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# Forum Review

# Fibrillin-1 Misfolding and Disease

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## ABSTRACT

**Fibrillin-1 is a 350 kDa calcium-binding protein which assembles to form 10–12 nm microfibrils in the extracellular matrix (ECM). The structure of fibrillin-1 is dominated by two types of disulfide-rich motifs, the calcium-binding epidermal growth factor-like (cbEGF) and transforming growth factor β binding protein-like (TB) domains. Disruption of fibrillin-1 domain structure and function contributes to the pathogenic mechanisms underlying two inherited diseases with very different etiologies: Marfan syndrome (MFS) and homocystinuria (HC). MFS is a connective tissue disease caused by mutations in the fibrillin-1 gene *FBN1*. Many missense mutations cause variable degrees of fibrillin-1 domain misfolding, which may affect the delivery of fibrillin-1 to the ECM and/or its assembly into microfibrils. HC is a metabolic disorder which affects methionine metabolism and results in raised serum levels of the highly reactive thiol-containing amino acid homocysteine. Patients with HC often exhibit ocular and skeletal defects resembling the MFS phenotype, suggesting that elevated homocysteine levels may lead to chemical reduction of disulfide bonds within fibrillin-1 domains resulting in the loss of native structure. Protein misfolding therefore is implicated in pathogenic mechanisms underlying MFS and HC.** *Antioxid. Redox Signal.* 8, 338–346.

## INTRODUCTION

Fibrillin-1 is a large (350 kDa) extracellular matrix (ECM) glycoprotein which forms the major structural component of 10–12 nm microfibrils in the ECM (42). Microfibrils adopt a $Ca^{2+}$-dependent architecture which has a "beads on a string" appearance when viewed by rotary shadowing electron microscopy (Fig. 1) (7, 20, 22). A large number of cell-matrix components have been identified which interact with fibrillin or microfibrils which include elastin (41), latent transforming growth factor β-binding proteins (LTBPs) (17), fibulin-2 (38), versican (16), MAGP-1 (18), MAGP-2 (31), and αVβ3, α5β1 integrins (5, 26, 33, 43). Microfibrils confer specific biological and biophysical properties on the ECM which include elastic fiber homeostasis, structural integrity and, though their association with the LTBP family, TGF-β storage (for review, see Ref. 36).

Fibrillin-1 has a modular organization and is dominated by 47 epidermal growth factor-like (EGF) domains interspersed with seven transforming growth factor β binding protein-like (TB) domains (Fig. 2a) (32). Forty-three of the EGF domains have a calcium binding (cb) sequence $(D/N)X(D/N)(E/Q)X_m(D^*/N^*)X_n(Y/F)$ where m and n are variable numbers of residues and * indicates a possible β-hydroxylation site (14, 37, 27). High resolution structures of cbEGF domains have identified a major and a minor anti-parallel β hairpin stabilized by three disulfide bridges organized in a 1–3, 2–4, 5–6 arrangement (Fig. 2b) (9, 12). Calcium coordination is via a pentagonal bipyramid arrangement of ligands, which may include up to seven intradomain oxygen ligands (26, 35). A calcium ion bound in the interdomain region and a conserved hydrophobic packing interaction both play a key role in stabilizing cbEGF-cbEGF domain interactions (12, 46). Analysis of different cbEGF domain pairs and triple domain fragments have identified a range of $K_d$ values for $Ca^{2+}$ from 350 μ*M* to 300 n*M*, measured under physiological conditions of $I = 0.15$ and pH 7.5 (23, 47). Under physiological conditions of $[Ca^{2+}]_{free}$, most calcium binding sites would be expected to be saturated. As a consequence, flexibility of the tandemly repeated cbEGFs is restricted and a rod-like appearance con-

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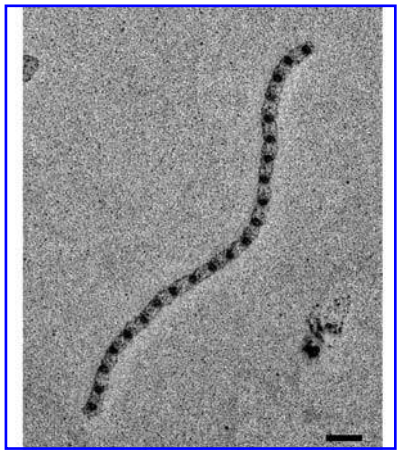

**FIG. 1. A fibrillin microfibril extracted in the presence of $Ca^{2+}$** and viewed by rotary shadowing electron microscopy. Scale bar =100 nm. Note the extended appearance of the fibril and the conserved bead to bead distance.

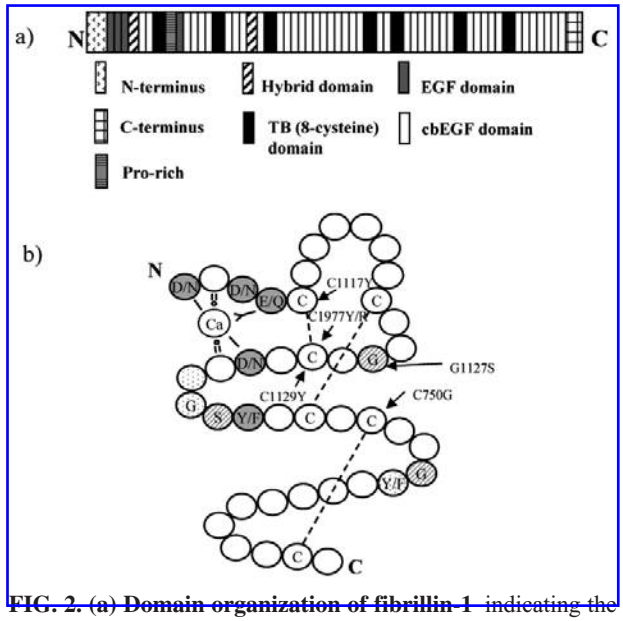


**FIG. 2. (a) Domain organization of fibrillin-1** indicating the multiple tandem repeats of cbEGF domains. (**b**) Schematic diagram of a single cbEGF domain showing the 1–3, 2–4, 5–6 disulphide bond organization and the location of the calcium binding site in the N-terminal region of the domain. Calcium-binding consensus residues are shaded in *gray*. Residues which form the cbEGF-cbEGF hydrophobic packing interaction are shown with *stippled shading*, other conserved residues are *shaded*. Missense mutations referred to in the text are indicated.

ferred to these regions within fibrillin-1. Calcium binding to these regions also confers resistance to proteolysis.

The TB domain has a novel fold comprising six antiparallel β-strands and two α-helices and is stabilized by four disulfide bonds and hydrophobic interactions (54). Six out of seven TB domains are covalently linked to cbEGF domains (Fig. 2a). Analysis of the calcium-binding properties of six TB-cbEGF pairs from fibrillin-1 shows that a wider variation in calcium binding affinity, from n*M* to m*M*, exists than for tandem repeats of cbEGF domains (19, 21). Further NMR studies indicate that different pairwise interactions between these domains modulate affinity (19). As for cbEGF domain pairs, most sites, with the exception of TB6-cbEGF32, would be saturated under physiological conditions and, as a consequence, protected against proteolysis. Furthermore, many protein–protein interactions mediated by fibrillin-1 are calcium dependent, consistent with the $Ca^{2+}$ playing a key role in stabilising intramolecular structure.

From the high resolution studies of fibrillin domain fragments performed so far, a homology model of cbEGF domains 11-TB5 has been constructed which shows an extended structure with a kink introduced by packing of cbEGF22 against TB4 (26).

## MARFAN SYNDROME AND SUBSTITUTIONS WHICH AFFECT DOMAIN FOLDING

Mutations in the fibrillin gene (*FBN1*) give rise to Marfan syndrome (MFS) and related disorders (11). Fibrillinopathies affect connective tissue and give rise to pleiotropic manifestations in the ocular, skeletal, and cardiovascular systems of the body (34). These include lens dislocation (ectopia lentis), tall thin physique, spinal curvature, pectus deformities, joint hypermobility, and dilation and dissection of the aorta. Inter- and intrafamilial variability in disease presentation is prevalent and diagnosis is made according to strict clinical criteria (10). In pulse-chase analyses of endogenous fibrillin-1 expressed in MFS fibroblasts, a variety of defects in synthesis, trafficking, and incorporation into the ECM can be seen, suggesting that different pathogenic mechanisms contribute to the disease (1–3, 13, 44). All are predicted to result in an impairment of microfibril function, either though a reduction in the amount of fibrillin produced from the cell, inhibition of microfibril assembly, or production of abnormal microfibrils.

Currently more that 500 mutations have been identified in *FBN1* (8). 60% of these are missense mutations. Within this group >40% of mutations either create or substitute one of the six conserved cysteine residues of the cbEGF domain (Fig. 2b). Since EGF domains do not possess a hydrophobic core, the disulfide bonds stabilize the fold of the cbEGF domain and therefore one would expect that the removal or addition of cysteine at these key positions within the domain would cause domain misfolding. A number of structural and functional investigations have recently revealed that this subgroup of mutations may lead to considerable structural heterogeneity with variable effects on the biosynthesis and trafficking of fibrillin-1. As a consequence the pathogenic mechanisms underlying this group of mutations are likely to be diverse (47, 48, 53).

## STRUCTURAL CONSEQUENCES OF CYSTEINE SUBSTITUTIONS IN FIBRILLIN cbEGF DOMAINS

Very few detailed studies have been performed which identify the effects of cysteine substitutions on fibrillin cbEGF domain structure. The extent of the effect is likely to

depend on the structural organization of the region affected and the particular disulfide bond affected. Methods which have been used recently to probe the effects of cysteine substitutions include limited proteolysis, 1H-NMR, and calcium chelation.

## *Limited proteolysis*

Previously, it was shown by comparative digestion of wild-type cbEGF domain fragments performed either in $Ca^{2+}$ or EGTA/EDTA that $Ca^{2+}$ greatly reduced the susceptibility of the protein to proteolysis (4, 6, 29, 39, 40, 47, 48, 52). This method was subsequently exploited to probe the structural effects of amino acid substitutions that cause MFS. Comparative analysis of wild type and mutant fibrillin domain fragments by SDS-PAGE, following limited proteolysis performed in the presence of $Ca^{2+}$, revealed enhanced digestion of the mutant fragments caused by the exposure of enzyme-specific cleavage sites. The identification of cleavage sites within a fragment by Edman degradation and subsequent mapping of these sites onto a three-dimensional model of the fragment yielded further information about the extent of the structural effect. These methods were used initially to identify the structural consequences of calcium binding substitutions in cbEGF domains (29, 40), and subsequently to identify the effects of other substitutions within the cbEGF domain (6, 48, 52). More recently these methods have been used to examine specific cysteine substitutions associated with MFS.

Two pathogenic mutations which result in amino acid changes C1977Y and C1977R in cbEGF domain 30 of fibrillin-1 were examined in a recombinant triple cbEGF domain fragment (cbEGF29–31) (Fig. 3a) (47). These substitutions affect the 1–3 disulfide bond which is in close proximity to the calcium binding site within the domain. Comparative tryptic digestion of wild-type and mutant fragments in the presence of $Ca^{2+}$ revealed greatly increased proteolytic susceptibility in the mutant fragments (Fig. 4). Following HPLC purification of digested material under nonreducing conditions and Edman degradation, two cleavage sites were revealed in the mutant domain 30 (Fig. 3a). Interestingly, a cleavage site in domain 31 that was revealed in the presence of EGTA in both mutant and wild-type fragments was fully protected in both fragments. This strongly suggested that although C1977R/Y had destabilized domain 30, the structural effects were not transmitted to domain 31. Limited proteolysis experiments had thus demonstrated that cysteine substitutions may be relatively localized in their effects. A limitation of theses studies is that the information that can be derived depends entirely on the distribution of cleavage sites within the fragment. Since this is sequence dependent, fragments may show an unequal distribution of sites. In the case of 29–31, no cleavage sites were present in cbEGF29 and therefore the effect of the cysteine substitutions on this domain had to be determined by other methods.

## *$^1$H-NMR*

One- and two-dimensional $^1$H-NMR analyses have previously been used to probe the structure and calcium binding properties of wild-type and mutant cbEGF-containing fragments (12, 21, 23, 45, 46, 49, 50–52). Initial inspection of such data can give insight into the degree of disruption introduced by a given substitution. In the case of cbEGF29–31, the presence of upfield shifted methyl resonances and downfield shifted $H^{\alpha}$ resonances characteristic of β-sheet suggested there were folded domains within the fragments (47). Further analysis of spectra on addition of $Ca^{2+}$ gave domain-specific information about the fold of the protein fragments. In these

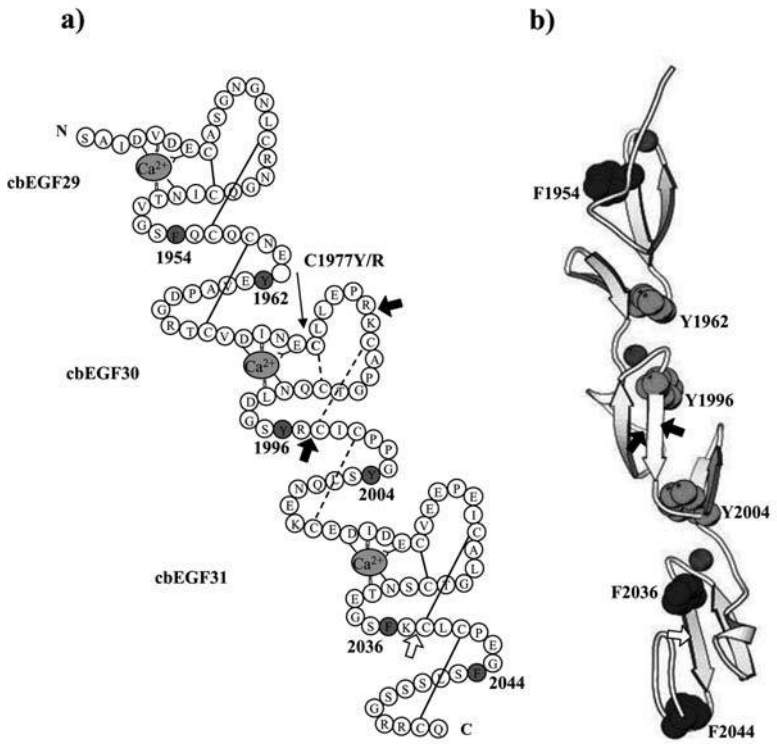


**FIG. 3. (a) Schematic diagram of a triple cbEGF domain fragment** (cbEGF29–31). A potential arrangement of $Ca^{2+}$ ligands is shown based on an earlier cbEGF domain structure, but has not been determined for this fragment. C1977 referred to in the text is indicated. Tryptic cleavage sites are shown. The *white arrow* indicates the site in cbEGF31 which is protected in mutant and wild-type fragments in the presence of $Ca^{2+}$. *Black arrows* indicate sites in the mutant fragments which lose protection. Aromatic markers used in NMR experiments for calcium binding measurements are *shaded* and *numbered*. (**b**) A three-dimensional model of cbEGF29–31 showing location of aromatic markers relative to each calcium binding site within the fragment. This figure was rendered from MOLSCRIPT (24, 28).

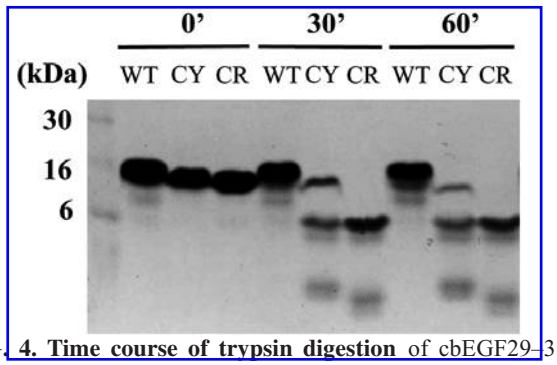


**FIG. 4. Time course of trypsin digestion** of cbEGF29–31 wild-type (WT), C1977Y (CY), and C1977R (CR) in 50 m*M* $CaCl_2$. Note the increased level of proteolytic degradation in the CY and CR fragments over time compared to the wild-type.

experiments the calcium-binding consensus aromatic residue (F1954, Y1996, and F2036 in domains 29, 30, and 31, respectively) was used to monitor intradomain calcium binding, while the aromatic residue located towards the C-terminus of the domain (Y1962 and Y2004 in domains 29 and 30) , which forms the hydrophobic packing interaction was used to monitor calcium binding to the following domain (Figs. 3a and 3b). For both wild-type and mutant cbEGF29–31 fragments, spectral changes were identified in low and high concentrations of $Ca^{2+}$, indicating the presence of low and high affinity sites. Assignment of aromatic residues within 2D-spectra gave domain specific information for the mutant and wild-type fragments. A peak assigned to F1954 in cbEGF29 (Fig. 3) was observed to shift at $Ca^{2+}$ concentrations up to 50 m*M* consistent with low affinity calcium binding to this domain in the wild-type and mutant fragments. Y2004 in cbEGF30, which acts as a marker for calcium binding for cbEGF31, was observed to shift at low concentrations of $Ca^{2+}$ suggesting that high affinity binding to cbEGF 31 is maintained in all three constructs. However, no calcium-dependent shift of Y1996 in domain 30, the marker for intradomain $Ca^{2+}$ binding, was observed in the mutant constructs nor was there a calcium-dependent shift observed for Y1962, the hydrophobic packing interaction residue and marker of $Ca^{2+}$ binding to domain 30. Collectively, these data suggest that the Cys substitutions disrupt the structure of domain 30, and the presence of multiple cross peaks for Y1996 suggest that this domain exists in multiple conformations in the mutant fragments.

### *Calcium chelation*

Whereas NMR analyses identified low and high affinity calcium binding properties of cbEGF domains within mutant and wild-type 29–31 fragments, slow exchange behavior of peaks characteristic of high affinity calcium binding made it difficult to derive quantitative $K_d$ information for these sites (47). To complement such studies calcium titrations were performed using chomophoric chelators such as 5,5-$Br_2$BAPTA. In these experiments calcium–free solutions of proteins are titrated with a $Ca^{2+}$ stock buffer in the presence of chelator which absorbs at $A_{263}$ (19, 47). As $Ca^{2+}$ binds to the chelator the $A_{263}$ value is lowered. A comparison of chelator only and chelator + protein curves gives an indication of the relative affinity of the protein for $Ca^{2+}$. $K_d$ values and number of sites can be derived using least squares fitting to the data. For wild-type cbEGF29–31, two high affinity sites were identified with $K_d$ values of ~0.3 and 3.7 μ*M*. These were assigned to domains 31 and 30, respectively, based on the calcium sensitivity of aromatic residues identified in NMR experiments. Both mutant fragments were found to have one high affinity site only, which was assigned to cbEGF31 based on NMR and limited proteolysis data.

In summary, a combination of biochemical and biophysical methods can be used to identify the local structural consequences of substitutions which cause domain misfolding. In the case of cbEGF29–31, although the two pathogenic cysteine substitutions caused intradomain misfolding and loss of calcium binding to domain 30, cbEGF29 and cbEGF31 appeared unaffected with the effects of the substitution confined to the N-terminal end of cbEGF30. However the structural effects of this large group of disease causing mutations are likely to be heterogeneous and influenced by the precise disulfide bond affected. A recent study by Vollbrandt et al., demonstrated that a substitution which affects the 5–6 disulfide bond of cbEGF7 (C750G) caused increased proteolysis in cbEGF8, and thus has a C-terminal effect (48). Future studies on cbEGF29–31 fragments with cysteine substitutions designed to disrupt the 2–4 and 5–6 disulfide bonds of cbEGF30 should reveal the extent of structural perturbation to fibrillin-1 associated with misfolding.

## STRUCTURAL CONSEQUENCES OF OTHER FOLDING SUBSTITUTIONS

Other missense mutations which affect key structural residues within the cbEGF domain have been studied. The structural consequences of a G1127S substitution in cbEGF13 were investigated by NMR and limited proteolysis (51, 52). G1127 is located in a turn at the end of a two-stranded β-sheet and substitution by another less flexible residue might be expected to interfere with domain folding. In the isolated domain, G1127S appeared to disrupt folding since the HPLC profile of the oxidised domain was heterogeneous, compared to a single peak observed for the wild-type domain. When examined in a cbEGF12–13 or 12–14 fragment, the effect of the G1127S substitution was less marked. Both limited proteolysis and NMR studies indicated that domain 13 retained the ability to bind $Ca^{2+}$ and a native-like fold was preserved. Recent calcium chelation studies of cbEGF12–13 and 12–14 G1127S constructs has confirmed that the affinity of domain 13 is slightly reduced compared to the wild-type domain, but flanking domains are unaffected (unpublished data).

## CELLULAR EFFECTS OF MISFOLDING SUBSTITUTIONS ON TRAFFICKING

The effect of cysteine substitutions on fibrillin-1 biosynthesis, processing, and matrix deposition have revealed heterogeneous effects. Most studies have reported normal synthesis but delayed secretion leading to reduced matrix deposition (1–3, 13, 44). However in some cell lines, a normal secretion profile has been reported. These studies are hampered by an inability to discriminate be-

tween the wild-type and mutant fibrillin-1, both of which are co-expressed in the patient fibroblast cells examined. In order to identify more clearly the fate of mutant fibrillin-1, a recombinant expression system has been developed using a fibroblast host cell (53). A cbEGF11–22 fragment covalently linked to the N-terminal region of fibrillin-1 (NterPro-cbEGF11–22) was successfully expressed in a human fibroblast line MSU-1.1 and shown to be secreted into the extracellular media. Its smaller mass (~100 kDa) allowed it to be easily distinguished from full length endogenous fibrillin when protein samples from cell lysates and extracellular media were examined by SDS-PAGE and Western blotting. A mutant fragment containing the G1127S substitution showed normal synthesis and secretion, and suggested an extracellular dominant negative effect of the mutant protein. This is consistent with the localized structural effects of G1127S and the normal synthesis and secretion profile observed in pulse-chase studies of MFS fibroblasts expressing G1127S. In contrast, mutant fragments containing cysteine substitutions associated with classic MFS, C1117Y and C1129Y in cbEGF13, were retained inside the cell (Fig. 5). This suggests that the pathogenic mechanism underlying these substitutions is either one of haploinsufficiency or a dominant negative intracellular effect of the mutant protein. Furthermore the mutant fragments containing cysteine substitutions undergo core, but not complex glycosylation, suggesting they are retained in the endoplasmic reticulum. This is consistent with abnormal protein folding. Further recombinant analysis of cysteine substitutions that do not appear to cause trafficking defects in MFS fibroblasts will identify the full spectrum of effects that these misfolding mutations may have on the secretory pathway. A combination of structural and cellular studies should enable the mechanisms which govern misfolding and the effects on trafficking to be identified.

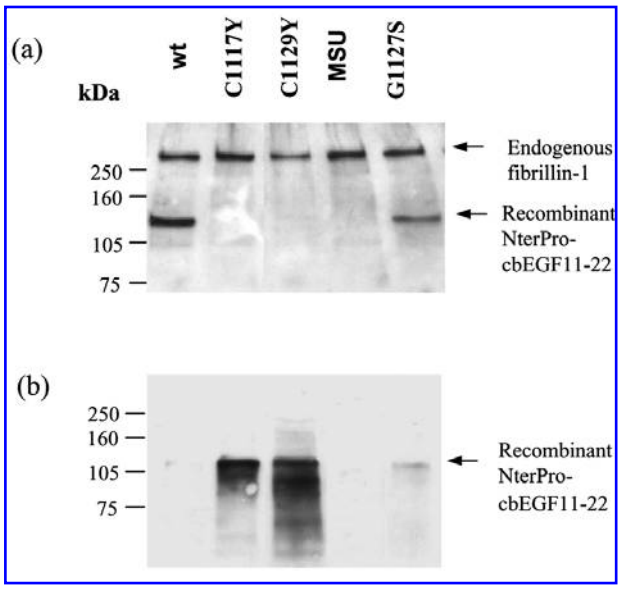


**FIG. 5. Western blotting** of (**a**) conditioned media, and (**b**) cell lysate samples from NterPro-cbEGF11–22 mutant and wild-type pools of clones after electrophoresis on a 4–15% gradient gel under reducing conditions. A polyclonal antiserum specific for the Pro-rich region of fibrillin-1 was used as primary antibody. MSU is the untransfected host cell line.

## FIBRILLIN-1 MISFOLDING IN THE PATHOGENESIS OF HOMOCYSTINURIA

Homocystinuria (HC) is an autosomal recessive disease affecting methionine metabolism. The enzyme cystathionine β-synthase (CBS) catalyzes the vitamin B6-dependent conversion of homocysteine to cystathionine in the trans-sulphuration pathway for the synthesis of cysteine (Fig. 6). The most common cause of homocystinuria is CBS deficiency and this causes elevated levels of the amino acids homocysteine and methionine (30). There is striking similarity in the clinical abnormalities seen in HC and MFS (30, 34), specifically those which affect the ocular and skeletal systems, suggesting that fibrillin abnormalities may be the cause of the connective tissue phenotype in HC patients. One possible mechanism by which fibrillin structure and function may be affected is though the reductive effect of homocysteine on the disulfide-stabilised domain organisation within the microfibril (15, 25).

## cbEGF DOMAIN MISFOLDING INDUCED BY HOMOCYSTEINE

Previous *in vitro* refolding experiments performed on cbEGF domain fragments from a variety of proteins demonstrated a consistent difference in the elution profile of oxidized *versus* reduced protein (27). Mass spectral analysis subsequently confirmed that each purified form had the expected mass. Reverse-phase HPLC analysis was therefore used to investigate the reductive effects of homocysteine on fibrillin domains (15). A triple domain fragment from fibrillin-1 cbEGF32–34 was incubated with 500 μ*M* homocysteine for 24 h at pH 7.5, 37°C, $I = 0.15$. This level of homocysteine was used as it represents the upper limit of total plasma levels detected in CBS-deficient homocystinurics. Following acidification, this sample was subjected to HPLC analysis where a longer elution time and broadening of peak, compared with a control oxidized sample, suggested partial reduction of the fragment (Fig. 7a). This effect was dependent of the concentration of homocysteine added, with lower levels

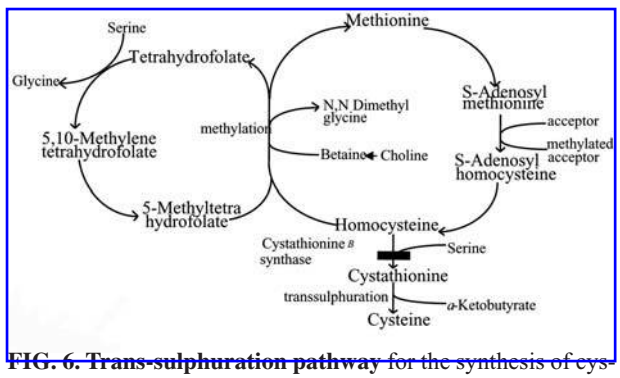


**FIG. 6. Trans-sulphuration pathway** for the synthesis of cysteine from methionine showing the reaction catalysed by cystathionine β synthase.

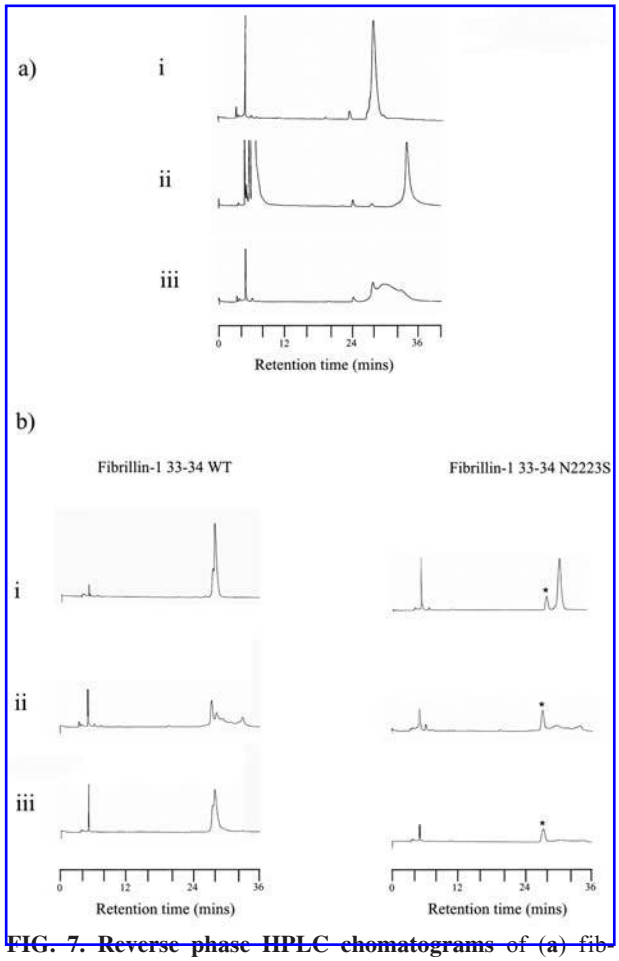


**FIG. 7. Reverse phase HPLC chomatograms** of (**a**) fibrillin-1 cbEGF32-34 treated with i) mock (0 μ*M*) homocysteine, ii) 100 μ*M* dithiotheitol, iii) 500 μ*M* homocysteine for 24 h at 37°C at pH 7.5 and eluted on a 20%–80% gradient of Buffer B (**A** is $H_2O$ containing 0.1% trifluoroacetic acid, **B** is acetonitrile:water (4:1 v/v) containing 0.1% trifluoroacetic acid) after acidification with 0.1% TFA. (**b**) fibrillin cbEGF33–34 wild-type and calcium binding mutant N2223S (which has a significantly lowered affinity for $Ca^{2+}$) treated with i) 0 μ*M* homocysteine, ii) 500 μ*M* homocysteine and 1.5 m*M* $Ca^{2+}$, iii) 500 μ*M* homocysteine and 50 m*M* $Ca^{2+}$ for 24 h at 37°C at pH 7.5. An *asterisk* indicates a protein contaminant unaffected by homocysteine addition which acts as an internal marker for the N2223S sample.

of homocysteine causing less pronounced changes in the HPLC chomatogram. Mass spectral analysis of purified homocysteine treated protein identified a mixture of species including fully reduced protein and protein with one or two homocysteine adducts.

## CALCIUM BINDING TO EGF DOMAINS PROTECTS AGAINST HOMOCYSTEINE REDUCTION

Further experiments were performed to identify whether bound $Ca^{2+}$ affected the reductive capacity of homocysteine to alter cbEGF domain structure. Inclusion of 1.5 m*M* $Ca^{2+}$ in the assay buffer was found to significantly protect the protein against homocysteine attack, although low levels of reduction were still observed. This was not due to a direct inhibitory effect of $Ca^{2+}$ on the reductive capability of homocysteine, since mutant cbEGF domains which have a significantly decreased affinity for $Ca^{2+}$ were reduced in the presence of calcium (Fig. 7b). These data suggest that some regions of fibrillin may be more susceptible to attack than others, specifically cbEGF domains with a low affinity for calcium such as cbEGF32, and non-calcium binding EGF and TB domains.

## HOMOCYSTEINE-TREATED FIBRILLIN-1 SHOWS INCREASED SUSCEPTIBILITY TO PROTEOLYSIS

An alternative assay to HPLC has also been used to demonstrate the homocysteine attack on fibrillin fragments (15). Moderate to high affinity calcium binding to EGF domains protects the fragments against proteolysis. In comparative analyses, removal of $Ca^{2+}$ by the use of a chelator such as EGTA or by the introduction of a missense mutation that diminishes calcium binding results in increased proteolysis (4, 6, 29, 39, 40, 47, 48, 52). Limited proteolysis was performed on control and homocysteine-treated cbEGF32–34 samples after HPLC purification. A time course of tryptic digestion revealed that homocysteine-treated cbEGF32–34 was significantly more susceptible to proteolysis than control peptide (Fig. 8). This confirmed that the effects of homocysteine were mediated though a change in the structural integrity of the domain.

Since many other cell surface and extracellular proteins contain multiple EGF domains, it is likely that these will also be susceptible to homocysteine attack, together with other disulfide-rich domains. Other potential targets are the Notch receptor and members of the latent transforming growth factor β binding protein family. However, the phenotype of the HC patient is likely to reflect changes to those proteins which have a long half life and are thus continually exposed to the reductive effects of this amino acid. Furthermore, the number of disulfide-rich domains that are targets for HC attack

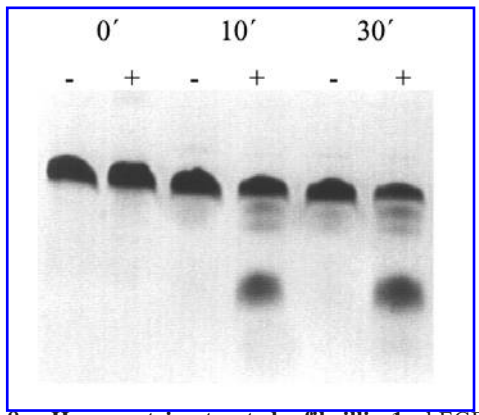


**FIG. 8. Homocysteine-treated fibrillin-1** cbEGF32-34 shows increased susceptibility to proteolysis by trypsin at pH 7.5, 10 m*M* $Ca^{2+}$ indicating a loss of native structure. Samples were incubated in the absence (−) or (+) presence of trypsin.

within a given protein may influence the degree of functional change seen. The clinical features of HC patients include additional abnormalities not seen in patients with MFS, consistent with the involvement of other proteins in disease pathogenesis (30).

## SUMMARY

Fibrillin-1 misfolding appears to be a major contributing factor to the pathogenic mechanisms underlying two inherited diseases with very different etiologies. The modular organization of fibrillin-1 makes it susceptible to primary effects of *FBN1* mutations which may destroy the native fold of individual domains, leading to effects on trafficking, assembly, and stability. In homocystinuria, secondary effects on fibrillin and/or microfibril structure may be caused by thiol attack. In both cases, domain misfolding is predicted to lead to changes in ECM function.

## ACKNOWLEDGMENTS

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## ABBREVIATIONS

cbEGF, calcium-binding epidermal growth factor-like; CBS, cystathionine β-synthase; ECM, extracellular matrix; HC, homocystinuria; HPLC, high performance liquid chomatography; MFS, Marfan syndrome; NMR, nuclear magnetic resonance; PAGE, polyacrylamide gel electrophoresis; TB, transforming growth factor β binding protein-like.

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