Autosomal Recessive Osteogenesis Imperfecta: A Puzzle for Bone Formation, Structure and Function

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Abstract Bone is a composite material that resembles reinforced concrete. The collagen matrix plays the role of reinforcement, whereas hydroxyapatite crystals are cementing material. Collagen fibers are responsible for the tensile strength of bones and prevent fractures from extending; the mineral phase is able to withstand compression loads. A right balance of these two parts synergistically provides the required stiffness for bone. Collagen abnormalities, such as reduced amount, disturbed composition, defects in structure and/or supra-molecular organization as well as insufficient or defective mineralization, lead to osteogenesis imperfecta (OI), also known as brittle bone disease. Until recently, mutations in the type I collagen genes *COL1A1* and *COL1A2* were the only known causes of the disease, which cover about 90 % of diagnosed OI. Within the last decade, we have witnessed a burst in the identification of new OI mutations in other genes. Here we summarize our knowledge of these mutations and their impact on bone quality.

Keywords Osteogenesis imperfecta · Autosomal recessive · Protein folding · Posttranslational modification · Hydroxylase · Hydroxyproline · Hydroxylysine · Glycosylation · Chaperone · Cross-linking

Introduction

Osteogenesis imperfecta (OI), a genetic disease, is primarily characterized by increased bone fragility and reduced bone mass [1]. Its overall frequency is about 10^{-4} . Bone fragility has led to the trivial name of ‘brittle bone disease.’ The most severe OI forms correlate with the strongest defects in collagen fibers. As collagen is an important component in other connective tissues, patients affected by OI generally exhibit an additional set of symptoms, including short stature, dentinogenesis imperfecta, loose joints, skin hyperlaxity, hearing loss, blue sclera, and neurological and pulmonary complications. Collagen matrix abnormalities also affect the mineral phase of bone. Bones from OI patients show a lower average bone mineral density and break more easily when deformed; in addition, fatigue damage accumulates much faster on repetitive loading of OI bones [2]. The material properties of the matrix are an important aspect in studying the mechanisms of OI.

In most OI studies, collagen production is examined in skin fibroblasts. Much less is known about the effects of mutations on osteoblasts, the primary sources of collagen in bone. Osteoblasts may differ with regard to post-translational modifications of collagen as well as in the propensity to incorporate collagen molecules into the extracellular matrix. Indeed, a recent study on 3-hydroxyproline sites in type I collagen from different tissues of the wild-type and *LEPRE1*-deficient mice showed not only the

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variability in the post-translational modification between skin, tendon and bone, but also demonstrated a change in the expression profile of other 3-hydroxylases [3]. Osteoblasts harboring an OI mutation may also have an abnormal expression pattern of other matrix proteins, such as proteoglycans, hyaluronan, asporin, decorin, fibronectin, thrombospondin, osteocalcin, SPARC and others.

Certain types of mutations leading to OI are associated with the induction of an endoplasmic reticulum stress-specific unfolded protein response involving upregulation of BiP, Hsp47 and Gadd153 with caspase-12 and -3 activation and apoptosis of osteoblasts both in vitro and in vivo [4]. Most of the newly identified OI mutations remain unexplored in terms of induction of the ER unfolded protein response.

Recently new OI mutations were identified in non-collagenous genes, i.e., *PLOD2*, *CRTAP*, *LEPRE1*, *PPIB*, *BMP1*, *SERPINH1*, *SERPINF1*, *FKBP10*, *TMEM38B*, *IFITM5*, *SMPD3*, *SP7* and *WNT1*, and the list is likely to be increased. All of these mutations are autosomal recessive, with the exception of an ‘activating’ dominant mutation in *IFITM5* [5•, 6•]. There are also recessive and dominant mutations found in *WNT1* [7, 8•, 9•, 10•, 11]. Here we review autosomal recessive OI mutations in genes not coding for collagen type I chains.

P3H1•CRTAP•CypB Complex

CRTAP was the first non-collagenous gene where human mutations were found to cause OI [12]. The primary indication of the existence of a complex of P3H1•CRTAP•CypB was previously reported by Vranka et al. [13] and further characterized in the CRTAP OI study [12]. Reports that alterations in *LEPRE1* (gene for P3H1) and *PPIB* (gene for CypB) cause very similar phenotypes as well as more cases of *CRTAP* mutations followed quickly [14•, 15–20]. Today, multiple cases of recessive OI due to mutations in components of the complex have already been described, and clinical cases and mouse models involving CRTAP, P3H1 and CypB keep accumulating (https://oi.gene.le.ac.uk/home.php?action=switch_db).

The molecular mechanism leading to OI in these cases remains speculative. In normal type I collagen, there is a single site, Pro986 in the $\alpha 1$ chain, which is fully 3-hydroxylated. Most mutations in *CRTAP*, *LEPRE1* or *PPIB* abolish this modification [14•, 15–17, 20, 21]. There are, however, a few exceptions [22, 23•] in which residual activity of P3H1 probably remains.

Mutations in all three genes usually result in overmodification (excess of lysyl hydroxylation and glycosylation) of collagen pro- α chains in the ER, presumably due to their delayed assembly into triple helices [12, 14•, 21, 23•]. This phenomenon is also observed in the dominant OI forms

with collagen mutations [24, 25]. A role of the P3H1•CRTAP•CypB complex in accelerating collagen folding is suggested, and an in vitro study has established that it is a potent molecular chaperone [26]. It has to be noted that CypB exhibits diverse functions and is involved in other interactions besides being a part of the P3H1•CRTAP•CypB complex. It was recently demonstrated that an N-terminal missense mutation in *PPIB* probably alters the activity of the lysyl hydroxylase 1 (LH1) without affecting the catalytic activity of CypB, which results in near normal collagen modifications regardless of the delayed folding rate [27•] (Table 1).

The absence of the 3-hydroxyl group at Pro986 or delayed folding leading to the excessive sugar modifications in type I collagen are two possible causes that lead to OI pathologies. Which one is the primary factor remains to be explored. The missing 3-hydroxyl group at Pro986 could also affect interactions of collagen with other matrix or signaling molecules, whereas extra sugars might adversely affect fibril assembly and the mechanical properties of bones as well as aberrantly influence fibril interactions in the bone milieu. It cannot be excluded that both factors contribute cooperatively in the disease. A more elaborate discussion of OI causative mutations in the P3H1•CRTAP•CypB complex components can be found in a recent publication [28•].

HSP47 (SerpH1)

HSP47, encoded by gene *SERPINH1*, is a collagen-binding protein and a collagen-specific chaperone in the ER. A recessive mutation in *Serpinh1* leading to an OI phenotype was originally identified in Dachshunds [29], and recently a human mutation in *SERPINH1* and its effect on type I procollagen production were reported [30•]. A knockout of *Serpinh1* in mice leads to embryonic lethality and has pleiotropic effects on collagen-containing tissues [31]. The mature form of type I collagen was significantly reduced in embryonic tissues. Type I procollagen secreted by a fibroblast cell line was protease sensitive at 37 °C in contrast to the control. Type I procollagen molecules formed aggregates in the ER and secretion was delayed. Cleavage of the pro $\alpha 1$ (I) N-propeptide was also deficient in *Serpinh1*^{-/-} fibroblasts.

Human autosomal recessive missense mutation (p.Leu78Pro) in *SERPINH1* results in degradation of HSP47 via the proteasome [30•]. Mutations of *SERPINH1* do not result in overmodified collagen chains and do not affect Pro986 3-hydroxylation, indicating that the protein interacts with type I procollagen at later stages of biosynthesis [30•]. In addition, secreted type I procollagen trimers have increased protease sensitivity. Although HSP47 can interact with individual chains, it appears that the preferred

Table 1 Autosomal recessive OI genes

Gene	Protein	Overmodification	Secretion	Thermal stability	Other defects	References
<i>LEPRE1</i>	P3H1	Yes	Delayed in ER	Higher	Lack of 3-hydroxylation	[14•, 15, 18, 19, 66–68]
<i>CRTAP</i>	CRTAP	Yes	Delayed in ER	Higher	Lack of 3-hydroxylation	[12, 15, 16, 18, 20, 69]
<i>PPIB</i>	CypB	Yes/no	Delayed/normal	Higher	Lack of 3-hydroxylation	[17, 21, 22, 70–72]
<i>FKBP10</i>	FKBP65	No	Delayed	Unknown	Telopeptide cross-linking	[37•, 39, 40, 41•, 73–76]
<i>PLOD2</i>	LH2	No	Normal	Unknown	Telopeptide cross-linking	[77, 78]
<i>SERPINH1</i>	HSP47	Yes/no	Delayed in ER/ delayed in Golgi	Higher/lower	Protease degradation of collagen	[29, 30•]
<i>SERPINF1</i>	PEDF	No	Normal	Normal	Reduced mineralization; excessive bone resorption?	[45•, 46•, 47, 48, 50•]
<i>BMP1</i>	BMP1	No?	No?	No?	C-propeptide cleavage	[42, 79]
<i>TMEM38B</i>	TRICB	Unknown	Unknown	Unknown	Mineralization?	[56, 57]
<i>SP7</i>	Osterix	Unknown	Unknown	Unknown	Reduced collagen transcription?	[63, 64]
<i>WNT1</i>	Wnt1	Unknown	Unknown	Unknown	Unknown	[7, 8••, 9•, 10•, 11]
<i>SMPD3</i>	SMPD3	No	Normal	Unknown	Reduced SPARC level; reduced mineralization; excessive bone resorption	[58–60]

substrate is the triple helical domain of intact procollagen trimers in the ER [32, 33]. Release of collagen from HSP47 takes place in the *cis*-Golgi or ER-Golgi intermediate compartment and probably is triggered by the lower pH [34, 35]. The missense mutation in *SERPINH1* leads to slightly delayed overall transit time from the ER to the extracellular space due to accumulation in the Golgi [30•]. It points to a possibility that the p.Leu78Pro mutation affects the pH-dependent release of HSP47 in the Golgi and retards collagen secretion, whereas the absence of Hsp47 in mice leads to ER-retention and stress.

A mutation in Dachshunds that also converted a leucine residue to a proline residue (p.Leu326Pro), but near the carboxy-terminal end of HSP47, resulted in a viable yet severe OI phenotype [29]. Again, the mutation probably does not represent a complete null allele but has some residual activity, which results in live-born dogs with OI instead of the embryonic lethality seen in *Serpinh1* knockout mice.

Both OI missense mutations occur in the hydrophobic core of HSP47, distant from the collagen binding site [36••]. These mutations most likely severely affect the overall structure, stability and functionality of HSP47.

FKBP65 and LH2

Two mutations have been reported in *FKBP10* (MIM 607063) in patients with moderately severe OI and recessive disease transmission [37•]. *FKBP10* codes for FKBP65, which is a protein with known type I procollagen chaperone function [38]. Investigation of the FKBP65 mutations in dermal fibroblasts from affected individuals

revealed normal 3-hydroxylation of proline 986 but delayed secretion of type I procollagen [37•]. Mutations in FKBP65 have also been described in patients with Bruck syndrome type I [39] and Kuskokwim syndrome [40]. Bruck syndrome type 2 is associated with mutations in *PLOD2* (lysyl hydroxylase 2, LH2). It has recently been shown that mutations in FKBP65 lead to a decrease in hydroxylysine residues in the telopeptides of type I collagen in bone [41•]. These hydroxylysines are involved in crosslinking of type I collagen, and LH2 is the enzyme catalyzing this modification. The activity of LH2 seems to be dependent on the presence of FKBP65, in a similar manner that the activity of LH1 seems to require CypB (26).

BMP-1 (mTld)

The major known function of bone morphogenic protein 1 (BMP-1) is the cleavage of the C-terminal propeptides of procollagen I, II and III. The other function that might contribute to the OI phenotype is the proteolytic activation of lysyl oxidase. Lysyl oxidase catalyzes the posttranslational formation of aldehydes from lysine and hydroxylysine residues in collagen precursors. These highly reactive aldehydes spontaneously form cross-links, which are essential for stabilization of collagen fibrils.

Two types of mutations causing OI were identified in *BMP1*. One mutation is located within the BMP-1 signal peptide and leads to impaired secretion and an alteration in posttranslational modification [42•]; the other causes an alteration in the protease domain of BMP-1. For both types, concomitant abnormal procollagen I C-propeptide

processing was observed. However, the following differences were discovered: the signal peptide mutation was accompanied by an increase in bone mineral density, whereas the catalytic domain mutation presented a decrease. No rational explanation exists for this discrepancy at the moment.

An interesting *COL1A1* mutation affecting the BMP-1 C-propeptide cleavage site was recently published [43]. The patients presented an increased-mineralization OI phenotype very similar to the signal peptide mutation in BMP-1.

PEDF (Serpin F1)

Pigment epithelium-derived factor (PEDF) is an ubiquitously expressed and secreted glycoprotein of the serpin superfamily, which is primarily known for its neurotrophic and antiangiogenic properties [44]. Multiple mutations were identified in the coding regions of the *SERPINF1* gene that lead to OI [45•, 46•, 47]. An in-frame duplication in *SERPINF1* was reported to be responsible for the OI phenotype in a patient who has the concurrent phenotype of two rare recessive diseases, cystinosis (caused by mutation in the *CTNS* gene) and OI [48]. In all reported cases so far, synthesis, folding, posttranslational modification and secretion of type I collagen seem to be normal, but bone mineral density is severely reduced. Serum levels of PEDF correlate with the OI caused by mutation in the *SERPINF1* gene and can be used for diagnostics [49].

Pedf null mice revealed reduced trabecular bone volume and the accumulation of unmineralized bone matrix. Fourier transformed infrared microscopy (FTIR) indicated an increased mineral:protein matrix ratio in mutant bones, which were more brittle than in controls. In vitro, osteoblasts from *Pedf* null mice exhibited enhanced mineral deposition as assessed by alizarin red staining and an increased mineral:protein matrix ratio determined by FTIR analysis of calcified nodules [50•].

PEDF was detected in osteoblasts, chondrocytes and, to a lesser extent, osteoclasts, allocated in areas of active bone remodeling [51]. Osteoblasts and osteoclasts express PEDF, VEGF-A isoforms and VEGF receptors, possible mediators of angiogenesis and matrix remodeling in the bone [52]. Type I collagen directly binds secreted PEDF [53] and restrains osteoclast precursor cell differentiation and hence bone resorption [54]. Decrease or loss of PEDF disturbs the balance between osteoblasts and osteoclasts, which leads to excessive bone resorption and development of OI.

TRICB (TMEM38B)

Trimeric intracellular cation channel type B (TRICB) protein is encoded by transmembrane protein 38B gene

(*TMEM38B*). It forms a multi-pass membrane cation channel that maintains rapid calcium release from intracellular stores [55]. Two reports of the same mutation in *TMEM38B* causing a recessive form of OI were published last year. The homozygous deletion mutation of exon 4 of *TMEM38B* in Saudi Arabian [56•] or Israeli Bedouin [57•] consanguineous families. The mutation results in translation of a truncated protein and a four-fold reduction of mRNA levels. The molecular mechanism leading to OI remains unknown. Aberrant function of TRICB might detrimentally affect calcium homeostasis in cell signaling pathways and/or in bone mineralization.

SMPD3 (nSMase2)

In 1981, a severe form of recessive OI (fragilitas ossium, fro) was discovered in a random-bred stock of mice that could not be attributed to a collagen defect [58]. Defects of type I or V collagen could not be detected from the electrophoretic patterns of α chains and cyanogen bromide-derived peptides [59]. Experiments with cultures of tissue-derived dermal fibroblasts demonstrated no abnormalities in the total collagen synthesis and secretion. A 30 % reduction in bone SPARC (osteonectin) was detected at the protein level, and a 50 % decrease in the mRNA level was found in the cultured fibroblasts. Significantly reduced mineral phase was observed in bones and teeth [59, 60]. In 2005, a deletion in *Smpd3* was identified as the causative mutation in fro mice [60]. *Smpd3* encodes sphingomyelin phosphodiesterase 3 (a.k.a. neutral sphingomyelinase 2), which is a Golgi- and cell-membrane associated enzyme that hydrolyzes sphingomyelin into ceramide and phosphocholine. Ceramide is a substrate for ceramidase that produces sphingosine. In turn, sphingosine is modified by a specific kinase and converted into sphingosine 1-phosphate, which has a mitogenic activity on osteoblasts [61]. Neutral sphingomyelinases are ubiquitously expressed enzymes with elevated expression in the brain and in the embryonic growth plate of bone. In fro mice, the activity of the neutral sphingomyelinases from brain extracts was severely deficient (~12 % of the control); this residual activity probably resulted from the other neutral sphingomyelinase, *Smpd2* [60]. Sphingomyelinase, ceramide and sphingosine 1-phosphate are inhibitory factors of bone resorption [62]. Impaired function of SMPD3 and a defective ceramide pathway could affect bone development and remodeling, leading to excessive bone resorption [60]. The detailed relationship between impaired function of SMPD3 and defective mineralized tissue is not yet fully understood.

Osterix (SP7, OSX)

SP7 encodes an osteoblast-specific transcription factor that is indispensable for bone formation. *Sp7* null mice are

deficient in osteoblast differentiation and have reduced expression of osteoblast markers, including Col1a1, bone sialoprotein and osteocalcin [63]. In an Egyptian child, a c.1052delA mutation was found responsible for recessive OI [64]. The clinical manifestations include recurrent fractures, mild bone deformities and delayed tooth eruption, but normal hearing and white sclera. The mutation introduces a premature stop codon within the final exon of the gene. The mutant mRNA transcript is predicted to escape the nonsense-mediated mRNA decay machinery [64]. A truncated Osterix protein lacks the last 81 amino acids, including the third Zn-finger domain, and carries instead 18 new residues. Its DNA-binding properties are expected to be altered, and, consequently, the transcriptional regulation should be impaired. The effect of the *SP7* mutation on type I collagen production in patient cells was not analyzed because of the unavailability of additional biological material from the patient [64].

Wnt1

A large number of recessive mutations in *WNT1* leading to OI were recently reported [8•, 9•, 10•, 11]. The WNT signaling pathway is not only involved in developmental processes, but also in bone homeostasis. Loss-of-function mutations in *LRP5*, encoding the WNT co-receptor for the canonical WNT- β -catenin pathway, lead to low bone mass, whereas gain-of-function mutations in *LRP5* lead to high bone mass. These high bone mass-causing mutations are linked to a decrease in binding to sclerostin [65]. The precise role of *WNT1* in the WNT- β -catenin pathway is still elusive, but it is interesting that Osterix is a downstream target of β -catenin-activated transcription factor TCF/LEF.

Conclusions

Human genome sequencing and rapid development of cheap and efficient sequencing techniques has allowed mapping of new genes and new mutations responsible for multiple inherited disorders at an unprecedented rate. Recent findings of mutations leading to OI in genes not encoding $\alpha 1$ and $\alpha 2$ chains of type I collagen significantly broadened the diagnostic repertoire for medical geneticists, which can now be used to counsel patients and families with OI. It is possible that the repertoire is still incomplete and more discoveries are to come. Unfortunately, detailed studies of molecular and cellular mechanisms underlying these new mutations are significantly lagging behind. Several OI-related genes encoding *TMEM38B*, *IFITM5*, *SERPINF1* and *SMPD3* are of unknown relation to disturbed bone tissue. Our understanding of the roles of

FKBP65 and HSP47 in collagen folding remains elusive. Functions of the P3H1•CRTAP•CypB complex are still not fully explored. There are “black boxes” hiding roles of Wnt1 signaling and collagen transcription regulated by Osterix. OI mutations are pieces of an exciting “puzzle” called “bone formation, structure and function” in a very broad meaning that includes multiple steps of biosynthesis of the collagen matrix and bone mineralization, as well as a sophisticated signaling network within the matrix and cells. By solving this puzzle we will benefit by uncovering new targets for OI treatment.

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Compliance with Ethics Guidelines

Conflict of Interest All authors declare no conflicts of interest.

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- Of importance;
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