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MULTI-AUTHOR REVIEW

# Genetic and functional linkage between ADAMTS superfamily proteins and fibrillin-1: a novel mechanism influencing microfibril assembly and function

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**Summary** Tissue microfibrils contain fibrillin-1 as a major constituent. Microfibrils regulate bioavailability of TGF$\beta$ superfamily growth factors and are structurally crucial in the ocular zonule. *FBN1* mutations typically cause the Marfan syndrome, an autosomal dominant disorder manifesting with skeletal overgrowth, aortic aneurysm, and lens dislocation (*ectopia lentis*). Infrequently, *FBN1* mutations cause dominantly inherited Weill–Marchesani syndrome (WMS), isolated *ectopia lentis* (IEL), or the fibrotic condition, geleophysic dysplasia (GD). Intriguingly, mutations in ADAMTS [a disintegrin-like and metalloprotease (reprolysin-type) with thrombospondin type 1 motif] family members phenocopy these disorders, leading to recessive WMS (*ADAMTS10*), WMS-like syndrome (*ADAMTS17*), IEL (*ADAMTSL4* and *ADAMTS17*) and GD (*ADAMTSL2*). An *ADAMTSL2* founder mutation causes Musladin–Lueke syndrome, a fibrotic disorder in beagle dogs. The overlapping disease spectra resulting from fibrillin-1 and ADAMTS mutations, interaction of ADAMTS10 and ADAMTSL2 with fibrillin-1, and evidence that these ADAMTS proteins accelerate microfibril biogenesis, constitutes a consilience suggesting that some ADAMTS proteins evolved to provide a novel mechanism regulating microfibril formation and consequently cell behavior.

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**Keywords** Fibrillin · ADAMTS · Marfan syndrome · Weill–Marchesani syndrome · Ectopia lentis · Fibrosis · Scleroderma

**Abbreviations**

| | |
|---|---|
| ADAMTS | A disintegrin-like and metalloprotease (reprolysin-type) with thrombospondin type-1 motif |
| ECM | Extracellular matrix |
| GD | Geleophysic dysplasia |
| HSPG | Heparan sulfate proteoglycan |
| IEL | Isolated ectopia lentis |
| MFS | Marfan syndrome |
| MIM | Mendelian inheritance in man |
| MLS | Musladin–Lueke syndrome |
| TGF$\beta$ | Transforming growth factor $\beta$ |
| WMS | Weill–Marchesani syndrome |

## Introduction

Fibrillin-1, -2 and -3 are large ($\sim$350 kDa) cysteine-rich, modular secreted glycoproteins that exemplify the structural and regulatory roles attributed to extracellular matrix (ECM) [1]. Fibrillin-1 forms beaded microfibrils of 10–12 nm diameter with a typical periodicity of 50–60 nm [2, 3] (Fig. 1). Fibrillin-3 is absent in rodents [4]. Fibrillins recruit several binding partners that may help microfibrils fulfill multiple tissue-specific physiological roles, such as providing a scaffold for elastic fiber biogenesis and regulating bioavailability of growth factors of the TGF$\beta$/BMP family [5, 6]. Some of these partners may modulate fibrillogenesis, whereas others may rely on proper formation of microfibrils for their activity. This review focuses on a group of new fibrillin-1 binding partners, ADAMTS [a disintegrin-like

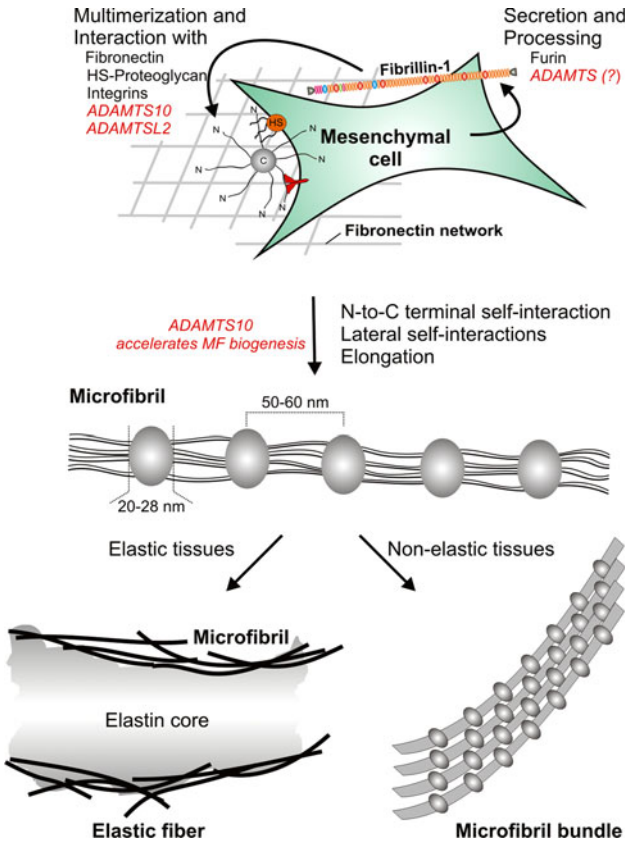


**Fig. 1** Scheme of microfibril assembly and potential points of involvement of ADAMTS proteins. The scheme illustrates the key points in fibrillin-1 secretion, initial assembly and then formation of microfibrils, at which the relevant ADAMTS proteins might act. It does not imply that all the relevant ADAMTS proteins work in an identical fashion or at all the indicated points in the pathway. The figure is modified from Hubmacher and Reinhardt [99], with permission from the publisher

and metalloprotease (reprolysin-type) with thrombospondin type-1 motif] proteins, whose relevance to microfibrils was identified principally through forward genetics, and discusses their emerging molecular mechanisms. Since ADAMTS research relating to fibrillins is still at an early stage, we summarize the genetic observations connecting fibrillin-1 to ADAMTS proteins and discuss those aspects of fibrillin-1 biology that may most productively direct future mechanistic studies of ADAMTS proteins.

## Fibrillin-1 and the Marfan syndrome

Fibrillins display an almost complete conservation of their domain organization, while their homology at the amino acid level ranges from 60 to 70%. The most frequent domains in fibrillins are epidermal growth factor-like (EGF) domains, most of which contain a consensus sequence for calcium binding (i.e., cbEGF domains) [1, 6] (Fig. 2). EGF and cbEGF domains are stabilized by disulfide-bonds formed by six highly conserved cysteine residues. The TGF$\beta$-binding protein-like (TB) domains are unique to fibrillins and to LTBPs [7]. Fibrillin-1 contains seven TB domains interspersed through the length of the protein, an RGD integrin-binding motif in the fourth TB domain (TB4) and four distinct heparin/HSPG binding sites [1, 6]. Other characteristic domains include the hybrid domain, which displays features of both the cbEGF and TB domains, and the proline-rich and glycine-rich domains in fibrillin-1 and -2, respectively (Fig. 2).

In tissues, microfibrils typically occur in groups or bundles that are often associated with elastic fibers, especially in the dermis, vasculature, and lungs. Microfibrils are widely distributed in the eye [8], and they constitute the ocular zonule, with fibrillin-1 being its chief molecular component [9]. The zonule is a cell-free structure comprising radially arranged microfibril bundles spanning the ciliary body and lens equator. The zonule positions the ocular lens in the center of the optic path and transmits contraction of the ciliary muscle to mediate accommodation. Thus, microfibrils are believed to have a mechanical role in the zonule.

Molecular defects of fibrillin-1 typically lead to the Marfan syndrome (MFS, MIM 154700), a fairly common autosomal dominant condition (incidence 2–3:10,000) [10] presenting with numerous anomalies, including skeletal overgrowth, arachnodactyly (long, slender fingers), dislocation of the ocular lens (*ectopia lentis*) and progressive dilatation of the aortic root (aortic aneurysm) [11–13]. The last-named anomaly is of serious medical concern, since it predisposes the aorta to dissection, an event in which blood splits the layers of the aortic wall and impairs circulation. This is a potentially catastrophic event with high morbidity and mortality. Several other anomalies are known in MFS: of relevance to this review, affected individuals frequently have hyperextensible joints and thin, stretchy skin. Mice with targeted inactivation of *Fbn1* or knock-in of a MFS mutation (Cys1039Gly) develop mild skeletal overgrowth, aortic aneurysms, valvular thickening, abnormal lungs and osteopenia, but do not have arachnodactyly or ectopia lentis [14–18]. In contrast to MFS in humans, which is an autosomal dominant disorder, *Fbn1* deficiency in mice manifests with equal severity only as a recessive phenotype [14].

*FBN2* mutations give rise to congenital contractural arachnodactyly (CCA, also known as Beals syndrome) [19] (MIM 121050), an autosomal dominant condition. This disorder is characterized by tall stature, kyphoscoliosis, osteopenia and arachnodactyly, but affected individuals very rarely develop cardiovascular problems and do not have ectopia lentis [19]. Instead, they typically have contractures of distal joints and a crumpled helix of the ear. Mice with targeted inactivation of *Fbn2* or naturally occurring *Fbn2* mutations do not show contractures, but

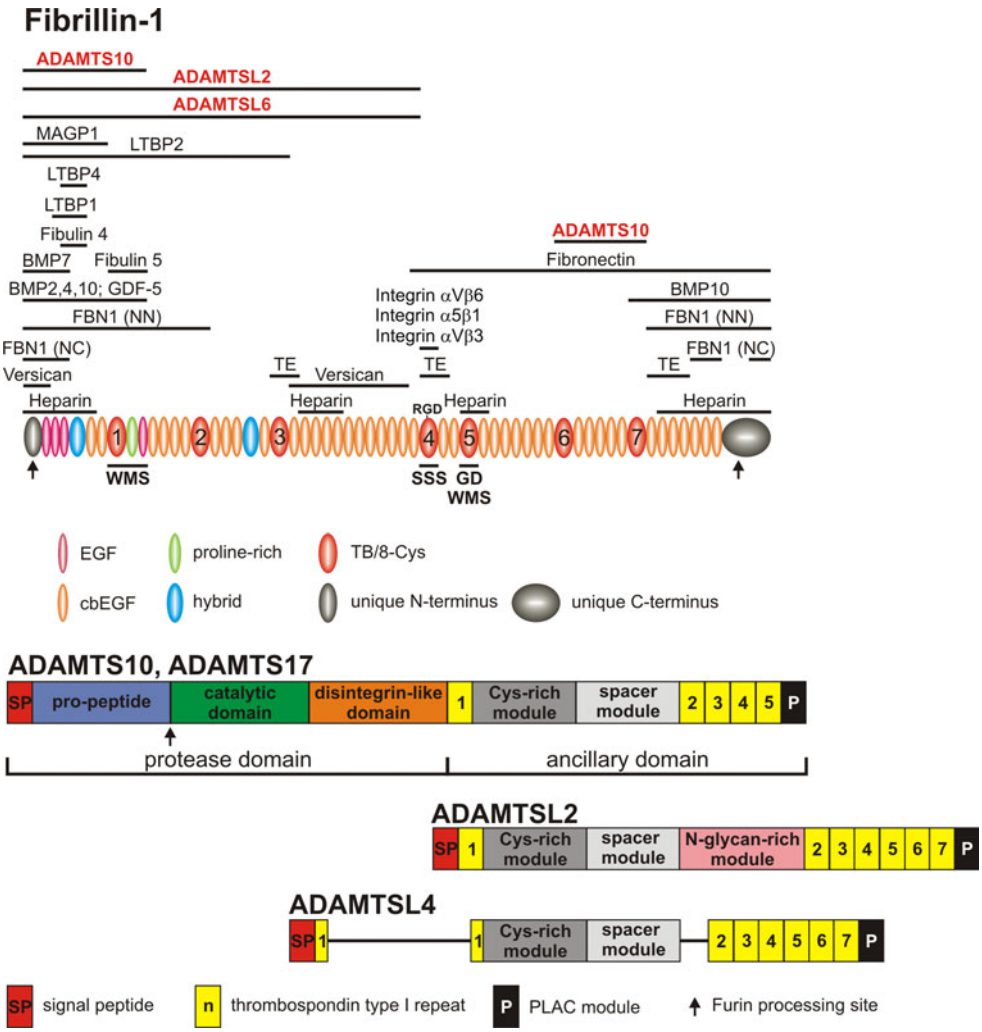


**Fig. 2** Domain structure of fibrillin-1 (*top*) and relevant ADAMTS proteins (*bottom*). Above the fibrillin-1 domain structure are illustrated binding sites for the indicated molecules. The locations of fibrillin-1 mutations identified in WMS, SSS and GD are shown below the fibrillin-1 domain structure. Abbreviations not explained in the text: *TE* tropoelastin, *NN* fibrillin-1 N-terminus to fibrillin-1 N-terminus interaction, *NC* fibrillin-1 N-terminus to fibrillin-1 C-terminus interaction

present a developmental anomaly, syndactyly, or fusion of the digits [20]. Recent work suggested involvement of fibrillin-3 in polycystic ovary syndrome [21, 22], but relatively little is known about this protein.

## Molecular pathology and mechanisms of Marfan syndrome

Mechanistically, disease progression in MFS appears to be the combined result of loss of structural function of microfibrils, altered tissue integrity and perturbed TGF$\beta$ signaling [6]. Loss of tissue integrity can result from impaired microfibril biogenesis (reduced number, poor quality) and/or from excessive degradation. The net result is a general reduction in the amount of functional microfibrils present in ECM. In juveniles or adults with MFS, ophthalmic examination under full pupillary dilatation often reveals broken or stretched zonule fibers [23].

*FBN1* haploinsufficiency is believed to make a significant contribution to the pathogenesis of MFS [24]. Through the use of mouse models of MFS, it became clear that many of the major extra-ocular features of MFS were a consequence of TGF$\beta$ dysregulation [15, 24–26]. TGF$\beta$ is regulated not by altered expression or synthesis of its three isoforms but primarily by activation of latent TGF$\beta$ stored within ECM [27]. However, fibrillins do not bind TGF$\beta$ directly. Fibrillins are closely related to the latent TGF$\beta$-binding proteins (LTBPs), which are involved in the secretion, tissue sequestration and activation of TGF$\beta$ [28, 29]. Together, fibrillins and LTBPs are the only proteins containing TB domains [7]. LTBP1, -3 and -4 bind the small latent complex (SLC), comprising a non-covalent association of the TGF$\beta$ dimer with its cleaved propeptides. The SLC binds to LTBPs to form the large latent complex (LLC), which binds to fibrillin-1 [27] as well as to other ECM molecules. In tissues, LTBP1 and latent TGF$\beta$1 were localized to fibrillin-1-containing microfibrils [30–32]. By interacting with LTBPs, microfibrils indirectly position TGF$\beta$ in the extracellular matrix as a reservoir from which rapid local activation, such as in wound healing, is possible.

LTBP1 and -4, but not LTBP3, interact via their C-terminus with purified fibrillin or with the fibrillin network produced by fibroblasts [31, 33, 34]. LTBPs interact with extracellular matrix components including fibronectin via their N-terminal region, and the interaction is covalently stabilized by transglutaminase cross-links [29]. The C-terminal interactions of LTBP with fibrillin-1 are of lower affinity and are mediated by non-covalent forces [31, 35]. Covalent and non-covalent anchoring in the ECM may be important for the physiological activation of TGF$\beta$, which occurs via various mechanisms including proteolytic cleavage, biomechanical stretching, and non-proteolytic displacement mechanisms proposed for integrins, thrombospondin-1, some fibulins and fibrillin-1 fragments (recently reviewed by [5]).

Demonstration of over-activation of TGF$\beta$ in mouse models of Marfan syndrome led to their treatment with TGF$\beta$ neutralizing antibodies or with losartan, an angiotensin II type 1 receptor blocker that reduces TGF$\beta$ activation [15, 25, 26]. These therapies rescued the phenotypes of mutant mice in the aorta, mitral valves and lung [15, 25, 26], and dramatically changed the prospects for Marfan syndrome, from being considered an untreatable condition caused by loss of a mechanical scaffold to one remediable by drug therapy. Initial analysis of a small patient cohort showed that losartan slowed progression of aortic dilatation [36], and it is undergoing evaluation in several clinical trials [37].

Similar to LTBP2, which does not bind TGF$\beta$, fibrillins are missing critical residues necessary for the binding of the LAP/TGF$\beta$ complex [38]. However, fibrillin-1 binds directly to several bone morphogenetic proteins (BMPs). BMP7 was localized to microfibrillar networks in skin and kidney, and direct interactions of the N-terminus of fibrillin-1 with the propeptides of BMP2, -4, -10, and with growth and differentiation factor GDF5, were established in vitro [39, 40]. In contrast to TGF$\beta$/LTBP complexes, BMPs are bound directly to fibrillin-1 and can be activated by competitive binding to BMP receptor II and activin receptors IIA and IIB [41]. In light of the fact that fibrillins are structurally related to LTBPs, it is possible that they serve essentially as BMP-binding proteins, as LTBPs do for TGF$\beta$.

Genetic validation of BMP regulation by fibrillins came from two sets of experiments. First, mice doubly heterozygous for *Bmp7* and *Fbn2* were shown to develop synpolydactyly, i.e. the phenotype of each null allele, polydactyly and syndactyly, respectively [20]. Second, *Fbn1*- and *Fbn2*-deficient mice were shown to have altered osteoprogenitor differentiation resulting from imbalance of BMP/TGF$\beta$ signaling [16]. Thus, the current view of microfibrils is that they provide a storage and dispensing mechanism for several TGF$\beta$ superfamily growth factors. During embryonic development, expression of fibrillin-2 precedes that of fibrillin-1, but mature microfibrils probably contain both fibrillin-1 and fibrillin-2 since heterotypic interactions have been demonstrated [42, 43]. Although fibrillin-2 is less expressed than fibrillin-1 in mature tissues, it was recently shown to be upregulated during wound healing [44], consistent with the possibility that its expression may be more closely related to the embryonic than adult state of tissues.

Microfibrils form a scaffold for several accessory proteins and form a structural network in ECM potentially comprising tropoelastin, versican, several fibulins, heparan sulfate proteoglycans and microfibril-associated glycoprotein-1 (MAGP1) and MAGP2 [6]. *Magp1* null mice show phenotypes consistent with loss of TGF$\beta$ function, i.e., they contrast with fibrillin-1 deficiency, which results in enhanced TGF$\beta$ signaling [45]. Elastin microfibril interface-located protein-1 (EMILIN-1), which is localized at the interface between microfibrils and the amorphous core of elastic fibers, inhibits TGF$\beta$ signaling by binding specifically to the TGF$\beta$ precursor and preventing its proteolytic maturation via proprotein convertases such as furin [46]. Fibulin-2, -4, -5, LTBP1 and LTBP4 have similar binding sites on fibrillin-1 [47], raising the possibility that the fine tuning of growth factors not only involves the growth factors themselves but a very delicate balance between several microfibril-associated proteins, to which category ADAMTS proteins now belong. In addition, the tissue and developmental stage-specific decoration of microfibrils with different subsets of associated molecules may prime the microfibrils for different tasks in different locations. This concept may help to understand why most of the accessory proteins (such as LTBPs, fibulin-1, fibulin-5, versican and MAGP-1) bind within a relatively confined region near the N-terminus of fibrillin-1, as well as the multifaceted phenotypes observed in various fibrillinopathies (described below).

Through its interaction with the C-terminal globular domain (G3) of the large aggregating proteoglycan versican, fibrillin-1 is connected to another cell-surface network comprising versican, hyaluronan and the hyaluronan receptor CD44 [48, 49]. Intriguingly, the N-terminal globular domain (G1) of versican binds to the N-terminal modules of the TGF$\beta$ activator thrombospondin-1, and fibrillar structures containing thrombospondin-1, versican and elastin were induced during an inflammatory response in aortic smooth muscle cells [50]. Of additional relevance to TGF$\beta$ activation, a recent report suggested that versican was necessary for binding of TGF$\beta$ to ECM in the developing skeleton [51].

In summary, the net function of microfibrils is likely determined by their fibrillin-1/fibrillin-2 ratios, which may vary according to embryonic developmental stage, tissue-specific decoration of microfibrils with accessory proteins,

interaction with other molecular networks in ECM, and the relative local concentrations of different TGF$\beta$ and BMP growth factors. With this plethora of interactions, one can speculate that the variability in the clinical presentation of MFS, even within affected families, could be due in part to differing expression of these interacting proteins, whose genes may act as key modifiers of the MFS phenotype.

## Rare disorders caused by *FBN1* mutations

Intriguingly, although the overwhelming majority of *FBN1* mutations cause MFS, some mutations, including at least one known to cause classic MFS, also lead to distinct, rare, dominantly inherited conditions. Together with MFS, these disorders are sometimes referred to as fibrillinopathies. Of these, four are relevant to this review.

Weill–Marchesani syndrome (WMS), autosomal dominant (MIM 608328) This is a generalized connective tissue disorder characterized by short stature, brachydactyly, thick skin, and stiff joints [52]. These characteristics are conspicuously the opposite of MFS. However, the primary clinical manifestation of WMS is ocular. Specifically, affected individuals develop glaucoma at an early age that eventually severely compromises their vision [53, 54]. In WMS, the lens is spherical, frequently small, and lacks evidence of microfibrils around its equator. Glaucoma results from a shallow anterior chamber, as well as reduced flow of aqueous humor as a result of pupillary block from anterior dislocation of the lens. Cardiac anomalies are present in WMS, but are generally not life-threatening [52]. The published WMS-causing *FBN1* mutation is an in-frame deletion of eight amino acids within exon 41 affecting the TB5 module [55]. A second WMS mutation in *FBN1*, recently reported in abstract form [56], identified an in-frame deletion near the N-terminus that removed the TB1 module, the proline-rich region and EGF-like domain 4.

Isolated (simple) ectopia lentis (IEL), autosomal dominant (MIM 129600) This entity describes dominantly inherited lens dislocation without any other accompanying major anomalies seen in MFS or WMS and may represent the mildest form of the highly variable clinical spectrum of MFS. In the pedigree in whom the first such mutation was detected, several individuals also had skeletal manifestations reminiscent of Marfan syndrome [57]. An Arg240Cys mutation in fibrillin-1 identified in dominant IEL is particularly intriguing because this mutation is also reported to cause classic Marfan syndrome [58].

Geleophysic dysplasia (GD), autosomal dominant Human GD is a severe condition whose autosomal dominant form was recently described [59] and attributed specifically to mutations affecting the TB5 domain of fibrillin-1 (Le Goff et al., Am J Hum Genet, in press) (Fig. 2). A high morbidity and mortality among affected children appears to be a consequence of progressive cardiac valvular disease and tracheal stenosis, which leads to repeated pulmonary infections [60]. GD presents with a characteristic "good-natured" facial appearance, short stature, small hands and feet, thick skin, and progressive contractures of the joints of the extremities, which lead to a characteristic "tip-toe" gait [60]. GD is related to WMS in having short stature, a "pseudomuscular appearance", thick skin and short fingers and toes (thus, these disorders belong to a group called acromelic dysplasias [61]), but lacks ectopia lentis, although ocular anomalies (glaucoma and strabismus) were reported in a significant proportion of cases [59]. Intriguingly, *FBN1* mutations are also responsible for a third member of the acromelic dysplasia group, acromicric dysplasia (MIM 102370), which features severe short stature, a muscular appearance, and characteristic radiographic features, but milder contractures, fewer respiratory complications, no cardioavascular anomalies and a relatively benign course compared to GD (Le Goff et al., Am J Hum Genet, in press).

Stiff skin syndrome (SSS, MIM 184900) The causative *FBN1* mutations in this condition were recently described in a small number of patients and are restricted to the TB4 domain in fibrillin-1, which contains the integrin-binding site [62] (Fig. 2). Affected individuals have short stature, stiff hard skin, and limitation of joint mobility, which have been attributed to altered cell–matrix interactions. One affected individual appeared to have a hybrid phenotype, with short stature and glaucoma additional to stiff skin. Intriguingly, partial *Fbn1* duplication in mice is the mechanism underlying the *Tight skin mouse 1* (*Tsk1*) phenotype, which has been used as a model for scleroderma [63–65]. Indeed, SSS is considered as a form of inherited scleroderma, since several histopathological changes observed in SSS skin were similar to scleroderma skin. These included giant, disorganized microfibrillar aggregates throughout the dermis that retained the ability to bind LTBP4, sparse elastin deposition on abnormal microfibrillar aggregates and electrolucent cores within elastic fibers [62].

## Overview of the ADAMTS superfamily

This superfamily of secreted proteins includes 19 zinc metalloproteases and 7 ADAMTS-like proteins, which lack catalytic activity. A recent review described the major structural characteristics, evolutionary considerations and several functions associated with this superfamily [66]. The ADAMTS repertoire of mammals represents a significant

gene expansion during chordate evolution, displaying neofunctionalization as well as subfunctionalization (within ADAMTS clades arising from duplication of precursor genes) [67]. The duplicated genes with identical domain organization appear to have functional overlap, but operate within different tissue contexts and may also each have unique functions. For example, a single, evolutionarily distinct ADAMTS, ADAMTS13, is required for molecular maturation of von Willebrand factor (an example of neofunctionalization). In contrast, a clade of three highly homologous members, the procollagen amino-propeptidases, is involved in procollagen maturation and a loosely related cluster of seven ADAMTS proteases appears to be involved in proteoglycan processing (examples of sub-functionalization) [66, 68]. These evolutionary relationships between ADAMTS family members are highly relevant to this review. ADAMTS10 and ADAMTS17 share a similar domain structure, but intriguingly, they arose from distinct gene duplication events, since ADAMTS10 is more closely related to ADAMTS6 and ADAMTS17 to ADAMTS19 [68]. Of the seven known ADAMTS-like proteins, four belong to two distinct clades [66], one of which contains ADAMTSL4 and ADAMTSL6, both functionally relevant to fibrillin-1, whereas ADAMTSL2 appears to have evolved as a unique member.

ADAMTS proteases have a characteristic domain organization consisting of a metalloprotease domain at their N-terminus, and a C-terminal ancillary domain (reviewed by [66]) (Fig. 2). The ancillary domain has a characteristic modular organization containing one or more thrombospondin type 1 repeats (Fig. 2). ADAMTS-like proteins resemble the ADAMTS ancillary domain (Fig. 2) but are the products of distinct genes and not the result of alternative splicing of ADAMTS genes. The close structural relationship of ADAMTSLs to ADAMTS proteases initially suggested a potential inhibitor or enhancer relationship. An in vitro study using purified proteins demonstrated a non-competitive inhibition of bovine ADAMTS2 by a *Drosophila* ADAMTSL named papilin [69]. Such a role is not yet supported by genetic evidence. However, it remains possible that an ADAMTSL could bind to similar partners as ADAMTS proteases, and compete with them; alternatively, it could operate in similar pathways or enable the formation of complexes containing other ADAMTS proteins. Post-translational modification has a critical impact on ADAMTS function. ADAMTS proteases are synthesized as inactive zymogens, and cleavage of the inhibitory N-terminal propeptide by proprotein convertases such as furin is necessary for their activation [66] (Fig. 2). In addition to N- and O-glycosylation, which are common in secreted proteins, specific residues in the thrombospondin type 1 repeats, occurring within a highly conserved sequence motif, are modified by *C*-mannosylation and *O*-fucosylation [70–72]. Most ADAMTS proteins undergo C-terminal proteolysis, which may generate ADAMTS fragments with novel bioactivities [73], or modify the function of the full-length protein [74]. Therefore, consideration should be given to three possible functional forms of an ADAMTS protease-the zymogen, mature protease and fragments resulting from degradation.

## Mutations affecting specific members of the ADAMTS superfamily phenocopy or resemble some fibrillinopathies

Since nonsense, mis-sense, and splice mutations are found in these recessive conditions, and appear to lead to an identical phenotype in each instance, it is likely that these conditions result from loss of function of the relevant ADAMTS proteins.

ADAMTS10 mutations cause WMS, autosomal recessive (MIM 277600) and ADAMTS17 mutations lead to a WMS-like syndrome, autosomal recessive (MIM 613195) The first hint of an association between the ADAMTS superfamily and fibrillin-1 came from the identification of *ADAMTS10* mutations in patients with recessively inherited WMS [53, 54]. The clinical pictures of WMS resulting from *FBN1* and *ADAMTS10* mutations were indistinguishable [52], suggesting that the products of these two genes were functionally coupled in a very specific way. A WMS-like syndrome with ocular manifestations and short stature, but without stiffness of the distal joints, brachydactyly, and cardiac valvular anomalies, was reported to result from *ADAMTS17* mutations [75]. *ADAMTS10* was also recently linked to a primary open angle glaucoma in dogs [76]. Several *ADAMTS10* variants were identified, and molecular modeling of one of these mutants, Gly661Arg, predicted disruption of folding of the cysteine-rich module. In biochemical analysis of this variant, it also appeared to be less stable than wild-type protein [76].

ADAMTSL4, IEL, autosomal recessive (MIM 225100), and ectopia lentis et pupillae (ELEP, MIM 225200) Recessively inherited *ADAMTSL4* mutations were linked to both these conditions [77–81]. ELEP is a disorder in which the pupil is displaced, usually in the opposite direction to the subluxated lens. Zonules are present in ELEP and the fibers are generally stretched or disrupted, but it is not clear from published reports whether the zonule is present or lacking in recessive IEL. Like ADAMTSL4, fibrillin-1 is also widely distributed in the eye [8]. Recently, an ADAMTS17 splice mutation was described in dogs of the terrier breeds with IEL (termed primary lens luxation in dogs) [82]. The mutation led to skipping of exon 10 and a frame-shift. Previously, analysis of mature affected dogs

had observed ruptured zonular fibers inserting at the lens equator, suggesting progressive breakdown of the zonule over a prolonged time period [83]. Scanning electron microscopy (EM) consistently showed zonule anomalies, most commonly a cobweb-like network of fibers lying between the ciliary processes [83]. Transmission EM revealed increased complexity of zonular insertions together with hypertrophy of the internal limiting membrane of the unpigmented ciliary epithelium. Within zonule fibers, the fibrils were sometimes disordered [83]. However, no abnormalities in structure, dimensions, or periodicity of microfibrils were seen. Increased intraocular pressure recorded in some affected dogs was thought to be secondary to lens subluxation.

ADAMTSL2, human geleophysic dysplasia (GD), autosomal recessive (MIM 231050) and canine Musladin–Lueke syndrome (MLS) The recessive form of GD is caused by *ADAMTSL2* mutations, which occur throughout the protein [84]. Interestingly, patients with recessive GD had a higher incidence of facial dysmorphism and tip-toe-walking than those with dominant GD [59], a phenotypic heterogeneity in this condition that contrasts with the homogenous clinical picture of dominant and recessive WMS. An as yet unexplained anomaly found in liver biopsies that were done owing to hepatomegaly in GD, was the electron microscopic identification of intracellular inclusions resembling a storage disorder [60]. Although the nature of the inclusions is unclear, it is noteworthy that ADAMTSL2 is a glycoprotein itself, with N- and O-linked glycosylation leading to a substantial increase in predicted molecular mass [85].

Recently MLS, a condition affecting beagle dogs was described; affected beagles are small, have thick hard skin, appear muscular and have severe distal extremity contractures [86]. The condition is caused by a founder mutation (Arg221Cys) that places a cysteine residue in the ADAMTSL2 spacer module, leads to formation of disulfide-bonded ADAMTSL2 dimers, and reduces secretion of the mutant protein [86]. Unlike GD, MLS is not a rapidly progressing disorder and appears to stabilize by about a year of age in affected dogs. Intriguingly, an Arg221Cys mutation was recently described in a patient having recessive GD [59]. Skin histology from an affected dog showed a dense network of collagen spanning the dermis to the subcutaneous tissue [86], although it was not as dense as that reported in SSS [62].

## Cellular and biological mechanisms of ADAMTS proteins in fibrillinopathies

The clinical evidence suggested that ADAMTS proteins interact with fibrillin-1 in some way to contribute to both the structural and regulatory roles of microfibrils. Emerging biochemical evidence suggests that the relevant ADAMTS proteins bind directly to fibrillin-1, providing a starting point for defining the molecular mechanisms underlying each phenotype. Recent work established that ADAMTS10 not only bound fibrillin-1 specifically and with high affinity in vitro, but showed that ADAMTS10 was associated with dermal and zonule fibrillin microfibrils in situ using confocal microscopy and immunoelectron microscopy [87]. Since the primary clinical feature of WMS is ectopia lentis, resulting from loss of fibrillin-1 microfibrils in the zonule, an attractive hypothesis was that rather than being involved in fibrillin-1 degradation, ADAMTS10 likely was involved in microfibril assembly. Indeed, ADAMTS10 cleaves fibrillin-1 inefficiently, since it is activated poorly by furin, whereas conditioned medium containing ADAMTS10 accelerates the assembly of fibrillin-1 microfibrils by fibroblast cultures [87]. Unlike ADAMTS10, ADAMTS17 has an appropriate processing site for excision of its propeptide by furin and is expected to be proteolytically active. It has not yet been expressed and analyzed and, therefore, its potential role in relation to fibrillin-1 has not been studied.

A detailed analysis of ADAMTSL6, to which ADAMTSL4 is closely related, previously provided several important insights; specifically, it was shown that ADAMTSL6 bound to fibrillin-1 microfibrils and enhanced their formation in cultured cells and in tissues of transgenic mice overexpressing ADAMTSL6 [88]. Recently, analysis of fibroblast cultures revealed co-localization of ADAMTSL4 with fibrillin-1 microfibrils [100]. Our recent work demonstrates widespread distribution of ADAMTSL4 in the eye, not only in the ciliary body but also in the trabecular meshwork, iris stroma, cornea, lens and retina [100]. This, taken together with subtle signs of anterior segment dysgenesis in ELEP, strongly suggests that ADAMTSL4 has a broad role in the anterior chamber of the eye, but that possibly owing to redundancy with other ADAMTS proteins, its deficiency is manifest primarily in the zonule and iris. In summary, these observations suggest that, in the eye, ADAMTSL4, ADAMTS10 and ADAMTS17 are involved with formation or maintenance of the zonule. It is also possible that these proteins are involved with proper attachment or insertion of the zonule to either the lens capsule or ciliary body.

On the other hand, organ anomalies and dysmorphism observed as a consequence of ADAMTS10, ADAMTS17 and ADAMTSL2 mutations suggest an influence on the regulatory role of microfibrils. The high levels of active TGF$\beta$, and evidence for increased TGF$\beta$ signaling in GD dermal fibroblasts [84], provides a convincing example of this function. ADAMTSL2 was shown to bind to LTBP1

[84], and, recently, to also bind fibrillin-1 (Le Goff et al., Am J Hum Genet, in press). Therefore, it is possible that ADAMTSL2 has a major, tissue-specific role in sequestering TGF$\beta$ in ECM or regulating its activation, which ADAMTS10 does not, and that ADAMTS10 may be primarily involved in assisting microfibril biogenesis. Taken together, these variable, but nonetheless consistent, relationships with microfibril defects suggest a dual role for ADAMTS proteins in microfibril formation and/or maintenance, as well as in fulfilling regulatory roles of microfibrils, e.g., in growth factor control.

At what points might ADAMTS proteins influence the process of microfibril biogenesis (Fig. 1)? During or immediately following secretion from cells, fibrillin-1 is proteolytically processed by proprotein convertases of the furin/PACE type, to excise its N- and the C-terminal propeptides [89, 90], which may prevent premature assembly in the secretory pathway. This and subsequent steps in fibrillin-1 assembly occur in the immediate proximity of the cell, i.e., at the cell surface or in the pericellular matrix, and indeed, ADAMTS10 and ADAMTSL2 were identified at these locations [85, 91]. Recapitulation of microfibril assembly from purified fibrillin-1 molecules in cell-free systems has not been successful, indicating that cell-derived helper molecules such as proteoglycans, fibronectin, integrins and others have an essential role in the process of microfibril biogenesis. Heparin/heparan sulfate has been shown to act as a potent inhibitor of microfibril assembly in cell culture pointing to an active role for cell-surface-associated proteoglycans in microfibril assembly [92]. The presence of a fibronectin network is indispensable for the formation of microfibrils in cell culture [93, 94]. Binding to fibronectin and/or to proteoglycans may provide a scaffold for fibrillin assembly by bringing together essential interaction domains and generating critical concentrations necessary for further steps in the assembly process. The interactions of fibrillins with heparan sulfate, fibronectin and integrins are likely dynamic in nature and may be designed to transiently "catalyze" and modulate microfibril assembly. ADAMTS proteins may work in this fashion through their direct interactions with fibrillin-1, or they may support the critical function of fibronectin and HSPGs. Understanding of the precise repertoire of ADAMTS interactions and of their interacting domains is at an early stage; the eventual goal should be to define intermolecular interactions and understand the consequences of disease-causing mutations at atomic resolution. Three-dimensional structure has not been determined for either a full-length ADAMTS or an ADAMTSL, but significant insight has been provided by analysis of the catalytic domains of ADAMTS4 and ADAMTS5, and the core ancillary domain of ADAMTS13, the TSR1-cysteine rich module-spacer region [95, 96]. This structural information can be used to model other ADAMTS proteins and together with biochemical analysis of loss-of-function mutants, may provide important insights on molecular mechanisms [76].

## Conclusions and unresolved questions

Collectively, the genetic and biochemical findings constitute a consilience, or a state in which findings obtained from disparate sources and approaches support a single solution. The consilience achieved is that several ADAMTS proteins partner with fibrillin-1 and are essential for fulfillment of its structural and regulatory roles (Fig. 3). These ADAMTS proteins act within a pathway that was unrecognized until revealed by human and animal mutations. Indeed, a significant number of ADAMTS proteins have evolved to operate within fibrillin-1 networks and render them optimally operational. The close evolutionary relationship of ADAMTS10 and ADAMTS17 to ADAMTS6 and ADAMTS19, respectively, suggests that the latter proteins may also be functionally related to fibrillins. ADAMTS6, ADAMTS19 and ADAMTSL6 should be considered as candidate genes for disorders resembling fibrillinopathies.

If all ADAMTS proteins related to fibrillin-1 act only to enhance microfibril biogenesis, then their phenotypes should be similar unless their expression patters are very different. The causative role of *ADAMTSL4*, *ADAMTS17* and *ADAMTS10* mutations in EL suggest that this may be true in the eye, and that these molecules might work cooperatively, i.e., they may have an additive effect on microfibril biogenesis. Alternatively, they might be part of a molecular complex required for zonule formation, such that, if any of them is missing, EL results. However, ADAMTS10 and ADAMTS17 clearly have additional roles in

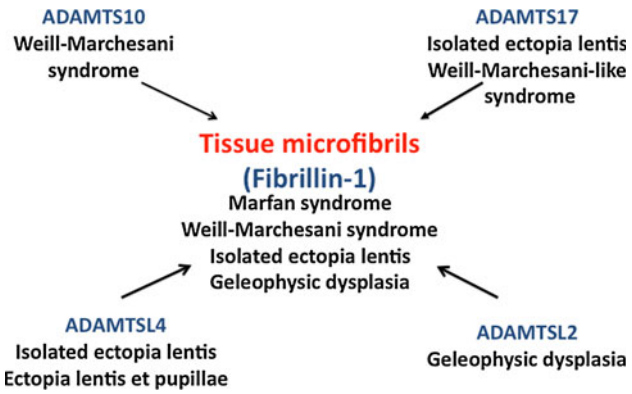


**Fig. 3** Representation of a consilience emerging from human and animal forward genetics that points to a role for ADAMTS10, ADAMTS17, ADAMTSL2 and ADAMTSL4 in the function of microfibrils via interaction with fibrillin-1

other organs, and each ADAMTS protein might build distinct tissue-specific complexes on fibrillin microfibrils.

What is the explanation for inter-species variability of phenotypes resulting from mutations affecting the same ADAMTS gene? *ADAMTSL4* and *ADAMTS17* mutations lead to recessive IEL in humans and canines, respectively, whereas in humans, *ADAMTS17* mutations lead to a WMS-like syndrome. One explanation could be that, since canine defects result from founder mutations (i.e., from a single mutation in all cases because all pure breeds are genetically homogenous) originating many years ago, they could be milder than the various mutations identified in corresponding human genes, some of which would have more severe consequences. For example, the founder MLS mutation in ADAMTSL2 leads to disulfide-bonded dimers, which are secreted, and this phenotype is milder than human GD, suggesting Arg221Cys is a hypomorphic mutation. Arguing against this possibility is the observation of an identical mutation (Arg 221 is highly conserved) in humans, which causes fully-fledged GD. This suggests essential differences in how human and canine physiology have evolved, in how individual ADAMTS proteins are deployed in each species, or in their expression levels relative to each other in different species. Mice lacking ADAMTS10 develop anomalies that only partially recapitulate human WMS (L. Wang, S.S. Apte, unpublished data). Thus, we conclude that while their broad role and relationship to fibrillin-1 is conserved, ADAMTS proteins may work slightly differently in various species.

ADAMTS10, ADAMTS17, and ADAMTSL4 are widely expressed [75, 77, 91, 97, 98], and there is a very strong likelihood, not yet rigorously tested, that the relevant cell types, dermal fibroblasts, valvular interstital cells, vascular smooth muscle cells and unpigmented ciliary epithelium, might express multiple ADAMTS genes. ADAMTS genes could be expressed at different levels in different cell types and give rise to different regulatory influences on microfibrils in the cell environment. This may be particularly true of ADAMTSL2, which appears to have the most limited expression pattern of the fibrillin-related ADAMTS proteins [85], yet its deficiency leads to the most severe phenotype. ADAMTSL2 binding to both LTBP1 and fibrillin-1, and the presence of very high levels of active TGF$\beta$ in geleophysic dysplasia [84] strongly suggest that this protein, but not other ADAMTS proteins, has an important role in 'locking down" TGF$\beta$ in ECM.

Since fibrillin-1 self-assembles to form microfibrils, ADAMTS proteins are probably not a prerequisite for microfibril assembly, but we propose that they might play a supporting role in this process, such as is played by fibronectin and HSPGs. Binding of ADAMTS proteins to fibrillin may provide a scaffold for docking of other proteins on microfibrils, such that context-specific functional assemblies are generated. ADAMTS proteins could bind to the same site on fibrillin-1 or overlapping sites and potentially compete for binding. Alternatively, they might bind cooperatively or independently to fibrillin-1, forming complexes with distinct composition in different cell types or tissues. Finally, they might function in a linear pathway, acting in sequence to enhance microfibril assembly or in parallel, such that their effects are additive. ADAMTS10 or ADAMTS17 could proteolytically modify an ADAMTS-like protein or another molecule bound to fibrillin-1, a possibility that merits investigation. Also requiring investigation are potential interactions of ADAMTS proteins with fibrillin-2 and fibrillin-3 and the mechanisms by which ADAMTS proteins such as ADAMTSL2 influence growth factor availability. These and other key areas will undoubtedly be the subject of much investigation in the future.

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