The fibrillin microfibril/elastic fibre network: A critical extracellular supramolecular scaffold to balance skin homeostasis

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Abstract
Supramolecular networks composed of fibrillins (fibrillin-1 and fibrillin-2) and associated ligands form intricate cellular microenvironments which balance skin homeostasis and direct remodelling. Fibrillins assemble into microfibrils which are not only indispensable for conferring elasticity to the skin, but also control the bioavailability of growth factors targeted to the extracellular matrix architecture. Fibrillin microfibrils (FMF) represent the core scaffolds for elastic fibre formation, and they also decorate the surface of elastic fibres and form independent networks. In normal dermis, elastic fibres are suspended in a three-dimensional basket-like lattice of FMF intersecting basement membranes at the dermal-epidermal junction and thus conferring pliability to the skin. The importance of FMF for skin homeostasis is illustrated by the clinical features caused by mutations in the human fibrillin genes (FBN1, FBN2), summarized as “fibrillinopathies.” In skin, fibrillin mutations result in phenotypes ranging from thick, stiff and fibrotic skin to thin, lax and hyperextensible skin. The most plausible explanation for this spectrum of phenotypic outcomes is that FMF regulate growth factor signalling essential for proper growth and homeostasis of the skin. Here, we will give an overview about the current understanding of the underlying pathomechanisms leading to fibrillin-dependent fibrosis as well as forms of cutis laxa caused by mutational inactivation of FMF-associated ligands.

Keywords
cutis laxa, extracellular matrix, fibrillin, fibrosis, growth factors

Abbreviations: 3D, three-dimensional; 8-cys, eight-cysteine; ACMICD, ACroMiCric Dysplasia; ADAMTS, A Disintegrin And Metalloproteinase with ThromboSpondin motifs; ADAMTSL, A Disintegrin And Metalloproteinase with ThromboSpondin motifs-Like; ADCL, Autosomal Dominant Cutis Laxa; AFM, Atomic Force Microscopy; ARCL, Autosomal Recessive Cutis Laxa; BM, Basement Membrane; BMP, Bone Morphogenetic Protein; cbEGF, calcium binding Epidermal Growth Factor-like; CCA, Congenital Contractural Arachnodactyly; CL, Cutis Laxa; CPLXs, TGF-β-like PD-GF complexes; DEJ, Dermal-Epidermal Junction; ECM, ExtraCellular Matrix; ECTOL, ECTOpia Lentis; EGF, Epidermal Growth Factor-like; EM, Electron Microscopy; EMILIN, Elastin Microfibril Interface Located proteIN; FGF, Fibroblast Growth Factor; FMF, Fibrillin MicroFibrils; FN, Fibronectin; FUN, Fibrillin Unique N-terminal domain; GF, Growth Factor; GPHYSD, GeleoPHYSic Dysplasia; GTB, C-terminely truncated and eGFP-tagged fibrillin-1; Hyb, Hybrid domain; LAP, Latency-Associated Peptide; LLC, Large Latent Complex; LOX, Lysyl Oxidase; LTBP5, Latent Transforming growth factor TGF-β1-Binding Proteins; MASS, Mitral valve prolapse, Aortic root dilatation, Skin striae, Skeletal features; MFAP, MicroFibrillar-Associated Protein; MFS, Marfan Syndrome; MYHRS, MYHRe Syndrome; PDs, ProDomains; RGD, Arginylglycylaspartic acid; SBF-SEM, Serial Block-Face Scanning Electron Microscopy; SH2, Src Homology 2; SSKS, Stiff Skin Syndrome; STED, Stimulated Emission Depletion; TB, TGF-β1-Binding like; TEM, Transmission Electron Microscopy; TGF-β, Transforming Growth Factors beta; tsk, tight-skin; WMS, Weill-Marchesani Syndrome.
Clinical data and evidence from mouse models clearly implicate fibrillin microfibrils (FMF) and their associated networks in the maintenance of skin homeostasis. Thereby, the phenotypes caused by mutations in building blocks or binding ligands of these extracellular supramolecular scaffolds are often times striking, ranging from stiff and fibrotic, to soft and hyperextensible skin.\[11,12\] FMF are viewed as crucial architectural scaffolds controlling the bioavailability of growth factors within the extracellular matrix (ECM). However, the exact mechanisms of growth factor targeting and utilization are only partially understood. Moreover structurally, FMF significantly contribute to the biomechanical properties of skin such as elasticity and pliability. Therefore, knowing the localization and assembly mechanism of FMF is essential to understand which particular mutation may impart a specific function and thereby trigger a specific pathomechanism in the skin. Here, we will give an overview about the current functional understanding of FMF and their associated ligands serving as critical determinants for cutaneous fibrosis and elastogenesis.

2 | FIBRILLIN DOMAIN STRUCTURE

Fibrillins are large, 350 kDa extracellular glycoproteins with a conserved domain structure. The fibrillin family consists of three members (fibrillins 1-3) which show the highest sequence homology to four related proteins called latent transforming growth factor TGF-β binding proteins 1-4 (LTBPs 1-4). The domain structure of fibrillins and LTBPs consists primarily of repeated units of epidermal growth factor-like (EGF) domains, dispersed by eight-cysteine (8-cys) domains also known as TGF-β-binding like (TB) domains (Figure 1). Fibrillins also contain hybrid domains, which are structurally similar to both EGF and TB domains, and harbour LTBP and fibrin-binding epitopes. TB domains are unique to fibrillins and LTBPs, and are not found in any other proteins. The third TB domains of LTBPs mediate binding with TGF-β prodomains (PDs) (also known as latency-associated peptide: LAP) to which they become covalently tethered via two disulphide bridges. In contrast, the TB domains of fibrillins have not yet been shown to be involved in growth factor (GF) binding. Fibrillin contains 47 EGF domains, of which 43 are calcium binding (cbEGF). The ability to bind calcium not only makes fibrillin monomers more stable against proteolytic degradation, but also appears to modulate its extensibility and thereby pliability of the skin. It was suggested that the interdomain linker regions between TB and cbEGF domains act like a molecular spring that unfolds in response to stretching force. Under normal physiological conditions, these domain interfaces are quantitatively saturated with calcium; however, upon extension the domain interfaces unfold, leading to a reduced binding affinity and calcium release. The RGD site in the fourth TB domain mediates fibrillin binding to the cell surface via \(\alpha\)5\(\beta\)1, \(\alpha\)\(\nu\)\(\beta\)3 and \(\alpha\)\(\nu\)\(\beta\)6 integrins. The fibrillin C-terminus is proteolytically processed by furin, which in turn enables efficient secretion and assembly of fibrillin into microfibrils.

3 | SUPRAMOLECULAR ORGANIZATION OF FIBRILLIN INTO MICROFIBRILS

It is still unclear how fibrillin molecules are organized into mature microfibrils with a diameter of 10-12 nm. Electron microscopy (EM) shows FMF with a “beads-on-a-string”-like appearance with the N- and C-termini of the fibrillin molecules contained in the bead region. Biochemical analysis revealed that the interbead region is stabilized by transglutaminase-derived cross-links that help to facilitate the correct alignment of monomers. FMF have a 56 nm repeating periodicity when extracted, which fits well with their periodicity of 50-60 nm in tissues. Upon stretching of the skin, the interbead distance can go up to more than 100 nm. However, the observed periodicity corresponds to a third of the measured length of extracted and recombinant fibrillin-1 monomers of about 150 nm. To reconcile this difference, three structural models of FMF have been proposed. In the pleated model, one back folded fibrillin monomer spans one 56 nm repeat. The two other models propose an extended conformation with each monomer spanning two or three interbead regions. To gain further information on the FMF ultrastructure, new imaging approaches employing EM and atomic force microscopy (AFM) were attempted. Serial block-face scanning electron microscopy (SBF-SEM) imaging and thick-section electron tomography showed that FMF contain a central cavity in the interbead region, where eight symmetrically arranged fibrillin monomers can be accommodated. Interestingly, AFM analysis in combination with mass spectrometry revealed that the bead morphology of skin FMF significantly differs from that of eye FMF due to their tissue-specific molecular composition.

4 | LOCALIZATION OF FIBRILLIN/ELASTIC FIBRE NETWORK IN SKIN

FMF decorate the surface of elastic fibres but they also form independent networks of stretchable bundles, making them indispensable for conferring elasticity to the skin. In normal dermis, elastic fibres are suspended in a three-dimensional (3D) basket-like lattice of FMF. FMF devoid of elastin intersect the basement membrane (BM) at the dermal-epidermal junction (DEJ) and thus conferring pliability to the skin (Figure 2A). It is commonly believed that degradation or loss of FMF at the DEJ—due to ageing or sun exposure—is the primary reason for wrinkling and sagging of the skin. Intrinsically aged skin shows fine wrinkles that are associated with a gradual fragmentation of the fibrillin/elastic fibre network, while photoaged skin appears deeply wrinkled and displays histologically a loss of FMF from the papillary dermis.

Also, in mouse models and patients with Marfan syndrome (MFS), which is characterized by hyperextensible skin, fragmentation of fibrillin fibres at the DEJ is observed by light microscopy (Figure 2B). Thick elastin-rich fibres in the reticular dermis form a continuum towards the lower papillary dermis, where the fibres become finer and contain less elastin. These fibres then appear to be
FIGURE 1  Schematic representation of the domain structures of fibrillin-1 and LTBP family members. All fibrillin family members have the same domain structure. Instead of a proline-rich region, fibrillin-2 harbours a glycine-rich region, and fibrillin-3 a glycine- and proline-rich region. The TB domain module is exclusively found in fibrillins and LTBP5, demonstrating the familial relationship. Multiple cbEGF modules are present in fibrillins and LTBP5.

Expression of a C-terminally truncated and eGFP-tagged fibrillin-1 in Fbn1^{GT8/GT8} knock-in mice gave insight into the structural organization of fibrillin-1 in the skin. Regions with identified functional interactions or mutations causing connective tissue disorders characterized by fibrotic skin features are indicated.

The process of elastic fibre formation in the skin follows a hierarchical order. Thereby in a primary step, fibronectin deposition is
considered mandatory to elaborate a proper FMF network.\textsuperscript{[45, 46]} It is believed that simultaneous assembly of FMF at both the papillary dermis (DEJ) and dermis in the skin is a prerequisite for elastin deposition.\textsuperscript{[47]} Thereby, FMF serve as a structural scaffold required for tropoelastin targeting and cross-linking into mature elastin.\textsuperscript{[3]} This complex process is facilitated by specific binding events among fibronectin, fibrillins, fibulin-4, fibulin-5, LTBP-4, tropoelastin and lysyl oxidase (LOX).\textsuperscript{[3, 48]} (Figure 3). In the current view, tropoelastin

\textbf{FIGURE 2} Localization of fibrillin and associated networks in the skin. A, FMF traverse as bundles with and without elastin from the deep reticular dermis to the papillary dermis where they insert perpendicular at the dermal-epidermal junction (DEJ) into the basement membrane (BM). Fibrillin-1 is also present within the BM, but the FMF characteristic 50 nm beads-on-a-string periodicity is lost, suggesting another assembly form.\textsuperscript{[36]} At the DEJ, FMF are decorated with EMILINs, which form fibrillar projections reaching beyond the BM to make contact with basal keratinocytes.\textsuperscript{[42-44]} B, C, Expression of a C-terminally truncated fibrillin-1 gives new insight into the architecture of the fibrillin network in the skin.\textsuperscript{[38]} B, In Fbn1\textsuperscript{GT8/GT8} neonatal skin (P1), intact fibrillin-1 fibres are detected at the DEJ, suggesting that truncated fibrillin-1 was deposited onto intact FMF composed of fibrillin-2. After one week of postnatal life (P7), fibrillin-1 fibres appear fragmented. C, BM localization of fibrillin-1 can be observed at time points when fibrillin fibre fragmentation is prevalent.\textsuperscript{[43]} E: epidermis, D: dermis, HF: hair follicle, co-localization with the BM marker nidogen-1 is marked with an asterisk. Scale bars: 50 µm
forms small aggregates (coacervation), which mediate interactions with fibulin-4 and fibulin-5. Subsequently, this complex is targeted to FMF-bound LTBP-4 which is required for the linear deposition along the FMF core structure.\textsuperscript{[3,48]} Thereby, it is thought that fibulin-4 and fibulin-5 specifically interact with LOX and lysyl oxidase like-1 (LOXL-1) respectively and thereby serve as carrier molecules required for their extracellular targeting to FMF\textsuperscript{[49,50]} (Figure 3). This view is based on more detailed studies for fibulin-4. Fibulin-4 interacts with the LOX propeptide and thereby mediates extracellular targeting of LOX to FMF where its activation occurs by BMP-1–mediated propeptide removal.\textsuperscript{[50]} LOX, which also establishes collagen cross-links in the ECM, generates together with LOXL enzymes the unique elastin-specific desmosine cross-links that are essential for providing elastic stretch and recoil to the skin.\textsuperscript{[48]}

Further, it was proposed that LTBP-2 may act as a negative regulator of elastogenesis. LTBP-2 interacts with fibrillin-1, tropoelastin and fibulin-5 and prevents tropoelastin binding to heparin.\textsuperscript{[51,52]} This suggests that LTBP-2 potentially displaces elastin microassemblies from complexes with fibulin-5 and/or cell surface heparan sulphate proteoglycans.

Interestingly, tropoelastin was also found to directly interact with the N-terminal region of fibrillin-1 via the TB2 domain and becomes covalently cross-linked to fibrillin-1 by transglutaminase-2 (TG2).\textsuperscript{[53,54]} Fibrillin-1 is also believed to promote coacervation of tropoelastin into larger and dense globules.\textsuperscript{[55]}

Biochemical interaction studies and functional gene ablation in mice were insightful in elucidating regulatory mechanisms of elastogenesis. It could be demonstrated that fibulin-4, fibulin-5 and LTBP-4 bind to residues contained within the EGF3/Hyb1 region of fibrillin-1,\textsuperscript{[6,7]} and that fibulins and LTBPs compete for this binding site suggesting a regulatory mechanism.\textsuperscript{[7]} EMILIN-1 may also serve as a modulator in this process as it also interacts with fibulin-4 and fibulin-5, and is targeted to FMF.\textsuperscript{[63,54,57]} Fibulin-4 ablation efficiently inhibits elastogenesis in all elastic tissues including skin leading to perinatal death.\textsuperscript{[58]} Fibulin-5 null mice have a severely disorganized elastic fibre system leading to loose skin (cutis laxa) but survive well into adulthood.\textsuperscript{[49,59]} LTBP-4 null mice die after the first week of life and show a much more severe elastic fibre phenotype than fibulin-5 null mice.\textsuperscript{[60]} EMILIN-1 null mice display only mild elastic fibre defects.\textsuperscript{[56]} In vitro cultures of human dermal fibroblasts showed that LTBP-4 recruits fibulin-5 to FMF and therefore enables the deposition of tropoelastin.\textsuperscript{[63]} However, recent data showed that Ltbp4S\textsuperscript{−/−};Fbln5\textsuperscript{−/−} mice, which still express the long isoform LTBP-4L but are deficient in LTBP-4S and fibulin-5, display structurally normal elastic fibres.\textsuperscript{[62]} This implies the existence of an alternative elastogenesis pathway involving presumably LTBP-4L and fibulin-4. We found that both LTBP-4L and LTBP-4S bind to fibulin-4 via their N-termini; however, we measured a significantly higher binding propensity for LTBP-4L.\textsuperscript{[60]} To also test a functional interaction between LTBP-4L and fibulin-4 in vivo, a hypomorphic fibulin-4 allele\textsuperscript{[63]} was introduced in Ltbp4S\textsuperscript{−/−} mice. Interestingly, compared to Ltbp4S\textsuperscript{−/−} or Fibulin-4\textsuperscript{R/R} control mice, which both can reach adulthood, Ltbp4S\textsuperscript{−/−};Fibulin-4\textsuperscript{R/R} mice displayed severe elastic fibre defects leading to a short lifespan.

**FIGURE 3** Simplified model of mechanistic sequence of elastogenesis. Proper elastic fibre formation requires assembly of fibrillin molecules into microfibrils. Secreted tropoelastin coacervates to form small aggregates at the cell surface, to which the pro-form of lysyl oxidase (proLOX) is tethered via fibulin-4.\textsuperscript{[50]} Elastin polymerization is catalysed by activated LOX upon BMP-1–mediated prodomain removal. Tropoelastin monomers are cross-linked to each other by mature LOX and LOXL proteins to establish the elastin characteristic desmosine cross-links (black lines). LTBP-4 interacts with FMF and fibulin-5 and promotes elastogenesis.\textsuperscript{[61]} In vivo data suggest that LTBP-4S interactions with fibulin-5 as well as LTBP4L interaction with fibulin-4 help to orchestrate the elastogenesis process.\textsuperscript{[60-62,64]} EMILIN-1 interactions with fibulin-5, tropoelastin and fibulin-4 are also important for the linear deposition of tropoelastin\textsuperscript{[56,57]}.\textsuperscript{[60-62,64]}
of around nine postnatal days. Together, these data clearly indicate a functional link between Ltbp-4L and fibrillin-4 as a crucial molecular requirement for survival and elastogenesis. Recently, biochemical studies provided insightful molecular data to understand the underlying mechanism of these findings in greater detail. It was shown that fibrillin-4 interaction with LTBP-4 induces an extended conformation that is required for linear deposition and fibre formation of LTBP-4. However, the full repertoire of FMF ligands involved in the formation of elastic fibres has still to be explored. Recently, we identified a new direct ligand of FMF, microfibrillar-associated protein 4 (MFAP-4), which is involved in elastic fibre formation by promoting tropoelastin self-assembly.

### 6 | FIBRILLIN MICROFIBRILS AS GROWTH FACTOR INTEGRATION PLATFORMS IN THE SKIN

Transforming growth factors beta 1-3 (TGF-βs 1-3) (hereafter collectively referred to as TGF-β) are targeted to FMF in skin and other tissues in form of a large latent complex (LLC) in which LAP is covalently linked to LTBP-1, LTBP-3 or LTBP-4. LAP inhibits access of TGF-β receptors to TGF-β GF, thereby ensuring latency of the prodomain-growth factor (PD-GF) small latent complex (SLC). Bone morphogenetic proteins (BMPs) also belong to the TGF-β superfamily of GFs. We could demonstrate that BMPs form TGF-β-like GF complexes (CLPXs) with their processed PDs, but interact with fibrillin-1 and fibrillin-2 via direct interactions with their PDs. Co-immunolocalization studies with specific BMP and fibrillin-1 or fibrillin-2 antibodies showed localization of BMPs to FMF in skin and other elastic tissues. We also determined the BMP PD interaction site on fibrillin-1 and fibrillin-2 to be the so-called "fibrillin unique N-terminal domain" (FUN). Recently, we demonstrated that BMP PD binding to fibrillin represents a BMP sequestration mechanism. In contrast to TGF-β, BMP PD-GF CLPXs are bioactive and have an open V-shape conformation. However, upon targeting to fibrillin a conformational change is induced, rendering the CLPX into a closed ring-shape conformation. In this fibrillin-bound state, the GF is latent since access of BMP type II receptors is denied due to blocking of its binding site on the GF by the PD. Moreover, EMILIN networks which structurally depend on FMF were also reported to have unique functions in GF regulation. EMILINs were shown to modulate processing of proTGF-β, activation of the extrinsic apoptotic pathway, and regulation of the bioavailability of Hedgehog ligands. Recently, EMILIN-2 was also suggested to serve as an extracellular modulator of the Wnt signalling pathway by exerting direct interactions with Wnt1. In addition to EMILINs, it was reported that LTBP-2, another mediator of elastogenesis which seems not to serve as TGF-β carrier, interacts with fibroblast growth factor-2 (FGF-2) and may thereby modulate its bioavailability in fibrotic reactions.

### 7 | FIBRILLINOPATHIES

The significant functional role of FMF in connective tissue growth and homoeostasis becomes evident by the various clinical features of patients with fibrillin mutations (termed “fibrillinopathies”). The fibrillinopathies represent multisystem connective tissue disorders with distinctive, common or each other opposing clinical features with an involvement of the musculoskeletal, cardiovascular, ocular, pulmonary and dermal system. In skin, fibrillin mutations result in features ranging from thick skin in Weill-Marchesani syndrome (WMS), geleophysic and acromicric dysplasia (GPHYSDD, ACMICD) to thin skin in congenital contractual arachnodactyly (CCA), and from stiff skin in Stiff skin syndrome (SSKS) to lax and hyperextensible skin in MFS. The most common skin manifestation resulting from FBN1 mutations is stria, also referred to as atrophic scars. Histological analysis of biopsies from atrophic lesions from a patient with MASS syndrome showed that reticular elastic fibres are aggregated, curled and fragmented. EM assessment revealed a moth-eaten appearance defined by numerous peripheral cracks and holes.

### 8 | FIBRILLIN-DEPENDENT FIBROSIS

Mutations in fibrillin-1 also trigger fibrotic reactions. This was first demonstrated by the identification of a large in-frame Fbn1 duplication in the tight-skin (tsk) mouse. This genetic insertion results in a larger than normal (418 kDa) mutant fibrillin-1 protein that assembles into FMF with altered molecular organization, probably resulting in a higher susceptibility to proteolytic degradation. Tsk mice show cutaneous fibrosis and have been used as a disease model for systemic sclerosis (scleroderma) in numerous pre-clinical studies. More recently, mutations in FBN1 were found in Stiff skin syndrome (SSKS), caused by fibrillin-1 mutations within the fourth TB domain harbouring the integrin-binding site. Studies employing the corresponding SSKS mouse model suggested that failed fibrillin-1-β1-integrin interaction is causative for the fibrotic skin phenotype.

#### 8.1 The acromelic dysplasia group

The acromelic dysplasia group consists of WMS, GPHYSDD, ACMICD and Myhre syndrome (MYHRS) which are characterized by short stature, short hands, stiff joints and thickened skin. Most feature characteristics for WMS appear to be "opposite to MFS." FBN1 mutations in the fifth TB domain are causative for autosomal dominant WMS (WMS2), while recessive WMS (WMS1) is caused by ADAMTS10 (A disintegrin and metalloproteinase with thrombospondin type 1 motif 10) mutations within its catalytic domain. This suggests a common genetic pathway involving both genes in the pathogenesis of WMS. Biochemical studies confirmed a
direct interaction between ADAMTS-10 and fibrillin-1. Further, it was shown that ADAMTS-10 is not only able to proteolytically cleave fibrillin-1, but also to promote fibrillin fibre assembly in fibroblast cultures. Mutations in ADAMTS-17, an enzyme from the same protease family, also lead to WMS (WMS4). Studies showed that ADAMTS-17 selectively interacts with fibrillin-2 but not fibrillin-1 and thereby prevents the incorporation of fibrillin-2 into FMF.

In this context, another functional interaction with FMF could be demonstrated for ADAMTSL-2 (ADAMTS-like-2). ADAMTSL-2
mutations prevent secretion due to an intracellular accumulation of the protein which leads to recessive GPHYSDD (GPHYSDD).\[102\] while fibrillin-1 mutations in the fifth TB domain are causative for GPHYSDD.\[84\] ADAMTSL2 exerts direct interactions with fibrillin-1,\[83,103\] as well as with LTBP-1,\[102\] which may help to keep the TGF-β(LLC) stable and thereby latent. It is thought that instable LLCs will eventually disintegrate leading to aberrant TGF-β activation.\[104\] Indeed, analysis of primary dermal fibroblasts isolated from GPHYSDD1 patients revealed increased amounts of bioactive TGF-β.\[102\] Despite this finding, a functional role of ADAMTSL2 in modulating TGF-β bioavailability seems plausible, since it was recently reported that LTBP-3 mutations also lead to GPHYSDD and ACMICD.\[105\]

### 8.2 Dysregulated growth factor signalling in acromelic dysplasias

In this context, it remains to be understood why also LTBP-2 mutations are causative for short stature, short hand and thick skin (WMS2),\[106\] although it was proposed that LTBP-2 does not interact with TGF-β.\[86\] However, as LTBP-2 exerts specific interactions with FGG-β, a growth factor with known antifibrotic activity,\[107\] LTBP-2 deficiency may downregulate FGG-β signalling and thereby promote myofibroblast activation resulting in skin fibrosis. It remains to be further investigated whether the clinical features of all acromelic dysplasias are solely due to dysregulated TGF-β activity. In primary fibroblasts isolated from WMS2 as well as mice carrying a corresponding knock-in mutation, we could not detect increased levels of active TGF-β.\[83\] In addition, mutations leading to defective TGF-β signal transduction were shown to cause MYHRS, also characterized by short stature and thick skin.\[108\] The mutations were identified in the SH2 domain of Smad4,\[108\] a co-Smad, which is essential for the efficient translocation of the TGF-β and BMP signalling intermediates phosphoSmad2/3 (pSmad2/3) and pSmad1/5/8 to the nucleus. Analysis of MYHRS patient fibroblasts showed an impaired Smad4 ubiquitination and TGF-β signal transduction indicated by decreased mRNA expression of TGF-β and BMP responsive elements.

### 8.3 Perturbation of fibrillin-dependent cellular microenvironment causes fibrosis

Future investigations may address the question why some fibrillin-1 mutations lead to MFS with thin and hyperextensible skin while others lead to acromelic features with thick and fibrotic skin. Our current understanding is that the 3D ultrastructure of FMF provides a cellular microenvironment which controls GF sequestration and bioavailability (Figure 4B). Any mutation-induced structural alteration within the FMF architecture may impact the presentation of GFs towards the cell surface. Targeted GF CPLXs may become instable, lose their sequestered status and become aberrantly activated. Structural deficiency of FMF may also inhibit GF activation mechanisms, since certain critical binding epitopes are not accessible anymore.

We gained some mechanistic insight into the orchestration of this microenvironment by the identification of a deletion mutation within the N-terminal region of fibrillin-1 causative for WMS2.\[83\] The mutation leads to deletion of the first TB domain, the proline-rich region and the fourth EGF-like domain.\[83\] Interestingly, although distant from each other in the primary structure of fibrillin-1 monomers, the WMS2 deletion region and the fifth TB domain (WMS1 region) localize both to the bead region in a 3D model of assembled MFMs (Figure 4B). Within this model also the fourth TB domain containing the integrin RGD binding site is found at the bead region (Figure 4B). Mutations in the fourth TB domain are causative for SSKS, presumably by disrupting integrin binding.\[86\] ADAMTSL2 mutations lead to recessive GPHYSDD,\[102\] while mutations in ADAMTS10 cause recessive WMS.\[97\] Interestingly, our interaction studies revealed that the WMS2 deletion site is found as a universal binding region to target ADAMTS proteins to FMF.\[83\] We also found that ADAMTS proteins are able to interact with ADAMTS10.\[83\] Since the WMS2 deletion site is in close proximity to the TGF-β LLC and BMP CPLX binding sites on fibrillin-1, it can be hypothesized that ADAMTS targeting to FMF enables ADAMTS enzyme recruitment for controlled GF activation via proteolytic FMF or PD degradation.\[7,71,72,83\] Such a mechanism may be

| Genetic disorder | Affected gene | OMIM#  | Inheritance | Reference |
|------------------|--------------|--------|-------------|-----------|
| ACMICD           | FBN1         | 102 370| AD          | [84]      |
| GPHYSDD1         | ADAMTSL2     | 231 050| AR          | [102]     |
| GPHYSDD2         | FBN1         | 614 185| AD          | [84]      |
| GPHYSDD3         | LTBP3        | 617 809| AD          | [105]     |
| MYHRS            | SMAD4        | 139 210| AD          | [108]     |
| SSKS             | FBN1         | 184 900| AD          | [86]      |
| WMS1             | ADAMTS10     | 277 600| AR          | [97]      |
| WMS2             | FBN1         | 608 328| AD          | [83,84,96]|
| WMS3             | LTBP2        | 614 819| AR          | [106]     |
| WMS4             | ADAMTS17     | 613 195| AR          | [101]     |

Abbreviations: AD, autosomal dominant; AR, autosomal recessive.
9 | CUTIS LAXA

Cutis laxa (CL) is a group of multisystem disorders that are characterized by loose and/or wrinkled skin that imparts a prematurely aged appearance. Face, hands, feet, joints and torso may be differentially affected. The skin lacks elastic recoil in marked contrast to the hyperelasticity apparent in, for example, classic Ehlers-Danlos syndrome.109

The underlying molecular defects in CL are caused by mutations in proteins that are essential for the mechanistic sequence of elastogenesis (Table 2). For example, autosomal dominant CL (ADCL) has been associated with mutations in elastin (ELN) resulting in abnormal branching and fragmentation of elastic fibres, as well as reduced elastin deposition and fewer FMF in the dermis.110 The characteristic loose skin may be accompanied by gastrointestinal diverticula, hernia and genital prolapse. The clinical spectrum of autosomal recessive CL (ARCL) is highly heterogeneous regarding organ involvement and severity. ARCL1A is caused by fibulin-5 mutations,111 ARCL1B by fibulin-4 mutations,112 and ARCL1C by LTBP4 mutations.113 ARCL1A and ARCL1B are characterized by the presence of severe systemic connective tissue abnormalities, including emphysema, cardiopulmonary insufficiency, birth fractures, arachnodactyly and fragility of blood vessels. All symptoms refer to disturbed elastic fibre formation and thereby initiated growth factor dysregulation. Patients with ARCL1C exhibit generalized CL in association with impaired pulmonary, gastrointestinal, genitourinary, musculoskeletal and dermal development.114

Usually, the histological examination of CL patient skin shows a reduced elastic fibre density. Electron microscopy analysis often times demonstrates abnormally fragmented elastic fibres with a build-up of amorphous deposits (moth-eaten appearance), and reduced FMF in the papillary dermis. Moreover, a reduced collagen bundle size in the dermis of CL patients was observed.115

Acquired CL often times occurs due to dysregulated ECM remodelling triggered by inflammatory responses, which results in elastic fibre degradation. For instance, we detected in primary dermal fibroblasts of a patient with acquired CL altered LTBP-4 glycosylation, and intracellular retention of LTBP-4 and fibulin-5. This may be caused by structural alterations in the Golgi apparatus which was most likely triggered by previous medical treatment. These defects may have led to severe impairment of elastic fibre regeneration in the affected skin of this patient.116

| Genetic disorder | Gene   | OMIM# | Inheritance | Reference |
|------------------|--------|-------|-------------|-----------|
| ADCL             | ELN    | 123 700 | AD          | [110]     |
| ARCL1A           | FBLN5  | 219 100 | AR          | [111]     |
| ARCL1B           | EFEMP2 | 614 437 | AR          | [112]     |
| ARCL1C           | LTBP4  | 613 177 | AR          | [113]     |

Abbreviations: AD, autosomal dominant; AR, autosomal recessive.

10.1 | MAJOR OPEN QUESTIONS

Despite their critical function for skin homoeostasis, several important questions regarding FMF function and assembly remain to be answered.

For instance, the mechanistic sequence of the FMF assembly process is not yet clear. In vitro studies with recombinantly expressed regions of full-length fibrillin-1 and fibrillin-2 showed that the N- and C-terminal ends interact in a head-to-tail arrangement.117 Furthermore, it could be demonstrated that the fibrillin-1 C-terminal region can spontaneously self-assemble into disulphide-linked bead-like structures with about 8 to 12 extensions that strongly interact with the fibrillin-1 N-terminus.118 In addition, it was reported that cell surface heparin sulphate proteoglycans assist in the assembly process that likely takes place in the pericellularly space around the cell surface.119-121 Other glycosaminoglycans (GAGs) such as heparin were also shown to sufficiently inhibit the assembly process in vitro.122 To what extent these processes occur in vivo remains to be studied. Thereby, the generation and analysis of new functional Fbn1 knock-in alleles for the systematic ablation of identified interaction sites will allow to gain a more detailed view of the in vivo assembly of FMF. Similarly, conditional knock-out and knock-in alleles would be helpful to investigate the contributions of keratinocytes and fibroblasts to the unknown fibrillin assembly process in the BM. According to some models of FMF,123 the head-to-tail arrangement of fibrillin monomers shows a 50% stagger. This suggests a complete overlap of the N-terminal half by the C-terminal half in normal FMF. Since the C-terminal half of fibrillin-1 is missing in Fbn1GT8/GT8 skin, anti-fibrillin-1 antibody epitopes within the N-terminal half cannot be masked. Although the assembly of fibrillin monomers within the BM does not show a 50-60 nm periodicity as in FMF, the stainability of N-terminal fibrillin-1 epitopes when the C-terminal half is ablated due to the GT8 mutation (Figure 2B) still points to a potential head-to-tail interaction with a considerable amount of stagger. Fibrillin monomers may be anchored within the BM via integral BM components such as perlecan. Specific interactions of fibrillin-1 and perlecan have already been shown,122 and there is also evidence that both may act in concert to target and sequester GFs.122 It is plausible that structural basement membrane defects are induced by the presence of mutant fibrillin leading to aberrant GF release and affecting basal keratinocytes. Future studies will...
address these questions. Subjection of skin biopsies from such transgenic mice to new powerful microscopy approaches such as super-resolution STED (Stimulated Emission Depletion) microscopy, AFM or cryo-EM in combination with sensitive mass spectrometry may reveal new insightful molecular information.

10.2 | Hierarchy of fibrillin deposition within the ECM

In addition to the question of FMF assembly, more information regarding the existence of a hierarchical interdependence of ligands within the elastic fibre/ FMF network is needed. Using dermal fibroblasts, it was shown that FMF assembly requires the prior deposition of a proper fibronectin (FN) network. This may suggest that during skin development, a similar dependence exists. However, evolutionary fibrillins emerge considerably before FN (first emergence in tunicates) prior to the split of cnidaria and bilateria, pointing to a FN-independent assembly process during embryogenesis. LTBP and EMILIN assembly was shown to be dependent on prior FMF assembly in dermal fibroblast; however, in embryonic fibroblasts a dependence on FN was identified. Overall, these findings indicate that deposition of elastic fibre/ FMF networks occurs in a context and cell-type-specific manner. The generation of new transgenic mouse models with inducible Fbn1 and Fbn2 knock-in or knock-out alleles will help to address the open questions regarding spatio-temporal expression (eg turn-over), as well as localization and composition of FMF during development and postnatal life in the skin.

10.3 | Mechanism of fibrillin-1 secretion

In this regard, it is also important to know which intracellular pathways regulate fibrillin secretion in the skin. It was shown that ablation of HSP47, a known collagen chaperon, in embryonic fibroblasts leads to over-glycosylation of fibrillin-1 probably due to a prolonged presence in the ER, which negatively impacts fibrillin fibre assembly. This suggests that collagen and fibrillin secretion pathways intracellularly intersect, which may open up new opportunities for treatment. It may be highly rewarding to further study which intracellular factors are critical for fibrillin secretion since it has the potential to cure many detrimental skin conditions. For instance, stimulation of fibrillin secretion or assembly may reverse skin ageing, a very desirable aim for society.

10.4 | Role of the fibrillin microfibrils in growth factor regulation

So far it is accepted that GFs are targeted to FMF, however, the pertaining activation mechanisms are not clear. Currently, there are two major concepts for GF activation and release from the FMF scaffold, mechanical pulling of the PD via integrins as well as proteolytical PD degradation. Several integrins are able to activate latent TGF-β through binding to an integrin-binding site (RGD) localized within the TGF-β1 PD. Although BMP PDs do not contain the classic RGD integrin interaction motif, it may be possible that integrins still interact with BMP PDs via non-conventional interaction sites. This may be also true for TGF-β2 which also does not contain a RGD motif. Since FMF are known to be stretchable extracellular scaffolds, it will be interesting to test whether fibrillin-bound BMPs can be released and activated upon exposition to extrinsic mechanical stretch forces. Another open question remains regarding a potential functional role of the displaced PD or PD fragments. Since PDs have been shown to compete for receptor binding sites on GFs, it is conceivable that they modulate signalling events at the cell surface by binding to co-receptors. Further, as PDs interact not only with fibrillins and LTBPs but also with other PDs and GFs, it may be that they assist in the recruitment of newly secreted GF CPLXs to the FMF/ elastic fibre scaffold. Also, replenishment of GFs may occur by targeting ECM molecules to the FMF/ elastic fibre scaffold that are already tethered to GFs during the secretory pathway. Currently, there is only limited knowledge about the secretory pathways GFs follow and how potential co-secretion with ECM molecules is facilitated. For instance, TGF-β could be recently shown to follow an unconventional pathway dependent on the autophagic machinery.

10.5 | Role of fibrillin microfibrils during wound healing

It is most plausible that FMF are functioning as potent sequestration scaffolds in the wound-healing process. FMF have been shown to be deposited into the wound matrix as an early event and were found to co-localize with LLCs of LTBP-1 during the earliest stages of skin regeneration from burn wounds. Also, integrins, which were identified to interact with fibrillin-1 as well as with LAP were found to be highly upregulated during the wound-healing process. However, for efficient activation of sequestered latent TGF-β from the wound matrix, appropriate localization of the LLC to specific binding sites within the sequestration scaffold is crucial. A corollary to this notion is that lost or inappropriate targeting of latent TGF-β due to pathological alterations in FMF ultrastructure will result in loss or insufficient activation of TGF-β signalling, leading to dysregulated wound healing as a consequence. Further studies need to be conducted to elucidate these aspects.

11 | CONCLUSIONS AND PERSPECTIVE

The FMF/elastic fibre network represents an intricate supramolecular scaffold required for maintaining skin development and homoeostasis. It not only provides biomechanical properties to the skin
but also regulates the bioavailability of essential growth factors. Understanding how the ultrastructure of these networks communicates with resident cells will allow us to develop treatment options for patients suffering from severe skin conditions such as fibrosis and CL. This will also shed light on the pathomechanisms leading to skin ageing. Thereby, new methods are required to recognize alterations in FMF/elastic fibre architecture in the skin at an early stage. New mass spectrometry protocols involving skin extractions will be very helpful in identifying new disease markers for the fibrillinopathies.\(^{[23]}\) We recently developed Raman microspectroscopy as a new minimal invasive method for the sensitive detection of molecular changes in the skin ECM before they can be monitored by standard microscopy.\(^{[41]}\) Such new tools will help us to develop new protocols to more precisely diagnose and monitor ECM changes in the skin.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**AUTHOR CONTRIBUTIONS**

A.V. Zuk conducted experiments shown in Figure 2 and provided some graphical support. C. S. Adamo and G. Sengle wrote the manuscript.

**DATA AVAILABILITY STATEMENT**

Research data are not shared.

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