HHS Public Access

Author manuscript

Curr Osteoporos Rep. Author manuscript; available in PMC 2020 January 28.

Published in final edited form as:

Curr Osteoporos Rep. 2010 June ; 8(2): 77–83. doi:10.1007/s11914-010-0010-7.

Role of Cartilage-associated Protein in Skeletal Development

Roy Morello, Ph.D.[1], **Frank Rauch, M.D.**[2]

[1]Department of Physiology & Biophysics and Division of Genetics, University of Arkansas for Medical Sciences, Little Rock, AR

[2]Genetics Unit, Shriners Hospital for Children and Department of Pediatrics, McGill University, Montreal, CANADA

Abstract

The past three years have been exciting for both collagen biologists and human geneticists studying the disease known as osteogenesis imperfecta (OI or brittle bone disease). Functional studies on Cartilage-associated Protein (Crtap) have identified it as an essential component of a heterotrimeric, endoplasmic reticulum resident complex responsible for both collagen prolyl 3-hydroxylation and chaperone function. Importantly, human mutations in the *CRTAP* gene have been associated with recessive forms of OI. Although the function and *in vivo* biological significance of the 3-hydroxyproline modification are still poorly understood, studies on *Crtap* have led to the identification of additional genes in which mutations also cause recessive forms of OI. These discoveries have now focused the interest of geneticists on the endoplasmic reticulum that will require the help of biochemists to unravel the molecular dynamics and complexities of collagen folding.

Introduction

Type I collagen is the most abundant protein in the human body and a fundamental component of bone matrix [1]. It is a heterotrimeric molecule consisting of two α1(I) chains and one α2(I) chain that are synthesized in the rough endoplasmic reticulum (rER) as preprocollagen chains and undergo several post-translation modifications, including signal peptide cleavage, disulfide linkage, prolyl- and lysyl-hydroxylation and glycosylation, before folding into a triple helical structure [2]. After transport through the Golgi apparatus and secretion to the extracellular matrix, additional processing takes place, which includes N- and C-propeptide cleavage, hydroxylysine oxidation and final assembly and cross-linking of fibrils [2]. Collagen type I is the prototypical example of a protein that is post-translationally modified and destined to the secretory pathway. An increasing number of rER enzymes and protein complexes act as ‘chaperones’, such as protein disulfide isomerase, peptidyl prolyl cis-trans isomerases, Hsp47, and the recently identified prolyl 3-hydroxylation complex. By interacting with the procollagen chains, these rER components

Corresponding author: Roy Morello, Ph.D., Assistant Professor, Department of Physiology & Biophysics, rm 211-2, University of Arkansas for Medical Sciences, 4301 W. Markham St., #505, Little Rock, AR 72205-7199, Office phone: 501 526-4090, rmorello@uams.edu.

Disclosure

The Authors have no conflicts of interest to disclose.

facilitate the process of chain recognition and assembly, folding of the triple helix and also inhibition of the inappropriate intracellular polymerization of collagen monomers into fibrils. Some of these molecules, like P3h1 (Leprecan) and perhaps Crtap, may accompany the collagen outside the cell and play a different function in the extracellular matrix.

Osteogenesis imperfecta (OI) is associated with mutations in the *COL1A1* and *COL1A2* genes but also in an increasing number of genes encoding rER-resident, collagen interacting proteins. Here we will focus our attention on *Crtap*, a member of the Leprecan family of genes and a key component of the collagen prolyl 3-hydroxylation complex and review its role in bone formation and human disease.

Crtap expression pattern and role during skeletal formation

Crtap was originally identified as a novel cDNA differentially expressed by chicken chondrocytes *in vitro* [3]. Initial evidence in the chicken confirmed by subsequent studies in the mouse identified expression of the Crtap transcript in several tissues and organs throughout development, although strongest protein expression was detected in the developing skeleton [3, 4]. The recent development of a newer Crtap polyclonal antibody allowed a better description of its distribution pattern: Crtap is expressed at all stages of mouse embryonic cartilage differentiation (especially in proliferating chondrocytes) and by osteoblastic and osteoclastic cells [5]. Furthermore its expression is detected in lung, kidney, testis, skin [6] and likely will be detected in all tissues expressing type I collagen.

To characterize its role during vertebrate development, a *Crtap*-null mouse was generated using a classic gene targeting inactivation strategy [5]. *Crtap*−/− mice from heterozygous crossings are born at the expected Mendelian ratio although they suffer some perinatal lethality; this becomes very frequent when the mutation is backcrossed in a pure C57BL/6J genetic background (Roy Morello, unpublished observations). Homozygous mutant pups are slightly smaller compared to their wild-type littermates, and both sexes remain significantly smaller in adulthood, weighing on average about 20–25% less than wild-type controls [5]. Their most striking phenotype is in the skeleton, with a profound generalized osteopenia, rhizomelia, mild cartilage dysplasia and progressive kyphosis of the spine. Craniofacial malformation and skin laxity are also present. Long-bone as well as vertebral histomorphometry showed about 50% reduction in trabecular bone volume and significant reduction in trabecular number and thickness with consequent increased trabecular separation. While both osteoblast and osteoclast numbers and surfaces were not different from wild-type controls, the bone formation rate was significantly decreased due to a reduced mineral apposition rate [5]. Moreover, all osteoid parameters including thickness, surface and volume, were reduced. Interestingly, the mineralization lag time, i.e. the time required for newly deposited matrix to mineralize, was also decreased, suggesting both a quantitative and qualitative defect of the osteoblast secreted matrix. Irrespective of the profound osteopenia, fractures were never observed in the *Crtap*−/− mice (Roy Morello, unpublished observations).

Concomitant to the phenotype delineation of the *Crtap*-null animals, Vranka et al. published the identification and initial characterization of the collagen binding protein prolyl 3-

hydroxylase 1 (P3h1) [7]. P3h1 was obtained to near purity from a chicken rER extract, but it always co-eluted with two ‘contaminant’ proteins, namely Crtap and Cyclophilin B (CypB). These were shown not to be required for the enzymatic activity of P3h1 *in vitro* [7]. Whether Crtap was necessary or not for collagen prolyl 3-hydroxylation *in vivo* was unclear and the *Crtap−/−* mice represented a useful model to evaluate this. Cyanogen bromide peptides derived from α1(I) and α1(II) chains extracted from bone and cartilage of *Crtap−/−* and wild type mice, respectively, were analyzed by tandem mass spectrometry for 3-hydroxyproline (3Hyp) content. A difference in peptide mass between *Crtap−/−* and wild type mice was revealed by mass spectrometry for the peptide sequence known to contain the primary proline (residue 986 of the triple helix) that becomes 3-hydroxylated in both α1(I) and α1(II) chains. These data showed a complete lack of the 3-hydroxyl group at Pro986 in α1(I) chains from bone or skin collagen and in α1(II) chains from cartilage of *Crtap−/−* mice and demonstrated that Crtap is required *in vivo* for fibrillar collagen proyl 3-hydroxylation [5].

In vitro collagen synthetic studies in *Crtap−/−* mouse osteoblasts revealed over-modification of procollagen chains, suggesting a slowing of triple-helix winding and an increased hydroxylation of proline and lysine residues in the rER. Ultrastructural studies showed significantly increased collagen fibril diameter in *Crtap−/−* skin and cartilage compared to wild type values. Consistent with a potential interaction with P3h1 which had been previously shown to be a rER resident protein [7, 8], the majority of Crtap was also localized by immunofluorescence within the rER in multiple cell lines, with only minor staining in the extracellular matrix shown in mouse tissue sections. Finally, several lines of experimental evidence, including co-sedimentation on a sucrose gradient, laser light scattering and Western analysis on affinity chromatography eluates, demonstrated that in the rER Crtap can complex with P3h1 and Cyclophilin B (CypB) in a 1:1:1 ratio [5].

At the amino acid level, Crtap and P3h1 are about 30% identical, even though Crtap completely lacks the half, C-terminal portion of P3h1 which contains the enzymatic domain. Together with *SC65* (coding for synaptonemal complex protein 65), *Leprel1* (coding for prolyl 3-hydroxylase 2) and *Leprel2* (coding for prolyl 3-hydroxylase 3), *Crtap* (or *Leprel3*) and *Lepre1* constitute the Leprecan family of genes.

CRTAP mutations cause recessive OI

OI is a hereditary disease characterized by bone fragility and short stature [9]. The clinical spectrum ranges from perinatal lethality to nearly asymptomatic individuals with occasional fractures and normal stature. The majority of individuals with a clinical diagnosis of OI have an identifiable mutation in *COL1A1* or *COL1A2*, the genes that encode the two type I collagen alpha chains [9]. OI patients with collagen type I mutations can be classified into four clinically defined types with varying severity, named OI types I to IV.

Apart from these ‘classical’ types of OI, three conditions, called OI types V, VI and VII, have been identified over the past decade [9]. OI types V to VII largely resemble OI types I, III or IV on clinical grounds, but also have some distinguishing features, and are not caused by *COL1A1* or *COL1A2* mutations. OI type VII was initially identified as an autosomal

recessive disorder in a Native ('First Nations') Canadian community in which eight members were affected with a moderately severe form of OI [10]. The disease locus was mapped to the short arm of chromosome 3, which contains the *CRTAP* gene [11]. When it became apparent that the phenotype of the *Crtap−/−* mouse model shared some characteristics with OI type VII, *CRTAP* emerged as an obvious candidate gene for OI type VII. Indeed, sequence analysis of the *CRTAP* gene in patients with OI type VII revealed a mutation in intron 1 that led to the introduction of a cryptic splice site and caused a marked reduction in the expression of CRTAP protein (see 'hypomorphic mutation' section below) [5]. Subsequently, *CRTAP* loss of function mutations were discovered in newborns and fetuses with lethal forms of OI [12–15].

As of now, 25 individuals with *CRTAP* mutations have been described in the literature [5, 10, 12–15]. The database of *CRTAP* mutations currently contains a total of 17 different mutations (http://www.le.ac.uk/ge/collagen/). These mutations affect either exon/intron 1 or exon/intron 4 of the CRTAP gene. All but the initially discovered 'hypomorphic mutation' in intron 1 are thought to lead to a complete inactivation of *CRTAP* [12, 13, 15]. More than half of the mutations are nonsense or frameshift mutations that introduce a premature termination codon. Three missense mutations have been described but only one reported patient is homozygous for a missense mutation (Table 1). Barnes et al. estimated that *CRTAP* mutations are responsible for 2–3% of cases of lethal OI [12]. This estimate is roughly in line with the observations by Bodian et al., who found *CRTAP* mutations in 1 out of 62 cases with lethal OI [14].

Clinical features of recessive OI due to *CRTAP* mutations

Hypomorphic Mutation.

Patients with hypomorphic mutations in *CRTAP* present the least severe phenotype associated with *CRTAP* mutations until now [10]. Patients were born with multiple fractures but had a normal birth weight (Table 1). During early childhood, all patients had recurrent fractures which led to bowing of femora and tibiae and necessitated intramedullary rodding surgery. In the four adult patients, fractures were rare after puberty, but by then they required wheelchairs for mobility. The four younger patients who received intravenous pamidronate treatment during childhood and adolescence were ambulatory without assistance at the end of puberty [16]. None of the patients with the hypomorphic mutation developed respiratory problems in the postnatal period and none was diagnosed with cardiac or other anomalies. Also, these patients did not have dentinogenesis imperfecta, ligamentous laxity, or facial dysmorphism. Skin and hearing were normal, and the sclerae were white.

Radiographically, the most striking findings were the presence of rhizomelia (selective shortening of proximal limb segments) and coxa vara. The presence of scoliosis and vertebral compression was variable. Skull radiographs revealed Wormian bones (accessory skull bones completely surrounded by a suture line) in half of the patients. Lumbar spine bone mineral density was within the normal range in the first year of life, but was below the normal reference range after the age of four years in all patients. There were no abnormalities in biochemical markers of bone and mineral metabolism, and bone

histomorphometric results in OI type VII were similar to those of OI type I, with a decreased amount of both cancellous and cortical bone and elevated bone turnover.

Loss-of-Function Mutations.

Apart from the individuals described in the preceding section, all other subjects with *CRTAP* mutations had presumed loss-of-function mutations and had a severe or lethal form of OI. Of the 17 cases with inactivating *CRTAP* mutations reported so far, five were aborted fetuses and eleven were born alive [12, 15, 17]. In one case, no information was provided on pregnancy and birth, but a diagnosis of lethal OI was reported [14]. Seven of the live-born children died of respiratory failure in the first year of life (Table 1). At the time of reporting, the oldest of the four surviving patients was nine years of age.

In most of the reported cases, birth weight was in the lower part of the reference range (Table 1). All children were born with multiple fractures, mostly of long bones and ribs. In a few cases, rhizomelia (short humeri and femora) was reported, but whether this was caused by a primary growth delay in the proximal limb segments or secondary to fractures is not clear. Apart from fractures and bowing, radiographic features included thin ribs and Wormian bones (Figure 1). Sclerae were white.

At birth, the clinical picture of patients with inactivating *CRTAP* mutations thus seems to be quite similar to that of patients with OI type II or III caused by structural collagen type I mutations. Possibly, the two disorders differ somewhat in the shape of the face, which is reported to be round in patients with *CRTAP* mutations and triangular in patients with collagen type I mutations. However, according to our experience, patients with severe OI caused by collagen type I mutations mostly develop a triangular face only after infancy. Scleral hue seems to be of some use in the differential diagnosis, as neonates with severe OI due to collagen type I mutations usually have grey sclerae whereas those with *CRTAP* mutations have white sclerae.

Regarding postnatal development, most patients with inactivating *CRTAP* mutations suffered from respiratory problems during infancy that eventually resulted in death in the majority of cases. These respiratory difficulties may be the consequence of thoracic cage skeletal deformities, but as *Crtap* is expressed in lung tissue it is also conceivable that there is a more direct effect of *CRTAP* mutations on lung function [5, 6].

Even though patients with *CRTAP* mutations reportedly have a normal skull shape at birth, it appears from the published cases that skull deformities can develop during infancy. As in other forms of severe OI, this is probably an indication of decreased mechanical resistance of the skull bone.

Reported children with *CRTAP* mutations have had severe growth failure. In addition, ‘popcorn epiphyses’, thought to contain material from disintegrated growth plates, were observed in an 8-year old girl with a homozygous missense mutation in *CRTAP* [13]. Possibly, growth plates are more severely affected in patients with *CRTAP* mutations than in patients with collagen type I mutations, because *CRTAP* is expressed in growth plates, whereas collagen type I is not. Nevertheless, growth failure and popcorn epiphyses are also

commonly observed in children with severe OI caused by collagen type I mutations and therefore are by no means specific signs of *CRTAP* mutations [18, 19].

Treatment

No treatment studies have been published in patients with inactivating *CRTAP* mutations, but it is clear that the lives of such patients are threatened by respiratory failure. They therefore require supportive respiratory therapy and aggressive treatment of pulmonary infections. Physiotherapy and, for children surviving infancy, orthopedic surgery, may also be required.

The manifestations of OI are mostly secondary to the extreme bone weakness. Bisphosphonate treatment is widely used to strengthen the bones of growing children with moderate to severe forms of OI [9]. The most frequently used approach is intravenous pamidronate, which has been reported to lead to increased lumbar spine bone mineral density and cortical thickness, improvements in vertebral shape, stronger muscles, and better mobility function. A recent study suggests that the effects of intravenous pamidronate treatment are similar in patients with hypomorphic *CRTAP* mutations as in patients who have OI of similar severity caused by collagen type I mutations [16]. Treatment with intravenous bisphosphonates is clearly beneficial in infants with OI caused by collagen type I mutations, and therefore might reasonably be used in infants with inactivating *CRTAP* mutations as well. It should be noted, though, that respiratory distress has occurred in infants receiving the first intravenous cycle of pamidronate [20], which may be a cause of concern in children with *CRTAP* mutations who already have respiratory problems.

Pathogenesis of recessive OI and collagen prolyl 3-hydroxylation

The identification of *CRTAP* and then *LEPRE1* mutations in probands affected with recessive OI has catalyzed a great interest in the function of collagen prolyl 3-hydroxylation. Until recently, lack of such modification at Pro986 of α1(I) in *Crtap−/−* mice as well as patients with *CRTAP* or *LEPRE1* mutations was held responsible for the dramatic osteopenia and bone defects. However, new evidence has demonstrated that the Crtap/P3h1/CypB prolyl 3-hydroxylation complex also exerts peptidyl-prolyl *cis-trans* isomerase (PPIase) activity, which is the ability to catalyze the bond preceding a proline residue between its *cis* and *trans* form, as well as collagen chaperone function in the rER [24]. Thus, a functional dysregulation of the complex not only affects collagen prolyl 3-hydroxylation but also collagen folding kinetics and/or ability which results in over-modification of additional lysine and proline residues. The importance of the latter versus the former function has now been demonstrated by the identification of mutations in *PPIB* (encoding Cyclophilin B) in severe recessive OI cases negative for mutations in *COL1A1*, *COL1A2*, *CRTAP* or *LEPRE1* [25]. Unlike *CRTAP* or *LEPRE1* mutations which cause a >90% reduction of prolyl 3-hydroxylation at Pro986 in α1(I), lack of *PPIB* causes a less severe reduction in 3Hyp at the same location but similar over-modification of type I procollagen chains made by cultured skin fibroblasts [25]. This suggests that the collagen chaperone function of the prolyl 3-hydroxylation complex may be more relevant as the cause of human disease than prolyl 3-hydroxylation itself. Moreover, homozygous *PPIB* mutations do not

affect the stability of CRTAP or P3H1 proteins while null mutations in *CRTAP* cause instability and lack of P3H1 protein and vice versa, but without affecting CypB [6, 25]. Hence CypB appears to enjoy a higher degree of 'independence' from the trimeric complex but the requirement for its prolyl isomerase activity in normal collagen folding is still unclear. Whether the stable CRTAP/P3H1 dimer associates with CypB and brings it to its substrate, the nascent triple-helix, at a position later marked by the 3-Hyp, is a possibility and will be the subject of further studies.

As a further sign of the complexity and importance of proper collagen folding versus prolyl 3-hydroxylation in OI pathogenesis, mutations in another rER resident PPIase with collagen chaperone activity, named *FKBP65*, have been reported in additional cases of recessive OI [26]. Furthermore, mutations affecting the *SERPINH1* gene, coding for HSP47, another collagen chaperone molecule, have also been associated with a recessive form of OI in dogs [27]. These data altogether suggest that any dysregulation of the highly regulated collagen folding process is a common mechanism for all recessive forms of OI and perhaps even for the classic dominant forms caused by *COL1A1* and *COL1A2* mutations.

Given the above evidence, the current role and significance of collagen prolyl 3-hydroxylation remain uncertain. Unlike prolyl 4-hydroxylation, which is a very common modification of proline residues in the Y position of the Gly-Xaa-Yaa triplet repeat, and confers stability to the triple helix [28], prolyl 3-hydroxylation in the X position is rarer. It is established at a single residue (Pro986 of the triple helix domain) in α1(I) and α1(II) chains but is more common in type IV collagen [5, 29]. While biophysical studies on synthetic peptides containing (Gly-3Hyp-4Hyp)(n) initially suggested that 3Hyp could confer a slight instability to the collagen triple helix [30], later studies actually showed that a 3Hyp in the Xaa position increases its stability [31]. The identification and characterization of primary (almost always fully 3-hydroxylated) and secondary (less frequently 3-hydroxylated) 3Hyp sites in various procollagen α-chains is a matter of active research. Several of these have being identified and are under further investigation (David Eyre, personal communication). From the study of the *Crtap−/−* mice we have now shown that a 3Hyp in α2(V) at the same P986 site as in α1(I) and α1(II) chains is >95% hydroxylated in wild type mice but unmodified in *Crtap* −/− bone or skin type V collagen [6]. Considering the role of type V collagen in regulating type I collagen fibril formation [32, 33] and its association with Ehlers-Danlos syndrome (EDS) when mutated [34], an abnormal α2(V) chain may explain the irregular fibrillogenesis and paucity of osteoid observed in *Crtap−/−* mice and also their skin laxity, a typical feature of EDS. These data also indicate that Crtap is required for the 3-hydroxylation of canonical proline sites within clade A (types I, II and V) collagen chains. Finally, given the normal 3-hydroxylation status of a previously described 3Hyp locus in α1(IV) extracted from *Crtap−/−* kidney [6] and the existence of three P3H genes in mice and humans, it is possible that multiple mechanisms of collagen 3-hydroxylation exist, some dependent and some independent of the Crtap function.

Conclusion

The discovery of *CRTAP* mutations as the cause of recessive OI was a major step forward in understanding the molecular basis of this rare disease. It led to four new genes (*CRTAP*,

LEPRE1, *PPIB* and *FKBP65*) being linked to recessive human OI (potentially five with *SERPINH1*). These findings are shifting current genetic research from the matrix to the rER compartment and highlight the complex cellular task of synthesizing and folding a collagen trimeric molecule. Clearly a range of rER resident chaperone proteins are essential.

Acknowledgements

The authors would like to thank David R. Eyre (Seattle, WA) and Dustin Baldridge (Houston, TX) for their insightful comments. This research was supported in part by the National Institute of Health grant AR051459 (R.M.), the Osteogenesis Imperfecta Foundation and the Rolanette and Berdon Lawrence Bone Disease Program of Texas (R.M.) and by the Shriners of North America (F.R.). F.R. is a Chercheur-Boursier Clinicien of the Fonds de la Recherche en Santé du Québec.

References and Recommended Reading

1. Gehron Robey P, and Boskey A: The composition of Bone. In Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. Edited by Rosen CJ. American Society for Bone and Mineral Research; 2008: 32–38.
2. Myllyharju J and Kivirikko KI, Collagens, modifying enzymes and their mutations in humans, flies and worms. Trends Genet, 2004 20(1): p. 33–43. [PubMed: 14698617]
3. Castagnola P, et al., Cartilage associated protein (CASP) is a novel developmentally regulated chick embryo protein. J Cell Sci, 1997 110 (Pt 12): p. 1351–9. [PubMed: 9217321]
4. Morello R, et al., cDNA cloning, characterization and chromosome mapping of Crtap encoding the mouse cartilage associated protein. Matrix Biol, 1999 18(3): p. 319–24. [PubMed: 10429950]
5••. Morello R, et al., CRTAP is required for prolyl 3- hydroxylation and mutations cause recessive osteogenesis imperfecta. Cell, 2006 127(2): p. 291–304. [PubMed: 17055431] This article characterizes the phenotype of Crtap−/− mice, the role of Crtap during skeletal formation, its interaction with P3h1 and CypB in the rER and the identification of the first CRTAP human mutations.
6. Morello R, Baldridge D, Lennington J, et al.: Comparative Phenotypic and Biochemical Analyses of Crtap−/− Mice and Patients with Recessive Osteogenesis Imperfecta. [abstract FR0141]. Presented at the ASBMR 31st Annual Meeting Denver, CO; September 11–15, 2009.
7. Vranka JA, Sakai LY, and Bachinger HP, Prolyl 3-hydroxylase 1: Enzyme characterization and identification of a novel family of enzymes. J Biol Chem, 2004.
8. Jarnum S, et al., LEPREL1, a novel ER and Golgi resident member of the Leprecan family. Biochem Biophys Res Commun, 2004 317(2): p. 342–51. [PubMed: 15063763]
9. Rauch F and Glorieux FH, Osteogenesis imperfecta. Lancet, 2004 363(9418): p. 1377–85. [PubMed: 15110498]
10. Ward LM, et al., Osteogenesis imperfecta type VII: an autosomal recessive form of brittle bone disease. Bone, 2002 31(1): p. 12–8. [PubMed: 12110406]
11. Labuda M, et al., Osteogenesis imperfecta type VII maps to the short arm of chromosome 3. Bone, 2002 31(1): p. 19–25. [PubMed: 12110407]
12•. Barnes AM, et al., Deficiency of cartilage-associated protein in recessive lethal osteogenesis imperfecta. N Engl J Med, 2006 355(26): p. 2757–64. [PubMed: 17192541] This article describes the finding of additional CRTAP mutations and confirms the role of CRTAP in recessive OI.
13•. Baldridge D, et al., CRTAP and LEPRE1 mutations in recessive osteogenesis imperfecta. Hum Mutat, 2008 29(12): p. 1435–42 [PubMed: 18566967] This article provides clinical and molecular information of patients with recessive OI and expands the phenotype of the disorder.
14. Bodian DL, et al., Mutation and polymorphism spectrum in osteogenesis imperfecta type II: implications for genotype-phenotype relationships. Hum Mol Genet, 2009 18(3): p. 463–71. [PubMed: 18996919]
15•. Van Dijk FS, et al., CRTAP mutations in lethal and severe osteogenesis imperfecta: the importance of combining biochemical and molecular genetic analysis. Eur J Hum Genet, 2009.This article

describes the phenotype of recessive OI due to CRTAP mutations and discusses the diagnostic work-up in such patients.

16. Cheung MS, Glorieux FH, and Rauch F, Intravenous pamidronate in osteogenesis imperfecta type VII. Calcif Tissue Int, 2009 84(3): p. 203–9. [PubMed: 19137231]
17. Baldridge D, et al., CRTAP and LEPRE1 mutations in recessive osteogenesis imperfecta. Hum Mutat, 2008 29(12): p. 1435–42. [PubMed: 18566967]
18. Zeitlin L, et al., Height and weight development during four years of therapy with cyclical intravenous pamidronate in children and adolescents with osteogenesis imperfecta types I, III, and IV. Pediatrics, 2003 111(5 Pt 1): p. 1030–6. [PubMed: 12728084]
19. Obafemi AA, et al., Popcorn calcification in osteogenesis imperfecta: incidence, progression, and molecular correlation. Am J Med Genet A, 2008 146A(21): p. 2725–32. [PubMed: 18798308]
20. Munns CF, et al., Respiratory distress with pamidronate treatment in infants with severe osteogenesis imperfecta. Bone, 2004 35(1): p. 231–4. [PubMed: 15207762]
21. Glorieux FH, et al., Type V osteogenesis imperfecta: a new form of brittle bone disease. J Bone Miner Res, 2000 15(9): p. 1650–8. [PubMed: 10976985]
22. Glorieux FH, et al., Osteogenesis imperfecta type VI: a form of brittle bone disease with a mineralization defect. J Bone Miner Res, 2002 17(1): p. 30–8. [PubMed: 11771667]

23••. Cabral WA, et al., Prolyl 3-hydroxylase 1 deficiency causes a recessive metabolic bone disorder resembling lethal/severe osteogenesis imperfecta. Nat Genet, 2007 39(3): p. 359–65. [PubMed: 17277775] This is the first article describing LEPRE1 mutations in recessive OI patients.

24••. Ishikawa Y, et al., Biochemical characterization of the prolyl 3-hydroxylase 1.cartilage-associated protein.cyclophilin B complex. J Biol Chem, 2009 284(26): p. 17641–7. [PubMed: 19419969] This study demonstrates that the prolyl 3-hydroxylation complex, formed by Crtap/P3h1/CypB also has collagen chaperone activity.

25••. van Dijk FS, et al., PPIB mutations cause severe osteogenesis imperfecta. Am J Hum Genet, 2009 85(4): p. 521–7. [PubMed: 19781681] This study demonstrates that mutations in the gene encoding the third component of the prolyl 3-hydroxylation complex,Cyclophilin B, also cause recessive OI.

26•. Alanay Y, Avaygan H, Camacho N, et al.: Mutations in a gene encoding a rough endoplasmic reticulum protein causes autosomal recessive progressive deforming osteogenesis imperfecta. [Abstract 212]. Presented at the ASHG 59th Annual Meeting Honolulu, HI; October 20–24, 2009 27.Identification of FKBP65 mutations, another collagen chaperone molecule, in recessive OI cases negative for mutation in COL1A1, COL1A2, CRTAP and LEPRE1 demonstrates the importance of collagen chaperone molecules in human disease.

27•. Drogemuller C, et al., A missense mutation in the SERPINH1 gene in Dachshunds with osteogenesis imperfecta. PLoS Genet, 2009 5(7): p. e1000579. [PubMed: 19629171] This article identifies the first mutations in the gene coding HSP47, a collagen specific chaperone, in a dog strain affected with recessive OI.

28. Myllyharju J, Prolyl 4-hydroxylases, the key enzymes of collagen biosynthesis. Matrix Biol, 2003 22(1): p. 15–24. [PubMed: 12714038]
29. Kefalides NA, Structure and biosynthesis of basement membranes. Int Rev Connect Tissue Res, 1973 6: p. 63–104. [PubMed: 4198817]
30. Jenkins CL, et al., Effect of 3-hydroxyproline residues on collagen stability. J Am Chem Soc, 2003 125(21): p. 6422–7. [PubMed: 12785781]
31. Mizuno K, et al., Effect of the -Gly-3(S)-hydroxyprolyl-4(R)-hydroxyprolyl- tripeptide unit on the stability of collagen model peptides. Febs J, 2008 275(23): p. 5830–40. [PubMed: 19021759]
32. Birk DE, et al., Collagen fibrillogenesis in vitro: interaction of types I and V collagen regulates fibril diameter. J Cell Sci, 1990 95 (Pt 4): p. 649–57. [PubMed: 2384532]
33. Wenstrup RJ, et al., Type V collagen controls the initiation of collagen fibril assembly. J Biol Chem, 2004 279(51): p. 53331–7. [PubMed: 15383546]
34. Wenstrup RJ, et al., A splice-junction mutation in the region of COL5A1 that codes for the carboxyl propeptide of pro alpha 1(V) chains results in the gravis form of the Ehlers-Danlos syndrome (type I). Hum Mol Genet, 1996 5(11): p. 1733–6. [PubMed: 8923000]

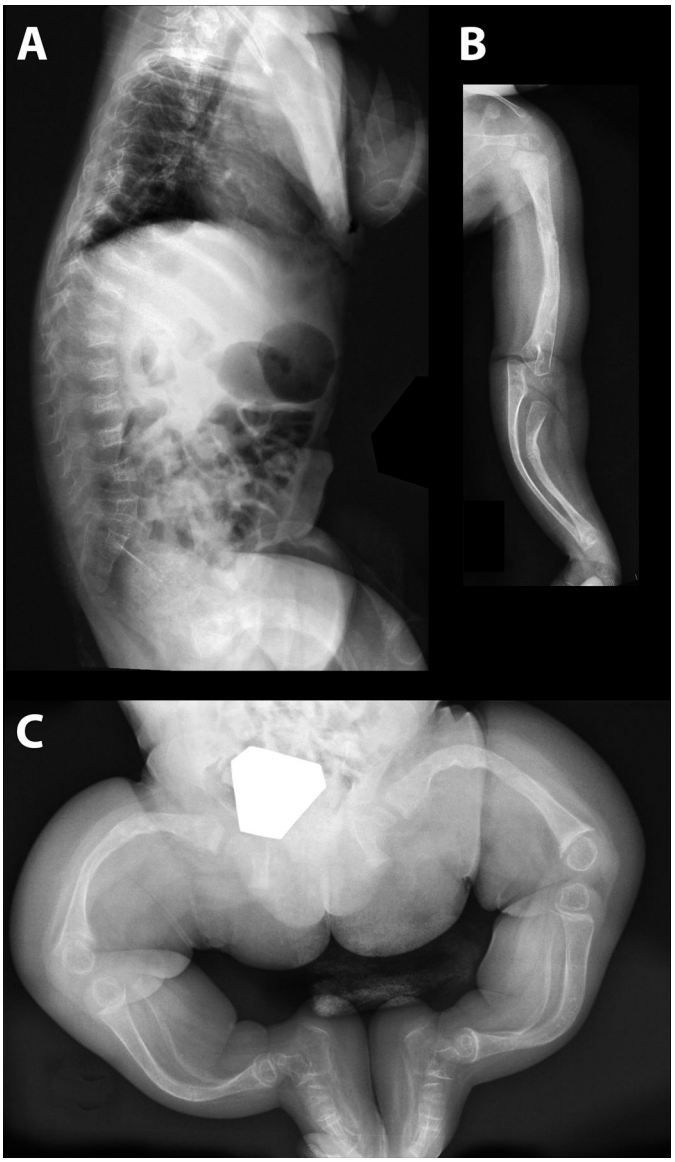

Figure 1.

Radiographs of a 15-months old girl with a homozygous p.Glu269_Val270del mutation in *CRTAP*. A. The lateral view of the spine shows multiple vertebral compression fractures. B. The long bones of the right forearm are deformed. C. There are severe deformities of the long bones of the lower extremities and cortices are very thin.

Table 1.

Characteristics of reported live-born individuals with *CRTAP* mutations

Source	Mutations	Sex	Gestational age	Birth Weight (g)	Status
Van Dijk, 2009	nonsense/missense	M	term		Died at 5 days
Van Dijk, 2009	nonsense/nonsense	F	term	2725	Died at 24 days
Van Dijk, 2009	nonsense/nonsense	M	term	2570	Died at 1 month
Van Dijk, 2009	nonsense/splice site	F	term	2710	Died at 2 months
Barnes, 2006	nonsense/nonsense	F	36 weeks	2435	Died at 3 months
Barnes, 2006	start codon/frameshift	F	term	2600	Died at 10 months
Barnes, 2006	splice site/splice site	M	35 weeks	2370	Died at 10 months
Baldridge, 2008	Frameshift/frameshift	F	NA	NA	Alive at 12 months
Van Dijk, 2009	splice site/splice site	F	term	3245	Alive at 2 years
Van Dijk, 2009	splice site/splice site	M	term	3110	Alive at 4 years
Baldridge, 2008	missense/missense	F	NA	NA	Alive at 9 years
Ward, 2002 / unpublished	Cryptic splice site/cryptic splice site	F	term	4005	Alive at 16 years
Ward, 2002 / unpublished	Cryptic splice site/cryptic splice site	F	term	2900	Alive at 18 years
Ward, 2002 / unpublished	Cryptic splice site/cryptic splice site	F	term	3830	Alive at 19 years
Ward, 2002 / unpublished	Cryptic splice site/cryptic splice site	F	term	3770	Alive at 20 years
Ward, 2002 / unpublished	Cryptic splice site/cryptic splice site	2F, 2M	NA	NA	Alive at 28–33 years