Bone 120 (2019) 70–74

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

Alterations in non-type I collagen biomarkers in osteogenesis imperfecta

Lindsey Nicol[a,*], Patrick Morar[b], Ying Wang[c], Kim Henriksen[d], Shu Sun[d], Morten Karsdal[d], Rosamund Smith[e], Sandesh C.S. Nagamani[f], Jay Shapiro[g], Brendan Lee[f], Eric Orwoll[h]

[a] Department of Pediatrics, Division of Endocrinology, Oregon Health & Science University, Portland, OR, United States of America
[b] George Fox University, Newberg, OR, United States of America
[c] Department of Medicine, Division of Biostatistics, Oregon Health & Science University, Portland, OR, United States of America
[d] Nordic Bioscience, Herlev, Denmark
[e] Lilly Research Laboratories, Indianapolis, IN, United States of America
[f] Department of Medicine, Division of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, United States of America
[g] Uniformed Services University of the Health Sciences, Dept. Endocrinology and Diabetes, Walter Reed National Military Medical Center, Bethesda, MD, United States of America
[h] Department of Medicine, Division of Endocrinology, Oregon Health & Science University, Portland, OR, United States of America

ARTICLE INFO

Keywords:
Osteogenesis imperfecta
Extracellular matrix
Collagen type I
Collagen type III
Collagen type IV
Collagen type V
Collagen type VI
Collagen type VII

ABSTRACT

Osteogenesis imperfecta [1] is a rare disorder of connective tissue caused by abnormalities in the synthesis or processing of type I collagen. Type I collagen is the most abundant type of collagen and is expressed in almost all connective tissues. Given that type I collagen interacts with other collagens based in the extracellular matrix (ECM), we hypothesized changes in type I collagen in OI would result in perturbations in the homeostasis of other collagen types. We measured serum biomarkers of several non-type I collagens in patients with mild (type I) and moderate-to-severe (type III/IV) OI. Compared to controls, those with moderate-to severe OI had a higher mean level of the synthesis markers of collagen III (ProC3) ($P = 0.02$), and levels of collagen V (ProC5) ($P = 0.07$) were slightly, but not significantly, higher. Degradation markers of collage type IV (C4M2) ($P = 0.04$) and type VI (C6M) ($P = 0.003$) were also higher. In each case, a test for trend suggested levels were higher in moderate-to-severe OI, intermediate in mild OI, and lowest in controls ($P = 0.06$–0.002). These changes supports the hypothesis that mutations in type I collagen induce a widespread alteration in the ECM, and that the diverse clinical manifestations of OI reflect an extensive disruption in ECM biology.

1. Introduction

Osteogenesis imperfecta [1] is a rare, heritable disorder of connective tissue that primarily presents with increased bone fragility. Short stature, immobility, skeletal deformities, and recurrent debilitating fractures are typical features in individuals with severe forms of the disease. Whereas over 90% of OI is caused by dominant pathogenic variants in *collagen type 1 alpha 1* (*COL1A1*) or *alpha 2* (*COL1A2*) genes, mutations in genes encoding proteins necessary for post-translational modification and cellular processing of type I collagen also result in recessively inherited, severe forms of the disease [2,3]. Type I collagen is the most abundant type of collagen and is expressed in almost all connective tissues, including bone, tendon, ligaments, sclera, and skin. Thus, not surprisingly, individuals with OI can also present with joint laxity, abnormal scar formation, pulmonary abnormalities, dentinogenesis imperfecta, thinning of the sclera, conductive hearing loss, and muscle weakness.

Type I collagen is one of a multi-functional molecular family of extracellular matrix (ECM) proteins that includes over 20 collagen types. Collagens differ in their distribution and function, but they share commonalities in structure and cellular pathways of processing, assembly, and degradation, and they participate in signaling functions involved in the control the ECM. Moreover, collagens, including type I, interact with an array of other proteins that are part of the ECM [4–7]. Thus, we hypothesized that abnormalities in type I collagen could affect the turnover and function of other EMC proteins, including other collagens. To explore the hypothesis, we examined serum levels of biomarkers reflecting the synthesis or degradation of collagen types III, IV, V, VI, and VII in adults with OI and compared them to a control population. In the first human study of its type, we demonstrate that the biomarkers of non-type I collagen are altered in individuals with OI. Our study suggests that disturbances in non-type I collagens may also

* Corresponding author.
E-mail address: nicol@ohsu.edu (L. Nicol).

https://doi.org/10.1016/j.bone.2018.09.024
Received 19 July 2018; Received in revised form 10 September 2018; Accepted 27 September 2018
Available online 02 October 2018

Table 1
Characteristics of OI patients and control participants (mean ± standard deviation).

	Controls (N = 101)	OI type I (N = 43)	OI type III/IV (N = 23)	*P* value OI type I vs. Control	*P* value OI type III/IV vs. Control	*P* value OI Total body type I verses OI type III/IV
Age (years)	46 ± 12	43 ± 12	37 ± 10	0.18	0.0006	0.03
Male (%)	40 (40)	21 (49)	6 (29)	0.30	0.41	0.15
White (%)	96 (95)	43 (100)	18 (86)	0.14	0.17	0.04
Height (m)	1.7 ± 0.1	1.6 ± 0.2	1.2 ± 0.3	< 0.0001	< 0.0001	< 0.0001
Weight (kg)	70.7 ± 12.5	67.3 ± 17.8	55.7 ± 16.0	0.20	< 0.0001	0.003
BMI ($kg/m^{2)}$	24.0 ± 3.0	28.6 ± 18.1	39.4 ± 17.2	< 0.03	< 0.0001	0.0006
BMC(g)[a]	1953 ± 454	1514 ± 388	1072 ± 458	< 0.0001	< 0.0001	0.001
Fat mass(kg)[a]	18.7 ± 7.4	19.6 ± 10.1	18.0 ± 8.3	0.61	0.84	0.56
Appendicular Lean mass (kg)[a]	21.9 ± 5.5	19.2 ± 5.7	12.5 ± 5.7	< 0.02	< 0.0001	< 0.0001

[a] Data available for 56 controls, 39 type I OI patients, and 21 type III/IV patients.

contribute to the pathophysiology of OI.

2. Materials and methods

2.1. Study design

Fasting serum samples were collected from adults with OI who were recruited for a clinical trial to study the effect of teriparatide therapy (NCT00131469) [8]. Samples were obtained before any therapy was instituted. Patients ≥18 years of age with a well-established clinical diagnosis of OI and fused epiphyses were enrolled at three academic centers: Oregon Health & Science University, Portland, OR, Kennedy Krieger Institute, Baltimore, MD, and Baylor College of Medicine, Houston, TX. Individuals with the following were excluded: therapy with anabolic or anti-resorptive therapy within one year prior to enrollment (26 had received bisphosphonates in the past, all for < 2 years and 14 for < 1 year); creatinine clearance < 30 ml/min; serum alkaline phosphatase > 1.5× upper limit of normal (ULN); aspartate aminotransferase and alanine aminotransferase > 3× ULN; hypercalcemia or hypocalcemia; and abnormal thyroid function. Subjects with serum 25-hydroxy vitamin D levels < 15 ng/ml (37.4 nmol/l) were eligible for enrollment after supplementation yielded levels that exceeded that threshold. As reported previously [8], fasting serum was obtained from a healthy control population of age- and sex-matched adult volunteers (n = 101) recruited from the Portland area. Whole body aBMD, bone mineral content (BMC) and body composition were measured by dual-energy X-ray absorptiometry (DXA) in the participants with OI as well as 56 control participants. All study procedures were approved by appropriate Institutional Review Board(s) including permission to use serum samples obtained in the study for future research analysis.

2.2. Biomarker assays

Serum samples were frozen at -80° C until analysis, and for each marker all samples were assayed together in a blinded and randomized fashion. Peptides were measured by ELISAs that have been well-validated for use in human serum. Samples were run in duplicate and inter- and intra-assay CV% values are available for review in Supplemental Table 1. The assays were used to measure peptides from the following regions: 1) the triple helix region (C3M) and pro-peptide region of type III collagen (ProC3) [9,10]; 2) the triple helix region (C4M2) and the 7S domain of type IV collagen (P4NP7S) [11–13]; 3) the triple helix region (C5M) and pro-peptide region of type V collagen (ProC5) [14–17]; 4) the triple helix region (C6M) and pro-peptide region of type VI collagen (ProC6) [18–21]; and 5) the triple helix region of type VII collagen (C7M) [22]. The pro-sequences peptides of collagens (e.g. ProC3) are derived from cleavage of the pro-peptide during post-translational processing, and are considered to reflect rates of protein synthesis. On the other hand, mid-portion (triple helix) peptides (e.g. C3M) likely reflect degradation through the actions of specific metalloproteinases or cathepsins, or via endocytic degradation pathways [23].

2.3. Statistical analysis

Statistical analyses Means and standard deviations for each group were calculated. Age and body mass index (BMI) adjusted least square means and 95% confidence intervals were computed for biomarker levels. Comparisons among groups were performed using analysis of variance (ANOVA), and were adjusted for age and BMI. Differences between the three groups were also evaluated using tests for trend. Models with no adjustments, or with adjustment for age and weight, yielded essentially identical results. A P value < 0.05 was considered to be significant. All analyses were performed using SAS version 9.3. SAS Institute, Cary, NC, USA. We adjusted for multiple comparisons using the Holm-Bonferroni (H–B) method to control the family-wise error rate (FWER). We applied the FWER to the association of biomarkers between controls and OI type I, and between controls and OI type III / IV, separately. Each biomarker – OI type association was ranked according to statistical significance, from 1 to 9 (Step 1). We then calculated the FWER by dividing the overall Type I error rate (α) by the total number of biomarkers (9) minus the rank (calculated in Step 1) plus 1 (Step 2). If the statistical significance (Step 1) was less than the FWER (Step 2) then the association continued to remain statistically significant at the FWER (Step 3).

3. Results

3.1. Participant characteristics

The characteristics of the OI and control participants are provided in Table 1 and have been previously reported [24]. Of those with OI, 39 had Sillence type I (due to collagen type I haploinsufficiency) and 23 had Sillence types III/IV (due to type I collagen structural abnormalities). Nearly all participants were Caucasian. Height and weight were similar between the control population and patients with OI type I; those with types III/IV OI had significantly lower height and weight. Total body BMC was lower in both the type I and type III/IV groups compared to controls, and total body BMC in those with type III/IV was significantly lower compared to the type I OI group.

3.2. Biomarkers of non-type I collagens are different in individuals with OI compared to unaffected individuals

Compared to controls, those with types III/IV OI had a higher mean level of the synthesis markers of collagen III (ProC3) ($P = 0.02$), and levels of collagen V (ProC5) ($P = 0.07$) were slightly, but not significantly, higher. Degradation markers of collage type IV (C4M2) ($P = 0.04$) and type VI (C6M) ($P = 0.003$) were also higher in type III/IV OI (Table 2). In each case, the test for trend across participant groups suggested levels of these markers were higher in type III/IV,

Table 2
Biomarker levels in type I and types III/IV compared to controls adjusted for age and BMI.

	Control (N = 101)	Type I (N = 39)	*P* value[a]	Type III/IV (N = 22)	*P* value[a]	*P* for trend
C3M	4.83 (4.53, 5.13)	5.06 (4.59, 5.53)	0.42	5.21 (4.53, 5.90)	0.33	0.26
PROC3	12.21 (11.22, 13.20)	13.57 (12.02, 15.11)	0.15	15.27 (13.03, 17.51)	0.02	0.01
C4M2	21.42 (20.04, 22.79)	22.27 (20.12, 24.41)	0.51	25.08 (21.97, 28.20)	0.04	0.06
P4NP7S	207.36 (190.74, 223.98)	230.89 (204.93, 256.85)	0.14	235.51 (197.78, 273.23)	0.20	0.10
C5M	3.42 (3.14, 3.70)	3.74 (3.30, 4.17)	0.23	3.63 (3.00, 4.27)	0.56	0.34
PROC5	358.42 (326.73, 390.11)	387.80 (338.30, 437.30)	0.33	433.15 (361.22, 505.09)	0.07	0.06
C6M	10.37 (9.17, 11.56)	12.39 (10.52, 14.26)	0.08	15.04 (12.33, 17.76)	0.003[b]	0.002
PROC6	8.14 (7.50, 8.79)	8.49 (7.49, 9.49)	0.57	9.08 (7.62, 10.54)	0.26	0.26
C7M	12.51 (11.22, 13.79)	10.39 (8.38, 12.39)	0.08	10.76 (7.85, 13.67)	0.30	0.12

[a] Verses control.
[b] Significant using H−B method of multiple comparisons.

intermediate in type I, and lowest in controls ($P = 0.06$–0.002). Because C6M is highly expressed in muscle, we further adjusted for lean mass in the type III/IV patients; levels remained higher in those with OI after adjustment (controls 9.95 (8.54, 11.36), cases (14.1 (11.43, 16.77): $P = 0.015$).

4. Discussion

Despite known effects of type I collagen mutations on bone, there are little data concerning how defects in collagen I may alter the homeostasis of other collagens in humans. In this study we demonstrate that plasma biomarkers of collagen types III, IV, VI and VII are altered in patients with OI compared to age- and sex-matched controls, providing evidence that the mutations in type I collagen underlying the pathophysiology of OI could be associated with disturbances in other collagens that are critical elements of the ECM. Understanding the basis of these abnormalities may help explain the genesis of both skeletal and non-skeletal manifestations of OI, and provide a broader understanding of the biological basis of this disease.

All the collagens we considered play important structural roles in the ECM. ECM maintenance is a dynamic process balanced by pathways controlling protein synthesis and turnover [23]. Biomarkers of non-type I collagens are elevated in other disorders characterized by increased connective tissue turnover [7,23].We found markers presumed to be associated with both collagen formation (e.g. ProC3, ProC5) and degradation (e.g. C4M2, C6M) to be altered in OI. However, how the rates of collagen turnover are specifically affected, and the nature of underlying perturbations in the ECM, must be determined in additional studies.

Whether the changes in collagens we describe are the result of abnormalities in bone vs other tissues is an interesting consideration. Disturbances in collagens prominently expressed in bone could result from the skeletal changes induced by type I collagen mutations, whereas changes in those collagens minimally expressed in bone could be attributed to the interactions with aberrant collagen I in other tissues. For example, in bone type V collagen acts as a core structural element in the assembly of heterofibrils that include type I collagen, and is present in other tissues such as corneal stroma, muscles, liver, lungs placenta (www.proteinatlas.org) [5,6]. Complete loss of collagen type V expression results in embryonic lethality in mice; collagen fibrils are absent leading to a series of significant abnormalities including in the spine (www.proteinatlas.org) [5,6,25,26]. Human mutations in collagen type V lead to Ehlers-Danlos syndrome, classic type, another multi-tissue disease with skeletal manifestations (www.proteinatlas.org) [5,6,25–29]. Therefore, it is likely that alterations in biomarkers of collagen type V are at least in part the result of skeletal abnormalities in OI. In our patients with OI, ProC5 levels were minimally higher than in controls and, similarly, the levels of other markers of type I collagen (NTX, P1NP) were in the normal range [8]. On the other hand, collagen type III is expressed in most extensible tissues (e.g. skin, lung, and vascular structures) [5,30–33] where it has been shown to interact with type I collagen [4]. Its RNA is expressed in mouse osteoblasts (www.biogps.org) and proteomic profiling of the ECM from human osteoblasts derived from mesenchymal stem cells identified the presence of the collagen III protein [34]. However, little is known about its direct expression in human bone. Pathogenic variants in *COL3A1* cause Ehlers Danlos syndrome, vascular type (MIM 130050) which can manifest with skin, visceral, and vascular fragility; however, other than acroosteolysis, involvement of bone is not a clinical feature of the disorder [30,31,35]. Hence, the alterations in type III collagen turnover biomarkers we describe may primarily reflect events in non-skeletal tissues. Type VI collagen has a more globular tertiary structure and is found in interstitial and microfibrillar structures that interweave with other collagen fibrils, including type I [36]. It is present in nearly all connective tissues but especially in muscle [1,5,37,38]. In fact, pathogenic variants in genes encoding the alpha chains of type VI collagen result in Bethlem and Ullrich congenital muscular dystrophy [39] and elevated levels of C6M has been shown to be related to lean mass [20,21]. Type VI collagen is also a component of articular cartilage and bone [40] and *Col6a1*$^{-/-}$ mice have osteoarthritic joint disease, reduced bone mineral density and abnormal trabecular structure [41,42]. RNA expression studies demonstrate several of the collagen VI genes are expressed in mouse osteoblasts (www.biogps.org) and its protein has been identified in proteomic studies of ECM derived from human osteoblasts [34]. Thus, the elevations we found in the levels of C6M could be multifactorial but may also reflect alterations in muscle or joints rather than bone. The structure and function of type IV collagen is different than the afore-mentioned collagens. It is a network-forming collagen, underlies the epithelium, and is the main collagen constituent of the basement membrane. Mutations in type IV collagen present as vascular or renal syndromes [43–45] and although it is expressed in most tissues [20,46–49], its presence in bone appears to be quite limited [34,46]. Thus, collagen IV biomarkers may also primarily reflect non-skeletal events. In sum, the alterations in the biomarkers we studied may reflect alterations in the biology of both skeletal and non-skeletal tissues in OI. If verified in additional studies, and if associated with manifestations of the disease, these measures may represent unique biomarkers with clinical and research usefulness.

This report is one of the first to describe alterations in non-type I collagens in OI. Thiele et al. [50] reported that in a mouse model of OI (AGA2) there was altered expression of other collagens in the heart (type VIII) and lung (type III, V). The changes in type I collagen in OI may alter the function of other collagens in several ways. In addition to changes in the structure of complex multi-collagen elements of the ECM that may be induced by type I collagen mutations, differences in the biomarker levels we report may also reflect perturbations in the cellular processing of other collagens. Mutations resulting in structural misfolding of collagen I can result in endoplasmic reticulum stress, changes in protein processing pathways and apoptosis. [51–55]. Since more than one collagen type can be produced by the same cell [56], and as

collagens share cellular biosynthetic pathways [7], dysregulation of intracellular processing of type I collagen in OI could affect the synthesis and processing of other collagens.

Alterations in type I and other collagens may also affect ECM signaling pathways. For example, collagen domains interact with integrin receptors that are not only important in cell adhesion but also in the regulation of collagen synthesis and degradation [3,57,58]. Collagens can be ligands for the dimeric discoidin receptors which regulate collagen deposition in the ECM through the inhibition of fibrillogenesis [59], and fragments of various collagens have important signaling functions; the type IV collagen fragments tumstatin and endotrophin, the type VIII collagen fragment vastain, the type XV collagen fragment restin as well as the type XVIII collagen fragment endostatin all have been shown do have direct signaling roles [60,61]. In OI, increased availability of mature TGFβ may result from altered interactions of fibrillar type I collagen and small leucine rich proteoglycans such as decorin [62].

The study has several strengths, including relatively large numbers of participants with OI, the inclusion of an age- and sex-matched control group, and the careful collection and analysis of serum samples. We used well-established assays of a variety of collagen biomarkers reflecting a spectrum of ECM functions. On the other hand, changes in the concentrations of circulating biomarkers are an indirect reflection of underlying molecular, cellular and tissue disturbances. Also,we do not know the effects of prior exposure to anti-resorptive therapy on collagen metabolism. Although unlikely, we can't eliminate the possibility of those effects in those few participants who had brief therapy more than one year before the study began. Additional basic studies are needed to understand the origins of changes in the handling of ECM collagens and their functional implications. Our results should be verified in studies of independent populations. We studied only adults with OI, and similar studies should be undertaken in children. Finally, although we examined several collagens involved in the ECM, there may be other proteins that are also disturbed, and further understanding of these processes, as well as how the alterations in the collagen turnover biomarkers respond to potential therapies, are of interest.

In summary, circulating biomarkers of collagen types III, IV and VI were increased in adults with types III/VI OI. These changes suggest that mutations in type I collagen induce a widespread alteration in the ECM, and raise the possibility that the diverse clinical manifestations of OI reflect an extensive disruption in ECM biology. Additionally, the presence of altered biomarker levels for collagen types IV, and VI which may not arise directly from bone perturbations, may reflect disruption in the function of the ECM that lead to some of the non-skeletal manifestations of OI.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2018.09.024.

Acknowledgements

This manuscript is the result of work supported with institutional resources at Oregon Health & Science University and research support from Eli Lilly. Collagen assays were performed by Nordic Bioscience. The authors would also like to thank Priya Srikanth for her support with the statistical analyses and the OHSU Biostatistics & Design Program (partially supported by UL1TR002369 [OHSU CTSA]).

Authors' roles

Study concept and design: LN, EO
Data collection: LN, EO, JS, SN, BL
Data analysis and interpretation: LN, YW, EO
Drafting manuscript: LN, EO, PM
Critical review and final approval of the manuscript content: all authors

Disclosures

Patrick Morar and Ying Wang have no conflicts of interest to disclose. Lindsey Nicol is a sub-investigator on a clinical research study sponsored by Mereo. Sandesh Nagamani and Brendan Lee receive research support from Genzyme for OI-related research. Kim Henriksen (KH), Shu Sun, and Morten Karsdal (MK) are employees of Nordic Bioscience and have patents on the biomarkers. KH and MK also hold stock in Nordic Bioscience. Rosamund Smith is a retired employee of Eli Lilly and owns Eli Lilly stock. Jay Shapiro is a medical consultant for Mereo. Eric Orwoll is a research consultant for Bayer and receives research support from Eli Lilly and Mereo.

References

[1] P. Iyengar, et al., Adipocyte-derived collagen VI affects early mammary tumor progression in vivo, demonstrating a critical interaction in the tumor/stroma microenvironment, J. Clin. Invest. 115 (5) (2005) 1163–1176.
[2] A. Forlino, J.C. Marini, Osteogenesis imperfecta, Lancet 387 (10028) (2016) 1657–1671.
[3] R. Morello, Osteogenesis imperfecta and therapeutics, Matrix Biol. (2018) 294–312.
[4] X. Liu, et al., Type III collagen is crucial for collagen I fibrillogenesis and for normal cardiovascular development, Proc. Natl. Acad. Sci. U. S. A. 94 (5) (1997) 1852–1856.
[5] K. Gelse, E. Poschl, T. Aigner, Collagens—structure, function, and biosynthesis, Adv. Drug Deliv. Rev. 55 (12) (2003) 1531–1546.
[6] D.E. Birk, Type V collagen: heterotypic type I/V collagen interactions in the regulation of fibril assembly, Micron 32 (3) (2001) 223–237.
[7] M.A. Karsdal, et al., The good and the bad collagens of fibrosis - their role in signaling and organ function, Adv. Drug Deliv. Rev. 121 (2017) 43–56.
[8] E.S. Orwoll, et al., Evaluation of teriparatide treatment in adults with osteogenesis imperfecta, J. Clin. Invest. 124 (2) (2014) 491–498.
[9] N. Barascuk, et al., A novel assay for extracellular matrix remodeling associated with liver fibrosis: an enzyme-linked immunosorbent assay (ELISA) for a MMP-9 proteolytically revealed neo-epitope of type III collagen, Clin. Biochem. 43 (10 − 11) (2010) 899–904.
[10] M.J. Nielsen, et al., Plasma pro-C3 (N-terminal type III collagen propeptide) predicts fibrosis progression in patients with chronic hepatitis C, Liver Int. 35 (2) (2015) 429–437.
[11] D.J. Leeming, et al., Enzyme-linked immunosorbent serum assay specific for the 7S domain of collagen type IV (P4NP 7S): a marker related to the extracellular matrix remodeling during liver fibrogenesis, Hepatol. Res. 42 (5) (2012) 482–493.
[12] J.M. Sand, et al., MMP mediated degradation of type IV collagen alpha 1 and alpha 3 chains reflects basement membrane remodeling in experimental and clinical fibrosis–validation of two novel biomarker assays, PLoS One 8 (12) (2013) e84934.
[13] D.J. Leeming, et al., Association of systemic collagen type IV formation with survival among patients undergoing hemodialysis, PLoS One 8 (8) (2013) e71050.
[14] E. Vassiliadis, et al., Immunological detection of the type V collagen propeptide fragment, PVCP-1230, in connective tissue remodeling associated with liver fibrosis, Biomarkers 16 (5) (2011) 426–433.
[15] D.J. Leeming, et al., Novel serological neo-epitope markers of extracellular matrix proteins for the detection of portal hypertension, Aliment. Pharmacol. Ther. 38 (9) (2013) 1086–1096.
[16] S.S. Veidal, et al., MMP mediated type V collagen degradation (C5M) is elevated in ankylosing spondylitis, Clin. Biochem. 45 (7–8) (2012) 541–546.
[17] D.J. Leeming, et al., Pro-C5, a marker of true type V collagen formation and fibrillation, correlates with portal hypertension in patients with alcoholic cirrhosis, Scand. J. Gastroenterol. 50 (5) (2015) 584–592.
[18] S.S. Veidal, et al., MMP mediated degradation of type VI collagen is highly associated with liver fibrosis—identification and validation of a novel biochemical marker assay, PLoS One 6 (9) (2011) e24753.
[19] M.J. Nielsen, et al., Markers of collagen remodeling detect clinically significant fibrosis in chronic hepatitis C patients, PLoS One 10 (9) (2015) e0137302.
[20] A. Nedergaard, et al., Type VI collagen turnover-related peptides-novel serological biomarkers of muscle mass and anabolic response to loading in young men, J. Cachexia. Sarcopenia Muscle 4 (4) (2013) 267–275.
[21] S. Sun, et al., Collagen type III and VI turnover in response to long-term immobilization, PLoS One 10 (12) (2015) e0144525.
[22] J.M.B. Sand, et al., Development of a neo-epitope specific assay for serological assessment of type VII collagen turnover and its relevance in fibroproliferative disorders, Assay Drug Dev. Technol. 16 (2) (2018) 123–131.
[23] W.C. Parks, R.P. Mecham, Extracellular matrix degradation, Biology of Extracellular Matrix, Springer, Berlin; Heidelberg; New York, 2011(1 online resource (x, 255 pages)).
[24] L. Nicol, et al., Serum Sclerostin levels in adults with osteogenesis imperfecta: comparison with normal individuals and response to teriparatide therapy, J. Bone Miner. Res. (2017) 307–315.
[25] S. Symoens, et al., A novel splice variant in the N-propeptide of COL5A1 causes an EDS phenotype with severe kyphoscoliosis and eye involvement, PLoS One 6 (5) (2011) e20121.
[26] K. Andrikopoulos, et al., Targeted mutation in the col5a2 gene reveals a regulatory

role for type V collagen during matrix assembly, Nat. Genet. 9 (1) (1995) 31–36.
[27] T.H. Milhorat, et al., Syndrome of occipitoatlantoaxial hypermobility, cranial settling, and chiari malformation type I in patients with hereditary disorders of connective tissue, J J. Neurosurg. Spine 7 (6) (2007) 601–609.
[28] P. Bouma, et al., COL5A1 exon 14 splice acceptor mutation causes a functional null allele, haploinsufficiency of alpha 1(V) and abnormal heterotypic interstitial fibrils in Ehlers-Danlos syndrome II, J. Biol. Chem. 276 (16) (2001) 13356–13364.
[29] A.C. Nicholls, et al., An exon skipping mutation of a type V collagen gene (COL5A1) in Ehlers-Danlos syndrome, J. Med. Genet. 33 (11) (1996) 940–946.
[30] A. Plancke, et al., Homozygosity for a null allele of COL3A1 results in recessive Ehlers-Danlos syndrome, Eur. J. Hum. Genet. 17 (11) (2009) 1411–1416.
[31] J.M. Bourhis, et al., Structural basis of fibrillar collagen trimerization and related genetic disorders, Nat. Struct. Mol. Biol. 19 (10) (2012) 1031–1036.
[32] X. Bao, et al., Developmental changes of Col3a1 mRNA expression in muscle and their association with intramuscular collagen in pigs, J. Genet. Genomics 34 (3) (2007) 223–228.
[33] L.T. Jensen, N.B. Host, Collagen: scaffold for repair or execution, Cardiovasc. Res. 33 (3) (1997) 535–539.
[34] M. Baroncelli, et al., Comparative proteomic profiling of human osteoblast-derived extracellular matrices identifies proteins involved in mesenchymal stromal cell osteogenic differentiation and mineralization, J. Cell. Physiol. 233 (1) (2018) 387–395.
[35] H. Kuivaniemi, G. Tromp, D.J. Prockop, Mutations in fibrillar collagens (types I, II, III, and XI), fibril-associated collagen (type IX), and network-forming collagen (type X) cause a spectrum of diseases of bone, cartilage, and blood vessels, Hum. Mutat. 9 (4) (1997) 300–315.
[36] H. von der Mark, et al., Immunochemistry, genuine size and tissue localization of collagen VI, Eur. J. Biochem. 142 (3) (1984) 493–502.
[37] D.R. Keene, E. Engvall, R.W. Glanville, Ultrastructure of type VI collagen in human skin and cartilage suggests an anchoring function for this filamentous network, J. Cell Biol. 107 (5) (1988) 1995–2006.
[38] Y. Zou, et al., Muscle interstitial fibroblasts are the main source of collagen VI synthesis in skeletal muscle: implications for congenital muscular dystrophy types Ullrich and Bethlem, J. Neuropathol. Exp. Neurol. 67 (2) (2008) 144–154.
[39] K.M. Bushby, J. Collins, D. Hicks, Collagen type VI myopathies, Adv. Exp. Med. Biol. 802 (2014) 185–199.
[40] M. Cescon, et al., Collagen VI at a glance, J. Cell Sci. 128 (19) (2015) 3525–3531.
[41] L.G. Alexopoulos, et al., Developmental and osteoarthritic changes in Col6a1-knockout mice: biomechanics of type VI collagen in the cartilage pericellular matrix, Arthritis Rheum. 60 (3) (2009) 771–779.
[42] S.E. Christensen, et al., Altered trabecular bone structure and delayed cartilage degeneration in the knees of collagen VI null mice, PLoS One 7 (3) (2012) e33397.
[43] B.G. Hudson, et al., Alport's syndrome, Goodpasture's syndrome, and type IV collagen, N. Engl. J. Med. 348 (25) (2003) 2543–2556.
[44] M. Mao, et al., Type IV collagens and basement membrane diseases: cell biology and pathogenic mechanisms, Curr. Top. Membr. 76 (2015) 61–116.
[45] M.A. Karsdal, et al., Biochemistry of collagens, laminins and elastin: structure, function and biomarkers, Elsevier/AP, London, United Kingdom; San Diego, CA, United States, 2016 (Academic Press is an imprint of Elsevier. xxxiv, 238 pages).
[46] J. Khoshnoodi, V. Pedchenko, B.G. Hudson, Mammalian collagen IV, Microsc. Res. Tech. 71 (5) (2008) 357–370.
[47] Y. Ninomiya, et al., Differential expression of two basement membrane collagen genes, COL4A6 and COL4A5, demonstrated by immunofluorescence staining using peptide-specific monoclonal antibodies, J. Cell Biol. 130 (5) (1995) 1219–1229.
[48] J.H. Miner, J.R. Sanes, Collagen IV alpha 3, alpha 4, and alpha 5 chains in rodent basal laminae: sequence, distribution, association with laminins, and developmental switches, J. Cell Biol. 127 (3) (1994) 879–891.
[49] S. Gunwar, et al., Glomerular basement membrane. Identification of a novel disulfide-cross-linked network of alpha3, alpha4, and alpha5 chains of type IV collagen and its implications for the pathogenesis of Alport syndrome, J. Biol. Chem. 273 (15) (1998) 8767–8775.
[50] F. Thiele, et al., Cardiopulmonary dysfunction in the Osteogenesis imperfecta mouse model Aga2 and human patients are caused by bone-independent mechanisms, Hum. Mol. Genet. 21 (16) (2012) 3535–3545.
[51] S.D. Chessler, P.H. Byers, BiP binds type I procollagen pro alpha chains with mutations in the carboxyl-terminal propeptide synthesized by cells from patients with osteogenesis imperfecta, J. Biol. Chem. 268 (24) (1993) 18226–18233.
[52] S.R. Lamande, et al., Endoplasmic reticulum-mediated quality control of type I collagen production by cells from osteogenesis imperfecta patients with mutations in the pro alpha 1 (I) chain carboxyl-terminal propeptide which impair subunit assembly, J. Biol. Chem. 270 (15) (1995) 8642–8649.
[53] T.S. Lisse, et al., ER stress-mediated apoptosis in a new mouse model of osteogenesis imperfecta, PLoS Genet. 4 (2) (2008) e7.
[54] J.F. Bateman, R.P. Boot-Handford, S.R. Lamande, Genetic diseases of connective tissues: cellular and extracellular effects of ECM mutations, Nat. Rev. Genet. 10 (3) (2009) 173–183.
[55] J.C. Marini, et al., Consortium for osteogenesis imperfecta mutations in the helical domain of type I collagen: regions rich in lethal mutations align with collagen binding sites for integrins and proteoglycans, Hum. Mutat. 28 (3) (2007) 209–221.
[56] S. Gay, et al., Simultaneous synthesis of types I and III collagen by fibroblasts in culture, Proc. Natl. Acad. Sci. U. S. A. 73 (11) (1976) 4037–4040.
[57] C.G. Knight, et al., Identification in collagen type I of an integrin alpha2 beta1-binding site containing an essential GER sequence, J. Biol. Chem. 273 (50) (1998) 33287–33294.
[58] Y. Xu, et al., Multiple binding sites in collagen type I for the integrins alpha1beta1 and alpha2beta1, J. Biol. Chem. 275 (50) (2000) 38981–38989.
[59] L.A. Flynn, et al., Inhibition of collagen fibrillogenesis by cells expressing soluble extracellular domains of DDR1 and DDR2, J. Mol. Biol. 395 (3) (2010) 533–543.
[60] M.A. Karsdal, et al., Serum endotrophin identifies optimal responders to PPARgamma agonists in type 2 diabetes, Diabetologia 60 (1) (2017) 50–59.
[61] M.A. Karsdal, et al., Novel insights into the function and dynamics of extracellular matrix in liver fibrosis, Am. J. Physiol. Gastrointest. Liver Physiol. 308 (10) (2015) G807–G830.
[62] I. Grafe, et al., Excessive transforming growth factor-beta signaling is a common mechanism in osteogenesis imperfecta, Nat. Med. 20 (6) (2014) 670–675.