ADAMTS and vascular remodeling in aortic aneurysms

Extracellular matrix (ECM) is a highly dynamic structure composed of a set of different molecules such as elastins, collagens, fibronectin (Fn), laminins, proteoglycans, and polysaccharides. ECM undergoes remodeling processes to regulate cell proliferation, differentiation, and adhesion (Lu et al., 2011). Abnormalities affecting the ECM can lead to alteration in cellular behavior and from this, this can lead to the development of pathologies. Metalloproteases play a key role in maintaining the homeostasis of ECM by mediating the cleavage of different ECM components. There are different types of metalloproteases: matrix metalloproteinases (MMP), disintegrin and metalloproteinases (ADAMs), and ADAMs with thrombospondin motifs (ADAMTSs). ADAMTSs have been found to participate in cardiovascular physiology and diseases and specifically in aneurysms (Santamaria and de Groot, 2020).

# Introduction

An aortic aneurysm (AA) is a dilatation that occurs in the aorta, a major blood vessel which comes out of the heart and carries blood throughout the body. Two types of aortic aneurysms can transpire: 1) abdominal aortic aneurysms (AAA) which occur in the descending aorta in the abdomen, and 2) thoracic aortic aneurysms (TAA) which occur in the aortic section of the chest cavity (ascending, cross, and early descending). Aneurysms from genetic origin usually appear in the thoracic aorta, and more specifically at the ascending aorta. With or without aneurysms, aortic dissections can also occur. A dissection is a loss of integrity between the media and adventitial layers of the aorta, which leads to the formation of a false lumen. Differing classifications of aortic dissections exist: DeBakey type 1 which involve ascending and descending aorta (*e.g.,* Stanford A); DeBakeay type 2 involving ascending aorta only (*e.g.,* Stanford A); and DeBakey type 3 involving descending aorta only, starting after the after the subclavian artery (*e.g.,* Stanford B).

Beyond cellular components such as smooth muscle cells, fibroblasts, endothelial cells and immune cells, the aortic extracellular matrix (ECM) has a crucial role in maintaining homeostasis and the physiopathological mechanisms of thoracic aortic aneurysms and dissections (TAAD). Almost all the thickness of the aorta is made up of ECM proteins, such as fibrillar proteins (*e.g.*, collagens, fibrillins, elastin), proteoglycans (*e.g.,* heparan-sulfates glycoproteins, perlecan), and metalloproteases. It must be emphasized that elastin fibers and fibrillar collagens make up to 50% of the dry weight of large caliber arteries. ECM protein- composing vessels are in permanent replacement due a large turnover of proteins with the help of metalloproteases, specifically matrix metalloproteases (MMP) and a disintegrin and metalloproteases with thrombospondin motifs (ADAMTS). The hallmarks of TAA are the progressive degradation of the media due to the decreased integrity of elastic fibers and increased elastolysis activity (through metalloproteases).

This study aims to decipher the potential role of ADAMTS proteins in the physiopathologic development of TAA and AAA. This study will focus on what is known from mice models, human tissues, and the first protein from ADAMTS involved in human aneurysms.

# ADAMTS

The ADAMTS family constitutes a group of proteins comprised of 19 enzymes and 7 ADAMTS-like. The ADAMTS share a specific domain organization with a signal peptide, a prodomain, a catalytic domain, and an ancillary domain. The catalytic activity involves zinc and three conserved histidine residues (Dubail et al., 2015). The ancillary domain may consist of a disintegrin-like domain, a first thrombospondin-type I motif, a cysteine-rich domain, a spacer domain, and other thrombospondin motifs. Certain ADAMTS have additional domains such as: the PLAC (protease and lacunin) domain, present in ADAMTS2, 3, 6, 10, 12, 14, 16, 17, 18 and 19; a Gon-1-like domain, found only in ADAMTS9 and 20; and a mucin domain, specific to ADAMTS7 and 12.

To be active, the zymogen form of ADAMTS proteases have an N-terminal propeptide which must be cleaved by proprotein convertases such as furin (Apte 2009). However, there are exceptions, such are ADAMTS9 and ADAMTS13 which stay active despite retention of the propeptide [30], [31], [32], [33], [34], [35], [36], [37]. The cleavage may occur in the trans-Golgi, or on the cell surface [35], [38]. Most proteases of ADAMTS are modified by post-translational modification, such as N- and O-glycosylation, addition of chondroitin–sulfate chains, C-mannosylation of tryptophane residues, and O-fucosylation of serine/threonine residues in the TSRs [39], [40], [41]. These enzymes are mainly regulated by endogenous inhibitors, TIMP-3, α2-macroglobuli, and by endocytosis mediated by the LRP-1 receptor 44,45,46[48], [49], [50].

Proteases of ADAMTS can be grouped according to their ancillary domains and then their substrate. They evolved likely by gene duplication that led to functional redundancy (Huxley-Jones et al., 2005; McCulloch et al., 2009; Mead et al. 2018; Mead et al., 2021). ADAMTS13 is the most well-known ADAMTS and is involved in von Willebrand factor (VWF) proteolysis and hemostasis (Furlan et al., 1996). The proteoglycanases ADAMTS1, 4, 5, 8, and 15, as well as ADAMTS9 and 20, form a group which can cleave proteoglycans. More specifically, ADAMTS4 and ADAMTS5 were shown to be responsible for cartilage aggrecan destruction in arthritis. Among the procollagen N-propeptidases (ADAMTS2, 3, and 14), ADAMTS2 is well characterized through its involvement in the rare human disorder, the Ehlers-Danlos syndrome (EDS) type VIIC. Recent data suggest that ADAMTS3 may be not an essential procollagen N-peptidase and it seems to have a role in lymphoangiogenesis through activation of VEGF-C. ADAMTS6, 10 and 17 can be considered as proteases associated with fibrillin and fibronectin. This group’s function was discovered through the identification of mutations in the spectrum of Weill-Marchesani syndrome. Interestingly, certain peptides issues from cleaved ADAMTS may have a specific function [47].

In this ADAMTS superfamily, ADAMTSLs are the products of distinct genes. They lack the catalytic domain as well as the propeptide and disintegrin-like domain present in all the proteases of ADAMTS. They have a similar structure to the ancillary domains of ADAMTS. However, none of the ADAMTSLs have the exact composition of the ADAMTS ancillary domains: This suggests that ADAMTSLs also contain specific extracellular binding proteins. Most of the ADAMTSL proteins bind to microfibrils such as ADAMTSL2, ADAMTSL4, ADAMTSL5, and ADAMTSL6 [12–15]. The data on *Drosophila* papilin and the structural similarities of ADAMTS proteases and ADAMTSLs suggest a potential functional link between these two types of proteins. This is an aspect that needs to be deepened.

1. ADAMTS/ADAMTSL and TAA

# *ADAMTSL6* mutations responsible for TAA

The *THSD4* gene encodes the ADAMTSL6 protein. ADAMTSL6 is known to be a microfibril-associated extracellular protein without any catalytic activity. In E16.5 mouse embryos, ADAMTSL6 proteins were observed in the dermis, perichondrium, aortic wall, and all elastic tissues (Tsutsui et al., 2010). The ADAMTSL6 protein was also present in adult kidney artery walls and the mitral valve in the adult heart. All these data suggest that ADAMTSL6 is an ECM protein associated with elastic tissues in its fibrillary components. ADAMTSL6 interacts directly with fibrillin-1 at its N-terminal and promotes early-stage fibrillin-1 microfibril assembly (Tsutsui et al., 2010). This role was confirmed using transgenic mice where ADAMTSL6 was overexpressed.

The recent identification of *THSD4* as a new gene involved in TAA led us to consider a new function of the ADAMTSL protein (El Bitar et al., 2021). In addition to human data, our recent data support that *Thsd4*+/- mice present progressive thoracic aortic dilation with disruption of elastic fibers and increased apoptosis of SMCs at eight-months-old. In human cellular models, the introduction of mutations in *ADAMTSL6* leads to impairments of FBN1 microfibril assembly. In the aorta of the TAAD patients, the disorganization of the FBN1 microfibril network was confirmed and was associated with an increase of TGF-β and overactivation of the TGF-β signaling pathway (El Bitar et al., 2021). In the context of a tumor, ADAMTSL6 also indirectly regulates the TGF-β signaling pathway (Liu et al., 2021). From these results, the role of ADAMTSL6 in the homeostasis of FBN1 in the aorta was highlighted. Another study suggests the possibility of using ADAMTSL6-mediated fibrillin-1 microfibril assembly as a therapeutic tool to rescue the cells of MFS (Saito et al 2011). Their findings highlight the improvement of the fibrillin-1 network after administration of ADAMTSL6 and the following attenuation of the overactivation of TGF b signaling.

# 1.2. Proteoglycanases involved in TAA

Proteoglycans are one of the major groups of ADAMTS substrates. Their composition is a core protein associated with various glycosaminoglycans. In vasculature, the proteoglycans are mainly expressed in endothelial cells and vascular smooth muscle cells of the intima and the media of the vessel wall. Due to their position in the ECM, they are involved in cell communication, signaling, and behavior. Despite the increase of proteoglycans as a hallmark of atherosclerosis plaques, recently it was demonstrated that enhanced aggrecan and versican are also features of aneurysms (Cikach et al., 2018). The level of proteoglycans is regulated by proteoglycanases such as ADAMTS. Considering the importance of proteoglycans in the vessels, we will highlight the role of these enzymes in vascular diseases.

## ADAMTS1

ADAMTS1 was discovered in 1997 as the first member of the ADAMTS. It possesses a large catalogue of substrates: aggrecan, versican, syndecan-4, TFPI-2, semaphorin 3C, nidogen-1, −2, desmocollin-3, dystroglycan, mac-2, gelatin (denatured collagen type I), amphiregulin, TGF-α, and heparin-binding EGF. Considering the number of substrates, this protease is also expressed in many different types of cells and then in endothelial and vascular smooth muscle cells.

ADAMTS1 seems important in specific developmental processes. During heart development, ADAMTS1 participates in stopping the proliferation of ventricular cardiomyocytes by cleaving versican. These data indicate that ADAMTS1 may be the principal ADAMTS which mediates versican V1 cleavage during ventricular morphogenesis ([Stankunas et al., 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3489172/%22%20%5Cl%20%22R37)) Fibulin-1 is the mediator of this function in ADAMTS1 during cardiac ventricular development (Cooley et al 2012). ADAMTS1 has also pro- and anti-angiogenic properties through either its nonenzymatic or catalytic functions, depending on the cellular context (Rodriguez Manzaneque et al 2015). It was demonstrated that the C-terminus of ADAMTS1 and its associated three thrombospondin motifs promote anti-angiogenic properties by sequestering VEGF (Luque et al 2003). In prostate tumors, expression of ADAMTS1 was directly correlated to the diameter of blood, thus proving a proangiogenic role of ADAMTS1.

More recently, a study demonstrated that ADAMTS1 may play a role in the pathophysiology of aorta remodeling. Enhanced expression of *ADAMTS1* was correlated with the occurrence of aortic dissection in humans, or in mice with angiotensin II (Ang II) (Gao et al., [2016](https://physoc.onlinelibrary.wiley.com/doi/full/10.1113/EP087018%22%20%5Cl%20%22eph12363-bib-0005)). These findings were confirmed via a study of ADAMTS1 deficiency in mice. This mouse model showed a decreased susceptibility to BAPN-induced TAAD formation and rupture. Furthermore, ADAMTS1 deficient mice had no inflammatory cell infiltration from inhibiting inflammatory cytokine levels and macrophage migration. Altogether, ADAMTS1 might be a suitable candidate as a potential therapeutic target for TAA (Wang et al 2018).

To be a good target, it is necessary to identify the regulatory molecules of ADAMTS1 expression. Different molecules implied in vascular remodeling (VEGF, angiotensin-II, interleukin-1β, and tumor necrosis factor α) enhance ADAMTS1 expression in two types of cells, endothelial and vascular smooth muscle cells. Intracellular signaling pathways are associated with enhanced ADAMTS1 expression by inducing pathological vascular remodeling. This upregulation is mediated by specific signal transduction pathways involving nuclear factor of activated T cells (NFAT) or CCAAT/enhancer binding proteins (C/EBPβ) transcription factors. These pathways are another possibility to target for treating vascular disease (Oller et al., 2015).

Oller et al., 2017 have shown that ADAMTS1 is an important mediator of vascular wall homeostasis and that its expression is decreased in individuals with MFS. Opposite results were reported by other groups. An increase of ADAMTS1 protein and mRNA expression was shown in TAAD tissues compared to control aortic tissues (Ren et al., 2013, Gunes et al., 2016). In parallel, one of their substrates called versican was found to be more cleaved in TAAD tissues than in control aortic tissues. The authors suggested then that increased level of ADAMTS1 may lead to TAA progression by degrading versican (Ren et al., 2013). These conclusions were established using descending aorta samples. These samples indicated a difference among ADAMTS1 regulation depending on the aorta type studied.

Untreated and angiotensin II-treated mice which targeted ADAMTS1 displayed aortic dilation. One study focused on a specific knock-down of ADAMTS1 expression in aorta (REF). By using a lentivirus encoding an ADAMTS1 specific siRNA, their results led to the confirmation of the potential role of ADAMTS1 in promoting TAA. Interestingly, the decrease of ADAMTS1 levels in a mouse model conduced medial degeneration with elastic fibers breaks and excessive collagen and proteoglycan accumulation – two hallmarks of TAA. As observed in MFS mice, the increase of TGFb activation was linked to media degeneration in aortic sections of ADAMTS1+/-. However, this activation is secondary to aortic dilation in this model. The knock-down of ADAMTS1 is immediately followed by the induction of elastolysis driven by MMP9 secreted from VSMCs (Oller et al., 2017). Elevated levels of aortic nitric oxide (NO) and nitric oxide synthase 2 (NOS2) were observed in ADAMTS1+/- and in a mouse model of MFS. NOS2 inhibition was found to protect both types of mice from aortic dilation or medial degeneration (REF). This mouse model revealed the critical role of NO and ADAMTS1 in syndromic forms of TAAD such as Marfan syndrome. These results also established a link between FBN1, ADAMTS, and NO.

## ADAMTS4

ADAMTS4 is a well-known proteoglycanase and it has angiomodulatory properties. Both pro- and anti-angiogenic activities have been attributed to ADAMTS4. The full length of ADAMTS4 favors tumor angiogenesis and then tumor growth. In opposite, the C-terminal ancillary domain possesses both pro- and anti-angiogenic properties (Rao et al., 2013). ADAMTS4 is expressed in endothelial cells (Kahn et al., 2000).

ADAMTS4 was found to be highly expressed in SMCs and macrophages in the aortic wall of challenged mice who were administered a high fat diet and angiotensin II infusion (Ren et al., 2017). ADAMTS4 expression was also enhanced in human aortic tissues (Ren et al., 2013). Deficient *ADAMTS4* mice did not develop TAAD when administered a high fat diet and angiotensin II infusion. Aortas in *Adamts4-/-* mice displayed reduced aortic diameter enlargement associated with a reduction of elastic fiber destruction. In addition, these mice had versican degradation, thus suggesting that *Adamts4* deficiency prevents aortic destruction and proteoglycan degradation (Ren et al., 2017). *Adamts4* seems to be involved in SMC apoptosis by directly interacting with and cleaving PARP-1, a nuclear protein that plays a role in DNA repair and cell survival. Surprisingly, ADAMTS4 as an enzyme is involved in turnover of ECM and has a role in the nucleus and induces SMC apoptosis. In the context of tumors, an indirect role in apoptosis has been shown (REFs). The inactive ADAMTS4 enzyme or only its C-terminus domain inhibits melanoma growth and its angiogenesis associated with tumor cell apoptosis. Its role in aortic aneurysms may be more connected to its apoptotic effect rather than its versicanase function.

One study found that the upregulation of ADAMTS4 was inhibited by miR-126a-5p. XX was shown to be downregulated 18-fold in AAA samples of mouse aortas. It was found that miR-126a-5p overexpression significantly improved survival rates and reduced aortic dilatation in Ang II-infused mice as well as elastic fragmentation and ECM degradation. There are many indicators that ADAMTS4 seems to be a new target for miR-126a-5p (Li et al 2020). This miRNA could be a good therapeutic target to modulate ADAMTS4.

## ADAMTS5

ADAMTS5 is one of the main enzymes involved in proteoglycan cleavage. Like ADAMTS4, ADAMTS5 protein expression was found to be significantly increased in TAAD tissues compared to control aortic tissues (Gunes et al 2016). More recently, other research demonstrated that ADAMTS5 mRNA and protein levels are reduced in TAAD tissues as well as in plasma (Cikach et al., 2018; Zeng et al., 2020). Mice data using *Adamts5*-deficient mice points to the same trend. Using Ang II as a model to induce TAAD, mice lacking the catalytic domain of ADAMTS5 (*Adamts5Δcat*) displayed a higher enlargement of the ascending aorta. These results show a distinct and non-redundant role between ADAMTS4 and ADAMTS5.

Versican seems central to the development of aortopathies as it was found to be the most upregulated ECM protein in *Adamts5Δcat* mice. In parallel, a clear reduction of versikin, a versican cleavage by specific ADAMTS, was observed (Fava et al 2018). Low density lipoprotein related protein 1 (LRP1) is a regulator of ADAMTS5 by promoting its endocytosis. LRP1 has been shown to be involved in aneurysm formation (Davis et al., 2015). LRP1 protein levels were found to be lower in Ang II-treated aortas from *Adamts5Δcat*mice. *Adamts5* deficiency was discovered to also increase ADAMTS1 protein levels. *ADAMTS1* expression was not able to compensate for the absence of versican cleavage: This suggests that Adamts5 is a major versicanase. To date, no data on ADAMTS4 expression levels are available. An additional mouse model was developed, an *Adamts5-/-* mouse model, and found aortic anomalies (n=19/19) compared with wild type (n=1/11). The aortas of *Adamts5-/-* displayed aggrecan accumulation, thus confirming the key roles of aggrecan/versican in aortopathies. Results from these studies suggest that ADAMTS5 may have a critical role in normal aortic wall development (Dupuis et al 2019).

ADAMTS16

The exact function and the potential substrates of ADAMTS16 are unknown except for the fibronectin (Schnellman 2018). The upregulation of ADAMTS16 was assessed using western blot on TAA aortic tissues (Güneş MF et al., 2016). It was suggested that enhanced expression of ADAMTS16 may promote the deceleration of TAA progression. This finding was not surprising considering that ADAMTS16 has been associated with hypertension, one of the main causes of aortic aneurysm development (Golapalakrishnan et al., 2012). Using a rat genetic model of hypertension with an *Adamts16* defect, the physiological observations established a linkage between *Adamts16* and blood pressure regulation. Heterozygote and homozygote *Adamts16* mutant rats presented a lower blood pressure along with a decrease in the aortic media layer thickness and pulse wave velocity. This last one is a measure of arterial stiffness. Furthermore, endothelial cell analysis showed the presence of elongated cilia. Elongated cilia establish contact from the apical surface of the cell with the collagen in the extracellular matrix. Even though the function of ADAMTS16 is unknown, it may play an important role in the vascular system (Golapalakrishnan et al., 2012).

1. ADAMTS linked to AAA

## ADAMTS8

ADAMTS8 is an aggrecanase with low efficiency (Santamaria et al 2020). To date, this enzyme has been poorly investigated. Its expression profile shows expression in the heart and lungs. Its expression in the lung was correlated to its role in pulmonary arterial hypertension (PAH) (Omura et al., 2019*).* In a mouse model using hypoxia to induce PAH, ADAMTS8 was found to be enhanced, as well as in the pulmonary arterial SMC (PASMC) in PAH patients. In a vascular SMC-specific mouse model, *Adamts8ΔSM22*, under hypoxia induced pulmonary hypertension it was found that PAH was reduced compared to controls*.* Even if ADAMTS8 is an extracellular matrix, it was shown that ADAMTS8 has a role in intracellular signaling using PASMCs.The proliferation of PASMC was decreased when human recombinant ADAMTS8 was deleted (REFs). The ADAMTS8-/- PASMCdisplayed increased phosphorylation of AMP-activated protein kinase (AMPK)/acetyl-CoA Carboxylase (ACC) signaling*.* ADAMTS8 also may have endothelial function through its secretion from PASMCs (Omura et al. 2019). Secreted ADAMTS8 from PASMCs might be the missing link between PAECs and PASMCs in PAH pathogenesis.A therapeutic approach was tested using a high-throughput screening method. This screening revealed that mebendazole, a broad-spectrum antihelminthic which is indicated for the treatment of parasite infections, decreased ADAMTS8 expression and ameliorated PH in PH rat models (Omura et al 2019).

In addition, a potential role of ADAMTS8 in abdominal aortic aneurysm (AAA) was established (Farrell et al 2019). ADAMTS8 was downregulated in SMCs issues from AAA patients. All other AAA hallmarks were observed such as lower elastin deposition and lysyl oxidase activity, or increased *Fibrillin-1* gene expression.

Considering the absence of NO signaling in AAA, an optimal NO dosage treatment delivered viaS-Nitrosoglutathione (GSNO) was used on SMCs from AAA patients (REFs). The GSNO was found to ameliorate the stiffness of SMCs, decrease MMPs -2, -9, and increased TIMP-1 release in AAA-SMC cultures. The study did not mention the impact of GSNO on ADAMTS8 expression. An analysis of a genome-wide association study (GWAS) using data from the UK Biobank revealed novel genetic loci that may susceptible for the development of thoracic and abdominal aortic aneurysms (Ashvetiya et al., 2021). In this GWAS research, ADAMTS8 was an identified loci associated with an increased risk to develop AAA.

## ADAMTS9

ADAMTS9 is an actor in ciliogenesis (Nasdasa Apte, 2019). This protease is also required for normal cardiovascular development. Heterozygous *Adamts9* mice were shown to have defects in the aortic wall, valvulosinus, and valve leaflets. These mice also displayed abnormal myocardial projections and “spongy” myocardium, consistent with non-compaction of the left ventricle*.* These mutant mice anomalies were correlated with abnormal accumulation of versican and a decrease in cleaved versican compared to WT mice. These findings suggest a potentially important role for ADAMTS9 cleavage of versican in heart development (Kern et al.,). In addition, ADAMTS9 expressed in vascular SMC may be a good candidate gene in hereditary thoracic aortic aneurysms. In fact, ADAMTS9 was identified as a marker of terminal abdominal aortic aneurysm. Analysis of aortic wall tissues from patients with elective or emergency repair of ruptured AAA were used to establish the molecular changes leading up to AAA rupture (Gabel et al., 2017). ADAMTS9 was found to only be upregulated in tissues from patients with an emergency repair of abdominal aorta.

# ADAMTS7 and COMP

ADAMTS7 is known to directly bind to, and possibly degrade, the ECM protein named cartilage oligomeric matrix protein (COMP) in cartilage. COMP has been implicated in the pathogenesis of arthritis(Liu et al., 2001). COMP was discovered to be expressed not only in skeletal tissue but also in aorta. It has also been implicated in the attachment and haptotaxis of VSMCs(Riessen et al., 2001). In addition, COMP has also been associated with human atherosclerotic lesions, which suggests its potential importance during pathological ECM remodeling and VSMC migration (Wang et al 2009) (circulation research and Bauer et al., 2015). ADAMTS7 has also been found to be involved in intimal thickening after vascular injury (REFs). Altogether these findings suggested that this is the consequence, at least in part, of ADAMTS7–dependent COMP degradation.

Considering the potential role of ADAMTS7 in pathogenesis of vascular atherosclerosis via degradation of COMP, the ADAMTS7/COMP pathway may therefore act as a potential therapeutic target for vascular disorders. Using human tissues from aortic aneurysms (AA), it was shown that ADAMTS7 expression was significantly increased in the AA group compared to controls. At the same time, conversely, the COMP protein level was decreased in AA samples (REFs). A novel axis of a potential therapeutic target in human AA is identified as being the ADAMTS7/COMP pathway (Qin et al., 2017).

ADAMTS7 has been implied in as participating in the inhibition of endothelial cell proliferation and migration in vitro. Moreover, *Adamts7* null mice showed an excessive reendothelialization in injured arteries (Kessler et al., 2015). These findings suggest that Adamts7 may retard endothelium repair independently of COMP and therefore may have other substrates. LTBP4 was identified as an ADAMTS7 substrate using a recent method, terminal amine isotopic labeling of substrates (TAILS) (Colige et al., 2019). Interestingly, LTBP4 is part of microfibrils and can interact with fibrillin-1 and fibulin-5, which are both involved in the formation of the elastic fibers. *Fibrillin-1* mutations are associated with Marfan syndrome which is characterized by thoracic aortic aneurysm. Lowered fibulin-5 levels have been linked to patients with aortic dissection: This may contribute to the pathogenesis of aortic dissection by impairing elastic fiber assembly (Wang et al., 2005). Results from these studies support the potential link of ADAMTS7 as playing a role in LTBP4 cleavage in aortic aneurysms. Recently, an efficient inhibitor of ADAMTS7 was identified as being TIMP-4. TIMP-4 may be an interesting therapeutic molecule to further research in its relational context to AA.

# Other ADAMTSL, ADAMTSL2 possibly involved in aorta pathology

Some ADAMTS(L) family proteins are involved in rare disorders. These disorders include Acromelic dysplasias regrouping the Weill-Marchesani syndrome (WMS), Geleophysic dysplasia (GD) with mutations in *ADAMTS10* (OMIM#277600), *ADAMTS17* (OMIM#613195), and *ADAMTSL2* (OMIM#231050), respectively. These disorders share similar clinical features such as short stature with shortened extremities, thick skin, and restricted joint mobility. These pathologies can also lead to cardiovascular defects that may be life-threatening conditions (Le goff et al., 2011 HMG).

GD was first described in 1971 in two patients presenting, among others, short stature, short hands and feet, and limited joint mobility (Spranger et al., 1971). Homozygosity mapping in two unrelated non-consanguineous families and four unrelated consanguineous families found *ADAMTSL2* to be the causal gene of the recessive form of GD (Le Goff et al., 2008). The first mutations were found either in the cysteine-rich module or in the sixth thrombospondin type I repeat (TSR6) of the protein (Allali et al., 2011).

Cardiovascular defects which affect GD patients evolves throughout their lifetimes. High mortality is observed due to cardiovascular/bronchorespiratory defects, making GD the most severe pathology among the Acromelic dysplasias (Le Goff et al., 2008). In addition, pulmonary stenosis, mitral and aortic valves stenosis and mild tricuspid regurgitation are also common cardiovascular manifestations found (Allali et al 2011, Ben-Salem et al., 2013; García-Ortiz et al., 2015; Mackenroth et al., 2016; Li et al., 2017). Also, thickened ventricle walls associated with limited ejection fraction were found, as well as pulmonary arterial hypertension, hypertrophy of the papillary muscles, and NYHA class III in acute chronic heart failure (Allali et al., 2011; Ben-Salem et al., 2013; Legare et al., 2018).

More recently, the defect in ADAMTSL2 was linked to a dilatation of ascending aorta in a 48-year-old patient. This patient also presented aneurysms in the brachiocephalic artery and main pulmonary artery (Legare et al., 2018). ADAMTSL2 is not known to be expressed in VSMC but in other cardiovascular cells such as cardiomyocytes and dermal blood vessels of human embryos (Le Goff et al., 2008). *Adamtsl2* was found to be overexpressed in microvascular endothelial cells of ventricles in a murine model of transverse aortic constriction (Trenson et al., 2021). Using mass spectroscopy, the ADAMTSL2 protein was found to be under-expressed in coronary heart disease with a congenital cold syndrome (Xie et al., 2021).

*Adamtsl2*−/− mice fail to survive postnatally and have cardiac developmental defects. This is likely due to lung anomalies associated with bronchial fibrillin microfibril accumulation (Hubmacher et al., 2015, Delhon et al., 2019). In heart ventricles undergoing heart fibrosis due to heart failure, all ADAMTSLs were overexpressed except ADAMTSL6. ADAMTSL2 was the most upregulated in cardiac fibroblasts (Rypdal et al., 2021). This increase resulted in reduced TGFβ production and signaling pathway activation. Increased ADAMTSL2 levels inhibited myofibroblast differentiation through the inhibition of *ACTA2* expression. This, in turn, caused an attenuation of important pro-fibrotic, phenotypic properties of cardiac fibroblasts such as proliferation, migration, and contractility. Taken together, findings indicate that ADAMTSL2 may be a negative regulator of TGFβ in cardiac fibroblasts. Collective research implies that ADAMTSL2 regulates ECM deposition and TGFβ signaling and may therefore have an important role in cardiac fibrosis and heart failure.

Belonging to the Acromelic dysplasia spectrum, ADAMTS10 and 17 have not yet be associated with aneurysms but they have been associated with cardiovascular defects. First described in 1932 by Weill (Weill G, 1932) and later in 1939 by Marchesani (Marchesani O, 1939), WMS has been suggested to be a “recessive condition” due to parental consanguinity of affected patients. It was not until the early 21st century that homozygosity mapping in consanguineous WMS families highlighted the homozygous splicing and nonsense mutations in *ADAMTS10*. This gene encodes the ADAMTS10 protein and specifically impacts the metalloprotease domain of the protein (Faivre et al., 2002; Kutz et al., 2008). Shortly after, the discovery of homozygous mutations in *ADAMTS17,* which encodes for the ADAMTS17 protein, has resulted in a new “Weill-Marchesani-like” phenotype (Morales et al., 2009; Khan et al., 2012). Both genes lead to the recessive form of WMS.

Cardiovascular defects affecting WMS patients are mainly localized in the human heart. These defects include: prolonged QTc (more than 0.46 seconds) with mitral valve prolapsus (Kojuri et al., 2007); hypertrophic obstructive cardiomyopathy; pulmonary stenosis with dysplastic valves (Dagoneau et al., 2004; Evans et al., 2020); and small muscular ventricular septal defect (Steinkellner et al., 2015). To date, little is known on the exact role of ADAMTS10 and ADAMTS 17 proteins in heart development and its homeostasis. ADAMTS10 is highly expressed in blood vessels and in the human heart (Somerville et al., 2004). ADAMTS10 is specifically expressed in aortic valve leaflets, leading to what many think that malfunctional ADAMTS10 proteins lead to mitral aortic prolapse (Wang et al., 2019). ADAMTS17 is also expressed in the human heart (Morales et al., 2009). ADAMTS10 and ADAMTS17 are also linked to fibrillin networks as ADAMTSL2 or ADAMTSL6. This suggests that they may have a role in the aorta where the fibrillin proteins are an important component of aortic elastic tissues.

# ADAMTS18 involved in aorta development

The role of ADAMTS18 in vascular development was researched by using an *Adamts18* knockout mouse model (Shuai Ye et al., 2021). During the development of the mice, ADAMTS18 mRNA was observed in cells surrounding the ascending aorta and carotid artery. *Adamts18* knockout mice presented malformations of the embryonic aortic arch and the carotid artery system including disordered elastic fibers and reduced carotid blood pressure. They also presented hypoplasia of the thymus and absence of the carotid body. These abnormalities could be explained by the deficiency of ADAMTS8, causing fibronectin accumulation and ECM remodeling. These phenomena would thus activate the NOTCH3 signaling pathway. This activation ends up affecting the differentiation of cranial neural crest cells into vascular smooth muscle cells (VSMC) (Shuai Ye et al., 2021). The repertoire of ADAMTS18 substrates needs to be further investigated in order to better understand the role of this enzyme in the aortic arch and carotid artery. Fibrillin-1 may be a substrate candidate. In *ADAMTS18-/-*, an increased level of fibrillin-1 was observed in a carotid artery with disorganized elastic fibers and lower blood pressure (Shuai Ye et al., 2021). It has been shown that ADAMTS18 is an actor of fibrillin-1 assembly in bronchial cells (Lu et al., 2020).

## ADAMTS19 implicated in valve development

Recent genetic data revealed a potential function of *ADAMTS19* in valve development. Loss of function *ADAMTS19* mutations have been identified as being responsible for non-syndromic heart valve disease (Wunnemabb et al., 2020). Patients with heart valve disease represent only 2% of the general population. Using whole exome sequencing, homozygous truncating nonsense variants in *ADAMTS19* were revealed in four affected cases of two distinct families. In order to understand the role of ADAMTS19 in valve development, a mouse model using the knock-out first allele tagged with a lacZ reporter cassette was generated. The follow up of the echocardiographic analysis displayed a progressive aortic valve disease at three months of age in 38% of the *Adamts19KO*/*KO* mice. The abnormal valves presented a disorganization of the ECM which could be a consequence of a mutant metalloproteinase such as ADAMTS19. Mutants displayed higher proteoglycan content as well as thinner collagen fibers. Using lacZ staining, the expression of *Adamts19* was strongly detected in all four valves, and specifically in the valvular interstitial cells (VICs). Single cell transcriptome data suggested that there is a defect in the mechano-transduction pathway from VIC to endothelial cells, implying Wnt signaling.

# Conclusion

The studies of human aortic tissues and the phenotypes of *ADAMTS*/*ADAMTSL* gene knockout mouse models revealed the important roles of this family in aortic aneurysms. Multiple ADAMTS or ADAMTSL seem to be involved in TAA as well as in AAA. However, from animal studies, it is possible to notice that there is no functional redundancy between these different ADAMTS. It is certainly necessary to further develop our knowledge on their spectrum of substrates. The linkages between ADAMTS proteases and ADAMTSL proteins remain to be elucidated. For example, the first ADAMTSL, namely ADAMTSL6 which is associated with TAA, may have a cooperative role with ADAMTS1. The ultimate challenge will be to develop specific therapeutic approaches using ADAMTS as an inhibitor targeting, for example, ADAMTS4 – a pro aneurysm molecule.