Musculoskeletal Pathology

An Inducible Cartilage Oligomeric Matrix Protein Mouse Model Recapitulates Human Pseudoachondroplasia Phenotype

Karen L. Posey,* Alka C. Veerisetty,* Pieman Liu,*
Huiqiu R. Wang,* Brian J. Poindexter,[†]
Roger Bick,[†] Joseph L. Alcorn,*
and Jacqueline T. Hecht*[‡]

From the Departments of Pediatrics* and Pathology,[†] University of Texas Medical School at Houston, Houston; and the Shriners Hospital for Children,[‡] Houston, Texas

Cartilage oligomeric matrix protein (COMP) is a pentameric extracellular protein expressed in cartilage and other musculoskeletal tissues. Mutations in the COMP gene cause pseudoachondroplasia (PSACH), a severe dwarfing condition that has a growth plate chondrocyte pathology. PSACH is characterized by intracellular retention of COMP and other extracellular matrix (ECM) proteins, which form an ordered matrix within large rough endoplasmic reticulum cisternae. This accumulation is cytotoxic and causes premature chondrocyte cell death, thereby depleting chondrocytes needed for normal long bone growth. Research to define the underlying molecular mechanisms of PSACH has been hampered by the lack of a suitable model system. In this study, we achieved robust expression of human mutant (MT) or wild-type (WT) COMP in mice by using a tetracycline-inducible promoter. Normal growth plate distribution of ECM proteins was observed in 1-month-old WT-COMP and C57BL/6 control mice. In contrast, the structure of the MT-COMP growth plate recapitulated the findings of human PSACH growth plate morphology, including (1) retention of ECM proteins, (2) intracellular matrix formation in the rER cisternae, and (3) increased chondrocyte apoptosis. Therefore, we have generated the first mouse model to show extensive intracellular retention of ECM proteins recapitulating the human PSACH disease process at the cellular level. (*Am J Pathol* 2009, 175:1555–1563; DOI: 10.2353/ajpath.2009.090184)

Cartilage oligomeric matrix protein (COMP) is an extracellular matrix (ECM) protein expressed primarily in car-

tilage, tendon, and ligament.^{1–10} COMP is the fifth member of the thrombospondin gene family (TSP-5) belonging to the TSP pentameric subgroup B, which consists of adhesive glycoproteins that are incorporated into the extracellular matrix of a variety of cells.^{2,11–13} Experimental evidence suggests that COMP has a variety of functions including interacting with other ECM molecules in the matrix, increasing cellular attachment, and regulating collagen fibril assembly and proliferation of chondrocytes.^{14–20}

Mutations in COMP cause two skeletal dysplasias pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED/EDM1), both of which are associated with a distinctive chondrocyte cellular pathology.^{2,16,21–42} This pathology was recognized long before mutations in COMP were identified and consists of giant rER cisternae that occupy the entire chondrocyte and contains lamellar appearing material.²⁶ COMP, type IX collagen and matrilin 3 (MATN3) are retained in the rER cisternae and form an ordered intracellular matrix.^{35,36,40,43} The presence of an insoluble intracellular matrix may explain why this material remains in the growth plate even after chondrocyte death.⁴³ Interestingly, mutations in MATN3 and type IX collagen, which are co-retained with mutant COMP (MT-COMP), also cause MED suggesting a common cellular pathology.^{21,44–47}

Information obtained from studies of the PSACH growth plate has been used to develop a pathophysiologic model of PSACH.⁴⁸ In PSACH, 3% or less of the COMP pentamers are postulated to contain all wild-type COMP (WT-COMP) subunits, so that most of the pentamers contain one or more mutant subunits.⁴¹ These mutant subunits are unable to fold properly and exert a dominant negative effect.^{41,48} Secondly, the mutant COMP cannot be processed through the rER to the Golgi and be-

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Address reprint requests to Jacqueline T. Hecht, Ph.D., Department of Pediatrics, University of Texas Medical School, 6431 Fannin, Houston, TX 77030. E-mail: Jacqueline.T.Hecht@uth.tmc.edu.

comes trapped along with other ECM proteins, initiating premature formation of matrix.^{16,24,29,32,33,39,40,43,48} The rER enlarges in response to the expanding retained matrix, eventually encompassing most of the cell volume and causing premature chondrocyte death.

Other PSACH growth plate findings include a large resting zone with fewer chondrocytes in the proliferative and hypertrophic zones.⁴³ The growth plate is divided into the following three zones: resting; proliferative; and hypertrophic. The chondrocytes in the resting zone are relatively inactive and supply the proliferative zone with chondrocytes as the cells in the proliferative zone divide and mature. In the hypertrophic zone chondrocytes enlarge and produce calcified cartilage that will be later converted into bone. The PSACH growth plate has few chondrocytes in the proliferative and hypertrophic zones that produce the cartilage anlagen necessary for linear bone growth. Chondrocytes that are normally found in ordered columns appear in clusters in the PSACH growth plate with many acellular gaps between clusters.⁴³ Many dead chondrocytes and cell remnants are present throughout the growth plate suggesting that the acellular gaps result from the loss of chondrocytes.⁴³ Depletion of chondrocytes from the growth plate results in decreased linear growth and growth retardation that manifests as dwarfism.^{37,42,49}

Although the outcome of COMP intracellular accumulation is well described, very little is known about the *in vivo* progression of the disease pathology. Most of the information about the disease pathology is from cross-sectional growth plate biopsy studies that provide information at only one time point, thus demonstrating the need for a physiological PSACH mouse model.⁴⁹ While several *in vivo* PSACH mouse models have been reported, none adequately mimics the characteristic PSACH chondrocyte pathology.^{49–51} An *in vivo* model that recapitulates the disease pathology is important to define the establishment and progression of the chondrocyte pathology, including information about the pre- and postnatal timing of protein accumulation and its effect on joint formation. This information cannot be obtained from human PSACH patients or from the *in vitro* chondrocyte cartilage models that do not emulate the growth plate. Here, we describe a transgenic inducible mutant COMP mouse model that recapitulates the cellular hallmark of PSACH pathology.

Materials and Methods

Generation of Constructs

Two plasmids, pTRE-COMP and pTET-On-ColIII, were generated and used to produce bigenic WT-COMP and MT-COMP mice. The pTRE-COMP construct contains the coding sequence of human COMP gene driven by the tetracycline responsive element (TRE) promoter. The WT and MT del469D constructs were generated by removing the COMP coding sequence with the FLAG tag (DYKDDDDK) from COMP-pcDNA 3.1 and placing it into the complementary NheI and EcoRV sites of pTRE-Tight vector (Clontech Inc.,

Mountain View, CA).²⁵ The orientation of the construct was confirmed by PCR and final constructs were verified by sequencing. The pTET-On-ColIII construct contains the tetracycline transactivator (rtTA) coding sequence driven by a type II collagen promoter.⁵² A fragment from pTET-On (rtTA) was amplified and NotI and SpeI restriction endonuclease sites were inserted by using the following primers: pTET-On-NotI 3'-CCGCGGCCGCGGATTTCATGTCTAGATTAGATAAAAGTAAAGTG-5' and pTET-On-SpeI 3'-CTCCATACTAGTTTCCTTAGCTCCTGAAAATCTCGCC-5'. The rtTA-SV40 polyA fragment was inserted into the NotI and XbaI restriction endonuclease sites of pNK185 vector replacing the β -galactosidase gene and verified by sequencing.⁵² Immunoblot analysis using antibodies directed against COMP or FLAG showed that the apparent molecular mass of WT- and MT-COMP-FLAG expressed from these plasmids was identical to the molecular mass of human COMP (data not shown). Vector sequences were removed from both constructs before injection into C57BL/6 mouse blastocysts.

Generation of Bigenic Mice

Founder mice were screened for the transgenes by using tail biopsy DNA and PCR primers specific for each transgene. COMP primers, 5'-GGAAGGGAGATCGTGACAGAC-3' and 5'-CTCGGCCACAGCACGGAAGG-3', were used to genotype the TRE-COMP transgenic mice. Custom commercial assay was used to determine whether animals were homozygous or heterozygous (Embark Scientific, Austin, TX). rtTA primers, 5'-ATGCCCTTGAATTGACGAGTACGG-3' and 5'-CGAGGCTTGACAGGATCATAATCAG-3', were used to genotype the Col II-rtTA transgenic animals and commercial assay was used to determine whether animals were homozygous or heterozygous (Charles Rivers, Wilmington, MA). Standard breeding was used to generate bigenic animals. Mice were administered 100 or 500 ng/ml doxycycline (DOX) (via drinking water + 5% sucrose) pre- and postnatally. Various organs (heart, lung, brain, and liver) from progeny of three positive lines were tested for transgenic human COMP expression by using real-time quantitative RT-PCR (qRT-PCR) as a means to verify tissue specificity of expression. No detectable expression was found in any of these tissues (data not shown). qRT-PCR of embryonic day 15 mouse embryos showed steady-state levels of human WT- and MT-COMP were approximately four to six-fold higher than steady-state levels of mouse COMP mRNA (data not shown).

Histology and Immunohistochemistry

Hind limbs from 1-month-old MT-COMP, WT-COMP, and C57BL/6 control mice were collected and tibial growth plates were analyzed. The limbs were fixed in 95% ethanol for COMP, calreticulin (CRT), FLAG, MATN3, types II and IX collagens immunostaining, or in 10% formalin for terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining. CRT is a resident rER protein and was used here to indicate whether protein accumulation was occurring in the subcellular rER com-

Table 1. Reagents Used in Growth Plate Immunostaining

Co-staining Parameters	Primary	Secondary	Tertiary
COMP and CRT co-staining COMP (human and mouse)	Rabbit polyclonal 1:300 (Kamiya)	Anti-rabbit Alexa fluor 488 1:400 (Molecular Probes)	—
CRT (ER)	Goat polyclonal calregulin (C-17) (Santa Cruz)	Anti-goat Alexa fluor 594 1:400 (Molecular Probes)	—
COMP and FLAG co-staining COMP (human and mouse)	Rabbit polyclonal 1:300 (Kamiya)	Anti-rabbit Alexa fluor 488 1:400 (Molecular Probes)	—
FLAG (Tg. human COMP)	Goat polyclonal FLAG 1:200 (Abcam)	Anti-goat Alexa fluor 594 1:400 (Molecular Probes)	—
COMP, Collagen Type IX, MATN3, and Collagen Type II co-staining Step I			
COMP (human and mouse)	Rabbit polyclonal 1:300 (Kamiya)	Anti-rabbit Alexa fluor 488 1:500 (Molecular Probes)	—
Collagen Type IX	Mouse monoclonal 1:300 (Iowa Hybridoma Bank)	Anti-mouse Alexa fluor 594 1:500 (Molecular Probes)	—
MATN3	Goat polyclonal 1:400 (R&D Systems)	Biotinylated anti-goat 1:600 (Chemicon)	Streptavidin Alexa fluor 405 (Molecular Probes)
Step II			
Collagen Type II	Goat polyclonal 1:200 (Santa Cruz)	Zenon Alexa fluor 647 goat IgG labeling kit (Molecular Probes)	—

Suppliers: Kamiya, Thousand Oaks, CA, Santa Cruz, Santa Cruz, CA, Abcam, Cambridge, MA, Iowa Hybridoma Bank, Iowa City, IA, and R&D Systems, Minneapolis, MN.

partment. The COMP polyclonal antibody cross-reacts with both endogenous mouse and transgenic human COMP (Table 1). FLAG specifies the recombinant human WT- or MT-tagged COMP. All samples were decalcified for 2 days in immunocal formic acid decalcifier solution (American Master Tech Scientific, Inc., Lodi, CA) and embedded in paraffin.

For immunostaining, 5 μ m sections were digested with pepsin A (Sigma-Aldrich, St. Louis, MO) at 1 mg/ml in 0.1 N HCl for 30 minutes at room temperature. The sections were rinsed in distilled H₂O and then washed in PBS containing 0.05% Tween-20 (PBS-T) for 5 minutes, followed by 20 minutes of blocking incubation with 10% donkey serum in PBS-T. All sections were then incubated overnight at 4°C with the primary antibodies listed in Table 1. Primary antibodies were detected by using fluorochrome conjugated secondary (and tertiary) antibodies listed in Table 1. Co-localization immunostaining was performed as previously described.^{35,36} Coverslips were mounted by using ProLong Gold anti-fade reagent (Molecular Probes, Carlsbad, CA). TUNEL staining was performed following the manufacturer's protocol (Promega, Madison, WI). DAPI (4'-6-Diamidino-2-phenylindole) pro-long gold anti-fade reagent staining and mounting solution was used to detect nuclei (Molecular Probes). All sections were examined and images were captured by using an Olympus (Melville, NY) BX51 inverted microscope attached to a Spot RT camera version 4.6 and images were merged by using Adobe Photoshop. To ensure consistency and so that samples could be compared, immunostaining of all samples was performed simultaneously and identical exposure times were used.

Human Chondrocyte Nodule Preparation

Primary human costochondral chondrocytes were collected from patients undergoing pectus excavatum surgery, isolated and cultured in a 3-D environment as previously described.^{2,36}

Protein Modeling of Subcellular Structure

Deconvolution microscopy was performed as previously described.³⁶

Results

Growth Plate Analysis of MT-COMP Mice

The bigenic WT- or MT-COMP lines were evaluated at 1 month of age, which corresponds to human adolescence and an age when the PSACH chondrocyte pathology is present.^{26,37,43,53} All analyses were performed on mice that were heterozygous for either the bigenic WT- or MT-COMP transgenes because the human PSACH disease is caused by a dominant COMP mutation.^{22,41} We first determined whether there were any growth plate abnormalities due to expression of the transgenes. Upper tibial growth plate sections were stained with H&E and were compared with the background C57BL/6 control strain (Figure 1, E and F). Growth plate organization was disrupted in mice expressing MT-COMP with the disorganization increasing as doxycycline (DOX) concentration increased from 100 to 500 ng/ml (Figure 1, A and B). Few hypertrophic chondrocytes were observed in the

Figure 1. Growth plate abnormalities are associated with overexpression of MT-COMP. H&E staining of growth plates from MT-COMP (A, B) and WT-COMP (C, D) mice at 1 month that were fed 100 or 500 ng/ml of DOX, respectively. C57BL/6 age-appropriate control growth plates exposed to 0 and 500 ng/ml DOX are shown in E and F. Increasing concentration of DOX is associated with more chondrocyte disorganization and acellular areas in MT-COMP growth plate. Scale bar = 25 μ mol/L.

MT-COMP growth plates (Figure 1, A and B). In contrast, mice expressing WT-COMP (Figure 1, C and D) showed more chondrocytes, which were better organized into chondrocyte columns and a wider growth plate (Figure 1). In addition, RT-PCR using human COMP-specific primers verified expression of the transgenic human MT- and WT-COMP in mice at embryonic day 15 (data not shown). Taken together, these observations suggest that MT-COMP expression is associated with fewer growth plate chondrocytes, disruption of chondrocyte columns, thinner growth plate, fewer hypertrophic chondrocytes and this cellular phenotype is modulated by DOX dosage.

MT-COMP Expression Is Associated with Increased Apoptosis

Several studies of MT-COMP suggest that apoptosis is increased in PSACH chondrocytes because intracellular retention negatively impacts chondrocyte function.^{30,43,54} To determine whether expression of MT-COMP stimulates a higher level of apoptosis in the growth plate, TUNEL staining was performed. The cells in each field were localized by using DAPI staining (Figure 2, B, D, F, and H). As shown in Figure 2, E and G, similar positive TUNEL staining in the MT-COMP growth plates exposed to 100 and 500 ng/ml of DOX is observed, whereas the WT-COMP and C57BL/6 control growth plates show neg-

Figure 2. Increased apoptosis in growth plate chondrocytes is associated with mutant COMP expression. TUNEL staining was performed on growth plates from 1-month-old mice fed 100 or 500 ng/ml of DOX. DAPI staining is shown to indicate all of the chondrocytes in the growth plate section (B, D, F and H). MT-COMP expression is associated with an increase in the number of TUNEL positive cells (E and G) in the growth plate compared with relatively few TUNEL positive cells in the WT-COMP (C) and the C57BL/6 control (A) mice. Scale bar = 25 μ mol/L.

ligible staining (Figure 2, A and C). Similar levels of apoptosis were observed in three different MT-COMP mouse lines. These results suggest that intracellular retention of MT-COMP induces increased apoptosis and this association is similar to that seen in human PSACH chondrocytes. Since exposure to 100 and 500 ng/ml of DOX elicits similar levels of apoptosis in the growth plates, the lower level of DOX was used in following studies to minimize any possible adverse physiological effects of high levels of COMP.

Human MT-COMP Expression Results in COMP Retention in Growth Plate Chondrocytes

We next determined if MT-COMP is retained in the rER of growth plate chondrocytes of the mice as observed in human PSACH.² In these studies, the COMP antibody recognizes both the endogenous murine and transgenic human COMP-FLAG protein, whereas the FLAG antibody binds only to the transgenic WT- or MT-COMP-FLAG protein. To delineate the rER, CRT, the resident chaper-

Figure 3. Mutant human COMP accumulates in the rER of mouse chondrocytes. Growth plates from MT-COMP (A–C), WT-COMP (D–F) and C57BL/6 (G–I) mice at 1 month were fed 100 ng/ml of DOX and were immunostained for COMP (green) and CRT (red) as described in the methods and the images were merged. CRT is a resident ER chaperone protein and the COMP antibody recognizes both transgenic human COMP and endogenous mouse COMP. Mice expressing MT-COMP show intracellular retention of COMP co-localizing with CRT indicating that they are in the rER of the growth plate chondrocytes (C). No co-localization is observed with WT-COMP and CRT (F). Scale bar = 20 μ mol/L.

one protein of the rER, was used as a marker.⁵⁵ As shown in Figure 3A, while some COMP is found in the ECM, strong intracellular localization is observed in the MT-COMP growth plate. When the COMP and CRT images were merged, co-localization was observed indicating that COMP is being retained in the rER (Figure 3, A–C). In contrast, neither human WT-COMP (Figure 3, D–F) nor endogenous mouse COMP (Figure 3, G–I) is retained in the chondrocyte, and both show similar pericellular localization.

Since human WT- and MT-COMP expressed in these animals has a FLAG moiety, FLAG immunostaining was used to confirm that MT-COMP was being retained and WT-COMP was being secreted. As shown in Figure 4, A–C, co-localization of COMP and FLAG was observed indicating that MT-COMP is being retained. Extracellular COMP was observed and did not co-localize with FLAG showing that endogenous mouse COMP was exported to the ECM (Figure 4C). In contrast, WT-COMP co-localized with FLAG in the ECM (Figure 4, D–F) indicating secretion to the ECM. This latter pattern is similar to that observed in the control C57BL/6 mice (Figure 4, G–I).

Figure 4. COMP and FLAG co-localize in the transgenic mouse growth plate. Growth plates from MT-COMP (A–C), WT-COMP (D–F), and control C57BL/6 (G–I) mice at 1 month were fed 100 ng/ml of DOX and were immunostained for COMP (green) and FLAG (red) as described in the methods. COMP antibody binds to both endogenous mouse and recombinant human COMP, whereas the antibody to FLAG is specific for the recombinant human COMP-FLAG protein. Endogenous and recombinant COMP expression is extracellular in the WT-COMP (D–F) and control mice (G–I). Both endogenous and recombinant COMP expression is observed extracellular and intracellular compartments of the MT-COMP mouse growth plate (A–C). Intracellular COMP is recognized by the FLAG antibody indicating that the retained material is recombinant MT-COMP (B and C). There appears to be less MT-COMP in the matrix as compared with the WT-COMP (B and E). Scale bar = 50 μ mol/L.

Type IX Collagen and MATN3 Are Retained with MT-COMP in rER of Growth Plate Chondrocytes

Both type IX collagen and MATN3 are retained in the rER of human PSACH chondrocytes.^{35,36,38,43} To assess whether MT-COMP affects the secretion of type IX collagen and MATN3, these proteins were immunolocalized in the mouse growth plates. As shown in Figure 5, A–D, MT-COMP immunolocalized with type IX collagen and MATN3 (white fluorescence foci in merge D) indicating that all three proteins are being retained in the rER. Less type IX collagen and MATN3 were found in the MT-COMP ECM (Figure 5, B and C). In comparison, human WT- (Figure 5, E–H) and endogenous mouse COMP (Figure 5, I–L) co-localized in the ECM with type IX collagen and MATN3.

Intracellular Matrix Is Formed in the rER of MT-COMP Mouse Growth Plate Chondrocytes

We have recently shown that mutant COMP retained in the rER of human chondrocytes supports the formation of intracellular matrix.³⁶ This intracellular matrix network,

Figure 5. MT-COMP co-localizes with type IX collagen and MATN3. Growth plates from 1-month WT-COMP, MT-COMP, and control mice were sectioned and stained for COMP (green), type IX collagen (red), and MATN3 (blue) as described in the methods. All mice were fed 100 ng/ml of DOX. In the MT-COMP growth plate, MATN3 and types II and IX collagen are observed intracellularly (**A–D**), whereas these proteins are observed in the ECM of the WT-COMP and control growth plates, respectively (**E–H** and **I–L**). Both WT-COMP and MT-COMP growth plates are disorganized compared with the C57BL/6 control (**A–L**). Scale bar = 50 μ mol/L.

comprised of mutant COMP, type IX collagen and MATN3, was organized around a type II pro-collagen core. This novel finding was previously shown in human PSACH chondrocytes with different COMP mutations.³⁶ To determine whether a similar mechanism occurs in these mice, we used the same fluorescent deconvolution microscopy technique to model the architecture of the intracellular COMP, types II and IX collagen, and MATN3 proteins.³⁶ The images shown in Figure 6 are from three different rER chondrocyte cisternae, two from MT-COMP mice (Figure 6, A–J) and one from a human PSACH patient (Figure 6, K–O). The results of the modeling in all three cisternae show that COMP, type IX collagen, and MATN3 co-localize and form a similar intracellular matrix surrounding a type II pro-collagen core (Figure 6). This result demonstrates that expression of MT-COMP supports intracellular matrix formation in the mouse growth plate chondrocytes and mimics the human PSACH chondrocyte disease pathology that we have previously described.³⁶

Discussion

In this study, we show that the characteristic human PSACH chondrocyte pathology of rER intracellular retention of COMP, types II and IX collagens and MATN3, premature intracellular ECM assembly and growth plate abnormalities are recapitulated in growth plate chondrocytes of transgenic mice expressing MT-COMP. Cartilage-specific expression of MT-COMP with the D469del mutation was accomplished by using heterozygous bigenic

TET-inducible transgenes. Unlike other reported mouse PSACH models, this one manifests the dominant negative effect of COMP mutations and has many of the cellular hallmarks of human PSACH chondrocytes.^{2,26,36,38,39,43}

The MT-COMP chondrocyte phenotype resulting from intracellular retention of MT-COMP and other ECM proteins is present throughout the growth plate by 4 weeks of age when the mice are fed DOX pre- and postnatally. In contrast, WT-COMP transgenic mice treated similarly do not demonstrate this chondrocyte phenotype because the endogenous mouse and transgenic human COMP is exported to the ECM. Three mouse models expressing mutant COMP have been reported; however, none have shown massive intracellular retention of MT-COMP in growth plate chondrocytes or any significant growth plate pathology.^{49–51} One model showed only minimal intracellular retention in the wild-type background expressing endogenous COMP but intracellular retention increased when mutant COMP was expressed in the COMP null background.⁵¹ No intracellular MT-COMP was found in two other models.^{49,50} Finally, a very mild MED phenotype was reported in a mouse expressing T585M mutant COMP from homozygous knock-in COMP mutation.⁵⁰ Based on these mouse models, we reasoned that expression levels of MT-COMP need to be very high to achieve the PSACH chondrocyte pathology in the context of murine physiology. Using an inducible system to facilitate MT-COMP expression, we observed significant intracellular retention of MT-COMP in the presence of endogenous wild-type mouse COMP, a phenotypic result that mimics the heterozygous human PSACH condition. In

Figure 6. Intracellular matrix is present in MT-COMP growth plate chondrocytes. Images of rER were captured by using fluorescence deconvolution microscopy and modeled as described in methods. Images are shown in solid or wire-frame renditions with type II collagen (yellow), type IX collagen (red), COMP (green), and MATN3 (blue). The retained proteins in the rER have a similar intracellular matrix structure (**E**, **J**, and **O**) composed of a central core of type II procollagen (**A** and **F**) that is surrounded by COMP (**B** and **G**), type IX collagen (**C** and **H**), and MATN3 (**D** and **I**). This intracellular matrix pattern is identical to that which we previously described³⁶ and show again in human PSACH chondrocytes with the D469del mutation (**K-O**).

comparison, we show that induced human WT-COMP expression has an extracellular matrix distribution and no intracellular retention of MATN3 and type IX collagen. Finally, we demonstrate that human MT-COMP induces apoptosis in the mouse growth plate.

Previous *in vitro* human chondrocyte studies have shown that types II and IX collagen and MATN3 are co-retained with mutant COMP, and interact prematurely thereby initiating matrix assembly in the rER.^{35,36} This novel observation was found in PSACH chondrocytes having different COMP mutations. We now demonstrate that human MT-COMP in the mouse background interacts with endogenous mouse collagens and MATN3. This premature interaction results in the same intracellular chondrocyte matrix architecture in the rER cisternae as that found in human PSACH chondrocytes (Figures 5 and 6). These results confirm our previous observation that MT-COMP appears to catalyze intracellular ECM matrix assembly, suggesting that this is an important and significant role of COMP in the matrix.^{36,43}

We had previously suggested that linear growth in PSACH was significantly impacted by the loss of chondrocytes in the growth plate.^{49,56} This was based on the *in vitro* observation that apoptosis was increased in patient chondrocytes expressing different COMP mutations.^{29,30,54,57,58} We have now shown that apoptosis is

significantly increased in growth plate chondrocytes expressing MT-COMP (Figure 2) and that there are acellular areas in these growth plates (Figure 1).

In other studies, we have shown that complete loss of COMP has minimal effect on the growth plate while loss of type IX collagen results in widened growth plates particularly at 1 month of age.⁵⁹ Compound loss of these ECM proteins results in limbs that are 11% shorter than the wild-type control mice. In the MT-COMP mouse, we find that there is diminished type IX collagen, and MATN3 in the ECM, which is due to intracellular retention and matrix assembly of these proteins, leading to their relative deficiency in the ECM. Based on these and previous results, the loss of chondrocytes, as well as the relative deficiency of these proteins in the matrix, may synergistically contribute to the profound short stature seen in PSACH. We can now assess the roles of COMP, type IX collagen, and MATN3 by crossing our MT-COMP mouse into these null mouse lines and evaluate the effect of MT-COMP in these single and compound knock-out mice. This will allow determination of the role of these proteins in matrix assembly.

The clinical phenotype of PSACH and MED can be quite variable. With our novel PSACH MT-COMP mouse model we can modulate the PSACH cellular phenotype on a homogenous genetic background, allowing us to

determine whether clinical phenotypic variability is due to different modifier genes or the amount of MT-COMP trapped in the rER and/or apoptosis in the growth plate. Additionally, the ability to regulate MT-COMP expression temporally by the addition or withdrawal of DOX at different stages of development will offer insight into the appropriate timing of delivery of future therapies.

Global expression of MT-COMP may have consequences beyond the PSACH phenotype and restricting expression to cartilage circumvents the potential side effects of inappropriate MT-COMP expression. Using this tissue-specific model, the PSACH phenotype can be examined pre- and postnatally to assess changes throughout development and at various ages. Furthermore, the effect of exercise on joints expressing MT-COMP can be studied in detail as the skeletal elements mature since joint disease/erosion is a serious and painful long-term problem in human PSACH. In summary, this PSACH mouse model displays the human PSACH phenotype *in vivo* and will allow experiments to be preformed that are not possible with *in vitro* systems.

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