas from Adamts1+/+, Adamts-1+/- and Adamts1+/- mice.

3.-Adamts-1 deficiency results aortic dilation, increase in aneurysm incidence and systemic hypotension



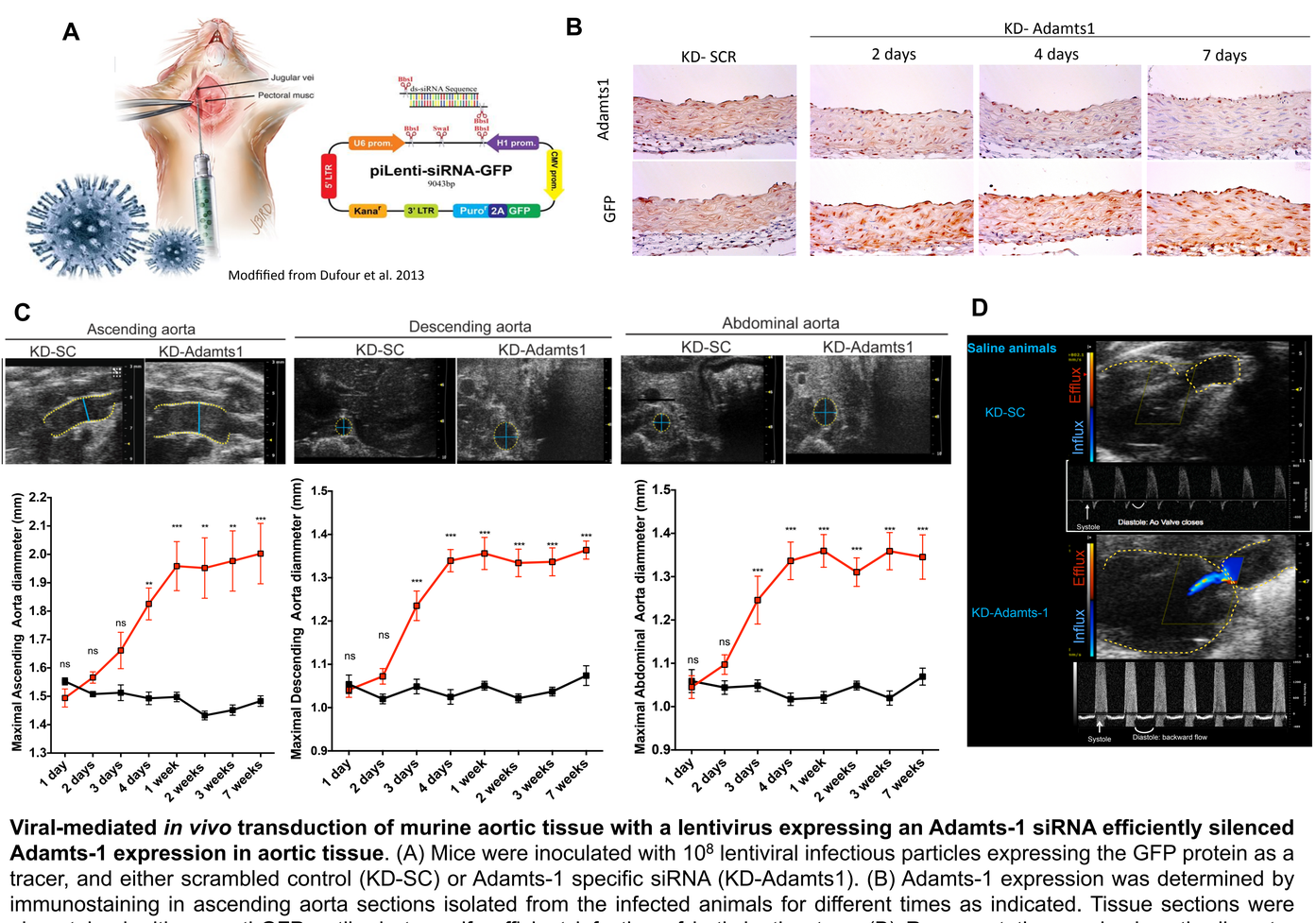
Adamts-1 deficiency results in aortic dilation. A) Representative aortic echographies from wild-type (Adamts-1$^{+/+}$) and Adamts-1-heterozygous mice (Adamts-1$^{+/-}$) in homeostasis (control) and Ang-II infusion for 28 days. B) Quantification of maximal aortic diameter from echographies of aortic ring, ascending, descending and abdominal aorta of Adamts-1$^{+/+}$ and Adamts-1$^{+/-}$ mice infused with or without Ang-II for 28 days. Data are shown as means ± maximun and minimum of 2 independent experiments with 6 animals per group. $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{****}P < 0.0001$ control Adamts-1$^{+/-}$ versus Adamts-1$^{+/+}$. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ and $^{\#\#\#\#}P < 0.0001$ Ang-II treated Adamts-1$^{+/-}$ versus Adamts-1$^{+/+}$ mice. C) Systolic and diastolic blood pressure from Adamts-1$^{+/+}$ and Adamts-1$^{+/-}$ mice infused with or without Ang-II for 28 days. $^{****}P < 0.001$ control Adamts-1$^{+/-}$ versus Adamts-1$^{+/+}$. $^{\#\#\#\#}P < 0.0001$ Ang-II treated Adamts-1$^{+/-}$ versus Adamts-1$^{+/+}$ mice. D) Representative images of heart and aorta from Adamts-1$^{+/+}$ and Adamts-1$^{+/-}$ mice without treatment or with Ang-II infusion for 28 days

4.- Adamts1 gene expression is necessary for the maintenance of vascular homeostasis and remodelling



Adamts1 deficiency results in an extensive vascular remodeing A)Representative images of aortic sections from Adamst1 wild-type or heterozygous mice stained with Elastin Van Gienson (EVG) and Alcian Blue to detect elastin and proteoglycans, respectively. B) Adamts-1, TGFB1, CTGF COl1A3 and PAI gene expression from aortas wild-type (+/+) or heterozygous *Adamts1* (+/-) mice infused with or without Ang-II for 28 days. Bar 40 μm C) Representative immunostaining for TGFβ, pSMAD2, total SMAD in aortas from wild-type (+/+) or heterozygous *Adamts1* (+/-) mice infused with or without Ang-II for 28 days. Specific isotyped-IgG was used as negative control.

5.- Adamts1 silencing induces early aortic dilation.



Viral-mediated *in vivo* transduction of murine aortic tissue with a lentivirus expressing an Adamts-1 siRNA efficiently silenced Adamts-1 expression in aortic tissue. (A) Mice were inoculated with 10^8 lentiviral infectious particles expressing the GFP protein as a tracer, and either scrambled control (KD-SC) or Adamts-1 specific siRNA (KD-Adamts1). (B) Adamts-1 expression was determined by immunostaining in ascending aorta sections isolated from the infected animals for different times as indicated. Tissue sections were also stained with an anti-GFP antibody to verify efficient infection of both lentivectors. (B) Representative maximal aortic diameter ultrasound images from ascending, descending and abdominal aorta of mice transduced with KD-SC and KD-Adamts1 for 7 weeks. Histograms represent maximal aortic diameter at different time-points in mice infected with control KD-SC (blue line) or KD-Adamts1 (red line). Data are shown as means ± SEM of 3 independent experiments with 9 animals per group. $^{**}P < 0.01$ and $^{***}P < 0.001$ versus KD-SC. D) Representative echo-doppler image of aortic regurgitation (in blue color) in animals transduced with KD-SC or KD-Adamts-1 for 7 weeks.

Conclusions :

- Adamts-1 is up-regulated by Ang-II in vascular cells and in aorta
- Adamts-1 null mice present in high lethality
- Adamts-1 heterozygous mice present aortic dilation, systemic hypotension, extensive aortic remodeling and activation of TGFβ pathway
- Ang-II infusion in Adamts-1 heterozygous mice induces aortic aneurysm and exacerbates vascular remodeling.
- Post-natal Adamts-1 silencing recapitulates early the vascular phenotype of Adamts-1 heterozygous mice.
- Both models, Adamts1 deficient mice and knocking-down present some vascular features that resembles aortic disorders
- **These data supports that Adamts1 expression is essential for vascular integrity, homeostasis and remodeling**

Bibliography:

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