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The molecular pathogenesis of the Marfan syndrome

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Abstract. The Marfan syndrome (MFS) is an autosomal dominant heritable disorder of connective tissue with highly variable clinical manifestations including aortic dilatation and dissection, ectopia lentis, and a range of skeletal anomalies. Mutations in the gene for fibrillin-1 (*FBN1*) cause MFS and other related disorders of connective tissue collectively termed type-1 fibrillinopathies. Fibrillin-1 is a main component of the 10- to 12-nm extracellular microfibrils that are important for elastogenesis, elasticity, and homeostasis of elastic fibers. Mutations in fibrillin-1 are hypothesized to exert their effects by dominant negative mechanisms, but recent work has also emphasized the potential role of proteases and disturbances in tissue homeostasis in the pathogenesis of the MFS. This article provides an overview of the clinical aspects of the MFS and current thinking on the pathogenesis of this disorder.

Key words. Marfan syndrome; fibrillin-1; microfibril; fibrillinopathy; pathogenesis.

Introduction

The Marfan syndrome (MFS) is an autosomal dominant heritable disorder of connective tissue with highly variable clinical manifestations including aortic dilatation and dissection, ectopia lentis, and a range of skeletal anomalies. Mutations in the gene for fibrillin-1 (*FBN1*) cause MFS and other related disorders of connective tissue, collectively termed type-1 fibrillinopathies [1] that range from neonatal MFS [2] to relatively mild clinical disorders lacking aortic dilatation [3].

Fibrillin-1 is a large (320 kDa) multidomain glycoprotein that is a main component of a class of 10 to 12-nm extracellular microfibrils found in a wide range of tissues both in association with elastin as elastic fibers and as elastin-free bundles. The microfibrils display a 'beads on a string' structure and consist of several distinct proteins in addition to fibrillin, and are thought to be important for elastogenesis, elasticity, and homeostasis of elastic fibers, although the molecular correlates of these functions have yet to be elucidated [4].

The pathogenesis of MFS was initially hypothesized to be due to a dominant negative effect, whereby mutant fibrillin-1 monomers interfere with the polymerization of fibrillin and the assembly of mature microfibrils [5]. More recent work has suggested that fibrillin defects may lead to impaired tissue homeostasis and increase the susceptibility of fibrillin to proteolysis. Each of these three aspects is likely important for understanding the pathogenesis of MFS. This article will provide an overview of the clinical aspects of MFS and the biology of fibrillin and fibrillin-rich microfibrils. The remainder of the review will summarize the evidence supporting each of the three most important theories concerning the molecular pathogenesis of MFS.

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Clinical aspects

MFS is a pleiotropic disorder of connective tissue with an incidence of approximately 1:5000, with about a quarter of cases representing sporadic mutations. Although involvement of the skeletal, ocular, and cardiovascular system is often responsible for the most prominent clinical manifestations, other organ systems may also display abnormalities, including the skin, fascia, lungs, dura, skeletal muscle, and adipose tissue [6].

The ocular features of MFS include myopia, which is often severe, and bilateral ectopia lentis. Affected patients are at risk of retinal detachment. Skeletal abnormalities include elongation of the extremities (dolichostenomelia) and fingers and toes (arachnodactyly), greater than average height, a long and narrow skull (dolichocephaly), scoliosis, and pectus deformities.

The leading cause of premature death in MFS is progressive dilatation of the aortic root and ascending aorta with resultant aortic incompetence and dissection [7]. Mitral valve disease may be the earliest cardiovascular manifestation of MFS, and may progress to cause significant mitral insufficiency in some patients. Progressive dilatation of the aorta generally starts at the aortic root but may extend into the ascending aorta, and the risk of aortic dissection is related to the extent of aortic root dilatation.

Despite recent advances in mutation detection, the diagnosis of MFS still relies primarily on clinical criteria as defined in the so-called Ghent nosology [8]. The phenotypes of affected individuals form a continuum of severity and include many features such as scoliosis or mitral valve prolapse that are relatively common in the general population. Other connective tissue disorders share features with MFS and may give rise to diagnostic dilemmas. The Ghent nosology attempts to address these difficulties by defining major criteria with high diagnostic specificity and minor criteria with less specificity; to make the diagnosis a constellation of findings including major criteria in two organ systems and the involvement of a third organ system are required (table 1).

The average life expectancy of individuals with MFS has risen significantly since 1972 [9], due mainly to improved management of the cardiovascular complications, including beta-adrenergic blockade [10], routine imaging of the aorta, and prophylactic replacement of the aortic root before the diameter exceeds 5.5–6.0 cm [11].

Fibrillin-1

The gene for fibrillin-1 (*FBN1*) spans about 200 kb of genomic DNA on chromosome 15q21.1 and possesses at least 65 exons corresponding to an mRNA transcript of about 10 kb. The protein product of *FBN1*, profibrillin-1, is a ~350-kDa cysteine-rich glycoprotein that undergoes amino- and carboxy-terminal processing to produce fibrillin-1, a major component of the 10 to 12-nm extracellular microfibrils.

Profibrillin-1 contains 2871 amino acids and displays a structure that can be divided into five distinct domains and a signal peptide (fig. 1). Fibrillin-1 consists primarily of repetitive motifs, the most common of which shows homology to the epidermal growth factor (EGF)-like motif and occurs 47 times in fibrillin-1. EGF motifs are approximately 45 amino acid residues long and display six highly conserved cysteine residues that together form three disulfide bonds in a characteristic manner (1–3, 2–4, 5–6). Forty-three of the 47 EGF repeats in fibrillin-1 contain a consensus sequence for calcium binding and are termed calcium-binding EGF repeats (cbEGF).

The consensus sequence for calcium binding can be summarized as x-D/N-x-D/N-E/Q-C_1-x_m-C_3-x-D/N*-x_4-Y/F-x-C_4, where m is variable,* indicates possible beta hydroxylation, and C_n indicates the nth cysteine residue of the cbEGF motif. In addition to the highly conserved residues denoted in the above consensus sequence, the other residues between the third and fourth cysteines display a non-random distribution of amino acids (three to six at any given position) in a sequence alignment of 154 cbEGF motifs, suggesting that these positions are also

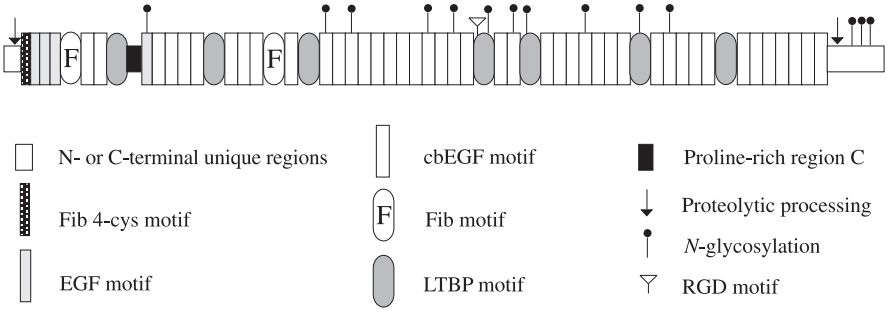

Figure 1. Domain structure of fibrillin-1. Fibrillin can be divided into a unique N-terminal sequence, followed by a stretch of cysteine-rich repeats, a proline-rich sequence, a second stretch of cysteine-rich repeats, and a unique C-terminal sequence.

Table 1. "Ghent" diagnostic criteria for the Marfan syndrome.

Skeletal system	Major criterion	Presence of at least four of the following manifestations: (i) pectus carinatum; (ii) pectus excavatum requiring surgery; (iii) reduced upper to lower segment ratio or increased arm-span to height ratio (>1.05); (iv) positive wrist and thumb signs; (v) scoliosis (>20°) or spondylolithesis; (vi) reduced extension of the elbows (<170°); (vii) medial displacement of the medial malleolus causing pes planus; (viii) protrusio acetabulae of any degree (ascertained on X-ray)
	Minor criteria	(i) Pectus excavatum of moderate severity; (ii) joint hypermobility; (iii) highly arched palate with dental crowding; (vi) Characteristic facial appearance (dolichocephaly, malar hypoplasia, enophthalmos, retrognathia, down-slanting palpebral fissures)
	Involvement	Presence of at least two of the components comprising the major criterion, or one component comprising the major criterion plus two of the minor criteria
Ocular System	Major criterion	Bilateral Ectopia lentis
	Minor criteria	(i) Abnormally flat cornea (as measured by keratometry); (ii) increased axial length of globe (as measured by ultrasound); (iii) hypoplastic iris or hypoplastic ciliary muscle causing a decreased miosis
	Involvement	Presence of at least two of the minor criteria
Cardiovascular system	Major criteria	(i) Dilatation of the ascending aorta with or without aortic regurgitation and involving at least the sinuses of Valsalva; (ii) dissection of the ascending aorta
	Minor criteria	(i) Mitral valve prolapse with or without mitral valve regurgitation; (ii) dilatation of main pulmonary artery, in absence of valvular or peripheral pulmonic stenosis or any other obvious cause, below the age of 40 years; (iii) calcification of the mitral annulus below the age of 40 years; (iv) dilatation or dissection of the descending thoracic or abdominal aorta below the age of 50 years
	Involvement	Presence of at least one of the minor criteria
Pulmonary System	Major criteria	none
	Minor criteria	(i) Spontaneous pneumothorax; (ii) apical blebs (ascertained by chest radiography)
	Involvement	Presence of at least one minor criterion
Skin and Integument	Major criterion	Lumbosacral dural ectasia by computed tomography or magnetic resonance imaging
	Minor criteria	(i) Striae atrophicae (stretch marks) not associated with marked weight changes, pregnancy or repetitive stress; (ii) recurrent or incisional herniae
	Involvement	Presence of at least one minor criterion
Family History	Major criteria	(i) Having a parent, child, or sibling who independently meets these diagnostic criteria; (ii) Presence of a mutation in *FBN1* known to cause the Marfan syndrome; (iii) Presence of a haplotype around *FBN1*, inherited by descent, known to be associated with unequivocally diagnosed Marfan syndrome in the family
	Minor criteria	none
	Involvement	For the family history to be contributory, one of the major criteria must be present

For the index case, major criteria must be present in at least two different organ systems, and a third organ system must be involved as defined in the table. The diagnosis can be made in a family member of a major criterion is present in one organ system, a second organ system is involved, and if the family history is positive for a major criterion [8].

important for calcium binding [12]. Including these consensus sequences and the six cysteine residues, 19 of the on average 42 residues of each cbEGF motif in fibrillin-1 are predicted to have some significance for calcium binding (fig. 2).

The molecular pathogenesis of MFS

To date, no comprehensive theory of the pathogenesis of MFS has emerged that could explain the development of the different disease manifestations in various organ systems and provide a rationale for the observed clinical variability.

The great majority of *FBN1* mutations identified to date have been unique to one affected individual or family. Although there is a clustering of mutations in exons 24–32 [13], disease-associated mutations are distributed throughout *FBN1*. The most frequently observed mutations are missense mutations affecting cbEGF motifs, which can be further classified according to their expected effects on fibrillin structure and function. Mutations of the highly conserved cysteine residues of the cbEGF motifs, or mutations introducing 'extra' cysteines are likely to cause domain misfolding, which in turn may have deleterious effects on the global structure of fibrillin. Mutations affecting residues of the calcium-binding consensus sequence may result in reduced calcium-binding affinity and cause destabilization of the interface between two cbEGF domains [14]. Mutations in cbEGF motifs not affecting cysteines or residues of the calcium-binding consensus sequence are rare, and have been pos-

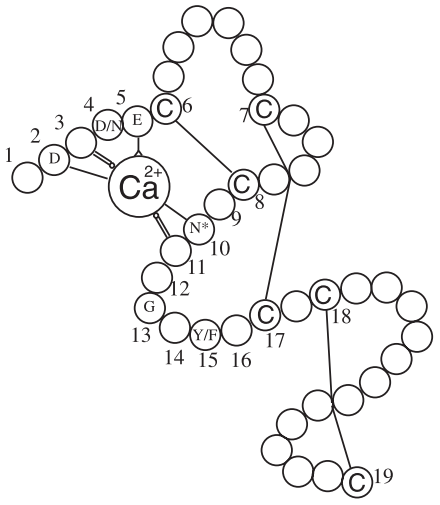

Figure 2. Schematic of a calcium-binding epidermal growth factor-like motif (cbEGF). Residues with putative significance for calcium binding are numbered sequentially [54], and highly conserved amino acids are identified by their single-letter amino acid code. The asparagine residue at position ten is marked with an asterisk to indicate possible beta hydroxylation.

tulated to affect intra- or intermolecular interactions [14]. In addition to missense mutations in cbEGF modules, missense mutations in other motifs, as well as premature truncation codon mutations and mutations associated with exon skipping have also been identified [15].

For a small group of *FBN1* mutations, more or less local consequences of the mutation, such as domain misfolding, reduced calcium affinity, or increased protease susceptibility have been shown. However, how these defects translate into the clinical phenotype of MFS is not known. Below, we provide an overview of current thinking on the molecular pathogenesis of MFS, including the dominant-negative model, disturbances of tissue homeostasis, and the potential effects of *FBN1* mutations on the protease susceptibility of fibrillin.

The dominant negative model of the pathogenesis of MFS

According to the dominant-negative model of pathogenesis, the product of the mutant allele interferes with the function of the wild-type gene product. Large, multiprotein aggregates may be especially sensitive to defects in monomeric components [16]. This model is particularly attractive for MFS, since fibrillin monomers form larger aggregates stabilized by intermolecular disulfide bonds [5, 17].

Several lines of evidence support a dominant-negative model for the pathogenesis of MFS. Premature truncation codon (PTC) mutations due to either a frameshift or nonsense mutation are usually associated with a reduced amount of mutant transcript. Several PTC mutations and the corresponding levels of mRNA expression have been characterized in the fibrillin-1 gene. In one study, the patient with the lowest amount of mutant transcript (6%) had a clinically mild fibrillinopathy termed the MASS phenotype [**m**yopia, **mi**tral valve prolapse, **a**ortic root dilatation without dissection, **s**kin abnormalities (striae), and **s**keletal involvement]. In contrast, another patient with a somewhat higher level of mutant transcript (16%) had classic MFS. The authors postulated on the basis of these observations that the reduced expression of the mutant allele due to PTC mutations leads to a preponderance of normal fibrillin monomers in the microfibrillar aggregates. Below a certain threshold of expression, there is mild disease. If an expression threshold is crossed (between 6 and 16% of wild-type levels), the mutant, truncated peptide disturbs the microfibrillar structure or assembly to such an extent that more severe disease occurs [5]. However, subsequent work suggests that transcript expression level alone is not enough to explain phenotypic severity. A patient with the mutation R529X with mutant transcript levels of 7% [18] and a patient with the mutation R1541X and mutant transcript levels of 2% [19, 20] were both reported to have severe manifestations of MFS. The exact relationship between mutant transcript expression and clinical disease therefore remains to be determined.

A series of studies on dermal fibroblast cultures from MFS patients also provides evidence suggestive of a dominant-negative model of pathogenesis. Fibrillin can be easily labeled with [^{35}S]cysteine owing to its high content of cysteine residues (ca. 14%), and isolated by immunoprecipitation following separation of the cell fraction, the medium, and a fraction representing the extracellular matrix (ECM). Pulse chase experiments in this system allow measurement of the synthesis, secretion, and ECM aggregation of fibrillin [21]. Patients with MFS display a variety of abnormalities in this system [22–25].

Some, but not all, cell cultures from MFS patients produce results consistent with interference by abnormal fibrillin molecules of normal microfibrillar assembly in a dominant-negative fashion, with total fibrillin deposition in the ECM reduced to less than 50% [21]. The formation of fibrillin aggregates is likely dependent on the proper conformation of participating monomers, and the mutant protein product may in some cases interfere with the mechanisms necessary for polymerization.

Aoyama and coworkers [24] analyzed 55 fibroblast strains from patients with MFS. Thirty-seven cell lines demonstrated extracellular fibrillin deposition of less than 35%. Although the causative *FBN1* mutations were not identified for all cell lines, both missense and nonsense mutations were identified in the cell lines showing ECM deposition below 50%. Interestingly, three nonsense mutations associated with expression levels of the

mutant transcript between 15 and 25% demonstrated ECM deposition levels between 7 and 25% of normal, whereas one nonsense mutation with an mRNA expression level of 6% showed ECM deposition of 54%, suggesting that C-terminally truncated products can also exert a dominant negative effect on fibrillin polymerization, but only if the mutant transcript exceeds a certain threshold.

Eldadah and coworkers [26] expressed a mutant allele from an MFS patient in normal human and murine fibroblasts by stable transfection. Pulse chase metabolic labeling and immunohistochemical analysis revealed substantially reduced ECM deposition of fibrillin. These results demonstrated that expression of a mutant fibrillin allele on the background of two normal alleles is sufficient to disrupt normal microfibrillar assembly and to reproduce an MFS phenotype at the cellular level.

There are several potential mechanisms by which a mutant fibrillin monomer could exert a dominant-negative effect [26]. First, the formation of fibrillin aggregates is likely to depend on the proper conformation of participating monomers, and the mutant protein product may in some cases interfere with the mechanisms necessary for polymerization. Second, mutant monomers may be incorporated into microfibrillar aggregates but may then act as a sort of Achilles' heel and destabilize the microfibrils with respect to proteolytic degradation or, third, lead to microfibrillar dysfunction (fig. 3).

Fibrillin-1 mutations and tissue homeostasis

Elastic fibers confer elasticity to connective tissue and are widely distributed; they consist of an amorphous core made primarily of cross-linked tropoelastin monomers surrounded by fibrillin-rich microfibrils. The microfibrils themselves are extensible and likely contribute to the elastic and mechanical properties of the elastic fibers. Although fibrillin-1 has been postulated to play a role in the deposition of elastin to form elastic fibers, results of experiments on *Fbn1*-gene-targeted mice suggest that the primary role of fibrillin-1 may be the maintenance of homeostasis of elastic fibers rather than elastic matrix assembly [27].

Two gene-targeted mouse models for MFS were created in the laboratory of F. Ramirez. In the first model [28], exons 19–24 of the fibrillin-1 gene were replaced with a neomycin-resistance (neo) expression cassette to yield a centrally deleted fibrillin-1 monomer lacking 272 amino acid residues. In addition, the expression of the mutant allele (mgΔ) was reduced by more than ten-fold, a phenomenon probably due to transcriptional interference by

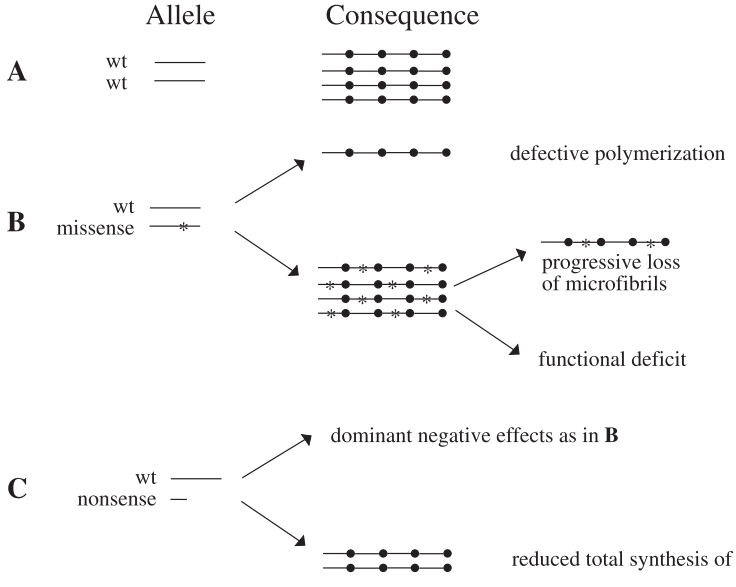

Figure 3. Potential implications of a dominant-negative model for the pathogenesis of Marfan syndrome. (*A*) Wild-type fibrillin monomers polymerize together with other proteins to form microfibrils with their characteristic bead-on-a-string structure. (*B*) Potential consequences of missense mutations. The formation of fibrillin aggregates likely depends on the proper conformation of participating monomers, and the mutant protein product may in some cases interfere with the mechanisms necessary for polymerization. Also conceivable is that mutant and wild-type monomers together form a relatively normal amount of microfibrils, but the resulting microfibrils may display either functional deficits or an increased rate of catabolism. (*C*) Potential effects of nonsense or other premature truncation codon mutations. Nonsense-mediated decay is often associated with reduced expression of the affected allele, so that total fibrillin synthesis is reduced. Alternatively, the truncated product of the mutant allele may interact with the wild-type monomer and cause dominant-negative effects similar to those postulated for missense mutations. The final common pathway of various types of mutation could therefore be a reduction in the amount and function of fibrillin-rich microfibrils.

the neo cassette. Heterozygous (mgΔ/+) mice expressed very low levels of mutant product and were morphologically and histologically indistinguishable from wild-type mice, a finding that appears to be consistent with the postulated dominant-negative model for MFS.

Because of the significantly reduced expression of mutant fibrillin-1, homozygous (mgΔ/mgΔ) mice were expected to produce only small amounts of mutant fibrillin-1. Although these animals appear normal at birth, they die of vascular complications shortly afterwards. Some mice showed thinning of the wall of the proximal aorta, suggesting aneurysmal dilatation not unlike that seen in human MFS. Fibrillin-1 immunohistochemical studies showed a substantial reduction of extracellular fibrillin-1, but a normal amount of elastin staining, suggesting that elastic fibers can accumulate even in the absence of normal fibrillin-1-rich microfibrils. This further suggested that the primary role of fibrillin-1 is tissue homeostasis rather than elastic matrix assembly, and that aortic dilatation may be due to a failure of the microfibril assembly of the aortic adventitia to sustain normal physiological hemodynamic stress, with disruption of the elastic network of the aortic media being a secondary event.

The authors proposed that since the adventitia sustains the bulk of hemodynamic stress, aortic dilatation in MFS may result primarily from loss of tensile strength by the adventitia, in which fibrillin-1 microfibrils may be required to properly organize the primarily collagenous connective tissue.

A second mouse model was accidentally created as a result of aberrant targeting in an embryonic stem cell by the vector that produced the mgΔ allele [29] resulting in the integration of the neo cassette between exons 19 and 18 of the *Fbn1* gene without loss of exonic sequence but with a fivefold reduction in gene expression (mgR). Similar to the mgΔ/+ mice, mgR/+ mice are normal at birth and throughout adult life. Homozygous mgR/mgR animals gradually develop severe kyphosis and die of MFS-like vascular complications at an average age of about 4 months. Histopathologic examination of newborn mgR/mgR mice revealed normal vascular anatomy and architecture including apparently normal elastic lamellae in the aortic media. Fibrillin hypomorphism in these mice appeared to trigger a secondary sequence of cell-mediated events, beginning with focal calcifications in the aortic elastic lamellae as early as 6 weeks of age, and progressing to intimal hyperplasia, monocytic infiltration of the medial layer, fragmentation of elastic lamellae, loss of elastin content, and finally aneurysmal dilation of the aortic wall. In addition, observations on mice carrying various combinations of hypomorphic (mgR) and antimorphic/hypomorphic (mgΔ) alleles suggested there may be a threshold of microfibril level and function, below which aortic dilatation and dissection occur [29].

The role of proteolysis in the pathogenesis of MFS

Calcium binding is essential for microfibrillar organization and integrity [30], causing multiple tandem cbEGF repeats in fibrillin monomers to take on a stabilized, rod-like form [31], and may also influence microfibrillar packing and folding in assembled microfibrils [32]. Calcium protects wild-type fibrillin-1 from proteolysis [33]; similar findings have been obtained for a range of other ECM proteins that contain cbEGF modules, including fibulin-1 and fibulin-2 [34], LTBP-1 [35], and LTBP-2 [36]. On the other hand, *FBN1* mutations can reduce the calcium affinity of cbEGF motifs in vitro [37, 38]. These observations provided the rationale for a series of recent studies that have convincingly demonstrated that *FBN1* mutations can increase the susceptibility of fibrillin peptides to proteolysis [39–42]. Fibrillin has proven to be difficult to express as a full-length molecule, and the above-mentioned experiments were performed on recombinant fibrillin fragments. Results of all studies on this topic published to date are summarized in table 2.

Several observations suggest that *FBN1* mutations increase protease susceptibility by exposing enzyme-specific cryptic cleavage sites. Analysis of proteolytic degradation products associated with the mutations N548I and E1073K revealed different specific sequences for degradation products following incubation in trypsin and chymotrypsin [40]. The protein-engineered mutation E2169K did not increase protease susceptibility to four MMPs, which could be explained if there were no cryptic cleavage site for MMPs exposed by the mutation [39]. In addition, the mutations E2447K [39], K1300E, C1320S [42], as well as D1406G and C1408F (see fig. 4) increase susceptibility to some but not all tested proteases. Finally, a protein-engineered mutation at the site of a cryptic trypsin cleavage site (K1317Q) rendered a peptide carrying the mutation K1300E resistant to proteolysis by trypsin [42].

Degradation patterns of peptides harboring mutations have shown no difference to the degradation patterns of the corresponding wild-type peptides following incubation in EDTA or EGTA [40, 42]. Calcium binding is predicted to introduce a local conformational change in the N-terminal portion of cbEGF domains [14]. Mutations that affect residues of the calcium-binding consensus sequence are therefore predicted to induce conformational changes due to reduced calcium affinity and also, perhaps, to the amino acid substitution itself. Mutations affecting the calcium-binding consensus sequence or one of the six conserved cysteine residues could therefore increase protease susceptibility by reducing steric hindrance for proteases to reach sensitive sites.

The effects of a given missense mutation in a cbEGF motif on protease susceptibility appear to depend on several factors. First, there must be a cryptic cleavage site for a

Table 2. Effects of *FBN1* mutations on protease susceptibility of recombinant fibrillin-1 fragments.

Mutation	Exon/module	Expression construct	Effect of mutation on protease susceptibility	Reference
E2447K	exon 59 (cbEGF38)	exon 50–65	MMP12 ↑ MMP13 ↑ MMP2 ± MMP9 ±	[39]
E2169K	exon 52 (cbEGF32)	exon 50–65	MMP2 ± MMP9 ± MMP12 ± MMP13 ±	[39]
K1300E	exon 31 (cbEGF17)	exon 29–34	trypsin ↑ CT ± PE ± HLE ±	[42]
C1320S	exon 31 (cbEGF17)	exon 29–34	trypsin ↑ CT ↑ PE ± HLE ±	[42]
D1406G	exon 34 (cbEGF20)	exon 32–36	CT ↑ trypsin ± PE ± HLE ±	Booms and Robinson, [unpublished data] (see fig. 4)
C1408F	exon 34 (cbEGF20)	exon 32–36	CT ↑ trypsin ± PE ± HLE ±	Booms and Robinson, [unpublished data] (see fig. 4)
N548I	exon 13 (cbEGF4)	exon 11–24	trypsin ↑ CT ↑ plasmin ↑	[40]
E1073K	exon 26 (cbEGF12)	exon 23–36	trypsin ↑ CT ↑ Glu-C ↑ plasmin ↑	[40]
N2144S	exon 52 (cbEGF32)	exon 50–52	trypsin ± PE ± plasmin ±	[41]
N2144S	exon 52 (cbEGF32)	exon 52–53	trypsin ± PE ± plasmin ±	[41]
N2183S	exon 53 (cbEGF33)	exon 52–53	trypsin ↑ PE ↑ plasmin ↑	[41]

↑ Constructs haboring the mutation significantly more susceptible to in vitro proteolysis by protease indicated (MMP, matrix metalloproteinase; CT, chymotrypsin; PE, pancreatic elastase; HLE, human leukocyte elastase); ± no difference in protease sensitivity compared with wild-type construct.

specific protease within the region that is structurally altered by the mutation. Second, the particular mutated residue within the affected cbEGF motif is likely to be important, and different mutations within a given cbEGF motif can have different effects on protease susceptibility [42]. Finally, the domain context of the mutated cbEGF motif is important [41]. Whereas binding of calcium by one cbEGF module can be influenced by calcium binding to other tandem cbEGF modules [43], covalent linkage of the sixth LTBP motif of fibrillin to cbEGF32 did not significantly alter the calcium-binding properties of cbEGF32 [44, 45]. This may explain why a mutation in cbEGF32 within a construct consisting of the LTBP6-cbEGF32 pair did not differ from the corresponding wild-type construct with respect to protease susceptibility [41].

The relevance of these findings concerning in vitro proteolysis of fibrillin fragments for the pathogenesis of MFS remains unclear. One study reported increased immunofluorescence for MMP-2 and MMP-9 in surgical thoracic aortic aneuryms of patients with MFS, with immunoreactivity being especially strong at the edges of areas of cystic medial necrosis, the hallmark histopathological lesion of MFS [46]. However, knowledge con-

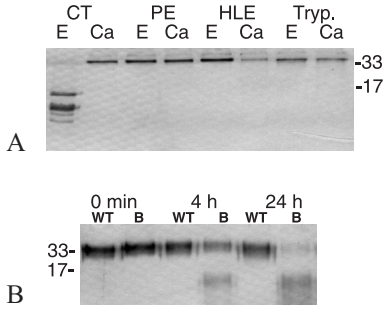

Figure 4. Analysis of the recombinant fibrillin construct rFib34^{wt}, which was designed to correspond to the five cbEGF modules encoded by *FBN1* exons 32–36 (amino acid residues 1323–1530) and was produced and tested as previously described [42]. (*A*) Influence of calcium on the protease susceptibility of rFib34^{wt}. Aliquots of (20 µl) recombinant peptide (ca 0.4 mg/ml) were incubated in the presence of 5 mM $CaCl_2$ (Ca) or 5 mM EDTA (E) for 20 min at room temperature. Trypsin (Tryp.), chymotrypsin (CT), pancreatic elastase (PE), and human leukocyte elastase (HLE) were added at an enzyme:substrate ratio of 1:80 (w/w). Samples were incubated at 37 °C for 16 h. The molecular masses of globular marker proteins are indicated in kDa to the right of the gel. (*B*) Influence of FBN1 mutations D1406G and C1408F on the protease susceptibility of rFib34. Two mutant constructs were produced by in vitro mutagenesis as previously described [42]. The mutations were found in individuals with manifestations of classic MFS [13]: patient D13 carried a mutation of the first cysteine residue of cbEGF20 (exon 34), C1408F (G4223T), and patient D36 carried a mutation at position 4 of the calcium-binding consensus sequence of cbEGF20, D1406G (A4217G). The corresponding constructs rFib34^{C1408F} and rFib34^{D1406G} were compared to the wild-type construct rFib34^{wt} (wt) with respect to susceptibility to proteolytic digestion by trypsin, chymotrypsin, PE, and HLE following incubation in calcium. rFib34^{C1408F} (*B*) displayed significantly increased susceptibility to proteolysis by chymotrypsin beginning at 4 h, but there was no observable effect upon susceptibility to trypsin, PE, or HLE (data not shown). Similar results were obtained for the construct rFib34^{D1406G} (not shown).

cerning the full range of proteases that may be involved in MFS and the early events involved in initiating progressive loss of microfibrils is lacking. In contrast, disturbances of MMP expression have been convincingly shown to play an important role in the pathogenesis of acquired abdominal aortic aneurysm (AAA). Local production of MMP-12 (macrophage elastase) is elevated in AAA tissues with distinct localization to residual elastic fiber fragments [47]. Additionally, the development of AAA in a Wistar rat model can be prevented by pharmacological MMP inhibition [48].

Conclusions and future directions

Significant progress has been made in understanding the pathophysiology of MFS since the discovery of fibrillin in 1986 [49] and the identification of the first *FBN1* mutation in an individual with MFS in 1991 [50]. Current thinking has emphasized three potentially complementary models for the pathogenesis of MFS. Many observations favor a dominant-negative model for the pathogenesis of MFS. Not known is whether *FBN1* mutations cause a reduction in fibrillin-rich microfibrils in individuals with MFS due to polymerization defects or if mutant fibrillin monomers act as a kind of Achilles' heel within microfibrils, leading to a progressive loss of microfibrillar structure and function. Observations on *Fbn1*-targeted mice suggest that a reduction in levels of fibrillin-1-rich microfibrils may trigger secondary events which in turn lead to a progressive loss of microfibrils and other components of the aortic wall; once a critical threshold is reached, aortic dilatation and dissection may occur [29]. We have speculated that proteolytic degradation products may trigger a type of vicious cycle by disturbing a hypothetical outside-to-inside feedback mechanism [42]. Fragments of ECM molecules can possess signaling properties that differ from those of the corresponding intact molecules. For example, in osteoarthritis, fibronectin fragments are present at increased concentration, and are able to up-regulate the expression of several MMPs by up to severalfold; increased MMP concentrations could possible cause continued damage of the matrix [51]. We have speculated that the production of low levels of proteolytic breakdown products of fibrillin or other microfibrillar components owing to *FBN1* mutations may be an early event in the pathogenesis of MFS [42]. These degradation products could in turn lead to increased MMP expression by cells such as medial smooth muscle cells of the aorta, which in turn could accelerate proteolytic degradation. Altered biomechanical properties of the aorta [52] could conceivably also contribute to altered gene expression patterns in the aorta. Finally, although the hallmark lesion of MFS in the aorta, cystic medial necrosis, is generally not associated with a pronounced inflammatory component [53], the role of cytokines and other inflammatory mediators in human MFS remains to be elucidated (fig. 5). Identification of the proteases likely to be involved in the pathogenesis of MFS and the factors that initiate their expression in tissues such as the aorta, and of other factors contributing to the progressive aortic dilatation often

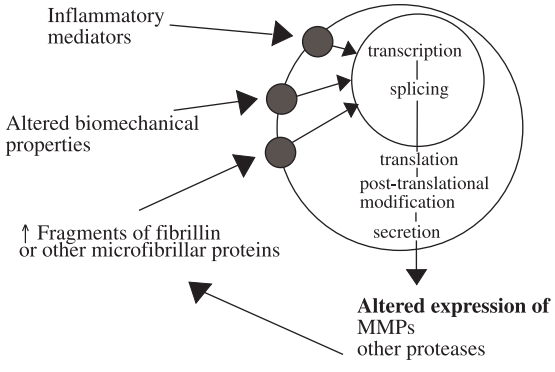

Figure 5. Hypothetical disturbance of feedback loops could lead to a progressive breakdown of microfibrils in tissue.

seen in individuals with MFS will represent an important step toward the development of new therapeutic strategies for this disorder.

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